

**THE 14TH INTERNATIONAL SYMPOSIUM ON BIOCONTROL  
AND BIOTECHNOLOGY**

**Saint-Petersburg – Pushkin, Russia**

**November 6 -9, 2016**

**ABSTRACTS AND PROGRAM**

**organized by**

**All-Russian Institute of Plant Protection**

**All-Russian Institute of Agricultural Microbiology**

**Harbin Institute of Technology (China)**

**King Mongkut's Institute of Technology Ladkrabang (KMITL, Thailand)**

**THE 14TH INTERNATIONAL SYMPOSIUM ON BIOCONTROL AND  
BIOTECHNOLOGY**

Abstracts are published in author's edition

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## Program

	<b>6 NOVEMBER 2016,</b> <b>Management Training Center, Radishcheva str., 4, Pushkin</b>
	<b>White hall</b>
<b>16.00-21.00</b>	Registration and poster hang up
<b>17.30-18.00</b>	<i>Welcome speeches</i>
	Foreword of Organizers
	<i>Assoc. Prof. Dr. Dusanee Thanaboripat</i> , Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Thailand
	<i>Prof. Vladimir Pavlyushin</i> , Director of All-Russian Institute of Plant Protection
18.00-20.00	Deserts and drinks
18.00-19.00	Concert
19.00-20.00	Excursions in Kochubey palace
20.00-20.15	Speech of <i>Prof. Yang Qian</i> , Harbin Institute of Technology, China,
20.15-20.30	Remarks of Organizers

	<b>7 NOVEMBER 2016,</b> <b>Management Training Center, Radishcheva str., 4, Pushkin</b>
<b>8.30-9.00</b>	Registration
	PLENARY SESSION, Congress-hall
9.00-9.30	<i>Prof. Vladimir Pavlyushin</i> , Director of All-Russian Institute of Plant Protection <b>«Breaking approaches in biological control»</b>
	<i>Endophytes</i>
9.30-10.00	<i>Dr. Adeline Ting</i> , Monash University, Malaysia, <b>«Endophytes as biocontrol agents for plant diseases: prospect, challenges and improvements»</b>
10.00-10.30	<i>Dr. Vladimir Chebotar</i> , All-Russian Research Institute for Agricultural Microbiology, Saint-Petersburg, <b>“Endophytic bacteria as promising biotechnological resource for biocontrol of phytopathogens”</b> ,
10.30-11.00	<i>Dr. Chokchai Kittiwongwattana</i> , King Mongkut's Institute of Technology Ladkrabang (KMITL), Thailand, <b>«Biodiversity and plant-growth-promoting activities of endophytic bacteria from rice (<i>Oryza sativa</i>)»</b>
11.00-11.30	Coffee break
	<i>Chemistry and biotechnology</i>
11.30-12.00	<i>Prof. Dr. Hartmut Laatsch</i> , University of Göttingen, Germany <b>«Computer meets Chemistry - Modern Aspects in Natural Products Chemistry and Biotechnology»</b>
12.00-12.30	<i>Dr. Vitaliy Dzhavakhia</i> , Institute of Phytopathology, Moscow <b>«Novel natural and synthetic blockers of aflatoxin B1 and melanin biosynthesis in <i>Aspergillus flavus</i>»</b>
12.30-13.00	<i>Prof. Hongmei Zeng</i> , Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing <b>«Two Novel Protein Elicitors from <i>Magnaporthe oryzae</i> Trigger Defense Response and Improve Plant Growth in Rice»</b>
13.00-14.00	Lunch

14.00-14.30	Poster session
	<i>Biocontrol</i>
14.30-15.00	<i>Prof. Viktor Glupov</i> , Institute of Systematics and ecology of animals, Novosibirsk, « <b>Immunosuppressive technologies for improvement of bioinsecticide performance</b> »
15.00-15.20	<i>Dr. Natalia Belyakova</i> , All-Russian Institute of Plant Protection, « <b>Biological control of pests in the modern greenhouses for protection of the virus-free potato</b> »
15.20-15.40	<i>Prof. Dr. Lyubov' Rimareva</i> , Federal Research Centre of Nutrition, Biotechnology and Food Safety, Moscow, « <b>New biological preservatives for storage of fruit and vegetable products</b> »
15.40-16.00	<i>Dr. Nattawut Rungjindamai</i> , KMITL, Thailand, « <b>Biodiversity of Ophiocordyceps nutans collected in Thailand</b> »
16.00-16.20	<i>Dr. Alexander Berestetskiy</i> , All-Russian Institute of Plant Protection « <b>Problems of development of bioherbicides based on plant pathogenic fungi and their metabolites</b> »
16.20-16.40	Coffee break
	<i>Plant pathogen detection and control</i>
16.40-17.00	<i>Dr. Alexander Golikov</i> , "Genbit" LTD, Moscow « <b>A matrix approach to simultaneous detection of multiple pathogens of potato by real-time PCR</b> »
17.00-17.20	<i>Prof. Alexander Ignatov</i> , Research Center "PhytoEngineering" LLC, Rogachevo, Moscow region, Russia, « <b>Emergence and spreading of Dickeya solani &amp; D. dianthicola on potato in Russia, and search for control measures</b> »
17.20-17.40	<i>Mr. Sergey Shebyakovsky</i> , All-Russian Institute for Biological Control, Krasnodar, « <b>Study of the influence disease resistance inducers on the development of wheat leaf rust under greenhouse and field conditions</b> »
17.40-18.00	<i>Dr. Vladimir Sendetskiy</i> , Podilsky State Agro-Technical University, Kamyanets'-Podilsky, Ukraine, « <b>The fight against pests during the degradation of straw in conjunction with the sowing of green manure</b> »
18.00-19.00	Poster session
19.00-20.00	Dinner

	<b>8 NOVEMBER 2016,</b> <b>Hotel "Natali", Malaya str., 56A, Pushkin</b>
	<i>Strain and cultivar improvement</i>
9.00-9.30	<i>Prof. Eddo Rugini</i> , Università degli Studi della Tuscia, Italy « <b>Genetic improvement of olive (Olea europaea L.) by conventional and in vitro biotechnology methods</b> »
9.30-10.00	<i>Dr. Sumet Treesaksri</i> , KMITL, Thailand, « <b>Callus Induction and Culture from Midrib of Anthurium cv. Chok Klao Chan by transverse Thin Cell Layer Technique (tTCL)</b> »
10.00-10.20	<i>Prof. Anatoliy Anisimov</i> , Saint-Petersburg Agrarian University,

	<b>«Entomophages and akariphages artificial selection is a promising way to improve their effectiveness in biological control of insect and might pests»</b>
10.20-10.40	<i>Prof. Dr. Tatiana Belozerskaya, Dr. Natalia Groza</i> , Federal State Institution “Federal Research Centre “Fundamentals of Biotechnology” of the Russian Academy of Sciences, Moscow, and Moscow Technological University Institute of Fine Chemical Technologies, <b>«Oxylipins in fungal development»</b>
10.40-11.00	Sponsor’s Presentation. <i>Mr. Eugeniy Vakhrushev</i> , AWTtech LTD, <b>«Technologies of artificial climate in biotechnology practice»</b>
11.00-11.20	Coffee break
	<i>Bioremediation</i>
11.20-11.50	<i>Prof. Yang Qian</i> , Harbin Institute of Technology, China, <b>“Biotechnology and Industry Waste Recycle”</b>
11.50-12.10	<i>Dr. Olga Maslova</i> , The M.V. Lomonosov Moscow State University, <b>«Stabilized forms of enzyme His<sub>6</sub>-OPH in hydrolysis of organophosphorus pesticides and N-N-acyl homoserine lactones»</b>
12.10-12.30	<i>Dr. Panutda Yodsang</i> , KMITL, Thailand, <b>«Molasses wastewater treatment by Bumileriopsis peterseniana isolated from ethanol-production wastewater»</b>
12.30-12.50	<i>Dr. Olga Kasaikina</i> , Semenov Institute of Chemical Physics RAS Moscow, <b>«Catalytic conversion of plant biomass into useful products»</b>
12.50-13.10	<i>Dr. Ivan Melnik</i> , Association "Bioconversion", Ivano-Frankivsk, the Ukraine, <b>«New biotechnology in modern agriculture of Ukraine»</b>
13.10-14.00	Lunch
	<i>Agro- and Food Biotechnology</i>
14.00-14.20	<i>Prof. Elena Serba</i> , Federal Research Centre of Nutrition, Biotechnology and Food Safety, Moscow, <b>«Perspective biotechnological products based on the direction conversion of secondary bioresources of agro-industrial complex»</b>
14.20-14.40	<i>Dr. Alexander Bunchak</i> , Podilsky State Agro-Technical University, Kamyanskyi-Podilsky, Ukraine, <b>«Biotechnology for organic products without the usage of pesticides and fertilizers»</b>
14.40-15.00	<i>Ms. Viktoria Poplevina</i> , Kuban agricultural technologies LTD, Krasnodar, Russia, <b>«Characteristics and critical points of biotechnological process small-volume production»</b>
15.00-15.20	<i>Mr. A.E.S. Kasem</i> , Saint-Petersburg Agrarian University, <b>“The effects of the last stages of nymphs <i>Podisus maculiventris</i> (Hemiptera: Aspionae) feeding adult of grain moth on predatory bug reproductive potential”</b>
15.20-15.40	Sponsor’s Presentation. <i>Ms. Kristina Yakovleva</i> . The Dia-M Company. <b>“Highly effective routine fermentation”</b>
15.40-16.00	Coffee break
	<i>Biorationals</i>
16.00-16.30	<i>Prof. Dr. Hartmut Laatsch</i> , University of Göttingen, Germany <b>«Dereplication Techniques using Databases»</b>
16.30-16.50	<i>Dr. Olga Kulikova</i> , Institute of Organoelement Compounds, Moscow, <b>«Biologically active compounds isolated from <i>Allium sativum</i> L. as a base for development of fungicides»</b>
16.50-17.10	<i>Dr. Ekaterina Poluektova</i> , All-Russian Institute of Plant Protection, <b>«Isolation, biological characterization and herbicidal potential of phytotoxins from <i>Paraphoma</i> sp. 1.46, a pathogen of <i>Cirsium arvense</i>»</b>
17.10-17.30	<i>Dr. Ekarat Detsri</i> , KMITL, Thailand, <b>«Synergistic antimicrobial activity of two-dimensional nanostructures of chitosan modified silver nanoparticles</b>

	<i>on Staphylococcus aureus»</i>
17.30-17.45	<i>Dr. Tatiana Khurshkainen</i> , Institute of Chemistry, Syktyvkar « <b>Technology of isolation of extractive matter from pine needles and production of biopreparations»</b>
17.45-18.00	<i>Ms. Anna Dalinova</i> , All-Russian Institute of Plant Protection, « <b>Structure and biological activity of secondary metabolites of <i>Alternaria sonchi</i> – a pathogen of sowthistle (<i>Sonchus arvensis</i>)»</b>
18.00-18.15	<i>Ms. Oratai Sukcharoen</i> , KMITL, Thailand, « <b>Contact and fumigant activities of <i>Cinnamomum porrectum</i> (Roxb.) Kosterm against aflatoxigenic <i>Aspergillus flavus</i> and <i>Aspergillus parasiticus</i>»</b>
18.15-18.30	<i>Ms. Karina Zinatullina</i> , N.N.Semenov Institute of Chemical Physics RAS « <b>Effect of immobilization on cellulose basis on the properties of biological active compounds»</b>
18.30-19.00	Clothing ceremony/concluding remarks
19.00-20.00	Dinner
20.00-22.00	Bowling party

	<b>9 NOVEMBER 2016,</b>
	<b>Excursion day Departure</b>

## **ORAL PRESENTATIONS**



# Salt tolerance of potato genetically engineered with *Atriplex canescens* BADH gene driven by 3 copies of CAMV35s promoter

Arfan Ali

*Institute of Molecular Biology & Biotechnology, The University of Lahore/ Centre of Excellence in Molecular Biology, University of the Punjab Lahore*

## Abstract

Potato (*Solanum tuberosum* L.) is ranked among the top leading staple foods in the world. Salinity adversely affects potato crop yield and quality. Therefore, increased level of salt tolerance is a key factor to ensure high yield. The present study focused on the agrobacterium-mediated transformation of *Atriplex canescens* betaine aldehyde dehydrogenase (BADH) gene, using single, double and triple CAMV35s promoter to improve salt tolerance in potato. Detection of seven potato lines harboring BADH gene, followed by identification of T-DNA insertions, determination of transgenes copies no through Southern Hybridization and quantification of BADH protein through Enzyme Linked Immuno Sorbent Assay were considered in this study. The results clearly depict that the salt tolerance of potato was found to be promoter-dependent, as the potato transgenic lines with triple promoter showed 4.4 times more glycine betaine production which consequently leads towards high resistance to salt stress as compared to transgenic potato lines with single and double promoters having least production of glycine betaine. Moreover, triple promoter transgenic potato lines have also shown lower levels of H<sub>2</sub>O<sub>2</sub>, malondialdehyde (MDA), relative electrical conductivity, high proline and chlorophyll content as compared to other two lines having a single and double promoter. Insilco analysis also confirmed that *Atriplex canescens* BADH has the tendency to interact with sodium ions and water molecules. Taken together these facts it can be concluded that over-expression of BADH under triple CAMV35S promoter with more glycine betaine, chlorophyll & MDA contents, high relative quantities of other metabolites results in an enhanced level of salt tolerance in potato.

**Keywords:** Potato, *Solanum tuberosum*, BADH, Salt tolerance, *Atriplex canescens*, and CAMV35s promoter

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\*Corresponding author: E-mail address: [arfan.ali@cemb.edu.pk](mailto:arfan.ali@cemb.edu.pk)

# Entomophages and akariphages artificial selection is a promising way to improve their effectiveness in biological control of insect and mite pests

Anisimov A. I.

*St. Petersburg State Agrarian University, Russia*

## Abstract

Currently achievements of biotechnology, particularly genetic engineering, not-withstanding the attempts of artificial restrictions are increasingly being implemented in the areas of human activity, which uses living organisms and their metabolic products. At the same time the traditional methods used by man for improving the living organisms does not exhaust itself, and in some areas are still little used. One such example is the oldest area of insect pests biological control protection of plants against - their suppression by natural enemies. The number of works (at least in the publications) dedicated to their artificial selection is very few. Unfortunately, quite often a detected in the field potentially beneficial entomophages and akariphages not find ways for a wide practical application for biological control of insect and mite pests because of insufficient expression of individual traits, which hampers their economical mass rearing or use under certain climatic conditions. A striking example of this is the sharp decline in the effectiveness of the predatory mite *Phytoseiulus persimilis* A.-H. (is used to control the spider mites) when the temperature in the greenhouses rises above a certain level. Targeted modification or improvement of such limiting characteristics and properties is quite possible with help of artificial selection.

In the present report provides an overview of research works conducted in this direction, are carried on in Russia (USSR) at the last approximately 50 years since the first studies of N.V. Voroshilov on the field of *P. persimilis* [1] and chine green lacewing *Chrysopa sinica* Tjed. [2] (is used to control the aphids) artificial selection to increase their resistance to high temperature; the predatory mite *Metaseiulus occidentalis* Nesbitt to make higher their resistance to insecticides [3], *P. persimilis* again to make higher their resistance to acaricides, to acaricides and high temperature together [4]. We consider the complex of works on successful attempts of predatory beetle *Cryptolaemus montrouziei* Muls (is used for mealybugs control) to increase fecundate at mass rearing [5] and the predatory bug *Macrolophus caliginosus* Wagner (is used for whiteflies control in greenhouses) to make possible their using in cucumber greenhouse [6]. The research works with aphid midge *Aphidoletes aphidimyza* Rond. (is widely used to control the aphids in greenhouses) to increase fecundate and decrease the time of development [7], as well as predatory bug *Orius laevigatus* (is used to control thrips) to increase fecundate during the eggs oviposition on Kalanchoe plants [8]. Particular attention is paid to the complex of work carried out in recent years to create the genetically homogeneous strains (animal varieties) of *P. persimilis* resistant to high temperatures [9].

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\*Corresponding author: E-mail address: anisimov\_anatoly@maul.ru

## Селекция энтомофагов и акарифагов – перспективный путь повышения их эффективности в биологической защите растений от вредителей

А.И. Анисимов

Санкт-Петербургский аграрный университет

В настоящее время достижения биотехнологии, в частности генной инженерии, несмотря на попытки искусственных запретов, все шире внедряются в те сферы деятельности людей, где используются живые организмы и продукты их жизнедеятельности. В тоже время и традиционные приемы совершенствования используемых человеком живых организмов отнюдь себя не исчерпали, а в некоторых областях пока еще мало используются. Одним из таких примеров может служить старейшая область биологической защиты растений от вредителей – их подавление с помощью энтомофагов и акарифагов, число работ (по крайней мере публикаций) по селекции которых очень и очень незначительно. Довольно часто, к сожалению, выявляемые в природе потенциально перспективные энтомофаги и акарифаги не находят путей для широкого внедрения в практику биологической защиты растений в следствии недостаточной выраженности отдельных признаков, что препятствует их экономически оправданному массовому разведению или применению при определенных климатических условиях. Ярким примером этого является резкое снижение эффективности хищного клеща фитосейулюса, применяемого для борьбы с паутинными клещами, когда температура в теплицах поднимается выше определенного предела. Целенаправленное изменение или улучшение таких лимитирующих признаков и свойств вполне возможно селекционно-генетическими методами.

В предлагаемом сообщении дается обзор исследований, проведенных в этом направлении в России (СССР) за последние приблизительно 50 лет, начиная с первых исследований Н.В. Ворошилова [1] по селекции *Phytoseiulus persimilis* А.-Н. и дальневосточной златоглазки - *Chrysopa sinica* Tjeder [2] на термоустойчивость, хищного клеща *Metaseiulus occidentalis* Nesbitt [3] на устойчивость к инсектицидам, фитосейулюса на устойчивость к акарицидам и комплексную устойчивость к акарицидам и повышенной температуре [4]. Рассматриваются комплекс работ по успешные попытки селекции хищного жука *Cryptolaemus montrouzie* Muls., применяемого для борьбы с червецами и пульвинариями, на повышение плодовитости [5] и хищного клопа - *Macrolophu caliginosus* Wagner, применяемого для борьбы с белокрылкой в теплицах, по признаку воспроизводимости на огурцах [6]. Анализируются работы по селекции хищной галлицы *Aphidoletes aphidimyza* Rond., широко используемой для борьбы с тлями в теплицах, на повышение плодовитости и скорости развития [7], а также хищного клопа *Orius laevigatus* , используемого для борьбы с трипсами, на повышение плодовитости при откладке яиц на растения каланхое [8]. Особое внимание уделено комплексу работ, выполненных в последние годы, по созданию чистых линий (даже пород) фитосейулюса, устойчивых к повышенной температуре [9].

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## Testing of oxylipins as autoregulators of fungal development

Belozerskaya Tatiana A.<sup>a\*</sup>, Gessler Natalia N.<sup>a</sup>, Filippovich Svetlana Yu.<sup>a</sup>, Bachurina Galina P.<sup>a</sup>,  
Groza Natalia V.<sup>b</sup>

<sup>a</sup>*Federal State Institution "Federal Research Centre "Fundamentals of Biotechnology" of the Russian Academy of Sciences, Moscow.*

<sup>b</sup>*Moscow Technological University, Institute of Fine Chemical Technologies, Moscow*

### Abstract

Oxylipins are a class of molecules derived from the incorporation of oxygen into polyunsaturated fatty acid (PUFAs) substrates. C18 PUFAs such as linoleic,  $\alpha$ -linolenic, and the monounsaturated oleic acid are the predominant oxygenase substrates used in fungi. Most fungi only produce minor amounts of C20 PUFA known as arachidonic acid (AA). Those fungi readily utilize and oxygenate AA from environmental sources. PUFA oxidation, leading to the formation of autoregulatory molecules in fungi, depends considerably on the environment and the developmental stage. These molecules regulate spore germination, morphogenesis, induce dimorphic transitions in fungi. They are regulators of secondary metabolites production closely coupled with asexual and sexual development. Over the last decade it has become apparent that oxylipins are a common means of communication among and between plants, animals, and fungi to control development and alter host-microbe interactions. These metabolites have been shown to contribute to virulence of human pathogens. Understanding the mechanism by which oxylipins of various structures regulate different ways of fungal development will have implications on both human health and global food production problems.

In the present investigation the regulatory role of linoleic and AA PUFA, and oxylipins on different ways of fungi morphogenesis was studied. Oxylipins of natural structure, derivatives of C14, C18 and C20 PUFA were prepared by previously elaborated methods based on the acetylene compounds. The model organism used for analysis was the ascomycete *Neurospora crassa*. Sexual and asexual reproduction in the fungus may be achieved by a change in the cultivation medium. Furthermore, light in the blue-violet region stimulates the processes of *N.crassa* differentiation towards the formation of either conidia or protoperithecia. Similarity of *N.crassa* genome profile with the main fungal parasites makes this universal fungal model an attractive one for investigation of the influence of oxylipins on fungal development.

Linoleic acid unlike AA had no influence on *N.crassa* reproduction, but affected fungal growth and branching. 3-hydroxy-(5Z,8Z)-octadecadienoic acid (C14 PUFA derivative) had only a slight effect on protoperithecia development without affecting conidia formation. C18 oxylipin – 18-hydroxy-(9Z,12Z)-octadecadienoic acid (18-HODE) influenced fungal reproduction most profoundly. The three double-bond C18 oxylipin – 18-(9Z,12Z,15Z)-octadecatrienoic acid (18-HOTrE) inhibited both conidia and protoperithecia formation under the influence of light. AA derivatives, especially 3(*R*)-hydroxyl-(5Z,8Z,11Z,14Z)-eicosatetraenoic acid (3-HETE) stimulated protoperithecia and conidia formation in the dark. Oxylipins effect on *N.crassa* differentiation depends on the number of carbon atoms and the double bonds in the molecule, and the position of the hydroxyl-group. The results obtained indicate that 18-HODE might be involved in light-dependent processes of differentiation in *N.crassa*.

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\*Corresponding author: E-mail address: tabinbi@mail.ru

# **Biological control of pests in the modern greenhouses for protection of the virus-free potato**

Belyakova N. A.

*All-Russia Institute of Plant protection (St.-Petersburg)*

## **Abstract**

Modern industrial greenhouses, which are widely used for cultivation of the virus-free potato by the degree isolation, are similar to the island ecosystems. However, isolation does not provide 100% guarantee against entering of pests into the greenhouse. This is evidenced by all the years of experience of the greenhouse plant growing. Pests invade the industrial greenhouses during the first 5-12 months after their commissioning, despite all isolation and regular activities for disinfection. The situation is complicated by the highest instability of the greenhouse agrocenoses. After contact with even single pests species, there exists the high risk of outbreaks of their reproduction on the background of the optimal climatic conditions, supported artificially in the greenhouses.

Migration of majority of harmful potato viruses occurs via aphids. The major share in the total vector activity of the aphids belongs to peach, bean, and buckthorn aphids that are the main carriers, including especially dangerous potato Y-virus. Aphids are effectively carried by the air currents; therefore, even at a considerable distance of seed greenhouses from plantations of the ware potato or other vegetable crops, a high likelihood of skidding of virus carriers exists, especially during the migration of aphids.

Our experience in protection of a number of vegetable and flower crops in industrial greenhouses indicates that the pest control must be complex. Along with a system of thorough isolation from the environment, the quarantine measures require preventive colonization of biological control agents that are able to find and destroy even the single individuals of pests, skidding of which into the greenhouses is inevitable. The regular destructive measures should be carried out using preparations based on entomopathogenic microorganisms that are safe for useful entomofauna and people.

The need to strengthen the potato virus protection in greenhouses is conditioned by the following reasons. Currently, expansion of the area of the planted potato in the sheltered ground by using mostly advanced technologies of hydroponic plant growing takes place in the Russian Federation. The largest domestic producers of seed potatoes are putting into operation the new industrial greenhouses for the year-round cultivation of potato. Applying of chemical method of protection against virus carriers in the greenhouses of this type has serious negative consequences. At the year-round cultivation of potato in a stable artificial climate, the regular insecticide treatment significantly increases the risk of development of resistance in insects-carriers, as compared to the open ground, where the growing season is limited and has unstable weather conditions. Furthermore, high pesticide loading adversely affects both plants and maintenance personnel working in these greenhouses.

Thus, to solve the problem of the virus carriers in the greenhouses, on the potato including, the necessary condition is the extensive use of the complex of biological plant protection products, i.e. entomophagous insects and biopesticides.

The objects of our research are entomophages, which are already used for biological protection of plants in the greenhouses, as well as new species, not previously tested in the practice of biocontrol. By analyzing the features of modern agrotechnology, new morphoecological screening criteria of entomophagous were identified for improving their effectiveness in conditions of intensive cultivation of potato in greenhouses. Results indicated

that the size sexual dimorphism, intraspecific variation in weight in the food-stress condition could be used to screen prospective species among Coccinellidae.

This research is carried out by a grant Russian Science Foundation (project № 16-16-04079).

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\*Corresponding author: E-mail address: [biocontrol@vizr.spb.ru](mailto:biocontrol@vizr.spb.ru)



# **Problems of development of bioherbicides based on plant pathogenic fungi and their metabolites**

Alexander Berestetskiy

*All-Russian Institute of Plant Protection*

## **Abstract**

Weedy vegetation is a continuous component of agroecosystems and a cause of up to 50% loss of crop yield. Therefore, any crop production technology includes weed management. Perennial and invasive species are marked in species composition of weeds because only repeated soil cultivation and increased doses of chemical herbicides provide their control. Soil erosion and chemical pollution of the yield elements are expected to increased expenses as a payment for eradication of the problem weeds. Development of novel biorational herbicides should be a solution of the problem.

Phytopathogenic fungi play an important ecological role in natural ecosystems reducing the number of plant populations and by maintaining their biological and genetic diversity. In the beginning of 1970 years, the investigations on use of phytopathogenic fungi in agricultural biotechnology for biological control of undesirable vegetation were started around the world. By the present moment, noticeable success was achieved: more than 10 mycoherbicides were registered in different countries. In recent years, registration tests of the new mycoherbicides developed in Canada ("Sarritor" on the basis of *Sclerotinia minor*, "Phoma" – on the basis of *Phoma macrostoma*, – both against Dicotyledon weeds) and Australia (Di-BakParkinsonia on the basis of three species of fungi against *Parkinsonia aculeata*) were passed. However, about two hundreds projects on use of fungi as mycoherbicides remained to be realized.

The main and well-known disadvantage of biopesticides, including the majority of potential mycoherbicides, is slowed-down action or/and insufficiently high biological efficacy comparing to chemical herbicides. Short period of storage, limited techniques for quality control and the requirement for utilization of waste substrate decrease practical attractiveness of some potential mycoherbicides despite on their good effects against target weeds. Therefore, there is need for approaches allowing to increase efficacy of potential mycoherbicides and to improve their production technology. These approaches should be based on deep understanding of ecology, biochemistry and physiology of biocontrol agents in the connection with their virulence and stress tolerance as well as on the knowledge of modern biotechnological processes.

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\*Corresponding author: E-mail address: aberestetskiy@vizr.spb.ru

## **Biotechnology for organic products without the usage of pesticides and fertilizers**

Bunchak A. M.

*Podolsky State Agricultural University, t. Kamenets-Podilsk, str. Shevchenko 13, Ukraine*

### **Abstract**

Presented the results of studies of the influence of organic fertilizers of universal action with a balanced content of trivalent chromium and bio stimulants «Biochrom» on the productivity of agricultural crops (corn, spring wheat, buckwheat, soybeans, oats) and for the maintenance of Cr+3 in the grain of investigated crops without usage of synthetic pesticides and fertilizers.

Scientifically proved and experimentally confirmed the need of Cr+3 for the life of humans, animals and plants. The US Academy of Sciences has established the need of Cr+3 for people (apr. 50-200 mg/day). In Ukraine unfortunately there is no provisions for trivalent chromium intake for humans. Bug even now many companies which produce mineral and vitamin supplements working at the pharmaceutical market of Ukraine, include Cr+3 («Multi-tabs. Classic», 50 micrograms Denmark, «Vitam», 30 micrograms Ukraine).

In general the researches made on animals and humans have demonstrated the effectiveness of additives of Cr+3 in order to increase the tissue sensitivity to insulin and also the correction of carbohydrate, lipid and protein exchanges and functions of the immune system. So the necessary amount of Cr+3 in the feeding of animals is very important.

To grow the grain with the required amount of Cr+3 we must fertilize the soil with organic fertilizers, which contain trivalent chromium (Cr+3).

Therefore, during 2010-2012 we have developed and patented the technology for producing the organic fertilizer of new generation «Bioproferm» with a balanced content of trivalent chromium for using it into the primary and secondary tillage and the technology of liquid bio-stimulator «Biochrom» with the necessary amount of Cr+3 for the pre-sowing processing of seeds of spring and winter crops and the foliar feeding of plants during the growing season.

The production of organic fertilizer «Bioproferm» with Cr+3 was arranged at our company «Leather Land» in Bolekhiv, and the production of bio stimulator «Biochrom» using the method of cavitation was made by the association «Bioconversion».

The main task of our researches (2012-2015) was to study the effect of organic fertilizers «Bioproferm» and bio-stimulator «Biochrom» with a balanced content of trivalent chromium on the productivity of corn, spring wheat, buckwheat, soybeans oats end the grain content of Cr+3 in the tested cultures.

On the basis of researches carried out by our company it was revealed that the usage of liquid organic «Bioproferm» fertilizer and bio stimulator «Biochrom» had positive effect on the growth and the development of crops during the whole period of their vegetation without using the synthetic pesticides and fertilizers providing the increase of harvest productivity up to 13-28 % and as a result of it we can get environmentally friendly products with the necessary amount of trivalent chromium.

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\*Corresponding author: E-mail address: leather@bigmir.net

## **Биотехнология для получения органической продукции без применения пестицидов и удобрений**

Бунчак А.М.

*Подольский государственный аграрный университет*

*Украина, г. Каменец-Подольский, ул. Шевченка 13*

*Представлены результаты исследований по изучению влияния органических удобрений универсального действия со сбалансированным содержанием трехвалентного хрома и биостимуляторы и «Биохром» на урожайность сельскохозяйственных культур (кукурузы на зерно, яровая пшеница, гречиха, соя, овес) и на содержание  $Cr^{+3}$  в зерне исследуемых культур без применения синтетических пестицидов и удобрений.*

Научно обосновано и экспериментально подтверждено необходимость  $Cr^{+3}$  для жизнедеятельности людей, животных и растений. Американская национальная академия наук установила потребность  $Cr^{+3}$  для людей - 50-200 мкг/сут. В Украине, к сожалению, еще отсутствуют нормы потребления трехвалентного хрома для человека. Однако уже сейчас многие фирмы производителей минерально-витаминных добавок, которые работают на фармацевтическом рынке Украины, включают в них  $Cr^{+3}$  («Multi-tabs. Classic», Дания - 50 мкг, «Витам», Украина - 30 мкг).

В целом исследование на людях и животных продемонстрировали эффективность добавок  $Cr^{+3}$  с целью повышения чувствительности тканей к инсулину, а также коррекции углеводного, липидного, протеинового обменов и функций иммунной системы. Поэтому актуальным является кормление животных кормами с необходимым количеством  $Cr^{+3}$ .

Чтобы вырастить зерно с необходимым количеством  $Cr^{+3}$  необходимо удобрять почву органическими удобрениями, которые содержат трехвалентный хром.

Поэтому в течение 2010-2012 г. нами разработана и запатентована технология получения органических удобрений нового поколения «Биоферм» со сбалансированным содержанием трехвалентного хрома для внесения его в основной и предпосевную обработку почвы и технология получения жидкого биостимулятора «Биохром» с необходимым количеством  $Cr^{+3}$  для допосевной обработки семян яровых и озимых культур и внекорневой подкормки растений в период вегетации.

Производство органического удобрения «Биоферм» с  $Cr^{+3}$  организовано на построенном цехе (биоферментаторе) в ООО «Мир кожи» (г. Болехов) и производство методом кавитации биостимулятора «Биохром» в ассоциации «Биоконверсия».

В основу наших исследований (2012 – 2015 г.) возложена задача изучить влияние органических удобрений «Биоферм» и биостимулятора «Биохром» со сбалансированным содержанием трехвалентного хрома на продуктивность кукурузы на зерно, яровой пшеницы, гречихи, сои, овса и на содержание в зерне исследуемых культур  $Cr^{+3}$ .

На основе выполненного нами исследования установлено, что применение жидкого органического удобрения «Биоферм», биостимулятора "Биохром" положительно влияло на рост и развитие сельскохозяйственных культур в течение всего периода их вегетации без применения синтетических пестицидов и минудобрений, обеспечивает увеличение урожайности на 13-28 % и получения экологически чистой продукции с содержанием необходимого количества трехвалентного хрома.

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\*Corresponding author: E-mail address: leather@bigmir.net

## **Endophytic bacteria as promising biotechnological resource for biocontrol of phytopathogens**

V.K. Chebotar, A. V. Shcherbakov, E. N. Shcherbakova, E. V. Voinov, A. N. Zaplatkin, N. V. Malfanova

*Federal state budgetary scientific organization All-Russian Research Institute for Agricultural Microbiology, 196608, Saint-Petersburg, Pushkin, shosse Podbelskogo 3*

Endophytic called bacteria that can colonize the internal tissues of plants without causing disease and not rendering negative influence on its development. There are great prospects for search, selection and study of new species of endophytic bacteria, improving the development of plants, with the aim of creating new microbial preparations for adaptive crop production. Since bacterial endophytes colonize the same ecological niches in the plant as phytopathogenic microorganisms, they are a promising agent for biocontrol of phytopathogens. Classical studies of biodiversity of endophytic bacteria based on a characterization of isolates obtained from internal plant tissues after surface sterilization. Endophytic bacteria are able to improve phosphorus nutrition of plants, to produce IAA and siderophores. It is shown that endophytic bacteria are capable of producing vitamins, have a number of additional properties necessary for the improvement of plant development, such as: regulation of osmotic pressure, regulation of stomata, modification of root development of plants, regulation of nitrogen nutrition of plants. Endophytic bacteria are able to reduce or prevent the negative effects of pathogenic microorganisms on plants. Inoculation of plants by endophytic bacteria is able to significantly reduce the harm caused to plants by pathogenic fungi, bacteria, viruses, insects and nematodes. Unique strains of endophytic bacteria can be used directly for inoculation of seeds or seedlings, reducing, thus, the influence of biotic and abiotic factors on the plant, due to the active colonization of internal tissues of plants and subsequent positive biochemical and physiological effect on the plant. While in endosphere, endophytes have a significant advantage over organisms that live in the rhizosphere and phyllosphere due to the stable pH, humidity, flow of nutrients and lack of competition from a large number of microorganisms. For the inoculation of plants with endophytic bacteria do not require large amounts of inoculum, taking into account high specificity of such plant-microbe symbiosis and competitiveness of endophytic bacteria. This technique can be very attractive for biotechnological productions, seeking the replacement of traditional chemical pesticides.

This work was supported by the Russian Science Foundation (project no. 14-16-00146).

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\*Corresponding author: E-mail address: vladchebotar@rambler.ru

## Эндофитные бактерии как перспективный биотехнологический ресурс биоконтроля фитопатогенов

В.К.Чеботарь, А.В. Щербаков, Е.Н. Щербакова, Е.В.Войнов, А.Н. Заплаткин,  
Н.В.Мальфанова

*ФГБНУ Всероссийский научно-исследовательский институт сельскохозяйственной микробиологии, 196608, Россия, г. Санкт-Петербург—Пушкин, шоссе Подбельского, 3*

Эндофитными называются бактерии, способные колонизировать внутренние ткани растения, не вызывая при этом заболеваний и не оказывая отрицательного влияния на его развитие. Открываются большие перспективы по поиску, выделению и изучению новых видов эндофитных бактерий, положительно влияющих на развитие растений, с целью создания новых микробиологических препаратов для адаптивного растениеводства. Бактериальные эндофиты колонизируют те же экологические ниши в растении, что и фитопатогенные микроорганизмы, поэтому являются перспективным агентом биоконтроля фитопатогенов. Классические исследования биоразнообразия эндофитных бактерий основаны на характеристике изолятов, полученных из внутренних тканей растений после их поверхностной стерилизации. Эндофитные бактерии способны улучшать фосфорное питание растений, продуцировать ИУК и сидерофоры. Показано, что эндофитные бактерии способны продуцировать витамины, обладают целым рядом дополнительных свойств, необходимых для улучшения развития растений, таких как: регуляция осмотического давления, регуляция работы устьиц, модификация развития корневой системы растений, регуляция азотного питания растения. Эндофитные бактерии способны уменьшать или предотвращать отрицательное воздействие фитопатогенных микроорганизмов на растения. Инокуляция растений эндофитными бактериями способна значительно уменьшать вред, наносимый растениям патогенными грибами, бактериями, вирусами, насекомыми и нематодами. Уникальные штаммы эндофитных бактерий могут быть использованы непосредственно для инокуляции семян или саженцев, уменьшая, таким образом, влияние биотических и абиотических факторов на растение, за счет активной колонизации внутренних тканей растений и последующего позитивного биохимического и физиологического воздействия на растение. Находясь в эндосфере, эндофиты имеют существенное преимущество перед организмами, обитающими в ризосфере и филлосфере за счет стабильного рН, влажности, потока питательных веществ и отсутствия конкуренции со стороны большого числа микроорганизмов. Для инокуляции растений эндофитными бактериями не требуется больших количеств инокулята, учитывая высокую специфичность данного растительно-микробного симбиоза и конкурентоспособность эндофитных бактерий. Этот прием может быть весьма привлекательным для биотехнологических производств, ищущих замену традиционным химическим пестицидам.

Работа выполнена при финансовой поддержке Российского научного фонда (проект №14-16-00146).

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\*Corresponding author: E-mail address: vladchebotar@rambler.ru

**Structure and biological activity of secondary metabolites of *Alternaria sonchi* –  
a pathogen of sowthistle (*Sonchus arvensis*)**

Dalinova A.A.<sup>1</sup>, Volosatova N.S.<sup>1</sup>, Prokofyeva D.S.<sup>2</sup>, Berestetskiy A.O.<sup>1</sup>

<sup>1</sup>*All-Russian Institute for Plant Protection, Saint-Petersburg, Russia*

<sup>2</sup>*Research Institute of Hygiene, Occupational Pathology and Human Ecology, Saint-Petersburg,  
Russia*

*Alternaria sonchi* is a pathogen of sowthistle (*Sonchus arvensis*). It causes foliar necrosis of sowthistle and has been evaluated as a candidate biological control agent for this weed. For some biopesticidal agents it was shown that solid-state fermentation is more preferable for obtaining of inoculum.

Wasted substrate can be used as a source of useful metabolites (pigments, biologically active compounds, surfactants). In our work, we showed that *A. sonchi* produced several biologically active secondary metabolites in solid culture on pearl barley. Five polycyclic ethanones, namely, alternethanoxins A–E were isolated from the fungal solid culture. Moreover, unusual  $\gamma$ -lactone derivative chloromonilicin and its biosynthetic precursor xanthone derivative chloropinselin were isolated from the solid culture of *A. sonchi*.

Alternethanoxins A and C demonstrated non-specific phytotoxic activity when assayed on punctured leaves of *Sonchus arvensis* and *Elytrigia repens*. Alternethanoxins A, C, D and chloromonilicin showed antimicrobial activity at different rates. Chloromonilicin also inhibited germination of conidia of phytopathogenic fungi. Alternethanoxins A and B displayed cytostatic growth inhibitory activity in five human and one mouse cancer cell lines. Chloropinselin and alternethanoxin D showed cytotoxic activity against human glioblastoma and lung adenocarcinoma cell lines. Alternethanoxins C, E and chloropinselin showed selective inhibitory activity of carboxylesterase.

We work out the optimal methods for extraction of these substances from solid culture of *A. sonchi*. Extraction of raw material with hexane gave the maximum yield of chloromonilicin. Extraction with Me<sub>2</sub>CO-H<sub>2</sub>O (50:50) and solid-phase extraction using Diaion HP-20 after Me<sub>2</sub>CO evaporation gave the maximum yield of alternethanoxins A–E.

These substances, their derivatives and synthetic analogues can be tested as new molecules for pharmacology and plant protection. Obtaining of these metabolites from wasted substrate can be an effective way of waste utilization after preparation of inoculum of *A. sonchi*.

The work was supported by RSF (projects № 14-26-00067, №16-16-00085).

Corresponding author: [toxbiotech@vizr.spb.ru](mailto:toxbiotech@vizr.spb.ru)

## **New natural and synthetic inhibitors of the aflatoxin b1 and melanin biosynthesis in *Aspergillus flavus***

Dzhavakhiya V.G., Voinova T.M., Popletaeva S.B., Statsyuk N.V., Mikityuk O.D., Nazarova T.A., Shcherbakova L.A.

*All-Russian Research Institute of Phytopathology, Bol'shie Vyazemy, Moscow region, 143050  
Russia*

Fungal polyketides represent a large group of biologically active compounds synthesized by enzymes from the polyketide synthase (PKS) family. Aflatoxins are secondary metabolites produced by *Aspergillus* fungi via the polyketide pathway; among them, aflatoxin B1 (AFB1) is the most common and dangerous. Fungal melanins are also synthesized via the PKS-depending pathway. The blocking of the melanin biosynthesis in pathogenic fungi can result in the loss of their pathogenicity and increased susceptibility to biotic and abiotic stresses. Therefore, the search of compounds able to block both aflatoxin and melanin biosynthesis seems to be of great theoretical and practical importance.

In this study we investigated the ability of some natural and synthetic compounds to block the biosynthesis of AFB1 and melanin in toxigenic *Aspergillus flavus* F11 strain. The studied natural compounds included lovastatin, thymol, and 3-hydroxybenzaldehyde (3-HBA), whereas synthetic compounds included (aminoethyl)thiophosphonic acid, (aminomethyl)thiophosphonic acid, alafosfalin, (1-aminoethyl)phosphonic acid, and N-hydroxyputrescine.

To reveal the effect of tested compounds on the melanogenesis, AF11 was cultivated on agar medium supplemented with these compounds at concentrations varied from 0.0001 to 0.1% depending on their activity. To determine the effect of the tested compounds on the AFB1 production, AF11 was cultivated for 170 h in a liquid Payne–Hagler medium followed by a comparison of the AFB1 content in the culture broth of experimental and control (pure medium) variants.

The performed screening allowed us to divide the tested compounds into three groups. Alafosfalin, (aminoethyl)thiophosphonic acid, and (aminomethyl)thiophosphonic acid (Group I), significantly decreased the AFB1 production, but did not influenced on the colony pigmentation. N-hydroxyputrescine, (1-aminoethyl)phosphonic acid, thymol, and 3-HBA (Group II) inhibited the melanin biosynthesis with the simultaneous increase in the AFB1 production. Finally, lovastatin (Group III) completely blocked both AFB1 and melanin production even at low concentrations (0.0005%). Therefore, compounds from the Groups I and II inhibited the AFB1 or melanin biosynthesis, respectively, via the blocking of stages located after the point of divergence of the corresponding biosynthetic pathways, whereas lovastatin either inhibited the polyketide biosynthesis prior the branching point, or simultaneously inhibited some stages of both AFB1 and melanin biosynthetic pathways after the branching point.

Thus, we first revealed the ability of lovastatin to efficiently inhibit both AFB1 and melanin biosynthesis in toxigenic *A. flavus*. The evaluation of the effect of lovastatin treatment of wheat grain contaminated with toxigenic *A. flavus* on the AFB1 accumulation in grain showed that the treatment of wheat grain with lovastatin at the concentrations of 0.25 and 0.5 mg/g of grain prior its contamination with AF11 reduced the AFB1 accumulation in 4 and 20 times, respectively. Taking into account the non-toxicity of lovastatin and the possibility of its highly-productive microbiological synthesis, the further study of the revealed new property of this natural compound intended to develop a new lovastatin-based preparation able to prevent the contamination of animal feed with AFB1 seems to be very promising. This work was financially supported by the Russian Science Foundation (project no. 14-16-00150).

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\*Corresponding author: E-mail address: dzhavakhiya@yahoo.com

## **Новые природные и синтетические блокаторы биосинтеза афлатоксина В1 и меланиногенеза у *Aspergillus flavus***

Джавахиya В.Г., Воинова Т.М., Поплетаева С.Б., Стацюк Н.В., Микитюк О.Д., Назарова Т.А., Щербакова Л.А.

*ФГБНУ Всероссийский НИИ фитопатологии, Большие Вяземы, Россия*

Поликетида грибов представляют собой группу биологически активных соединений, синтезируемых при участии семейства ферментов поликетидсинтаз (ПКС). К этой группе относятся афлатоксины – вторичные метаболиты грибов рода *Aspergillus*, среди которых наиболее распространенным и токсичным является афлатоксин В1 (АФВ1). Меланиновые пигменты грибов также являются продуктом ПКС-зависимого биосинтеза. Известно, что блокирование биосинтеза меланина может приводить к снижению патогенности и повышению восприимчивости грибов к биотическому и абиотическому стрессу. В этой связи поиск веществ, способных одновременно блокировать биосинтез АФВ1 и меланина у токсиногенных грибов представляется вполне актуальным.

В настоящей работе представлены результаты исследования способности ряда природных и синтетических соединений блокировать биосинтез АФВ1 и пигмента меланина штаммом *Aspergillus flavus* AF11. В качестве потенциальных ингибиторов использовали вещества природного происхождения - ловастатин, тимол и 3-гидроксибензальдегид (3-НВА), а также различные синтетические вещества - (аминоэтил)тиофосфоновую кислоту, (аминометил)тиофосфоновую кислоту, алафосфалин, (1-аминоэтил)фосфоновую кислоту и N-гидроксипутресцин.

Для определения влияния тестируемых соединений на меланиногенез штамм AF11 культивировали на агаризованной среде с добавлением этих соединений в концентрации от 0.0001 до 0.1% в зависимости от активности соединения. Для определения влияния исследуемых веществ на биосинтез АФВ1, штамм культивировали в течение 170 ч в жидкой питательной среде Пейна-Хеглера, после чего сравнивали содержание АФВ1 в культуральной жидкости в опытных вариантах и контроле (питательная среда без добавок тестируемых соединений).

Проведенный скрининг позволил разделить исследуемые вещества на три группы. Алафосфалин, (аминоэтил)тиофосфоновая кислота и (аминометил)тиофосфоновая кислота (группа I) существенно снижали продукцию АФВ1, однако не влияли на пигментацию колоний AF11. N-гидроксипутресцин, (1-аминоэтил)фосфоновая кислота, тимол и 3-НВА (группа II) блокировали биосинтез меланина и одновременно существенно увеличивали продукцию АФВ1. Наконец, ловастатин (группа III) полностью блокировал биосинтез АФВ1 и меланина даже в низких концентрациях (0.0005%). Таким образом, вещества из I и II группы блокировали биосинтез АФВ1 и меланина, соответственно, на стадиях, расположенных после точки разветвления этих биосинтетических путей, в то время как ловастатин либо действовал на стадии, предшествующей точке ветвления, либо одновременно блокировал какие-то стадии цепей биосинтеза АФВ1 и меланина после их расхождения.

В результате проведенного исследования впервые была выявлена способность ловастатина эффективно блокировать биосинтез АФВ1 и подавлять биосинтез меланина у *A. flavus*. Оценка влияния ловастатина на накопление АФВ1 в зерне пшеницы, зараженном *A. flavus* AF11, показала, что обработка зерна ловастатином в концентрациях 0.25 и 0.5 мг/г зерна перед заражением AF11 снижала накопление в нем АФВ1 в 4 и 20



раз, соответственно. Обнаруженные свойства ловастатина в сочетании с его нетоксичностью и возможностью высокопродуктивного микробиологического синтеза делают перспективными дальнейшие исследования, направленные на разработку на его основе препарата, обеспечивающего снижение контаминации АФВ1 фуражного зерна и других кормов для сельскохозяйственных животных.

Работа выполнена при финансовой поддержке Российского Научного Фонда (грант № 14-16-00150).

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\*Corresponding author: E-mail address: [dzhavakhiya@yahoo.com](mailto:dzhavakhiya@yahoo.com)

## **Immunosuppression as a new way of improving bioinsecticides activity**

Glupov V.V., Kryukov V.Yu.\*, Dubovskiy I.M., Yaroslavtseva O.N., Grizanova E.V., Akhanaev Yu.B., Polenogova O.V., Tomilova O.G., Kryukova N.A.

*Institute of systematics and ecology of animals SB RAS, Novosibirsk, Russia*

### **Abstract**

Immunosuppressive effect of sublethal bacterial infection (*Bacillus thuringiensis*), synthetically and natural insecticides (avermectins, chlorogenic acid, usnic acid, *Cordyceps militaris* extracts) and venom of parasitoid *Habrobracon hebetor* on defense systems of Colorado potato beetle *Leptinotarsa decemlineata* and wax moth *Galleria mellonella* have been studied. These immunosuppressors change of fungistatic properties of integuments, phenoloxidase activity, detoxicative enzymes activity (nonspecific esterases, glutathion-S-transferases), as well as ratio of hemocytic types, necrosis/apoptosis relation and encapsulation response. These dramatic depression in hosts defense systems led to increased susceptibility of insects to bioinsecticides (fungi *Beauveria bassiana*, *Metarhizium robertsii* and bacteria *Bacillus thuringiensis*) from ten to several thousand fold. Taken together, the results indicate the disturbance of the insects immune defenses under the influence of different immunomodulators that lead to synergy between these agents. Combine using of bacteria and fungi together with immunosuppressors provides a strong insecticidal effect in the shortest possible time. Such an approach constitutes a new perspective for the development of multi-component bioinsecticides. The study was supported by the Russian Science Foundation (Grant №15-14-10014).

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\*Corresponding author: E-mail address: [krukoff@mail.ru](mailto:krukoff@mail.ru)

# Synergistic antimicrobial activity of two-dimensional nanostructures of chitosan modified silver nanoparticles on *Staphylococcus aureus*

Kanrayasiri Kamhom<sup>1</sup>, Ekarat Detsri<sup>1,2\*</sup>

<sup>1</sup>*Department of Chemistry, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand*

<sup>2</sup>*Advanced Materials Research Unit, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand*

## Abstract

In this study, we established a simple chemistry method to fabricate two-dimensional nanostructures of silver nanoparticles decorated with chitosan (AgNPs) using Layer-by-Layer self-assembly technique. The presence of the charge is fundamental for build up of the nanostructures thin films using the advantage of the electrostatic interaction between oppositely charged species of cationic AgNPs and anionic poly(sodium 4-styrene sulphonate). The formation of nanostructures thin films were exhibited the striking yellow color due to the nanoparticles adsorption onto the surface of the substrate. UV-visible spectrophotometer, contact angle measurement, field emission scanning electron microscope and X-ray diffraction analysis were employed to characterise the formation and interaction of the prepared nanostructures thin films. Antimicrobial effect of AgNPs nanostructures thin films was evaluated against gram-positive *S. aureus* pathogen bacteria. The AgNPs nanostructures thin films have excellent antibacterial activity with  $97.85 \pm 1.74\%$  of bacteria removed. We confirmed that AgNPs nanostructures thin films had considerable inhibition of the growth of *S. aureus*.

**Keywords:** *Staphylococcus aureus*; silver nanoparticles; layer-by-layer; chitosan

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\*Corresponding author: E-mail address: Ekarat.de@kmitl.ac.th

## **The Overview about Application and Regulation of Chitinase**

He Liu, Qian Yang\*

*School of Life Science and Technology, Harbin Institute of Technology, 150001, China*

### **Abstract**

Chitin, a homopolymer of  $\beta$ -(1,4)-linked N-acetyl-d-glucosamine (GlcNAc), is one of the most abundant polymers in nature, second only to cellulose. It is a major structural component in a wide variety of organisms; it is found in the exoskeletons of many invertebrates and in the cell walls of most fungi and algae. Chitin degradation is, therefore, important to a wide variety of applications, ranging from the seafood industry and environmental clean-up of chitinous wastes like shrimp and crab shells, to human therapy, plant-defence systems and biological control. Chitinases, the chitin degrading enzymes, are known to be produced by several groups of organisms and have been shown to have various physiological functions in many fields such as industry, agriculture, medicine, biotechnology. The regulation of this expression, however, is still not completely known. On some level, the regulation of chitinase is similar to the regulation of cellulose, especially in negative pathway. Though the activity of enzyme isolated from nature is insufficient to be applied in industry, chitin and chitinase-produce microorganism still process a bright future as carbon nitrogen source, plant-pathogen inhibitor, enzymic preparations.

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\*Corresponding author: E-mail address: shadowshade@163.com

# **Plant Virus Expression Vectors for Biopharmaceutical Production**

Kathleen Hefferon

*Cornell University*

## **Abstract**

Plant made biologics have elicited much attention over recent years for their potential in assisting those in developing countries who have poor access to modern medicine. Additional applications such as the stockpiling of vaccines against pandemic infectious diseases or potential biological warfare agents are also under investigation. Plant virus expression vectors represent a technology that enables high levels of pharmaceutical proteins to be produced in a very short period of time. Recent advances in research and development have brought about the generation of superior virus expression systems which can be readily delivered to the host plant in a manner that is both efficient and cost effective. The following presentation describes recent innovations in plant virus expression systems and their uses for producing biologics from plants.

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\*Corresponding author: E-mail address: [klh22@cornell.edu](mailto:klh22@cornell.edu)

## **Technology of the extraction of needles compounds and biologics obtaining**

T.V. Hurshkainen, A.V. Kutchin

*Institute of Chemistry Komi Science Center of Ural Division of RAS, Syktyvkar, Russia*

### **Abstract**

Chemical treatment with the extraction of biologically active substances is a promising area of needles using. Needles are the large-scale waste produced by the logging companies.

One of the innovative activities of the Institute of Chemistry of KSC UB RAS is development of the technology which allows processing of vegetable raw materials by the new environmentally friendly method without the use of organic solvents. This method doesn't have any analogues now.

The studies provided a method of producing a complex of natural biologics. A pilot manufacturing was made in order to obtain experimental batches. Biologics for plants and animals were tested. The plant growth regulator "Verva" with fungicide action was derived from a fir needles. Long-term tests of "Verva" were performed with vegetables and industrial crops, state registration was made. The biologics is produced at the pilot plant of LLC "Scientific and technological enterprise of the Komi Institute of Chemistry SC UB RAS".

Natural compounds of the biologics increase plants resistance to extreme environmental influences. Active ingredients of the biologics are triterpene acids with lanostane structure which were not found in other coniferous tree species. Triterpene acids contribute to enhanced biological and bioimmune processes in plants.

A biologics with soft influence on the plants and environment are becoming increasingly important in the protection of crops against diseases. Needles of a spruce is a raw material for a new plant protection biologics. Due to the strengthening of the natural protective mechanisms of plants, this product acts as a systemic broad spectrum fungicide. The active ingredients of the biologics are the natural phenolic compounds.

According to the studies performed in the Saint-Petersburg Forestry Academy, the phenolic compounds of the spruce needles demonstrates high activity against viruses, filamentous fungi and yeast, as well as helps the growth of plants. Studies of the insecticidal activity of the spruce extractives have showed biological activity against the Colorado potato beetle, codling moth and other garden pests. Medical and toxicological studies of a new biologics were performed in the Kirov State Medical Academy. It was found that the spruce extractives are low-hazard compounds which have no skin-irritant, cumulative and sensitizer effects.

*This work is carried out with the financial support of the project 15-15-34-68 of the program of UB RAS.*

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\*Corresponding author: E-mail address: hurshkainen@chemi.komisc.ru

## Emergence and spreading of *Dickeya solani* & *D. dianthicola* on potato in Russia, and search for control measures

Alexander Ignatov<sup>1\*</sup>, Maya Khodykina<sup>1</sup>, Fevzi Dzhililov<sup>2</sup>, Alexander Karlov<sup>2</sup>, Vladimir Karandashov<sup>1</sup>, Fedor Shirshikov<sup>3</sup>, Konstantin Miroshnikov<sup>3</sup>

(1) Research Center “PhytoEngineering” LLC, Rogachevo, Moscow region, Russia

(2) Russian State Agrarian University-MSKHA, Moscow, Russia,

(3) M.M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences, Moscow, Russia

### Abstract

Several regions of Russia were assayed for potato bacterial diseases at 2001-2014. Strains of pectolytic bacteria isolated at Lipetsk, Novgorod, Moscow, Voronezh, Bryansk, and other regions were tested for biochemical traits, virulence and genetic properties, including MLST for 8 genes: *acnA*, *dnaX*, *gapA*, *icdA*, *mdh*, *mtID*, *pgi*, and *pelD*. Bacteria causing black leg and soft rot diseases belonged to three genera: *Pectobacterium* (75% of all isolates), *Serratia* (~10%), and *Dickeya* (~15%). The stains isolated from diseased potato plants in 2001-2008 belonged to *Pectobacterium* spp. (75% of all isolates) and *Serratia* spp. (~25%). The first isolates of *Dickeya dianthicola* and *D. solani* were revealed on summer 2009 [1]. According to MLST, the Russian strains of *D. dianthicola* were similar to *Erwinia chryzanthemi* isolated in US by R.S. Dickey from sweet potato [2], and to strains obtained at 1999-2000 in the South of Russia (Krasnodar and Stavropol regions) from corn plants by E.V. Matveeva (unpublished). Some different isolates were identified later as a new species *D. solani*. All the *Dickeya* strains were virulent for potato, tomato, tobacco and iris plants, and could be identified by real-time PCR with ADE1/ADE2 primers with original ADE3 fluorescent Taqman® probe [3]. Surveys of several regions of Russian Federation at 2009 showed low abundance (<3% of tested potato stocks) of the *Dickeya* pathogens. However, at 2010-2015 we have documented 8-10 fold increased frequency (up to 26-30% of assayed samples) of *D. dianthicola* and *D. solani* infection found in green plants and in harvested tubers in the assayed regions. We assumed that *Dickeya* became the main causing agent of potato black leg disease in Russia. We can speculate that large part of potato seeds collected at 2016 was infected by *Dickeya* spp., too. Ten representative strains of *D. dianthicola* and *D. solani* were tested for response to bactericides and fungicides permitted in Russia for potato protection [4] and found very limited efficiency of them against the pathogens both *in vitro* and *in vivo* assays. We initiated a project for development of new biological control agents based on bacteriophages and antagonistic bacteria of genus *Bacillus*, specifically efficient against pectolytic enterobacteria, including *Dickeya* spp.

The work was supported by the project RSF 15-15-00073

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\*Corresponding author: E-mail address: an.ignatov@gmail.com

## Catalytic conversion of plant biomass into useful products

Kasaikina O. T., Pisarenko L. N., Zinoviev, I. V.

*Semenov Institute of Chemical Physics RAS*

*119991 Moscow, Kosygin str., 4*

### Abstract

Processing and utilization of vegetable wastes is a relevant scientific and technical task. In nature, waste undergoes gradual degradation due to the influence of enzymes, produced by microorganisms. A necessary condition for the development, reproduction, and functioning of microorganisms is the presence of water and mineral salts. Biochemical processes, such as anaerobic digestion, ethanolic and acetone-butanolic fermentation are used in practice for processing of biomass with high moisture content. However, microbiological processing is time-consuming and may involve the risk of breeding undesired microorganisms.

We have developed a simple biomimetic model of the enzyme complex and used it for the accelerated catalytic oxidative degradation of plant crop residues. Colloidal dispersion of iron (III) oxide and hydroxide with carbon and silicon inclusions has been used as a catalyst that simulates the active site of enzyme. Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and/or atmospheric oxygen have been used as environmentally friendly oxidants.

The oxidative degradation of the biomass (sawdust, peat, olive bagasse, rice and oat straw) results in the formation of low molecular weight oxidation products of lignin, cellulose, lipoproteins, and sugars; the solid residue is a cellulose and its derivatives [1,2]. The yield of solid residue depends on the biomass nature, the ratio of the reactants' concentrations (biomass, catalyst, hydrogen peroxide) and the time of the processing. At sufficient concentrations of H<sub>2</sub>O<sub>2</sub>, pure cellulose (E 460), suitable for food and pharmaceutical industries can be obtained from pine and spruce sawdust. Along with cellulose, an aqueous solution of organic acids, vitamins, sugars, etc., suitable for the preparation of dietary supplements and preservatives for animal feed, is produced as a by-product. Recently, it was shown [3] that water-soluble products after neutralization to pH 5-6 can serve as a useful nutritional Supplement in soil and a stimulant for plant growth. For agriculture, it is possible to create mobile systems of small volumes used directly by the consumers of these products in the area of its application.

The oxidative catalytic method is quite versatile and fundamentally suitable for processing of different kinds of plant residues, including for the processing of the leaves. In this case, the solid residue is mainly a condensed product of the transformation of suberin and cutin, the main component of the protective sheath of leaves, which are hard oxidizable and non-combustible. The liquid phase containing organic acids, after neutralization can be used for seed treatment and plant nutrition.

Thus, the accelerated catalytic processing of vegetable waste allows solving two problems at once: recycling and decontamination of waste of agricultural production and the improvement of agricultural and greenhouse soils by returning to the soil chemicals needed for plant life.

This work was partially supported by RFBR grant No. 14-03-00757

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\*Corresponding author: E-mail address: okasai@yandex.ru



## Каталитическая переработка растительной биомассы в полезные продукты

Касаикина О.Т., Писаренко Л.Н., Зиновьев И.В.

*Федеральное государственное бюджетное учреждение науки Институт химической физики им. Н.Н. Семенова РАН, 119991 Москва, ул. Косыгина д. 4*

Переработка и утилизация растительных отходов являются актуальной научно-технической задачей. В природе биомасса медленно разлагается под действием ферментов, вырабатываемых микроорганизмами, для размножения и функционирования которых необходимо наличие воды и минеральных солей. Биохимические процессы - анаэробная переработка, этанольная и ацетоно-бутанольная ферментации, применяют в случае биомассы с высокой влажностью. Однако микробиологическая переработка требует времени и сопряжена с опасностями размножения нежелательных микроорганизмов.

Нами разработана простая биомиметическая модель ферментного комплекса для ускоренной каталитической окислительной деструкции растительных остатков. Катализатор - коллоидная дисперсия гидроксида и оксида железа (III) с включением кремния и углерода, моделирует активный центр фермента. В качестве окислителя используются экологически безопасные пероксид водорода ( $H_2O_2$ ) и/или кислород воздуха.

В результате термоокислительной деструкции биомасса (древесные опилки, торф, оливковый жом, рисовая и овсяная солома) перерабатывается в низкомолекулярные продукты окисления лигнина, целлюлозы, липопротеидов и сахаров; твердый остаток представляет собой целлюлозу и ее производные [1,2]. Выход твердого остатка зависит от природы биомассы, соотношения концентраций реагентов (биомасса, катализатор, пероксид водорода) и времени проведения деструкции. При достаточных концентрациях  $H_2O_2$  из сосновых и еловых опилок получается чистая целлюлоза квалификации Е 460, пригодная для пищевой и фармацевтической промышленности. Кроме получения целлюлозы, сопутствующим продуктом является водный раствор органических кислот, витаминов, сахаров и т.д., пригодных для приготовления биодобавок и консервантов кормов для животноводства. В [3] показано, что водорастворимые продукты после нейтрализации до pH 5-6 могут служить полезной питательной добавкой в почво-грунт и стимулирующим средством для роста растений. Для сельского хозяйства возможно создание мобильных комплексов малых объемов, используемых непосредственно потребителями этой продукции на местах её применения.

Каталитический окислительный способ является достаточно универсальным и принципиально пригодным для переработки разного рода растительных остатков, в том числе для переработки листьев. В этом случае твердый остаток представляет собой конденсированный продукт трансформации трудно окисляемых и негорючих суберина и кутина, основных компонентов защитной оболочки листьев. Жидкая фаза, содержащая органические кислоты, после нейтрализации может быть использована для обработки семян и подкормки растений

Таким образом, применение ускоренной каталитической переработки растительных отходов позволяет решать сразу две задачи: утилизация и обеззараживание отходов сельскохозяйственного производства и оздоровление сельскохозяйственных и

тепличных грунтов путем возврата в почву химических веществ, которые нужны для жизнедеятельности растений.

Работа частично поддержана грантом РФФИ № 14-03-00757.

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\*Corresponding author: E-mail address: [okasai@yandex.ru](mailto:okasai@yandex.ru)

## Development and reproductive potential of a generalist predator, *Podisus maculiventris* (Hemiptera: Aspionae) feeding adult of grain moth

A.E.S. Kasem, A.I. Anisimov\*

St. Petersburg State Agrarian University, St. Petersburg, Russia

It is well known that the larvae of greater wax moth *Galleria mellonella* L. are enough good food for polyphagous predator spined soldier bug, which is used for biological control of gypsy moth, Colorado potato beetle, Mexican bean beetle, and some other insect pests. But greater wax moth mass rearing expensive, the diet includes useful components for human, dynamics of laboratory populations are not always stable in artificial breeding conditions.

Our experiments carried out to detect possibility of feeding the late instars bug nymphs or adults with adults of Angoumois grain moth *Sitotroga cerealella* Ol., as mixed factitious preys system (MFPS) to keep rearing under laboratory conditions, the possibility evaluated based on some biological characteristics. The Angoumois grain moth eggs are widely used in Russia as a food for many beneficial insects reared for insect pests biological control. By technology of breeding the adult moths discarded after oviposition, so they are very cheap food.

The adult Angoumois grain moth resulted from mass rearing collected, dated and separated in labeled sieve. Sticky cards (3.5 × 4.5 cm) to collect adult Angoumois grain moth (2 or 4 days) were used to feed the predator bug starting from 4<sup>th</sup>, 5<sup>th</sup> nymphal instars or adults. At the same time the greater wax moth only was used as control. Plastic cup 500 ml provided with wetted cotton and filter paper were used as a replication (three for each treatment) and also 500 ml plastic container used for pairs mating and oviposition.

The biological effects of feeding late nymphal and adult stages of *P. maculiventris* with Angoumois grain moth were evaluated under laboratory facilities.

There are no significant differences in mortality when feeding the adults of bug predator with adults 2 or 4 days grain moth compared with control, at the same time, no significant differences in survival of predator nymphs (4<sup>th</sup> instars) fed grain moth adults (2 days) compared with greater wax moth only. The average of development time for both males and females didn't affect negatively with the diet. Weights of females or males showed no significant differences compared with control when reared on 2 or 4 days grain moth. Emerged females of 4<sup>th</sup> instars nymphs which fed 2 or 4 days grain moth weighed 66,3± 1,85, and 62,1± 1,62 mg, respectively. While females' weight (which fed 2 or 4 days grain moth adult in 5<sup>th</sup> nymphal instars) was 62,1± 2,86 and 54,7± 2,58, respectively, predator males' weight which emerged from nymphs 4<sup>th</sup> or 5<sup>th</sup> instars didn't reflect any significant differences. The average of weight for both females and males reached to the lower level in case of using 4 days adults grain moth as a prey for 5<sup>th</sup> instars of bug predator. On the other hand, females' fecundity revealed differences in most cases compared with control. The results of fecundity indicated slightly differences between 2 or 4 days grain moth adults as a prey for adult of bug predator, while there is no significant difference between fecundity of feeding 4 days adult grain moth to fed adults' predator bug compared with control. The fecundity decreased to -3.8 fold when the 4<sup>th</sup> instars of the predator fed with prey 4 days. No significant differences in egg viability percentages of both grain moth prey 2 and 4 days, but 4<sup>th</sup> nymphal instars which fed 2 or 4 days prey moth badly affected. However, significant differences in preoviposition period within most treatments, the first eggs' count hadn't been negatively affected. Although, there are significant differences in number of egg batches within treatments, no significant differences recorded in case of feeding predatory females Adults with grain moth adults compared with control.

We can conclude that using Angoumois grain moth adults as alternative factitious prey such to fed late nymphs and adults of predatory soldier bugs need more attention in mass rearing technology especially, in mixed factitious prey system MFPS.

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\*Corresponding author: E-mail address: anisimov\_anatoly@mail.ru

## Antifungal substances of *Allium sativum* L.: new approach to plant protection

Kulikova O.G.<sup>1</sup>, Maltsev D.I.<sup>2</sup>, Kartashov M.I.<sup>3</sup>, Yamskova V.P.<sup>2</sup>, Yamskov I.A.<sup>1</sup>

<sup>1</sup>*A.N. Nesmeyanov Institute of Organoelement Compounds of Russian Academy of Sciences,  
Moscow*

<sup>2</sup>*Koltzov Institute of Developmental Biology of Russian Academy of Sciences, Moscow*

<sup>3</sup>*All-Russian Research Institute of Phytopatology, B. Vyazyomy, Odintsovo distr.*

### Abstract

Nowadays, a wide range of chemical products is used to protect plants against different phytopathogenes. The main disadvantages of chemical agents are multistage production processes using reagents that are difficult to procure, multicomponent composition, as well as accumulation of harmful substances in the soil, water and groundwater when they are widely used. As an alternative to chemical pesticides, the agents containing natural substances, or biopreparations, are sometimes used. In the literature there is no data on the use of any substances extracted from the garlic *Allium sativum* in agriculture for the protection of plants. *Allium sativum* L., commonly known as garlic, is one of the most widely and frequently used vegetables around the world. The most common method of use of this plant is in cooking, but due to its medicinal properties *Allium sativum* is applied both in conventional and modern medicine.

In this study we investigated biological activity of the complex derived from the garlic extract and formed by allinase and lectin subunit of garlic. A number of researchers demonstrated that a wide range of biologically active substances exhibit some form of activity in ultralow concentrations. New approaches developed on the base of ultralow doses concept application allow using both drugs and pesticides in much smaller doses, which is very important in terms of ecological and economic issues. Based on the above, in this study we evaluated the activity of obtained complex at  $10^{-11}$  mg/ml.

Experiments to determine activity of the allinase-lectin complex against phytopathogenic fungus *B. sorokiniana* and *Puccinia recondita* on wheat and barley leaves *in vitro* were carried out on isolated plant leaves. The biological efficacy of the use of complex was 80 and 85% appropriately.

We studied complex activity against the pathogen of rice blast *Magnaporthe grisea*. The complex did not affect *M. grisea* directly, but protected plants upon their infection by this pathogenic fungus (reduced the number of necrosis on treated leaves of rice).

Investigation of complex activity against *Cladosporium cucumerinum* revealed its ability to inhibit this fungus. The biological efficacy of the use of complex for the first leaf was 73.2%, for the second leaf was 62.1%.

The obtained complex does not possess phytotoxicity, and due to its high activity at low concentrations shows very low discharge at its application. Moreover, by virtue of its origin it is absolutely harmless for humans and animals and is environmentally safe technology of wheat and barley protection, which is especially important in modern agriculture.

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\*Corresponding author: E-mail address: [koulikova\\_olga@mail.ru](mailto:koulikova_olga@mail.ru)

# Computer meets Chemistry - Modern Aspects in Natural Products Chemistry and Biotechnology

Hartmut Laatsch

*Institute of Organic and Biomolecular Chemistry,*

*Göttingen, Germany*

## Abstract

Chemists and biologists are having different views and different approaches on biotechnology and chemistry, respectively: For chemists, biotechnology is perhaps an essential requirement to access new microbial products or to get sufficient amounts of penicillin, and for biotechnologists, chemistry may be just a tool to understand a metabolome. I realized in the past decades, however, that both disciplines are more and more understanding each other now and are coming closer together. However, what we still need is also to understand the language of our "lab animals", e.g. of the microbes.

In my presentation, I will illuminate the different approaches of biologists (in a wider sense) and chemists to optimize e.g. a fermentation, to extract and to purify metabolites, to identify known compounds (a process, which is called dereplication), to elucidate a structure and to look for biological activities. In most of these steps, computational techniques are applied, and the differences and advantages of some of these concepts will be discussed. Key phrases are "chemicals are words in the microbial language", the OSMAC method and neuronal networks for strain optimization, separation techniques, dereplication by databases or molecular networking, or enzymatic versus *ab-initio* configuration determination, just to name a few. It will come out that both approaches are more or less synergistic.

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\*Corresponding author: E-mail address: hlaatsc@gwdg.de

## ***In silico* approaches to identify the NAD<sup>+</sup>-dependent DNA ligase gene (*sll1209*) from cyanobacterium *Synechocystis* sp. PCC6803**

Cherdsak Maneeruttanarungroj<sup>1,2\*</sup>, Khanittha Samasil<sup>3</sup>, Panutda Yodsang<sup>4</sup>

*1*Bioenergy Research Unit and *2*Department of Chemistry, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand.

*3*Laboratory of Cyanobacterial Biotechnology, Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok, 10330, 10520, Thailand.

*4*King Mongkut's University of Technology Thonburi (Ratchaburi Campus), Ratchaburi 70150, Thailand

### **Abstract**

NAD<sup>+</sup>-dependent DNA ligase from model organism cyanobacterium *Synechocystis* sp. PCC6803 was characterized using bioinformatics tools to investigate the deep understanding of the protein function. The gene *sll1209* encoded for ligase enzyme was retrieved from Cyanobase website (<http://genome.microbedb.jp/cyanobase/>). This gene was predicted for protein primary sequence containing 669 amino acid residues with a molecular size of 74.62 KDa and pI of 5.59. The bootstrap phylogenetic tree could separate the DNA ligase enzyme into 3 groups including cyanobacterial, bacterial and eukaryotic groups. DNA ligase from *Synechocystis* sp. PCC6803 could be placed into cyanobacterial group as predicted. The ligase enzyme contains 5 domains including adenylation domain, OB domain, zinc finger domain, helix hairpin helix domain and BRCT domain. In adenylation domain, the K-X-D-G amino acid sequence was observed as the important characteristic for ligation catalysis reaction in NAD<sup>+</sup>-dependent DNA ligase class. Protein primary sequence was further modeled for three dimensional structure using the Raptor X program available online at <http://raptorx.uchicago.edu/>. The modeled result showed the high similarity to DNA ligase from *E. coli* (PDB code 2OWOA) with the lowest p-value of 1.25e-22 for the overall first 4 domains except BRCT domain which conserved among the cyanobacterial group. Superimposition view showed that most secondary structures of these enzymes, both from *E. coli* and *Synechocystis* sp., could be aligned in the same configuration especially at the active site in which the K-X-D-G amino sequence located. This is the first preliminary study for NAD<sup>+</sup>-dependent DNA ligase from the model organism cyanobacterium *Synechocystis*.

**Keywords:** NAD<sup>+</sup>-dependent DNA ligase, *Synechocystis* sp. PCC6803, protein modeling, protein structure, KXDG amino acid sequence

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\*Corresponding author: E-mail address: cherdsak.ma@kmitl.ac.th

# Stabilized forms of enzyme His<sub>6</sub>-OPH in hydrolysis of organophosphorus pesticides and N-N-acyl homoserine lactones

Maslova O.V.\*, Senko O.V., Lyagin I.V., Stepanov N.A., Efremenko E.N.

*The M.V. Lomonosov Moscow State University*

## Abstract

Organophosphorus hydrolase (OPH) is the most well studied enzyme catalyzing the hydrolysis of different organophosphorus pesticides (OPP) containing P-O and P-S bonds. Polyhistidine-containing polypeptide with the catalytic activity of OPH was obtained as genetically modified form of OPH (His<sub>6</sub>-OPH) and it possesses notably improved catalytic properties in reactions with a number of substrates as compared to native enzyme.

In investigations of Ecobiocatalysis laboratory of Chemical Department of the M.V. Lomonosov Moscow State University, the high efficiency of hydrolytic action of His<sub>6</sub>-OPH was revealed in hydrolytic processes of OPP under various conditions and in different media.

The possible application of His<sub>6</sub>-OPH in practice seems to be realistic if the obtained results of optimization of conditions that should be used for production of the enzyme in soluble form someone can take into account, especially in a case when the conditions application can be scaled up concurrently with shortening of time necessary for accumulation of target protein in the maximum intracellular concentration.

It was discovered that same enzyme His<sub>6</sub>-OPH has lactonase activity in relation to N-acyl homoserine lactones (AHL) differed in chemical structures between each other. It is known that AHL trigger the formation of quorum-response of gram-positive bacteria including pathogen agents provoking development of diseases in animals. These results provide an interest to the practical use of the enzyme in agriculture in various directions.

The use of His<sub>6</sub>-OPH in agriculture demands the development of stabilized forms of the enzyme enabling the increase in duration of its effective functioning.

To reach this goal a number of stabilized forms of the enzyme were obtained. The used approach to that was based on obtaining of polyelectrolyte complexes (PEC) between enzyme and specially used polymer. Various poly-anions were used as partners for the enzyme in PEC. Used polymers were characterized by different chemical structure and molecular weight. The formation of PEC was realized at pH 7.5 and 10.5.

High enzymatic activity of PEC was detected in reactions with OPP and AHL Independently on the type of prepared samples. The samples demonstrated effective catalytic hydrolysis of various AHL containing from 4 to 12 carbon atoms. With the elongation of the acyl-radical in the AHL-molecule the effective constant of enzyme action increased. The main reason of that was the decrease of K<sub>m</sub>- value, characterizing the improvement of substrate specificity of His<sub>6</sub>-OPH to corresponding AHL.

The obtained results open the perspectives for the further investigation of properties just developed PEC based on His<sub>6</sub>-OPH for the degradation of OPP present in agricultural sources as well as for the application as additives to antimicrobial agents used against gram-positive pathogens of animals allowing improvement of effective action of the agents. *The work was financially supported by RSCF (project 16-14-00061).*

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\*Corresponding author: E-mail address: olga-still@mail.ru

## Стабилизированные формы фермента His<sub>6</sub>-ОРН в гидролизе фосфорорганических пестицидов и N-ацилгомосеринлактонов

Маслова О.В., Сенько О.В., Лягин И.В., Степанов Н.А., Ефременко Е.Н.

*Московский государственный университет имени М.В. Ломоносова*

Органофосфатгидролаза (ОРН) является сегодня наиболее охарактеризованным ферментом, осуществляющим гидролиз различных фосфорорганических пестицидов (ФОП), содержащих Р-О и Р-S связи. Полигистидин-содержащий полипептид со свойствами органофосфатгидролазы является генетически модифицированной формой органофосфатгидролазы (His<sub>6</sub>-ОРН) и обладает значительно улучшенными каталитическими характеристиками по ряду субстратов в сравнении с природным ферментом.

В исследованиях лаборатории эковиокатализа Химического факультета МГУ имени М.В.Ломоносова установлена высокая эффективность действия His<sub>6</sub>-ОРН в процессах гидролиза ФОП в различных условиях и средах.

Возможность широкого применения фермента His<sub>6</sub>-ОРН на практике представляется реализуемой в свете результатов оптимизации условий получения данного фермента в высокоактивной растворимой форме в легко масштабируемых условиях с сокращённым временем накопления в клетках целевого белка в максимальной концентрации.

Было обнаружено, что тот же фермент обладает лактоназной активностью по отношению к отличным по структуре ацилгомосеринлактонам (АГЛ), которые являются сигнальными молекулами кворум-зависимых бактерий (в том числе возбудителей болезней животных и птиц). Это обеспечивает основу для его практического применения с целью решения различных задач агропромышленного комплекса.

Практическое использование His<sub>6</sub>-ОРН в сельском хозяйстве требует разработки стабилизированных форм данного фермента с целью увеличения длительности его эффективного функционирования.

В работе были получены стабилизированные формы фермента в виде разнообразных фермент-полиэлектролитных комплексов (ФПК). В качестве партнёров фермента по ФПК использовались разные полианионы, характеризующиеся разнообразной химической структурой и молекулярной массой. Формирование ФПК осуществлялось при рН 7,5 и 10,5.

Не зависимо от типа полученных образцов ФПК была установлена их высокая ферментативная активность в отношении как ФОП, так и АГЛ. При этом образцы ФПК, полученные на основе His<sub>6</sub>-ОРН, одинаково эффективно катализировали гидролиз различных по строению АГЛ, содержащих 3-оксо-группу в ацильном радикале (С<sub>8</sub>, С<sub>10</sub> и С<sub>12</sub>), и не содержащих данную группу (С<sub>4</sub>, С<sub>6</sub>). С увеличением длины ацильного радикала в молекуле АГЛ с 4 до 12 атомов углерода константа эффективности действия фермента также увеличивалась. Основной причиной такой закономерности было уменьшение константа Михаэлиса, характеризующей сродство His<sub>6</sub>-ОРН к АГЛ, при переходе от лактона С<sub>4</sub> к С<sub>12</sub>.

Полученные результаты открывают перспективы для дальнейшего исследования свойств полученных ФПК на основе His<sub>6</sub>-ОРН для разложения ФОП, содержащихся в сельскохозяйственном сырье, а также для применения ФПК в качестве добавки к антимикробным препаратам, применяемым в животноводстве против грамположительных патогенов, с целью повышения эффективности действия этих препаратов. *Работа выполнена при финансовой поддержке РФФИ (грант 16-14-00061).*

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\*Corresponding author: E-mail address: olga-still@mail.ru



## **New biotechnology in modern agriculture of Ukraine**

Melnik IA, Sendetsky VN

*Association "Bioconversion" Ivano-Frankivsk (Ukraine)*

### **Abstract**

The results of long-term fundamental and applied research on the development and introduction of innovative technologies for improving soil fertility for sustainable functioning of agro-ecosystems, the production of environmentally friendly products and environmental protection in modern agriculture.

On the basis of comprehensive long-term studies to resolve the issues of improving soil fertility and balanced development of agro-ecosystems, we achieved the following results:

- Developed and implemented technology and industrial vermi-cultivation vermicomposting using red Californian worms;
- Developed manufacturing technology and methods of application of organic fertilizer "Biohumus" obtained by vermi-cultivation in agribusiness, and in suburban backyards;
- Developed, patented and introduced the technology of accelerated biological fermentation of organic animal waste and plant and other origin in bio-fermentators that organo-defecators and organic fertilizers "Mikroorganik" by the method of accelerated biological fermentation in open areas using the mixer-aerator;
- Developed, patented and introduced technology for processing organic waste in leather industry and sewage sludge by accelerated biological fermentation;
- Developed technology to produce and organize production in PP "Bioconversion" humic complex biological products "Vermistim", "Vermimag", "Vermiyodis" vermi-composts and organic tea, bio-degrader straw and plant residues of winter and spring crops "Vermistim-D";
- Developed and implemented technology in the production of 18 biologic drugs, isolated and deposited 7 strains of microorganisms in a microbiology laboratory PP "Agrofirma" Kolos "of the Kiev region.

During 2011-2015, complex humic biological products "Vermistim", "Vermimag", "Vermiyodis" vermi-composts "teas", destructor "Vermistim-D" was used in the technology of cultivation of winter and spring cereals, soybean, canola, sunflower, corn, sugar beet, rice, potatoes, vegetables and other crops for pre-sowing treatment of seeds, root and foliar feeding crops in agricultural enterprises, in the farmlands in the Ivano-Frankivsk, Odessa, Kirovograd, Chernigov, Nikolaev, Kiev and other areas on the area of more than 500,000 hectares while providing increase of yields by more than 1.5 billion. UAH. (In 2015 prices).

Scientific-theoretical and experimental research and the widespread introduction of new technologies of organic fertilizers by vermi-culture and accelerated biological fermentation, complex humic biologic preparation, straw degradation with simultaneous sowing green manure crops for the efficient functioning of modern adaptive-landscape agriculture is a significant and integral part of a national program to improve fertility of soil, and increase of the inexpensive production of environmentally friendly products for the domestic and foreign markets, to minimize the negative impact on the environment.

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\*Corresponding author: E-mail address: [vermos2007@ukr.net](mailto:vermos2007@ukr.net)

## Новые биотехнологии в современном земледелии Украины

Мельник И.А., Сендецкий В.Н.

*Ассоциация «Биоконверсия», г. Ивано-Франковск (Украина)*

Представлены результаты многолетних фундаментальных и прикладных исследований по разработке и внедрению инновационных технологий улучшения плодородия почв для устойчивого функционирования агроэкосистем, производство экологически чистой продукции и охраны окружающей среды в современном земледелии.

На основе комплексных многолетних исследований нами разработаны научные и практические основы решения проблемы улучшения плодородия почв и сбалансированного развития агроэкосистем и увеличения производства экологически чистой сельскохозяйственной продукции высокого качества и охраны окружающей среды, а именно:

- В Украине и бывшем Союзе впервые разработана и внедрена технология промышленного вермикультивирования и вермикомпостирования с помощью завезенных нами из за границы красных дождевых калифорнийских червей.

- Разработана технология производства и способы применения органических удобрений «Биогумус» полученных методом вермикультивирования в агропредприятиях, на дачных и приусадебных участках.

- В Украине впервые разработана, запатентована и внедрена технология ускоренной биологической ферментации органических отходов животного и растительного и иного происхождения в биоферментаторах та органо-дефекатных удобрений и органических удобрений «Микроорганик» методом ускоренной биологической ферментации на открытых площадках с помощью смесителя-аэрата.

- В Украине и странах бывшего СНГ впервые разработаны, запатентованы и внедрена технология переработки органических отходов кожевенного производства и осадка сточных вод методом ускоренной биологической ферментации.

- Разработана технология производства и организовано в ПП «Биоконверсия» производство комплексных гуминовых биопрепаратов «Вермистим», «Вермимаг», «Вермийодис», технология производства и организован процесс производства вермикомпостных и биочайов, производство биодеструктора соломы и растительных остатков озимых и яровых культур «Вермистим-Д».

- Разработана технология и внедрен процесс производства на базе микробиологической лаборатории ПП «Агрофирма» Колос »Киевской области 18 биопрепаратов, изолированно и задепонировано 7 штаммов микроорганизмов.

В течение 2011-2015 гг., комплексные гуминовые биопрепараты «Вермистим», «Вермимаг», «Вермийодис», вермикомпостный «чай», деструктор «Вермистим-Д» применяли в технологиях выращивания озимых и яровых зерновых, сои, рапса, подсолнечника, кукурузы, сахарной свеклы, риса, картофеля, овощных и других культур для допосевной обработки семян, корневой и внекорневой подкормки посевов в агропредприятиях, на дачных и приусадебных участках в Ивано-Франковской, Одесской, Кировоградской, Черниговской, Николаевской, Киевской и других областях на площади более 500 000 гектаров и обеспечили увеличение урожайности на сумму более 1,5 млрд. грн. (По ценам 2015 года).

Научно-теоретические и экспериментальные исследования и широкое внедрение новейших технологий производства органических удобрений методом вермикультивирования, ускоренной биологической ферментации, комплексных гуминовых биопрепаратов, деструкции соломы с одновременным посевом культур на сидераты для эффективного функционирования современного адаптивно-ландшафтного земледелия является весомой и неотъемлемой составляющей общенациональной программы

улучшение плодородия почв, удешевление и увеличение производства экологически чистой продукции для внутреннего и внешнего рынков и минимизации негативного воздействия на окружающую среду.

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\*Corresponding author: E-mail address: [vermos2007@ukr.net](mailto:vermos2007@ukr.net)

## Breaking technologies in biological control

Vladimir Pavlyushin

*All-Russian Institute of Plant Protection*

### Abstract

In ecologically friendly plant protection, the role of biotechnological aspects of the phytosanitary is very important. They include molecular diagnostics of phytosanitary objects, the use of resistant cultivars, biopesticides and entomophagous organisms.

During the last years, research institutes (All-Russian Institute of Plant Protection, All-Russian Institute of Agricultural Microbiology) and some companies (“Agrobiotechnology LTD”, “Pharmbiomed LTD”, “Sibbiopharm LTD”) introduced more than 30 bioproducts for plant protection. Their application in more than 50 crops allows decrease use of chemical pesticides.

All-Russian Institute of Plant Protection offers 20 species of selected entomophagous insects for protection of vegetables against aphids, white flies, and trips in the greenhouse. The sets of entomophagous insects and useful microorganisms were established for control of more than 10–15 greenhouse pests. To get the guaranteed protection effect following rules should be performed:

- permanent monitoring for pest and useful organisms;
- preventive use of biostimulants and biopesticides;
- coincidence of optimums for biocontrol activity and crop production;
- simultaneous action of different biocontrol agents;
- compatibility of biocontrol measures and other crop protection techniques.

For the successful development of biopesticides a number of characteristics of microorganisms should be taken in account: high activity, adaptability, ecological plasticity, competitive ability and wide functionality. Biopesticides based on *Bacillus subtilis* (Alirin-B, Gamair etc) effectively control bacterial and fungal diseases and stimulate plant growth. Biofungicide Sternifag based on *Trichoderma harzianum* fastens degradation of plant residues. The selection of entomopathogenic fungi from genera *Beauveria* and *Metarhizium* allowed finding strains that caused high level of death of *Calliptamus italicus* and *Dociostaurus maroccanus* within 2 weeks.

Priority studies in development of biopesticides include:

- construction of recombinant strains with higher insecticidal toxicity;
- development of multi-function biopesticides that show plant growth stimulation effects and optimize microbe communities;
- establishment of epizooties in the natural reservations of locusts.

**Isolation, biological characterization and herbicidal potential of phytotoxins from  
*Paraphoma* sp. 1.46, a pathogen of *Cirsium arvense***

Ekaterina Poluektova, Alexander Berestetskiy

*All-Russian Research Institute for Plant protection (VIZR)*

*Saint-Petersburg, Russia, 196608*

**Abstract**

The fungal genus *Phoma* is known for its diverse spectrum of secondary metabolites including phytotoxins (*P. herbarum*, *P. macrostoma*). Our bioassays had shown that extracts of *Paraphoma* sp. VIZR 1.46, a pathogen of *Cirsium arvense*, are phytotoxic. In this study, two phytotoxically active metabolites were isolated from *Paraphoma* sp. 1.46 liquid and solid cultures. These compounds were identified as curvulin and phaeosphaeride A (PhA), respectively. Both metabolites were assayed for phytotoxic, antimicrobial and zootoxic activities. Curvulin and PhA were tested on weedy and agrarian plants, fungi, Gram+ and Gram- bacteria, and on paramecia. None of two metabolites showed antimicrobial activity when assayed on the eight tested bacteria and two fungi at concentration up to 100 µg/disc. Curvulin showed a weak nonselective phytotoxic activity when assayed on ten plant species in concentration of 2 mg/ml, what is corresponding with early reported data for this compound (Kenfield et al., 1989). PhA demonstrated high level of nonselective toxicity on all the tested plants. Phytotoxic activity of this compound was reported for the first time, earlier PhA was determined to inhibit signaling by the transcription factor STAT-3 (Maloney et al., 2006), located in a nucleus of cell. This protein seems to be unknown in plants. For further investigations method of *Paraphoma* sp. 1.46 solid state fermentation was optimized and it allowed to increase PhA production from 130 mg/kg to 1.9g/kg. To evaluate PhA herbicidal efficacy, phytotoxic activity of compound in combination with five different adjuvants was studied. Our results have shown that addition of some adjuvants, including Hasten, Trend-90 and Biopower, allowed to increase PhA activity on intact leaf discs of the tested plants. Treatment of *Cirsium arvense* plants with extract of solid state culture of *Paraphoma* sp.1.46 in combination with Hasten caused necrosis of 70-80% of leaves. It was 2.5 fold more effective than the effect of extract without adjuvants and caused 3fold decrease of leave biomass. These results could be useful for devising new natural products with potential application as a herbicide in agriculture, otherwise, these data can be used when studying the mode of action of PhA.

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\*Corresponding author: E-mail address: catcatwow@mail.ru, toxbiotech@vizr.spb.ru

# Biodiversity and plant-growth-promoting activities of endophytic bacteria from rice

(*Oryza sativa*)

Wilasinee Raweekul<sup>1</sup>, Sukritta Wuttitummaporn<sup>1</sup>, Wanrudee Sodchuen<sup>1</sup>

and Chokchai Kittiwongwattana<sup>1,2\*</sup>

<sup>1</sup>Department of Biology, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang  
Bangkok, Thailand

<sup>2</sup>Actinobacteria research unit, Faculty of Science, King Mongkut's Institute of Technology  
Ladkrabang Bangkok, Thailand

## Abstract

Endophytic bacteria are defined as bacteria that colonize internal tissues of plants without causing diseases to the plant host. Research on endophytic bacteria has been focused on two particular topics on the diversity as well as their positive effects towards plant growth. Plant internal tissues represent a specialized environment that may affect the diversity of bacterial communities. Recently, my lab isolated and characterized 126 endophytic bacteria from rice plants that were grown in organic farms. Based on the analysis of partial sequences of the 16S rRNA gene, these bacteria were members in four different phyla and classified into 31 different genera. A distinction between the diversity of endophytic bacteria in roots and stems was observed. While *Firmicutes* was found dominant (66.7%) in the roots, *Proteobacteria* represents the majority (53.7%) in the stems. Additionally, the nearly complete sequences of the 16S rRNA gene and fatty acid profiling of isolates 1017 and 1048 suggested that they were potential candidates for novel bacterial species in genera *Pedobacter* and *Sphingomonas*, respectively. Their closest relatives were *Pedobacter tournemirensis* TF5-37.2-LB10<sup>T</sup> (96.6% sequence similarity) and *Sphingomonas changbaiensis* V2M44<sup>T</sup> (97.4%). For direct plant growth promotion, all endophytic bacteria were co-cultivated with rice seedlings for 16 days. The fresh weight of the seedlings that were inoculated with twelve bacterial isolates was at least two-fold higher than that of the water-treated control group. Various plant-growth-promoting traits were examined in these isolates. While seven isolates displayed at least one trait, the remaining isolates were tested negative for every trait. For the indirect effect on plant growth, 14 isolates displayed the antagonistic activities against either *Xanthomonas oryzae* or *Pyricularia grisea* while four isolates were able to inhibit the growth of both pathogens. However, only seven isolates were found effective in controlling the leaf blight and leaf blast diseases *in vivo*. All seven isolates produced protease whereas only one isolate was positive for chitinase. Taken together, the results obtained from this study signify the importance and potential of endophytic bacteria as biofertilizers and biocontrol agents.

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\*Corresponding author: E-mail address: chokchai.ki@kmitl.ac.th

## New biological preservatives for storage of fruit and vegetable products

Rimareva L.V., Volkova G.S., Kuksova E.V., Pogorzshelskaya N.S.\*

*The Russian Research Institute of food biotechnology – branch of Federal Research Centre of Nutrition, Biotechnologie and Food Safety, Moscow*

### Abstract

Population requirements satisfaction in high-quality products of food – one of the most important problems of modern science and practice. In our country special attention is paid to questions of the solution of this problem. Improvement of supply of the population with fruit and vegetable products requires not only further increase in production and improvement of quality, but also reducing losses of products along the line from the field to the consumer. It is possible thanks to implementation of progressive technologies of handling and storage of fruits and vegetables.

It is known that at the heart of all practical actions for storage of fresh fruits and vegetables management of the fiziologo-biochemical processes happening in fruit and vegetable products for the purpose of prevention of losses of nutrition value, protection against microbiological spoil and premature germination lies.

On the basis of modern methods of food biotechnology the new natural biopreservatives allowing to provide long-term storage of fruits and vegetables, and also to reduce irreversible changes of valuable substances are created.

Natural biopreservatives are products of an organic origin and represent the solutions received after fermentation of acid-forming bacteria, containing organic acids and biomass. Thanks to availability of bakteriotics such biopreservatives have antimicrobial properties.

Characteristics of the used natural biopreservatives is their organic origin, harmlessness for a human body, lack of a pungent smell and influence on tastes of the processed raw materials, and also the minimum time of impact for achievement of protective effect.

For receipt of biopreservatives fermentations of the selected strains of *Lactobacillus plantarum* B-578/2 and *Propionibacterium freudenreichii subsp shermani* Ac-103/12 – producers of organic acids and bakteriotics for the purpose of receipt of biopreservatives. The indicators characterizing them protective and preventive and antimicrobial properties are determined. Researches on assessment of effect of biopreservatives for storage durations and quality of fruit and vegetable products are conducted.

Handling of fruit and vegetable raw materials solution of biopreservatives was carried out after a raw materials sink by flowing water by immersion or spraying, with the endurance providing necessary efficiency. In particular, handling of carrots solutions of biopreservatives allowed to reduce losses to 4-5%. At the same time safety of fresh root crops without signs of microbiological spoil up to 10 months and compliance to their requirements the SanPiN 2.3.2.1078 was reached.

The offered method of storage of fruit and vegetable products showed outstanding performance and can be demanded in the conditions of the fruit and vegetable warehouses and the entities which don't have the equipped storages of industrial amount. Use of natural biopreservatives allows to refuse preservatives of a synthetic and chemical origin.

*The researches are executed with the support of the Russian Science Foundation grant No. 16-16-00104.*

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\*Corresponding author: E-mail address: [ferment\\_vniipbt@mail.ru](mailto:ferment_vniipbt@mail.ru)

## Новые биологические консерванты для хранения плодоовощной продукции

Римарева Л.В., Волкова Г. С., Куксова Е.В., Погоржельская Н.С.\*

*Всероссийский научно-исследовательский институт пищевой биотехнологии – филиал ФГБУН Федерального исследовательского центра питания, биотехнологии и безопасности пищи, г. Москва*

Удовлетворение потребностей населения в высококачественных продуктах питания – одна из важнейших проблем современной науки и практики. В нашей стране вопросам решения этой проблемы уделяется особое внимание. Для улучшения снабжения населения плодоовощной продукцией необходимо не только дальнейшее увеличение производства и повышение качества, но и сокращение потерь продукции на пути следования от поля до потребителя. Это возможно благодаря внедрению прогрессивных технологий обработки и хранения плодов и овощей.

Известно, что в основе всех практических мероприятий по хранению свежих плодов и овощей лежит управление физиолого-биохимическими процессами, происходящими в плодоовощной продукции, с целью предотвращения потерь пищевой ценности, защиты от микробиологической порчи и преждевременного прорастания.

На основе современных методов пищевой биотехнологии созданы новые натуральные биоконсерванты, позволяющие обеспечить длительное хранение плодов и овощей, а также сократить необратимые изменения ценных веществ.

Натуральные биоконсерванты являются продуктами органического происхождения и представляют собой растворы, полученные после ферментации кислотообразующих бактерий, содержащие органические кислоты и биомассу. Благодаря наличию бактериоцинов такие биоконсерванты обладают антимикробными свойствами.

Характерными особенностями используемых натуральных биоконсервантов является их органическое происхождение, безвредность для организма человека, отсутствие резкого запаха и влияния на вкусовые качества обрабатываемого сырья, а также минимальное время воздействия для достижения защитного эффекта.

Для получения биоконсервантов были проведены ферментации селекционированных штаммов *Lactobacillus plantarum* B-578/2 и *Propionibacterium freudenreichii* subsp. *shermani* Ac-103/12 – продуцентов органических кислот и бактериоцинов с целью получения биоконсервантов. Определены показатели, характеризующие их защитно-профилактические и антимикробные свойства. Проведены исследования по оценке действия биоконсервантов на сроки хранения и качество плодоовощной продукции.

Обработку плодоовощного сырья раствором биоконсервантов проводили после мойки сырья проточной водой путем погружения или разбрызгивания, с выдержкой, обеспечивающей необходимую эффективность. В частности, обработка моркови растворами биоконсервантов позволила сократить потери до 4-5%. При этом достигалась сохранность свежих корнеплодов без признаков микробиологической порчи до 10 месяцев и соответствие их требованиям СанПиН 2.3.2.1078.

Предлагаемый способ хранения плодоовощной продукции показал высокую эффективность и может быть востребован в условиях плодоовощных баз и предприятий, не имеющих оборудованных хранилищ промышленного объема. Применение натуральных биоконсервантов позволяет отказаться от консервантов синтетического и химического происхождения.

*Исследования выполнены при поддержке гранта Российского научного фонда № 16-16-00104.*

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\*Corresponding author: E-mail address: [ferment\\_vniipbt@mail.ru](mailto:ferment_vniipbt@mail.ru)



## Genetic improvement of olive (*Olea europaea* L.) by conventional and *in vitro* biotechnology research methods

Eddo Rugini\*, Cristian Silvestri, Valerio Cristofori, Marco Cirilli, Rosario Muleo, Bruno Ruggiero,

Ciro De Pace

University of Tuscia, Department of Agricultural and Forestry Science, Via S.C. De Lellis,  
01100 Viterbo, Italy

### Abstract

Over 90% of Olive trees (*Olea europaea* L. ssp. *europaea* var. *europaea*) are cultivated in the Mediterranean Basin, where they coexist with their wild ancestor, the oleaster (-var. *sylvestris*). Recently, due to the impact of the healthy properties of the fruit components, the cultivation began to expand to new countries, which adopt intensive and super-intensive plantation practices which require however new suitable varieties or rootstocks. The olive groves are made up of selected clonal varieties and ancient olives from oleaster ecotypes. Among more than 1,200 cultivated accessions, only two-or three-dozen contribute to more than 90% of the total olive production. Oil and fruits sustain the incomes of many farmers and are key ingredients of the *mediterranean diet*. Many of olive's chemical compounds provide salutary properties to humans and are used for external application as antimicrobial agents, while several of them also act as biocontrol agents for the olive fruit itself, protecting it from pathogens and insects. Other plant organs are important for the development of new plant extracts for medicines (i.e. bark and leaf infusions for treat diarrhea and blood pressure) and agricultural products.

Exploration of the olive genetic resources allows for the identification of accessions that accumulate functional chemicals in a different way and concentration. New spectroscopic methodologies allow for the detailed chemical fingerprinting of the olive fruit metabolome and olive oil components, useful in the typification of accessions with significant biomarkers of resistance to stresses, as well as of the functional chemicals with bioactive and antimicrobial effects. Conventional plant breeding approaches based on intercrosses of the fingerprinted accessions and clonal selections within their progeny support the development of new olive varieties suitable for mechanical pruning and harvesting, having high oil and metabolites (bioactive phenols, antioxidant, oleuropein, vitamins) content, that correspond to social acclaims in health, nutrition and sustainable agriculture.

Metabolomic results complement the genomic and transcriptomic information on genes related to the regulation of metabolic pathways. High throughput RNAseq analysis is also helpful to identify epigenetic regulative elements of gene expression (i.e. smallRNA) and, in combination with genomic and EpiHRMAssay system, screen the olive germplasm for an improvement of the biochemical synthesis in modern olive varieties.

Biotechnology techniques have been developed for the rapid clonal propagation of new olive genotypes and for shoot and somatic embryo regeneration from mature tissues, allowing the production of genetically-modified (GM) plants. In GM-olive, the constitutively overexpressed *Osmotin* tobacco gene, which encodes the protein belonging to the PR5 family, induces protection from freezing, drought and fungal diseases. These plants are now being considered as *biofactories* in view of the effects of this protein in humans and animals for the control of various diseases including diabetes, cancer and disorders of the central nervous system, comprising Alzheimer's disease. Transgenic *rolABC* olive plants resulted instead in candidates for dwarfing rootstocks. With the advent of new technologies, such as the "clustered regularly interspaced short palindromic repeats (CRISPR)-Cas" genome editing system, the boundaries between GM and non-GM techniques will become increasingly blurred, and a policy

window is being opened to improve rules for meaningful and impactful change for safe, responsible, legitimate, and appropriate use of future varieties.

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\*Corresponding author: E-mail address: [rugini@unitus.it](mailto:rugini@unitus.it)

## Biodiversity of *Ophiocordyceps nutans* collected in Thailand

Wuttikorn Pooissarakul<sup>1,2</sup>, Nattawut Rungjindamai<sup>1\*</sup>, Suchada Mongkolsamrit<sup>2</sup> and Janet Jennifer Luangsa-ard<sup>2</sup>

<sup>1</sup>Department of Biology, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang (KMIL), Chalongkrung Road, Ladkrabang, Bangkok 10520 Thailand

<sup>2</sup>Microbe Interaction Laboratory (BMIT), National Center for Genetic Engineering and Biotechnology (BIOTEC) 113 Thailand Science Park, Phahonyothin Road, Khlong Nueng, Khlong Luang, Pathum Thani 12120 Thailand

### Abstract

Entomopathogenic fungi are a group of fungi that live in insect hosts as a parasite and later kill their hosts. They are well known as producers of diverse biologically active secondary metabolites. Thailand, which is situated in a tropical region, harbours a huge diversity of insect fungi with many genera were recorded in many parts of Thailand. In this study *Ophiocordyceps nutans* is focused. This fungus was identified mainly based on its morphology. *O. nutans* was collected from three different locations including Phu Khiao Wildlife Sanctuary, Khao Yai National Park and Phaothai Community Forrest. The samples of insect fungi were incubated in a moist chamber to facilitate ascospore discharge. The fungi were isolated from its host using a single spore isolation method and a spore shooting method. The cultures of *O. nutans* were studied morphologically and phylogenetically. Morphological characteristics of *O. nutans* comprising of its colours, size of fruiting body and host were recorded and photographed. The DNA barcoding region Internal Transcribed Spacer (ITS) rDNA and partial sequences of two genes including Large Subunit (LSU) and RNA polymerase II (RPB2) were amplified and phylogenetic trees were constructed. Biodiversity of *O. nutans* and relationship between *O. nutans* and its hosts are discussed.

**Keywords:** Biodiversity, Cryptic species, *Ophiocordyceps nutans*, phylogeny

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\*Corresponding author: E-mail address: nr74@kentforlife.net; nattawut.ru@kmitl.ac.th

## **Study of the influence disease resistance inducers on the development of wheat leaf rust under greenhouse and field conditions**

Volkova G.V., Shebyakovsky S.V.\*, Vaganova O.F., Dyadyuchenko L.V.

*FGBNU "All-Russian Research Institute of Plant Biological Protection", Krasnodar*

The aim of our study was to evaluate the effectiveness of a number of known and new disease resistance inducers against the causative agent of wheat leaf rust under greenhouse and field conditions.

To study the drugs were selected: Bioduks, M (active ingredient: arachidonic acid 0.3 g/l, consumption rate: 3 ml/ha.); Biosil, WE (active ingredient: triterpene acids 100 g/l, consumption rate: 30 ml/ha); Immunotsifit, P (active ingredient: arachidonic acid ethyl ester, 0.167 g/kg, consumption rate: pill/100 ml of water); Ribav Extra, L (active ingredient: L-alanine, L-glutamic acid, 0.00152 + 0.00196 g/l, consumption rate: 1 ml/ha); Ekogel, WS (active ingredient: lactate chitosan, 30 g/l, consumption rate: 4 g/l); Appin - Extra, S (active ingredient: 24-epibrasinolide, 0.025 g/l, consumption rate: 50 ml/ha), and compound L-241, L-222, DA-59, L-226, L-191, L-194 first synthesized in VNIIBZR. Biological standard Zircon, S (active ingredient: hydroxycinnamic acid, 0.1 g/l, consumption rate 20 ml/ha), Chemical standard – Amistar Extra, SC (active ingredient: azoxystrobin + cyproconazole, 200 + 80 g/l, consumption rate - 0.5 l/ha).

We used two methods of treatment - at the very first signs of the disease and during the mass development of the disease. Artificial infection Urediniospores leaf rust pathogen was carried out by existing methods (Anpilogova, Volkova, 2000).

It was found that under conditions of the greenhouse effect of the disease resistance inducers on sort Vershina the first signs of the wheat leaf rust was higher than in processing during the mass development of the disease, which confirms previous studies (Volkova, Kolbin 2012). The effectiveness of the use inducers on the first signs of the disease ranged from 20% to 49% with the maximum rates for the L-241 (49%) and Bioduks (49%). Biological Standard Zircon holding back progression of the disease by 58%, the chemical standard Amistar Extra - 100%. Treatment of disease resistance inducers in the mass development of *Puccinia triticina* reduce disease development from 6% (Ribav Extra) to 36% (L-241). The effect of the biological standard 38%, the chemical - 79%.

In the field, on the sort Batko, effectiveness of disease resistance inducers applied for the very first signs of the disease ranged from 14% to 52%. The maximum effect was observed for the drug L-241, synthesized in VNIIBZR. The effectiveness of the biological standard Zircon was 43%, the chemical Amistar Extra - 92%.

We believe that the use of inductors of plant disease resistance is one of the most effective methods of phytosanitary agroecosystem optimization, including winter wheat. Preparations of this type have a low toxicity for the fauna are not inferior chemical fungicides on the nature of phytopathogens on effectiveness, cost less and have low consumption rates, which makes their use environmentally advantageous.

This work was supported by Russian Foundation for Basic Research grant and administration of the Krasnodar Territory №16-04-230459

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\*Corresponding author: E-mail address: shebiakovsky@gmail.com

**Direction: biotechnological approaches to develop new crop protection agents and drugs.**  
**Characteristics and critical points of biotechnological process small-volume production**

Sedinina Natalia, Kotlyarov Vladimir, Kotlyarov Denis, Donchenko Dmitry,

Poplevina Viktoria

*OOO Kuban agricultural technologies, Krasnodar city*

**Abstract**

The basic concept of biotechnology in crop production, the method of OOO Kuban agricultural technologies based on the reproduction or translation of microbiological preparations in a vegetative state. To do this, use low-tonnage production in the farms under the supervision of specialists of the company. This approach is associated with the production of high-quality products for plant protection with low cost and the idealization of logistics, and confirmed that the practice-small-scale production has fully justified itself in the number of farms in the Krasnodar and other regions of Russia.

Small scale production of microbiological preparations as any biotechnological production is based on certain conditions: the cleanliness and the correct preparation of raw materials, sanitary conditions of water, temperature, pH etc. The difference between the production of microbiological preparations on the basis of low-tonnage enterprises is that the process is not a sterile environment. It is periodic and seasonal process. The sterilization of raw materials is not always feasible and technologically justified. This is due to the loss of nutritional properties of raw materials (grain, bran, molasses etc.) for microorganisms. In addition to the main microflora may develop outside.

In violation of the recommended process conditions the cultivation of extraneous microflora can start active development, and even to prevail over the target. Accumulated in the culture medium, the metabolic products of foreign microorganisms can halt the further propagation of the main culture. Insufficient consumption of sugars by microorganisms and their balance in the final drug after its spill will lead to the continuation of the process of reproduction in packing, which causes deformation of the commodity form of packaging. An important condition is the consumption of the components of the nutrient medium and the completion of the process in the fermenter. Usually, small scale production does not have its own laboratory. Therefore, methods and means of production quality control should be simple. This is a pH meter, thermometers, burettes, microscopes. One of the ways of control of readiness of the product - determining the content of reducing sugars by the method of Bertrand titration. Timely removal of waste production and its utilization ensures clean and necessary sanitary condition in the workshop premises. The use of activators and growth factors in the cultivation of microorganisms is one of the ways to stimulate the development of target microflora.

Thus, to obtain high-quality biotechnological product should the following events and conditions on small-scale production:

- incoming control of raw materials
- disinfection, filtering and control over the sanitary condition of the premises
- sanitization of equipment, inventory

- control temperature and pH of the fermentation process
- provide aeration for aerobic microorganisms or the isolation of oxygen for anaerobic
- determination of content of reducing sugars in the environment
- the dilution or destruction of non-targeted metabolic products of microorganisms.

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\*Corresponding author: E-mail address: [sedinanv@mail.ru](mailto:sedinanv@mail.ru), [vladimir.v.kotlyarov@rambler.ru](mailto:vladimir.v.kotlyarov@rambler.ru), [denis.kotlyarov@rambler.ru](mailto:denis.kotlyarov@rambler.ru), [d.donchenko@mail.ru](mailto:d.donchenko@mail.ru), [Vika\\_ximik@mail.ru](mailto:Vika_ximik@mail.ru)

## **The fight against pests during the degradation of straw in conjunction with the sowing of green manure**

Sendetskyy VN

*Podilsky State Agro-Technical University, Ukraine, Kamyanets'-Podilsky, st. Shevchenko, 13*

### **Abstract**

In order to improve soil fertility, reduce pests in them, we have developed and introduced into production a new technology bio-destructor "Vermystym-D" for processing straw and plant residues into organic fertilizer with simultaneous sowing green manure.

During harvesting cereals, maize or sunflower, straw and other plant remains are crushed in combine's choppers and evenly spread on the soil surface. After that, vegetable residues are sprayed and incorporated into the soil. In traditional agriculture during spraying, a working solution except for bio-destructor "Vermysym-D" (depending on the amount of straw per hectare) includes 8.12 kg / ha of ammonium nitrate or urea, or Nitrate urea-ammonium. In organic farming instead of nitrogen fertilizers, liquid organic fertilizers are used: manure (7-10 t / ha) or "Biohumus" (3-4 t / ha), or "Bioproferm" (5-6 t / ha).

Accelerated destruction of stubble residues by the bio-destructor "Vermystym-D" improves soil fertility by providing them with fungicide and bakteriot microflora, natural vitamins, plant growth hormones, amino acids and trace elements.

Making Biodestructors "vermistim-D" remains on plant grain, corn, sunflower accelerating decomposition residues, pathogens are destroyed. The coefficient of expansion straw 3-4 months is 70-90% (depending on culture), the number of microorganisms, is the stimulation of growth and development of soil microbiota, tselyulozorunyuyuchyh, azotofiksuyuchyh, fosfatmobilizuyuchyh and other microorganisms that zaselyvshys on plant debris with indigenous microflora destroy them, that feed on them, multiply, forming up to 4-6 t / ha own biomass a year, which is after the death of a valuable source of power for these microorganisms and plants. The result is a soluble humus and available for plants form plants necessary macro- and micronutrients.

One ton of grain straw after the destruction of the content of organic matter, nitrogen, phosphorus and potassium equivalent to 3-5 tons of manure with humidity up to 75%, provides increased productivity by 25-35% while reducing the cost of purchasing fertilizers by 40-50%.

The use of straw sowing green manure has significant value in the rotation as a means of inhibiting weeds and diseases. This is caused both timely tillage and improving conditions for the germination of weed seeds under cover of green manure subsequent suppression and destruction due pryoryuvannya green manure and straw.

Our research showed that permanent crops and crop rotation grain saturation to 85% accompanied by a high degree of affection plant barley and winter wheat root rot diseases caused by fungi *Helminthosporium*, *Fusarium*. Pryoryuvannya green manure with straw reduced the destruction of plants in 1.5-2 times, preventing harvest shortfall of plant diseases. This is due to the fact that after the destruction of green manure straw green mass causes rapid development of saprophyte microflora, which accelerates mineralization of plant residues - the main substrate on which pathogens develop root rot, snow mold and others. In addition, due pryoryuvannya green mass of straw in the soil several times growing number of actinomycetes, which are antagonists

of pathogens in the soil. Root selection radish and mustard oil, stimulated output larvae of beet nematode populations in an earlier spring and insufficient amount of active temperatures they could not reach sexual maturity and give the next generation, so that the density of their population decreased by 30-60%.

Consequently, destruction straw while sowing crops syderalnyh enhances soil fertility and provides a significant reduction of pests.

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\*Corresponding author: E-mail address: vermos2007@ukr.net



## **Perspective biotechnological products based on the direction conversion of secondary bioresources of agro-industrial complex**

Serba E.M., Rimareva L.V., Kurbatova E.I., Overchenko M.B., Volkova G.S.

*The Russian Research Institute of food biotechnology – branch of Federal Research Centre of Nutrition, Biotechnologie and Food Safety, Moscow*

### **Abstract**

Filamentous fungi - one of the most important biotechnology objects used in the industry. They not only have the ability to secrete substances having commercial importance (such as enzymes, antibiotics, organic acids and etc.), but also synthesize biomass containing proteins with a high content of essential amino acids, vitamins and valuable polysaccharides.

*Aspergillus* is of great interest to the industry due to its ability to synthesize complex enzymes of different spectrum necessary for the hydrolysis of agricultural raw materials in processing industries of agro-industrial complex. The most promising are the species *A. oryzae*, *A. awamori*, used to prepare enzyme preparations amyolytic and proteolytic activities. In connection with the existing trend of increasing volume and range of biotechnological products obtained using micromicetes, are particularly important issues of waste management of these industries and especially the mushroom mycelium. Fungal biomass is not subject to long-term storage and is a source of environmental pollution. Therefore, studies aimed at the creation of technologies of rational processing of this valuable secondary resource - fungal biomass, are very promising.

Development of technology based on the multipurpose use of microorganisms: the culture fluid - for production of industrially important enzymes and biomass - structural elements of cells containing proteins, nucleic acids, polysaccharides, cell wall - for the production of biologically active food additives and fodder. It is shown that the nutritional value of the microbial biomass is determined by its rich protein-carbohydrate and vitamin complex. The fungal biomass is the high content of essential amino acids and B vitamins, which play an important role in metabolic processes.

Complex processing of secondary resources is biocatalytic degradation of the residual microbial biomass in protein and amino acid dietary supplements. The degree of protein destruction depends on the substances biomass composition enzymatic system, enzyme dosage and time degradation.

The results confirm the possibility of a controlled process of obtaining biological products with the specified structural and fractional composition based on the enzymatic hydrolysis of polymers mycelial biomass. These fermentative of fungal biomass can be used for the enrichment of food and feed digestible low molecular weight peptides, essential amino acids, vitamins and carbohydrates.

Resource-saving technologies allow, recycling waste of biotechnological production in biologically active products, create new types of functional biotechnology products, implement import substitution, contributing to the solution of environmental problems.

*The researches are executed with the support of the Russian Scientific Foundation grant No. 16-16-00104.*

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\*Corresponding author: E-mail address: serbae@mail.ru

## **Перспективные биотехнологические продукты на основе направленной конверсии вторичных биоресурсов АПК**

Серба Е.М., Римарева Л.В., Курбатова Е.И., Оверченко М.Б., Волкова Г.С.

*Всероссийский научно-исследовательский институт пищевой биотехнологии – филиал ФГБУН Федерального исследовательского центра питания, биотехнологии и безопасности пищи, г. Москва*

Мицелиальные грибы — одни из важнейших объектов биотехнологии, применяемые в промышленности. Они обладают способностью не только секретировать вещества, имеющие промышленное значение (такие как ферменты, антибиотики, органические кислоты и др.), но и синтезировать биомассу, содержащую белковые вещества с высоким скором незаменимых аминокислот, витамины и ценные полисахариды.

Грибы рода *Aspergillus* представляют большой интерес для промышленности благодаря способности синтезировать комплекс ферментов различного спектра действия, необходимых для гидролиза сельскохозяйственного сырья в перерабатывающих отраслях АПК. Наиболее перспективными видами являются *A. oryzae*, *A. awamori*, используемые для получения ферментных препаратов протеолитического и амилалитического действия. В связи с имеющейся тенденцией увеличения объема и расширения ассортимента биотехнологических продуктов, получаемых с использованием микромицетов, особое значение приобретают вопросы утилизации отходов этих производств и в первую очередь грибного мицелия. Грибная биомасса не подлежит длительному хранению и является источником загрязнения окружающей среды. Поэтому исследования, направленные на создание технологий рациональной переработки этого ценного вторичного ресурса - биомассы грибов, являются весьма перспективными.

Разработка технологии базируется на многоцелевом использовании микроорганизмов: культуральной жидкости - для получения промышленно значимых ферментов, и биомассы - структурных элементов клетки, содержащих белковые вещества, нуклеиновые кислоты, полисахариды клеточных стенок - для получения биологически активных добавок пищевого и кормового назначения. Показано, что питательная ценность микробной биомассы определяется ее богатым белково-углеводным и витаминным комплексом. В грибной биомассе высокое содержание незаменимых аминокислот и витаминов группы В, играющих важную роль в обменных процессах.

Комплексная переработка вторичных ресурсов заключается в биокаталитической деструкции остаточной микробной биомассы в белково-аминокислотные биологически активные добавки. Установлено, что степень деструкции белковых веществ биомассы зависит от состава ферментативной системы, дозировки ферментов и времени деструкции.

Результаты исследований подтверждают возможность проведения регулируемого процесса получения биопрепаратов с заданным структурно-фракционным составом на основе ферментативного гидролиза полимеров мицелиальной биомассы. Полученные ферментоллизаты грибной биомассы могут быть использованы для обогащения продуктов питания и кормов легкоусвояемыми низкомолекулярными пептидами, незаменимыми аминокислотами, витаминами и углеводами.

Ресурсосберегающие технологии позволяют, перерабатывая отходы биотехнологических производств в биологически активные препараты, создавать новые виды функциональной биотехнологической продукции, осуществлять импортзамещение, способствуя решению экологических проблем.

*Исследования выполнены при поддержке гранта Российского научного фонда № 16-16-00104.*

**Contact and fumigant activities of *Cinnamomum porrectum* (Roxb.) Kosterm  
against aflatoxigenic *Aspergillus flavus* and *Aspergillus parasiticus***

\*Sukcharoen, O., \*\*Sirirote, P., and \*\*Thanaboripat, D.

*\*Department of Biotechnology, Faculty of Science,*

*Ramkhamhaeng University, Bangkok 10240, Thailand*

*\*\* Department of Biology, Faculty of Science,*

*King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand*

**Abstract**

The essential oil of the bark of *Cinnamomum porrectum* (Roxb.) Kosterm was obtained by hydrodistillation using a Clevenger-type system for 3 hr and identified by gas chromatography-mass spectrometry. The compounds were safrene (93.9%), elemicine (4.3%) and methyl eugenol ether (1.79%). Essential oil of *Cinnamomum porrectum* (Roxb.) Kosterm was evaluated against *Aspergillus parasiticus* IMI 283883 and *Aspergillus flavus* IMI 242684 for their effects on mycelial growth, sporulation and aflatoxin B1 production, using direct contact method and vapor contact method. Aflatoxin B1 was determined using the Enzyme-linked immunosorbent assay (ELISA). The results showed that the inhibition activity of the *Cinnamomum porrectum* (Roxb.) Kosterm essential oil was a strong exhibited inhibition of mycelial growth, sporulation and aflatoxin B1 production of both strains ( $p < 0.05$ ) as compare with control. The fumigation activities via vapor contact method showed higher inhibition than direct contact method. This study suggests that *Cinnamomum porrectum* (Roxb.) Kosterm essential oil represents a good alternative to eliminate aflatoxigenic strains on food and agricultural commodities.

Keywords : *Cinnamomum porrectum* (Roxb.) Kosterm , essential oil, aflatoxin, *Aspergillus flavus*, *Aspergillus parasiticus*

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\*Corresponding author: Tel: 662-3194385 Fax: 662-3108239

Email: osukcharoen@hotmail.com (Sukcharoen, O)

# Endophytes as biocontrol agents for plant diseases: prospect, challenges and improvements

Ting ASY

*School of Science, Monash University Malaysia,*

*Jalan Lagoon Selatan, 47500 Bandar Sunway, Selangor Darul Ehsan, Malaysia*

## Abstract

Endophytes are microorganisms found inhabiting plant tissues. They colonize plants asymptotically, and form a symbiotic relationship with the host plant. Endophytes have therefore been investigated for their many beneficial associations with host plants, i.e. promoting plant growth, improving tolerance to abiotic and biotic stress, and developing resistance to herbivores. As a result, they have excellent prospect for development into biocontrol agents, whereby they are known to confer disease tolerance via strengthening of plant growth and vigour, production of inhibitory compounds, and competitive exclusion of pathogens from colonization niche. The role of endophytes as biocontrol agents are identified based primarily on laboratory assessments and greenhouse evaluations. The biocontrol expression is however, often diminished or rarely reproduced under field conditions. It has long been suggested that soil conditions play a major role in influencing endophyte growth and survival in the field. Under field conditions, introduced endophytes compete with indigenous soil microflora, and are susceptible to the soil physico-chemical factors. These challenges limit the use of endophytes. Therefore, to address these limitations, attempts have been made by improving strategies in endophyte application (bioformulations, frequency). In recent years, new hypothesis emerged that endophyte-host plant interaction may also be a contributing factor to poor biocontrol expression. As such, studies into endophyte-host plant interaction are attempted where investigations into endophyte growth and colonization behavior *in planta* are conducted. This presentation would discuss the prospect, challenges and improvements in biosourcing endophytes as biocontrol agents, including preliminary results on our investigations on endophyte-host plant interaction as a possible factor influencing endophyte growth and their subsequent biocontrol expression.

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\*Corresponding author: E-mail address: [adeline.ting@monash.edu](mailto:adeline.ting@monash.edu); [adelsuyien@yahoo.com](mailto:adelsuyien@yahoo.com)

## The Research Progress and Application of Pectinase

Xueying Sun, Qian Yang\*

*School of Life Science and Technology, Harbin Institute of Technology, 150001, China*

### Abstract

Pectin polysaccharide is a kind of macromolecule material, linked by alpha 1, 4 glycosidic bond of galacturonic acid oligomeric polymer chains, exists in plant cell walls widely. It is widely used in food, medicine, chemical and other major areas due to its good thickening, coherence and stability. Modified pectin is used in the field of anticancer, immune, materials in recent years. The degradation of pectin also is a kind of modification.

Pectin is degraded by the method of chemical and enzymatic hydrolysis mainly. Pectin can be degraded into galacturonic acid, rhamnose, arabinose, xylose, mannose and galactose and so on. Pectinase was divided into original pectinase, pectin enzyme hydrolysis, pectin lyase (PL) and pectin esterase (PE) originally. But the most widely used pectin enzyme hydrolysis, which is widely studied and applied in the production of life is poly galacturonic acid enzyme (PG). Poly galacturonic acid enzyme (PG) will degrade pectin macromolecular into small molecules, and absorbed by organisms.

The study of pectinase is very popular in the domestic and overseas in recent years, mainly concentrated in citrus peel, apple residue, sugar beet, peduncle of microbial fermentation to produce pectinase. As the industrialization process, the application of pectinase is becoming more and more widely, it can be applied to food industry, feed industry, papermaking industry, textile industry, pharmaceutical industry, fruit juice clarification and the extraction of natural substances.

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\*Corresponding author: E-mail address: 14745157934@163.com

**Callus Induction and Culture from Midrib of Anthurium cv. Chok Klao Chan by  
transverse Thin Cell Layer Technique (tTCL)**

Sumet Treesaksri

*Department of Agricultural Education, Faculty of Industrial Education, King Mongkut's  
Institute of Technology Ladkrabang, Bangkok Thailand*

**Abstract**

The study of callus induction and culture were divided in two experiment. The first experiment is to study the effect of  $\frac{1}{4}$  MS medium supplement with NAA and BAP on callus induction from midrib of Anthurium cv. Chok Klao Chan by using transverse Thin Cell Layer (tTCL). Leaf segment of Anthurium cv. Chok Klao Chan were sterilized by using 70 % ethanol for 1 minutes followed by 10 % clorox solution for 10 minutes and 5% clorox solution for 40 minutes and finally washed 3 times in sterile distilled water. Midrib segments were transverse cut at a thickness of 1-1.5 mm. and cultured on  $\frac{1}{4}$  MS medium supplemented with 0,0.1,0.5 and 1 mg/l NAA combined with 0,0.1,0.5,1,1.5 and 2 mg/l BAP. The explants were cultured in dark at  $25\pm 3^{\circ}\text{C}$  for 8 weeks. It was found that friable white callus , fast growing callus were found and gave the 100 % of callus induction after 1 weeks on  $\frac{1}{4}$  MS+0.1 mg/l NAA ,1.5 mg/l BAP

The second experiment is to study the effect of  $\frac{1}{4}$  MS medium supplement with NAA and BAP on callus culture. The explants of this experiment is the transverses midrib segments were cut at a thickness of 1-1.5 mm and cultured on  $\frac{1}{4}$  MS + 0.1 mg/l NAA , 1.5 mg/l BAP in dark at  $25\pm 3^{\circ}\text{C}$ . for 4 week. At that time, transfer the callus and cultured on  $\frac{1}{4}$  MS medium supplemented with 0,0.1,0.5 and 1 mg/l NAA combined with 0,0.1,0.5,1,1.5 and 2 mg/l BAP. It was found that very fast growing callus of the latter was obtained when midrib callus was cultured on MS + 1 mg/l NAA and 1.5 mg/l BA. Unsuccessful attempts to regenerate callus and induce adventitious shoots on the 2 explants are discussed.

**Keywords:** transverse Thin Cell Layer, callus induction , callus culture

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\*Corresponding author: E-mail address: kunsumet@yahoo.com

## **The development of bio-zeolite and its application in wastewater treatment.**

Meiling Xiong, Jinzhu Song\*

*School of Chemistry and Chemical Engineering, Harbin Institute of Technology, 150001, China*

### **Abstract**

Zeolites, crystalline porous solids, are readily available tectosilicates consisting of corner-sharing  $AlO_4$  and  $SiO_4$  tetrahedra, resulting in their molecular sieve, ion exchange, and catalytic features, which determine their ability to remove pollutants from industrial, processing waters and wastewaters. Bioremediation, the use of microorganisms or microbial processes to degrade and detoxify environmental contaminants, is becoming quite an attractive alternative to consume and break down environmental pollutants. Based on these properties and advantages, it is possible to attach the microbial cells to the surface of zeolites, hoping to exert a combined removal effect of pollutants.

Bio-zeolite, which uses zeolite as a microbe carrier, can not only maximize the removal capability of zeolites but also function to regenerate used zeolites, exhibiting a superior removal capability and reusability than zeolites alone because of microbes' intensive immobilization on the zeolite surface and relative ease of collection. Lots of studies focused on the use of bio-zeolite in the biological wastewater treatment process are reported in recent years, showing a favourable removal efficiency.

Thus, the bio-zeolite carrier could be applied as a novel, highly efficient capping technique for bioremediation in contaminated water resources. Thus, it is amongst the new technologies that derive their scientific justification from the emerging concepts of green chemistry and green engineering. This fast growing and highly promising remediation technique is increasingly being studied and applied practically for removing pollutants.

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\*Corresponding author: E-mail address: 13895749154@163.com

## Protoplast fusion as a tool for improving bioremediation in bacteria

Mohamed M. Yacout

*Department of Genetics, Faculty of Agriculture, Alexandria University, Alexandria- Egypt*

### Abstract

Bioremediation is the use of organisms to remove or neutralize pollutants from a contaminated site. Bioremediation may occur on its own, meanwhile recent advancements have also proven successful via the addition of matched microbe strains to the medium to enhance the resident microbe population's ability to break down contaminants. Currently among the various groups of pesticides that are being used the world over, organophosphorus group forms a major and most widely used group accounting for more than 36% of the total world market. Approximately 3 million poisonings and 300,000 human deaths occur per year owing to OP ingestion, and OP poisoning is a global clinical problem. Present work is dealing with bacterial biodegradation of two organophosphorus pesticides i.e. Paraxon (diethyl- *p*-nitrophenyl phosphate) and Chlorpyrifos® 48 % ( 0.0- diethyl 0-(35.6- Trichloro-2- Pyridyl) Phosphoro-thioate). Three local isolates *Rhizobium leguminosarum* in addition to *Pseudomonas Fluorescens* and *Bacillus Megaterium* and their protoplast fusants (hybrids) were tested for biodegradation capability mentioned pesticides. Total protein and DNA were applied using SDS-PAGE and RAPD techniques, respectively, for bacterial isolates and their hybrids.

Keywords: Bioremediation Organophosphorous pesticides - SDS-PAGE - RAPD - biodegradation – Paraxon (diethyl- *p*-nitrophenyl phosphate)- Chlorpyrifos – *Rhizobium*-*Bacillus*- *Pseudomonas*.

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\*Corresponding author: E-mail address: mohamed.abdyacout@alexu.edu.eg



# Genome-Wide Screening of Stress Granule Related Genes in *Saccharomyces cerevisiae*

Xiaoxue Yang<sup>1</sup>, Beidong Liu<sup>2</sup>, Per Sunnerhagen<sup>2</sup>, Zhenyu Wang<sup>1\*</sup>

*1 School of Chemistry and Chemical Engineering, Harbin Institute of Technology, 150001, China*

*2 Department of Chemistry and Molecular Biology, Lundberg Laboratory, University of Gothenburg, Gothenburg, Sweden*

## Abstract

When faced with stressful conditions, eukaryotic cells respond by forming large cytoplasmic granules consisting of complexes of mRNAs, ribosomal components, and RNA-binding proteins including translation factors. Stress granules (SGs), which are the main subject of this research, larger with an irregular shape, are consisted of messenger ribonucleo protein particle (mRNP) and formed under severe stress, including heat shock, deprivation of carbon source, peroxidation, hyperosmosis, virus infection and ultraviolet radiation.

Formation of stress granules provides a molecular explanation of stress ability of *Saccharomyces cerevisiae* Hansen, at the same time, the references of survival selection, even the formation of cancer cell for other eukaryotic cells. So far, the overall composition of SGs, as well as their assembly requirements and regulation through stress-activated signaling pathways remain largely unknown, the purpose of this research is to explain the mechanism of them.

The genome DNA sequence of *S. cerevisiae* is acquired already. Also, the technology of automated yeast synthetic genetic array (SGA) and high throughput or high content microscopy (HCM) are successfully developed. Base on the technical platform above, this research transforms a red fluorescent protein with stress granule marker *PAB1-RFP* into *S. cerevisiae* genome, 4600 single gene knockout mutants and 800 temperature sensitive mutants were treated with stress, mainly deoxy glucose, as stress granule induction. Difference in stress granules abundance of mutant and wild type is the way to pick out the candidates, with analyzing the interaction between stress granule and bio-function of the mutant missing gene, to make clear the metabolic pathways and mechanisms of cell process in related in the formation of stress granules.

The screen revealed numerous genes not previously implicated in stress granule formation. The GO analysis shows that stress granule formation required genes group in endosomal components, ribosomal components, GAPR complex, EGO-GSE complex and protein folding. Missing gene lead to increase of stress granule, which working on resolution, is related to biological membrane structure, vesicle transport and autophagy.

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\*Corresponding author: E-mail address: xiaoxue\_yang@qq.com

## **Bio-technology and Industry Waste Recycle**

Qian Yang

*Harbin Institute of Technology*

*Harbin 150001, P R China*

### **Abstract**

The fundamental way of protecting the environment from the pollution by the wastes from different industries is recycling those wastes. The key words here are how to work out the technique for the waste recycle. Comparing with various techniques, bio-tech is the best way for developing the technique to recycle the wastes. It is known to all that the wastes produced from different industries have already serious damaged the environment everywhere in the world. In China such problems appear in different industries such as food industry, medicine industry etc. In this paper the examples of recycling the wastes from food industry by bio-tech is introduced. The significance of those techniques is discussed.

**Key words:** bio-technology, industry waste, recycle

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\*Corresponding author: E-mail address: yangq@hit.edu.cn

## Molasses wastewater treatment by *Bumileriopsis peterseniana* isolated from ethanol-production wastewater

Panutda Yodsang<sup>1\*</sup>, Cherdsak Maneeruttanarungroj<sup>2,3</sup>, Rungroj Piyaphanuwat<sup>1</sup>

<sup>1</sup>King Mongkut's University of Technology Thonburi (Ratchaburi Campus), Ratchaburi 70150, Thailand

<sup>2</sup>Bioenergy Research Unit and <sup>3</sup>Department of Chemistry, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok, 10520, Thailand.

### Abstract

Sewage is a kind of the water pollution issues occurred from molasses wastewater in ethanol production in industry. Microalgae, a type of the biological controls, can be used in the wastewater treatment process. This research focused on the isolation of microalgae in molasses wastewater from Ratchaburi Ethanol Production Industry. *Bumileriopsis peterseniana* isolated from wastewater-aerated pond was identified by using morphology observation under the light microscope. Growth of *B. peterseniana* was studied in various molasses wastewater concentration in both water and tri-acetate-phosphate (TAP) medium. The results showed that *B. peterseniana* could not use molasses wastewater as a nutrient source. When wastewater concentration increased, increasing of algal growth inhibition was observed. Furthermore, *B. peterseniana* was studied the aspects of wastewater treatment by decolorization, chemical oxygen demand (COD) and biological oxygen demand (BOD) measurement in the period of 4 days. The ability of *B. peterseniana* in decolorization of wastewater represented in 9.37 %. The algae could also reduce COD and BOD, representing of 47.83 % and 80.65 %, respectively. These results indicated that the microalgae *Bumileriopsis peterseniana* isolated from ethanol-production wastewater, showed the promising potential to treat molasses wastewater.

**Keywords:** microalgae, isolation, wastewater treatment, *B. peterseniana*, molasses, BOD, COD, decolorization

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\*Corresponding author: E-mail address: panutda.yod@kmutt.ac.th

# **Towards Industrially Feasible Treatment of Potato Starch Processing Waste by Mixed Cultures**

Liang Zhang<sup>1</sup>, Guangli Cao<sup>1</sup>, Bingnan Liu<sup>2</sup>, Ying Li<sup>1</sup>, Qian Yang<sup>1\*</sup>

*1 School of Life Science and Technology, Harbin Institute of Technology, 150001, China.*

*2 School of Biological Engineering, Dalian Polytechnic University 116034, China*

## **Abstract**

The present study aimed at reducing the pollution of the waste generated by the potato starch industry to the environment and transform the potato pulp and wastewater into single-cell protein (SCP) to be used as animal feed. The chemical oxygen demand of the wastewater was reduced from 26,700 to 9,100 mg/L by batch fermentation with mixed cultures in an aerated 10-L fermenter. The SCP products, with a crude protein content of 46.09% (higher than soybean meal), were found palatable and safe for mice. During the treatment process, the microbial community was analyzed using the terminal restriction fragment length polymorphism for bacterial 16S rRNA genes. The results of the analysis suggested that *Curcubacter/Pseudoalteromonas* and *Paenibacillus/Bacillus* were the main microorganisms in treating potato starch processing wastes. The 150-m<sup>3</sup>-scale fermentation demonstrated a potential for treatment in industrial applications. Fermentation of potato pulp and wastewater without adding an extra nitrogen source was a novel approach in treating the potato starch processing waste.

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\*Corresponding author: E-mail address: zltj729@163.com

## Two Novel Protein Elicitors from *Magnaporthe oryzae* Trigger Defense Response and Improve Plant Growth in Rice

Hongmei Zeng

*State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100081, China*

MoHrip1 and MoHrip2, two novel protein elicitors inducing a hypersensitive response and early events in tobacco cell, were isolated and purified from the culture filtrate of *Magnaporthe oryzae*. The encoding gene *mohrip1* and *mohrip2* were isolated and expressed, and the expression proteins also form necrotic lesions in tobacco. The deduced proteins had molecular weight of 14.32 kDa and 16.25 kDa, separately. Both of them could induce the early events of the defense response, including hydrogen peroxide production, callose deposition, and alkalization of the extracellular medium, in tobacco. Moreover, elicitor-treated rice seedlings possessed significantly enhanced systemic resistance to *M. oryzae* compared to the control seedlings. The realtime PCR results indicated that the expression of pathogenesis-related gene *OsPR-1a* and *OsPR-10a* could be induced by the two elicitors.

After screening and optimization of crystallization condition, well-diffracting crystals of the two proteins were obtained and good diffraction patterns of X-ray were yielded. The structures of the two elicitors were successfully determined. Simultaneously, the interacting proteins of MoHrip1 in the rice were screened by Yeast two-hybrid. The interaction between MoHrip1 and its interacting protein will be further confirmed.

By gene knockout and gene complimentation, the function of the two elicitors in *M. oryzae* was studied. The data demonstrated that both proteins could inhibit the growth of *M. oryzae* and reduce its pathogenicity. It is proved that the elicitors have the potential to be developed as biopesticides to control plant disease.

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\*Corresponding author: E-mail address: zenghongmei@hotmail.com

## Effect of immobilization on cellulose basis on the properties of biological active compounds

Zinatullina K.M.<sup>1,2\*</sup>, Khrameeva N.P.<sup>2</sup>, Kasaikina O.T.<sup>1,3</sup>

<sup>1</sup>*N.N.Semenov Institute of Chemical Physics RAS,*

<sup>2</sup>*N.M.Emanuel Institute of Biochemical Physics RAS,*

<sup>3</sup>*Moscow State University, Chem. Dep.*

*Moscow, Russia*

### Abstract

Oxidative stress is characterized by a high content of reactive oxygen species (ROS), and reflects an imbalance between the rates of ROS and their utilization. There are biologically active compounds in all living organisms, which produce ROS (generating of free radicals) and those scavenging ROS by reduction of peroxides and by formation inactive products with radicals (bioantioxidants).

Glutathione (GSH) is the intracellular thiol (–SH) molecule presents in virtually all mammalian tissues in high intracellular concentration (millimolar levels). High electron-donating capacity of GSH made it one of the major endogenous bioantioxidant produced by the cells, participating in various physiological processes. Glutathione plays an important role in preventing oxidative damage to the skin; it has skin lightening ability and protects the skin from UV radiation and other environmental impacts, as well as internal stressors that generate free radicals that cause skin damage and hyperpigmentation [1,2]. In this study, the behavior of GSH towards peroxy radicals and hydrogen peroxide has been studied. We have established the free radical formation in reactions of GSH with H<sub>2</sub>O<sub>2</sub> and the kinetics of this reaction has been determined.

Acetylcholine (ACh) is an organic chemical that functions in the brain and body of many types of animals, including humans, as a neurotransmitter. Acetylcholine plays a major role in the processes of life: it takes part in the transmission of nerve excitation in the central nervous system, autonomic nodes, the endings of the parasympathetic and motor nerves. Acetylcholine is associated with memory functions. Similarly to other quaternary ammonium compounds ACh catalyzes the hydroperoxide and H<sub>2</sub>O<sub>2</sub> decomposition into free radicals [3].

In this work the immobilization of glutathione and acetylcholine on cellulose basis was made. Two types of cellulose were used: one, purchased from company “Evalar”, and second – cellulose obtained by catalytic treatment of plant biomass. The samples were characterized by means of IR spectroscopy. The adsorption of glutathione and acetylcholine on cellulose are equal to  $6 \cdot 10^{-4}$  and  $8 \cdot 10^{-4}$  mole/g respectively. It must be noted that both types of cellulose demonstrated equal properties. Immobilized GSH saves its ability to reduce peroxide and scavenge peroxy radicals. However, GSH activity in free radical initiating decreases. In the case of immobilized ACh, its activity in the catalysis of hydroperoxide decomposition resulted in radical initiation is found to be rather high.

These results might be useful for medicine and cosmetology, i.e. to create disinfecting and medical materials with biologically active compounds adsorbed on cellulose basis.

This study is partly supported by the Russian Foundation for Basic Research, grant No 14–03–00757.

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\*Corresponding author: E-mail address: karinazinat11@gmail.com

## **POSTER PRESENTATIONS**



## **Strawberry bud weevil (*Anthonomus rubi* Herbst) biological control at the low density of pest**

A.I. Anisimov, K.N. Fursov, S.A. Dobrohotov

St. Petersburg State Agrarian University, St. Petersburg, Russia

### **Abstract**

From 2013 at smallholding situated near St. Petersburg the efficiency of some microbiological preparations are investigated in strawberry bud weevil biological control on 3 varieties of strawberry (Polka, Surprise to Olympiada, Tsarskoselskaia). At 3 years the efficiency of microbiological preparation Batsikol (5 %) created at All Russian Institute of Agricultural Microbiology on the basis of bacterium *Bacillus thuringiensis* Berliner (pathotype *BT H<sub>10</sub>*) in comparison with biochemical preparation Fitoverm (1 %) - natural avermectin complex from soil fungus *Stereptomyces avermitilis*. At the season 1915 another one microbiological preparation Turengin (*B. thuringiensis*, serotype 1) and two repellents (Dachnik - substance fragrant pine oil-based and food additive - Liquid smoke) was tested. As a standard we used the chemical preparation Alatar (active drug substances: malathion - 22.5 %, cypermethrin - 5.0%). All buds damaged by strawberry weevil ore not were counted on each plant separately one time per 4 days.

It was founded that at 2015 the strawberry bud weevil harmfulness on the strawberry was negligible (1 – 2 % of damaged buds). For comparison, in 2013 at the areas without treatments level of damaged buds varied from 20 to 30 % [1-3] and in 2014 - from 10 to 20 %. Most likely this is due to climatic terms of the year affecting the population density of the weevil in the season 2015.

In our experiments 3 treatments by Batsikol and Fitoverm demonstrated approximately the same biological efficiency (55-60 %) in strawberry (Surprise to Olympiada and Tsarskoselskaia varieties) protection from strawberry bud weevil, though at Polka variety efficiency was somewhat lower (45-50 %).

At the low density of pest in 2015 the highest efficiency (60-70 % at different strawberry varieties) demonstrated the microbiological preparation Turengin slightly less (50 – 60 %) chemical standard Alatar. The efficiency of biological preparation Batsikol was lower (40 – 50 %). Unexpectedly low efficiency of Fitoverm (20 – 30 %) does not coincide with the results of previous studies [1-3]. Regarding repellents we can only talk about the predominance Liquid smoke preparation (efficiency about 17 %) on Dachnik (negative efficiency).

Evaluating the overall results we conclude that the first preventative strawberry treatment by Batsikol at the beginning of blooming buds reduces the level of strawberry bud weevil harmfulness but do not protect the crop from this pest completely. Two subsequent treatments with 4-7 days interval prevent the increase of strawberry bud weevil damages until the harvest.

We can recommend microbiological preparation Batsikol so as Turengin for inclusion in the State pesticides and agrochemicals permitted for use in the territory of Russian Federation catalog for control the strawberry bud weevil on strawberry in particular in organic farming along with the already included there Fitoverm.

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\*Corresponding author: E-mail address: anisimov\_anatoly@mail.ru

## Possibility to use aphids as available diet for some nymph instars of *Podisus maculiventris* (Hemiptera: Aspionae)

<sup>1</sup>A.I. Anisimov, <sup>1</sup>A.E.S. Kasem, <sup>2</sup>E.G. Kozlova

<sup>1</sup>St. Petersburg State Agrarian University, <sup>2</sup>All-Russian Institute for Plant Protection

### Abstract

Possibility to use three species of aphids (green bug - *Schizaphis graminum* Rond., vetch aphid - *Megoura viciae* Buck., green peach aphid - *Myzus persicae* Sulz.) as available food for some nymphal instars of *Podisus maculiventris* Say at laboratory conditions was investigated to make the technology of mass rearing more secure and application of the predatory bug in insect pests control more wide. We offered to spined soldier bug nymphs as only source of food aphid each species in four combinations: 1) at the period of 1<sup>st</sup> instar (about first 5 days after hatching) then grater wax moth (*Galleria mellonella* L.) larvae until the adult stage; 2) at the period of 2<sup>nd</sup> instar (from the 6<sup>th</sup> to the 10<sup>th</sup> day after hatching approximately) then grater wax moth larvae until the adult stage; 3) at the period of 2<sup>nd</sup> and 3<sup>rd</sup> instars (from the 5<sup>th</sup> to the 15<sup>th</sup>, day or more after hatching) then grater wax moth larvae until the adult stage; 4) at the period of 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> instars (from the 5<sup>th</sup> to the 20<sup>th</sup> day or more after hatching) then grater wax moth larvae until the adult stage. At the control variant we fed the spined soldier bug nymphs grater wax moth larvae only. In each variant the nymphs survival and duration of development, imago weight, their fertility, preoviposition period, the average volume and the number of egg-batches, total fecundity (or per 30 days) and longevity of adult bugs were evaluated.

The results of experiments showed that: 1) Adding the aphids in the period of 1<sup>st</sup> instar nymphs development does not affect the examined biological characteristics of bugs because they do not eat at this stage and need only water.

2). Feeding the bag nymphs green bug and vetch aphid at the 2<sup>nd</sup> instar significantly reduces their survival rate for 42 – 43 % and green peach aphid for 14 %. This increases the nymph developmental time for 10 days if vetch aphid was used and for 2 - 2.5 days when green bug and green peach aphid were used. The weight of newly emarginated adult bugs slightly decreases when nymphs feeding vetch aphid (only females for 12% in average). Their fecundity (per 30 days) decreases for 43 % and 49 % when the vetch aphid and the green peach aphid were used as a food for 2<sup>nd</sup> instar bug nymphs, respectively.

3. Feeding the bag nymphs green bug and vetch aphid up to the 3<sup>rd</sup> instar reduces their survival for 65 % and 58 %, respectively. Their developmental time increases significantly: when feeding green bug on 5 to 7 days, vetch aphid on 4 to 6 days and green peach aphid for 3-4 days. The weight of the imago affected slightly. The females weight reduced for 12.5 % only when feeding green bug, and males when feeding all examined species of aphids also in an average of 12 %. Average total fecundity (per all life) of predatory bugs decreased for 15 %, 32 % and 49 % when feeding green bug, vetch aphid and the green peach aphid, respectively. The longevity of females in the same conditions decreased (but statistically not significant) for 15 %, 32 % and 42 %, and males to a much lesser extent: 9 %, 2 % and 4 % for green bug, vetch aphid and the green peach aphid, respectively. The eggs hatching also reduced, but not too much: for 8 %, 7 % and 14 % the green bug, vetch aphid and the green peach aphid, respectively. The average volume of egg-batches reduces significantly decreases on 32 % only if green peach aphid was used. Preoviposition period, the volume of first egg-batches and average number of egg-batches was not affected.

4. The spined soldier bag nymphs feeding aphids up to 4<sup>th</sup> instar in most cases enhances the negative effects of such diet on bags biological characteristics. Nymph survival reduced for 53 %, 82 % and 73 % when feeding green bug, vetch aphid and the green peach aphid, respectively. Their developmental time increases females on 7.5, 6.1 and 7.8 days, for males on 6.3, 8.3 and 10.1 days, respectively. The bags female and mail weight reduced significantly (on 7-18 % and 14-21 %, respectively) if all examined spaces of aphids were used etc.

Nevertheless, some individuals survive and give progeny even at nymphs feeding aphids up to 4<sup>th</sup> instar. This gives the possibility to adapt the special strains of spined soldier bag to aphid diet by the artificial selection.

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\*Corresponding author: E-mail address: anisimov\_anatoly@mail.ru

# The using of entomopathogenic nematodes and nematode–bacterium complex against harmful organisms

Agansonova N.E.

*All-Russian Institute of Plant Protection, St. Petersburg-Pushkin*

## Abstract

The data obtained have proved the possibility to increase biological effectiveness and to reduce the number of treatments by using preparations (insecticides spintor 240 SC, actara WG and new biopreparation aleycid PS), or their tank mixtures, and biological agents in the system of cucumber protection against Western flower thrips *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae). Foliar application of insecticides and aleycid PS, or their tank mixtures, should be accompanied by application of biopreparation of entomopathogenic nematodes *Steinernema feltiae* (Filipjev) entonem–F (Nematoda: Steinernematidae) against soil stages of the pest development and by the release of predatory *Orius majusculus* (Reuter) (Heteroptera: Anthocoridae). Combined application of insecticides (spintor 240 SC, actara WG), aleycid PS, or their tank mixtures, entonem–F and orius *O. majusculus* proved to be highly effective (98–99%) for 75 days. The methods of using insecticides, new biopreparation and entomopathogenic nematodes together with predatory Orius recommended for integrated pest control.

Combined application of tank mixtures and entomopathogenic nematodes proved to be highly effective (99%) for 60 days (Agansonova, 2014).

The protective action of nematode–bacterium complex on potato *Solanum tuberosum* L. and cucumber *Cucumis sativus* L. was established.

High effective using entomopathogenic nematodes *Steinernema feltiae*, *St. carpocapcae* and *St. feltiae protense* subsp. N. against wireworms (Elateridae, Agriotes) was obtained. The using of metabolism products of entomopathogenic nematodes symbiotic bacteria (Xenorhabdus, Enterobacteriaceae) suppressed the development of the phytophthora *Phytophthora infestans* (Mont.) de Bary (Agansonova, 2015), root rot *Fusarium oxysporum* Schlecht. (Agansonova, 2016) and common scab *Streptomyces scabies* Waks. & Henr., increased the activity of enzyme peroxidase in plants and the yield, improved quality, increased the level of enzymes activity in the soil at the end of the growing season of plants. At induction by potato cyst nematode *Globodera rostochiensis* Woll the increase of plant attraction degree for Colorado beetle *Leptinotarsa decemlineata* Say and the increase of the pest number are found. Results showed that studied metabolism products of entomopathogenic nematodes symbiotic bacteria decrease the attractivity of potato plants to Colorado beetle (Agansonova, 2015).

The metabolism products of entomopathogenic nematodes symbiotic bacteria and entomopathogenic nematodes were obtained from the All-Russian Institute of Plant Protection (St. Petersburg–Pushkin, Russia).

The results can be used to improve plant integrated protection systems against harmful organisms.

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\*Corresponding author: E-mail address: [aghansonova@mail.ru](mailto:aghansonova@mail.ru)

## **Efficacy of Nano-ZnO on Growth Inhibition of *Phytophthora* spp. Causing Root and Stem Rot of Durian Tree**

Sakrin Boonlum<sup>1\*</sup>, Wasinee Thamsathit<sup>1</sup>, Pornprapa Kongtragoul<sup>2</sup>, Pramote Sirirote<sup>3</sup> and  
Dusanee Thanaboripat<sup>3</sup>

<sup>1</sup>*Scientific Instrument Centre, Faculty of Science, King Mongkut's Institute of Technology  
Ladkrabang, Bangkok 10520, Thailand.*

<sup>2</sup>*Department of Horticulture, Faculty of Agricultural Technology, King Mongkut's Institute of  
Technology Ladkrabang Prince of Chumphon Campus, Chumphon 86140, Thailand.*

<sup>3</sup>*Department of Biology Faculty of Science, King Mongkut's Institute of Technology Ladkrabang,  
Bangkok 10520, Thailand*

### **Abstract**

Nano-ZnO at twelve concentrations of 0.1, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10 g l<sup>-1</sup> were tested for an ability to inhibit the growth of *Phytophthora* spp. Using the poisoned food technique on potato dextrose agar (PDA). The culture were incubated at 25 °C for 7 days. The results showed that concentration of nano-ZnO inhibitory could inhibit fungal growth of *Phytophthora* spp. When these nano-ZnO were used for the control of growth, nano-ZnO at 10 g l<sup>-1</sup> up showed to give the best inhibition.

**Keywords :** ZnO, Zinc oxide, root rot, stem rot, *Phytophthora* spp., durian.

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\*Corresponding author. E-mail: Truemove2524@gmail.com, kbsakrin@kmitl.ac.th

## **Biotechnological aspects of the use of whey in food technology**

Burova T. Ye.\*, Rachevskaya O. Ye.

*St. Petersburg Polytechnic University of Peter the Great Graduate School of  
Biotechnology and Food Science, St. Petersburg*

### **Abstract**

When separation of milk, production of cream, butter, natural cheese, cottage cheese and milk protein on traditional technology produces by-products-skimmed milk, buttermilk and whey, which are called secondary dairy raw materials. Composed of these products contain all components of milk, but only in certain ratios.

The main way to solve the problem of complex processing of raw milk is seen in the creation of non-waste technologies of cheese, cottage cheese and casein. Currently, there are two approaches for processing whey: full utilization of dry substances; separate use of components.

The first approach is implemented in two ways. The first is to use natural whey as an ingredient of bakery products, beverages and jellies, desserts and ice cream, as well as for the preparation of nutrient feed and fertilizer, Wednesday, detergents, cosmetics and Wellness baths. The second direction makes it possible to obtain a condensed and dry whey concentrates, dry demineralised whey, dry bezlaktoznuû whey, whey with fillers, block serum (products with intermediate moisture), granular serum. Full use of serum components based on dewatering by vacuum evaporation and drying.

In recent years, increased attention has been paid to the whey processing-receiving derived from individual components (butterfat and protein hydrolysates derived lactose-lactulose. lactitol, tagatose, galacto etc.).

At the Graduate School of Biotechnology and Food Science over the years, work is underway on the use of whey in the manufacture of food products. Special attention is paid to the manufacturing technology of sauces, desserts and drinks.

The technology of manufacture of egg-oil, vegetable and fruit sauces based on whey, which is used as a liquid Foundation. Traditionally the liquid basis for sauces served a variety of broths. Use whey instead of broths significantly reduces the cost to manufacture the product and simplifies the technology. Has developed recipes sauces, study their organoleptic, physico-chemical and microbiological indicators. It has been established that the use of whey leads to the creation of products with high consumer properties, expanding the range of sauces, enriches the product. For egg-oil sauces offered quality and safety management system.

One of the principal tasks important for the dairy industry of the RUSSIAN FEDERATION, is considered to be the provision of products of functional purpose. These include are low lactose milk products that have all the chances to provide good nutrition for people with lactase deficiency.

In the work were used sourdough liofilizirovannaya "Cottage cheese" is manufactured by technology and order "Bioproduct" ("Own brand yogurt") and sourdough "EKOKOM" – dry cultures of microorganisms for the production of cheese dairy product (manufacturer: OOO "Lactina", Bulgaria).



Has developed recipes and are made of milk-fruit desserts and drinks on the basis of experimental samples of whey low lactose fermentation of milk produced by a variety of sourdough. Desserts and drinks had high organoleptic characteristics. All dairy and fruit drinks are consistent with the requirements of GOST R53914, GOST 2010-3624-92, ГОСТ 3626-73; desserts – GOST r 55462-2013.

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\*Corresponding author: E-mail address: [burova-tatyana@list.ru](mailto:burova-tatyana@list.ru)

## **Биотехнологические аспекты использования молочной сыворотки в технологии продуктов питания**

Бурова Т.Е.\*, Рачевская О.Е.

*Санкт-Петербургский политехнический университет Петра Великого*

*Высшая школа биотехнологии и пищевых технологий*

*г. Санкт-Петербург*

При сепарировании молока, производстве сметаны, сливочного масла, натуральных сыров, творога и молочного белка по традиционной технологии получают побочные продукты – обезжиренное молоко, пахта и молочная сыворотка, которые называют вторичным молочным сырьем. В своем составе эти продукты содержат все составные части молока, но только в иных соотношениях.

Основной путь решения проблемы комплексной переработки молочного сырья видится в создании безотходных технологий сыра, творога и казеина. В настоящее время известны два подхода по переработке сыворотки: полное использование сухих веществ; раздельное использование составных компонентов.

Первый подход реализуется по двум направлениям. Первое – использование натуральной сыворотки как ингредиента хлебобулочных изделий, напитков и желе, десертов и мороженого, а также для приготовления питательных сред, кормов и удобрений, моющих средств, косметики и оздоровительных ванн. Второе направление дает возможность получать сгущенные и сухие сывороточные концентраты, сухую деминерализованную сыворотку, сухую безлактозную сыворотку, сухую сыворотку с наполнителями, блочную сыворотку (продукты с промежуточной влажностью), гранулированную сыворотку. Полное использование компонентов сыворотки базируется на обезвоживании путем выпаривания под вакуумом и сушки.

В последние годы уделяется усиленное внимание глубокой переработке молочной сыворотки – получению производных из отдельных компонентов (гидролизаты молочного жира и белков, производные лактозы – лактулоза, лактитол, тагатаза, галактоолигосахариды и др.).

В Высшей школе биотехнологии и пищевых технологий на протяжении ряда лет ведутся работы по использованию молочной сыворотки при изготовлении продуктов питания. Особенное внимание уделяется технологии изготовления соусов, десертов и напитков.

Предложена технология изготовления яично-масляных, овощных и фруктовых соусов на основе молочной сыворотки, которая используется в качестве жидкой основы. Традиционно жидкой основой для соусов служат разнообразные бульоны. Использование молочной сыворотки вместо бульонов существенно снижает затраты на изготовление продукта и упрощает технологию. Разработаны рецептуры соусов, проведено исследование их органолептических, физико-химических и микробиологических показателей. Установлено, что использование молочной сыворотки приводит к созданию продуктов с высокими потребительскими свойствами, расширяет ассортимент соусов, обогащает продукт. Для яично-масляных соусов предложена система менеджмента качества и безопасности.

Одной из принципиальных задач, важной для молочной индустрии РФ, считается обеспечение населения продуктами функционального назначения. К таковым относятся

низколактозные молочные продукты, которые имеют все шансы обеспечить полноценное питание людям с лактозной недостаточностью. Разработаны рецептуры и изготовлены молочно-фруктовые десерты и напитки на базе экспериментальных образцов молочной сыворотки с пониженным содержанием лактозы, полученной путем сквашивания молока различными заквасками.

В работе были использованы закваска лиофилизированная «Творог» изготовлена по технологии и заказу ООО «Биопродукт» (торговая марка «Свой йогурт») и закваска «ЕКОКОМ» – сухие культуры микроорганизмов для производства молочного продукта творог (производитель: ООО «Лактина», Болгария).

Десерты и напитки имели высокие органолептические показатели. Все молочно-фруктовые напитки соответствовали требованиям ГОСТ Р53914-2010, ГОСТ 3624-92, ГОСТ 3626-73; десерты – ГОСТ Р 55462-2013.

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\*Corresponding author: E-mail address: burova-tatyana@list.ru

**Zinc, lead and cadmium contaminated soil remediation by durian and  
mangosteen shell-derived biochar**

Chompoonut Chaiyaraksa\*, Tamonwan Jaipong, Prakaikarn Tamnao, Anujit Imjai

*Department of Chemistry, Faculty of Science,*

*King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand*

**Abstract**

The target of this research is on studying of the possibility of using biochar from durian and mangosteen shell as natural adsorbed materials to adsorb zinc, lead and cadmium in soil. The durian and mangosteen shell were firstly passed the pyrolysis process. The chemical characterisation of these biochars was then performed. It was found from the characterisation process that biochars could be used as adsorbed materials. The neutralization capability and acid neutralization capability (ANC) were examined by adding 0.1 M nitric acid and 0.1 M sodium hydroxide until the pH reached 2-12. These two biochars had high neutralization capability. The acid neutralization capability of biochar from durian and mangosteen were 1,464.80 meq.kg<sup>-1</sup> and 1,328.98 meq.kg<sup>-1</sup>, respectively. The study included an adsorption isotherm. By using biochar from durian shell, Freundlich isotherm was a suitable isotherm to explain the adsorption of all three metals, whereas Langmuir isotherm is better to explain the adsorption of metals by biochar from mangosteen shell. The adsorption capacity of both biochars was not much different. They gave >95% zinc, lead and cadmium adsorption capacity.

**Keywords:** Biochar, Soil Amendment, Cadmium, Lead, Zinc

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\*Corresponding author: E-mail address: kcchompoonut@gmail.com

## Studies on non-wood cellulose of red algal, *Gracilaria fisheri* for papermaking

Weena Choochote\*, Pattharapol Janprapa, Rattapol Sartongyae and Anchana Chamniprasertkul

*Department of Biology Faculty of Science King Mongkut's Institute of Technology*

*Ladkrabang (KMITL) Bangkok Thailand*

### Abstract

The red alga, *Gracilaria fisheri* is one of seaweeds which produces phycocolloid agar. Cellulose as a byproduct from agar extraction was studied on possibility for papermaking. The effect of treatments using water, hydrochloric acid and sodium hydroxide on agar extraction were investigated in comparison with physical properties of paper. The results show that sodium hydroxide was the best solvent to extract agar from *Gracilaria fisheri* by yielding 23.17 % while distilled water gave the highest yield of cellulose from at 17.74 %. Physical properties including color measurement, thickness, wettability and tensile strength were studied. The results show that paper made from cellulose extracted with sodium hydroxide has the brightest value at  $L^* = 89.72$  and  $b^* = 4.56$ . The thickness of this paper was 0.32 mm and weakly hydrophobic. While paper made from cellulose extracted with distilled water has higher tensile strength at 8.37 MPa and 4.40 sec.

**Keywords:** *Gracilaria fisheri*, red algae, papermaking , algal paper, byproducts

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\*Corresponding author: E-mail address: weena.ch@kmitl.ac.th

## Allelopathic effects of xanthoxyline-derived chalcones on tested plants

Nawasit Chotsaeng<sup>a\*</sup>, Patchanee Charoenying<sup>a</sup>, Chamroon Laosinwattana<sup>b</sup>

<sup>a</sup>*Department of Chemistry, Faculty of Science, King Mongkut's Institute of Technology  
Ladkrabang, Bangkok-10520, Thailand.*

<sup>b</sup>*Department of Horticulture, Faculty of Agricultural Technology, King Mongkut's Institute of  
Technology Ladkrabang, Bangkok-10520, Thailand*

### Abstract

Twelve chalcones were prepared by Claisen-Schmidt condensation reaction under basic conditions between xanthoxyline (an allelochemical isolated from *Zanthoxylum limonella* Alston fruit) and a variety of aromatic aldehydes containing both electron-withdrawing and electron-donating substituents. Allelopathic effects of these chalcones together with xanthoxyline on two tested plants, Chinese amaranth (*Amaranthus tricolor* L.) and Barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.), were then investigated under laboratory conditions. The results revealed that 2,4,6-trimethoxy-substituted derivative had the highest inhibitory activities on seed germination and seedling growth of Chinese amaranth. At a concentration of 400  $\mu$ M, this compound inhibited seed germination, shoot and root growths of Chinese amaranth by 82%, 66% and 78%, respectively. Except for xanthoxyline, all synthesized-chalcones had no inhibitory effects on Barnyardgrass.

**Keywords:** Allelopathic, Xanthoxyline, Chalcone, Chinese amaranth, Barnyardgrass

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\*Corresponding author: E-mail address: nawasit.ch@kmitl.ac.th

**Effect of temperature on mycelium growth, conidia germination and pathogenicity of *Phoma complanata*, causal agent of *Heracleum sosnowskyi* phomosis**

Gasich E.L.\*, Khlopunova L.B, Berestetskiy A.O.

*All-Russian institute of plant protection, St. Petersburg, Pushkin*

**Abstract**

*Heracleum sosnowskyi* is invasive weed which is quickly occupying various habitats and forcing out local vegetation. *Heracleum sosnowskyi* can harm human health, being a contact allergen. At present to control this weed generally mechanical and chemical methods which are quite labor-consuming are used, and also they are not always effective and safe. Use of the organisms constraining the number of weed plants under natural conditions can become a new contribution to resolve this problem. Among them phytopathogenic fungi are studied most actively as they are capable to cause epiphytoses in plant populations and by that to control the number of weed plants at the certain level. We revealed potential of *Phoma complanata* (Tode) Desm., as possible agent of biological control of *Heracleum sosnowskyi*. One of the most important ecological factors influencing growth, sporulation and pathogenicity of phytopathogenic fungi is temperature. In our investigation the growth of *P. complanata* on potato-saccharose agar, and also conidia germination in water at temperatures 6, 9, 16, 20, 24, 28, 32 °C were studied. It was established that for *P. complanata* colonies growth the optimum temperature ranges from 20 to 24 °C. At 16 °C small reduction in the growth rate was observed, at 6 and 9 °C diameter of colonies was 3 times less, in comparison with optimum temperature range. At 32 °C the colonies growth was not noted, the subsequent incubation at 24 °C didn't lead to renewal of growth. *P. complanata* conidia germinated in the studied temperature range with formation of 1-2 growth tubes. At 20 – 24 °C after 5 hours about 20% of conidia germinated, at 9, 16, 28 and 32 °C – about 8%, after 16 hours at 20 – 24 °C – more than 90%, at 16 and 28 °C – about 85%, at 9 °C – 65%, at 32 °C – 18%. After 5-7 hours influence of temperature on the average length of growth tubes wasn't revealed. After 16 hours the maximum length of growth tubes was observed at temperature of 20 °C, minimum – at 9 and 32 °C. For studying of temperature effect on pathogenicity of *P. complanata*, containers with leaf disks, inoculated with mycelium (100 mg/ml) or conidia ( $1 \times 10^7$  spores/ml) suspensions, incubated at a constant temperature 12, 16, 20, 24, 28 °C. For preparation of mycelium suspension 4-day culture on the soy medium was used, for conidia suspension – 2-week culture on oat agar. Inoculum (10 µl) applied on the lower intact surface of leaf disk. At the temperature range between 12 – 24 °C essential effect of temperature on pathogenicity of *P. complanata* mycelium (necrosis diameter for 3 days reached 4 – 5 mm) wasn't revealed, at 28 °C development of necroses wasn't noted. Conidia were characterized by quite low pathogenicity. Despite high percent of conidia germination in water in the wide range of temperatures, after 3 days development small necroses were registered only at 16 °C.

Work is performed with financial support of the Russian Science Foundation (project 16-16-00085).

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\*Corresponding author: E-mail address: elena\_gasich@mail.ru

# Phytochemical and antibacterial efficiency of extract compounds from *Nymphaea* spp. on *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis*

Marisa Jatupornpipat<sup>a\*</sup>, Aree Rittiboon<sup>b</sup> and Arisara Khunprama<sup>c</sup>

<sup>a, b, c</sup> Department of Biology, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10500, Thailand

## Abstract

In the course of our investigation about Red Water Lily Flower strain, *Nymphaea* spp., from Red Lotus Sea in Kum Pavapi district Udon Thani province of Thailand. Part of sepal, petal and pollen in *Nymphaea* spp. were extracted with two organic solvents, distilled water and ethanol. The crude extracts were tested for antimicrobial activities against *Staphylococcus aureus* ATCC 1466, *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 6633 and *Pseudomonas aeruginosa* ATCC 27853 by agar disc diffusion. The results revealed that the crude extracts of *Nymphaea* spp. using in this study inhibited the growth of tested microorganisms and it could be inhibited to gram-negative bacteria were better than gram-positive bacteria. And then, all crude extracts of *Nymphaea* spp. were analyzed for studied about antioxidant against the total phenolic content in Folin Ciocalteu method, flavonoid content in Aluminium colorimetric method and anthocyanin content in pH differential method. The results revealed that the fresh extracts with distilled water of *Nymphaea* spp. had the highest total phenolic content, 4741.23 mg. gallic acid/100 g sample, while the dry extracts with ethanol of *Nymphaea* spp. had the lowest total phenolic content, 3481.98 mg. gallic acid /100 g sample. According to flavonoid content averaged, the fresh extracts with distilled water of *Nymphaea* spp. had the highest flavonoid content, 1840.07 mg/100 g sample, while the dry extracts with ethanol of *Nymphaea* spp. had the lowest total phenolic content, 1295.40 mg/100 g sample. Anthocyanin content in the fresh and dry extracts with ethanol of *Nymphaea* spp. had 30.78 and 19.87 mg/100 g sample respectively.

**Keywords:** Phytochemical , Antimicrobial, *Nymphaea* spp., Flavonoid, Phenolic compound, Anthocyanin

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\*Corresponding author: E-mail address: kjmarisa@gmail.com



## Suppression of microorganisms quorum sensing by ruminal fluid molecular factors

Karimov I.F.<sup>1\*</sup>, Duskaev G.K.<sup>2</sup>

<sup>1</sup>*Orenburg State University, Orenburg*

<sup>2</sup>*All-Russian Research Institute of Beef Cattle, Orenburg*

### Abstract

Intercellular communication in bacterial communities realized by a variety of low molecular weight factors, for example, homoserine lactone, providing density-dependent regulation of cell activity (Quorum sensing). Pathogenesis elaboration proceeded with a sufficient number of bacterial cells, which increases the chances for infection efficiency. On the other hand, the macroorganisms (animals or plants) tend to suppress Quorum sensing system by binding or cleavage of regulatory molecules (autoinducers). This mechanism of disease defense realize as bacterial virulence control and in some cases better than bactericidal factors production. The aim of our work was the study of the ability of the molecular factors rumen fluid to ensure suppression of intercellular communication bacteria.

Used bacterial strains *E.coli* K12, transformed by plasmids, carrying receptor genes (*rhlR*, *lasR* from *Pseudomonas aeruginosa* and *luxR* from *Vibrio fischeri*) and *Photobacterium luminescens luxCDABE*-genes fusions. Cultivation was carried out on LB-agar supplemented with doxycycline and then transferred to a LB-broth and again incubated for 90 minutes at 37 °C. As autoinducer used C4-HSL, oxo-C6-HSL and C12-HSL. Rumen fluid of Kazakh white breed cows was centrifuged at 5000 rpm for 10 minutes. Evaluation of the ability to inhibit activity of autoinducer was performed by incubating rumen fluid with HSL in the range of 10<sup>-8</sup> to 10<sup>-4</sup> M in equal volumes for 15 minutes at 37 °C, after which it was added an appropriate biosensor. Bacterial luminescence dynamics registered by plate luminometer LM-01T for 120 minutes.

The first phase is estimated own toxic activity of rumen fluid on used biosensors. Strains lost 50% of baseline luminescence using native samples but emission quenching due to colored samples saturated, leading to absorption of light in bioluminescence of bacterial cells. It was found that the dilution of rumen fluid to 12.5% ensures leveling of luminescence quenching effect, so this concentration was used in the follow experiments.

In the second phase we investigated the ability of rumen fluid components to reduce HSL activity. It was found that the highest efficiency of suppression autoinducer activity is carried out with respect to oxo-C6-GSL, which is fully inactivated with the concentration of 10<sup>-8</sup> M. The efficacy of blocking autoregulatory molecules observed at a concentration of 10<sup>-6</sup> M, over which the effect is negligible. However, inhibition of the activity of C4 and C12-HSL observed at the maximum concentrations of these molecules is associated with features of reacted to them biosensors (firstly intensity of luminescence).

The development of this approach will allow for the regulation of the activity of unwanted or pathogenic microbiota by selective or total suppression of their communication signals.

This work was supported by grant of Russian Scientific Fund (project No. 16-16-10048).

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\*Corresponding author: E-mail address: ifkarimov@yandex.ru

## **Protease form Actinomycetes isolated from mangrove forest soil and salt field soil samples in Thailand with their culture characterization and antimicrobial activity**

Khanungkan Klanbut<sup>1,2</sup>

<sup>1</sup>Department of Biology and <sup>2</sup>Actinobacterial Research Unit, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand 10520

### **Abstract**

Gram-positive, soil dwelling actinobacteria has been receiving more attention since actinomycetes displays a complex secondary metabolites and important industrial enzymes, which have an active role in biotechnological processes. Ninety nine strains were isolated from mangrove forest soil collected from Chonburi province and salt field soil collected from Samutsakorn province, Thailand. Cultures were grown in ISP 2 medium to study the morphological, cultural characteristics and different NaCl concentration. 9 isolated strains were studied for proteolytic enzyme production on skim milk agar medium and azocasein colorimetric assay. Effects of environmental conditions on protease enzyme production were also studied at different pH, temperature and metal ions (PMSF, 1,10-phenantroline, KMnO<sub>4</sub> and EDTA). Five isolates obviously produce the antimicrobial activity on agar plate. Further study was carried out on disc diffusion assay, the 14 days fermented crude extraction was extracted by ethyl acetate solvent. This crude extract were test for antimicrobial activity against *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Micrococcus luteus* ATCC 9341, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 1466 and *Candida albicans* ATCC 9779. The results revealed that crude extract inhibited the growth of 4 microorganisms; *Bacillus subtilis* ATCC 6633 (24%), *Micrococcus luteus* TISTR 2374 (29%), *Staphylococcus aureus* TISTR 118 (18%) and *Escherichia coli* ATCC 25922 (29%) respectively. The current results confirm that for this strains have a great ability to produce protease, which support the use of applications in industry.

**Keywords:** Actinomycetes, Protease, Antimicrobial activity, Mangrove forest soil, salt field soil

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\*Corresponding author: E-mail address: khanungkan@hotmail.com

# Agrotechnical and biological methods of fighting against pests in the technology of growing of flax

Lubov Knignitskaya

*Transcarpathian State Agricultural Research Station*

*Ukraine, Ivano-Frankivsk*

## Abstract

Precarpathian region promote frequent and intense clogging farmland, which negatively affects the quality and productivity of crops. Especially there is the influence of weed germination and maturation of flax, which rise to a greater or lesser extent, slower than the growth of weeds. Therefore, the technology of growing of flax is necessary to apply a complex agro-technical and biological measures aimed at cleaning of the potential and actual weeds, reduce diseases of flax plants.

A study on the ways of the basic soil cultivation and fertilizers in the cultivation of flax in conditions of Precarpathian region was held. They studied plowing at 20-22 cm, plowing at 14-16 cm, plowing at 14-16 cm 35-40 cm loosening, disking 8-10 cm, 8-10 cm disking with loosening to 35-40 cm. In these methods of tillage studied such backgrounds fertilizers without fertilizer (control), green manure (oilseed radish), green manure + N30P45K60, green manure + N45P90K120.

Soil of experimental plot of sod-podzolic surface gleyed medium-characteristic for flaxen zone of Ukraine, and is suitable for the cultivation of flax with such agrochemical indexes: humus content in the arable layer of 2.6%, pH of salt extraction - 4.0-4.5; availability of soil mobile phosphorus and potassium (for Chirikov) respectively of 10,2-11,0 and 10,0-12,0, easily hydrolyzed nitrogen (for Kornfield) - 9,2-10,9 mg / kg soil Investigation of the effect of different tillage methods on weed crops showed that at small tillage - tillage and stubble - infestation was more than deep tillage, which is associated with the placement of the main quantity of weed seeds in the 0 -5 cm processed layer, whereas at a deep plowing they earn on the depth and germinate later.

The results of these studies showed that all tillage methods and embodiments are directed to fertilizers maximum control of weeds, but causes less deep loosening infestation of crops due to the occurrence of weed seeds deeper than the shallow tilling when the seed lies in the surface portion of the processed layer. The largest infestation was traced to variant without fertilizers, whereas the use of green manure and fertilizers provided a reduction in contamination of cultivated flax area.

The smallest morbidity of flax plant (8-12%) was in the variants where they spent plowing to a depth of 14-16 cm with a loosening of 35-40 cm and use green manure (oilseed radish). Thus in the conditions of Precarpathian region of Ukraine in order to decrease the incidence contamination of fields and plants should be carried out on a shallow plowing or disking 14-16 cm by 8-10 cm soil loosening to 35-40 cm sowing green manure on oilseed radish.

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\*Corresponding author: E-mail address: vermos2011@ukr.net

## Antibacterial and Antioxidation Activities of Peanut-Based Yoghurt

Thanavadee Kor-arnan\*, Patchareewan Poonpipattanakul, Phichayada Pakdee and Lita Sudachan

*Department of Biology, Faculty of Science, King Mongkut's Institute Technology of Ladkrabang (KMITL), Bangkok, Thailand 10520*

### Abstract

This study interested to improve phytonutrients in yoghurt with peanut (Fabaceae). The peanut milk was mixed with distilled water (ratio = 8:1) and milk powder at 4 %. Lactic acid bacteria (LAB) starter (e.g. *Lactobacillus acidophilus*, *Lactobacillus* supsp. *bulgaricus*, *Streptococcus thermophilus* and *Streptococcus thermophilus* mixing with *Lactobacillus* supsp. *bulgaricus*) were inoculated at 5% and incubated at 40°C until pH decreasing to 4.0-4.5. It was found that, peanut-yoghurt was a white color and homogeneous curd. Fermentation with *L. acidophilus* showed higher acidity (pH = 3.95) and *S. thermophilus* showed higher total acid (0.43%). Number of vialble LAB in peanut-yoghurt was similar commercial yoghurt range ( $10^7$ - $10^8$  CFU/mL). Supernatants of symbiotic fermentation between *S. thermophilus* and *L. bulgaricus* showed higher antibacterial activity against *Salmonella typhimurium*, *Escherichia coli* and *Staphylococcus aureus*. That may increase metabolites by mixing cultures. *Streptococcus* sp. can provide antibiotics inhibiting bacterial growth and also provided CO<sub>2</sub> environment. Moreover, yoghurt from *L. bulgaricus* concentration at 1.0 ug/mL showed higher antioxidation activity (56.23%). Among LAB in this study, *L. bulgaricus* improved the proteolytic protein source from peanut.

**Keywords:** peanut yoghurt, lactic acid bacteria and proteolytic activity

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\*Corresponding author: E-mail address: chotiros8@hotmail.com

## Morpho-physiological and genetical changes of entomopathogenic ascomycete *Beauveria bassiana* during passaging through four various hosts

V. Yu. Kryukov<sup>1</sup>, U. N. Rotskaya<sup>1\*</sup>, O. N. Yaroslavtseva<sup>1</sup>, E. A. Elisaphenko<sup>2</sup>, V. V. Glupov<sup>1</sup>

<sup>1</sup>*Institute of Systematic and Ecology of Animals SB RAS, Novosibirsk*

<sup>2</sup>*Institute of Cytology and Genetic SB RAS, Novosibirsk*

### Abstract

Phenotypic and genetic characteristic of parasites are changes under epizootic outbreak. Particularly facultative parasites can change virulence level during passaging through hosts significantly. The ascomycete *Beauveria bassiana* is endophytic and entomopathogenic fungi. It has a wide range of hosts and using for biological control of phytophagous and blood-sucking insects. The continuance of epizootic which is induced by entomopathogenic ascomycetes, are 1 – 3 years. During this period the fungi has several passaging through host. In our investigation we simulated this process in laboratory conditions.

Six passages of entomopathogenic ascomycete *Beauveria bassiana* (strain Sar-31) through four hosts (laboratory populations of *Galleria mellonella* and *Tenebrio molitor*, larvae from nature populations *Leptinotarsa decemlineata* and *Locusta migratoria*) and following estimation of phenotypic and genetic differences of the initial strain and reisolated cultures were conducted. The passaging of strain through certain host led to increasing of virulence for both this host and other test-insects. Unidirectional changes of morpho-cultural characteristics: colonies pigmentation and relief strengthening, increasing of conidia production and lipolytic activity were registered in all passaged cultures. Genetic analysis with 6 ISSR markers revealed some variations between initial and reisolated cultures in 3 markers. But these variations were small and did not change individual fingerprint of strains. Taken together, the results of this study help us understand potential ways of applying of entomopathogenic fungi strains in nature, it changes during epizootic process and possibilities of ISSR assay for investigation of pathogen qualitative variability.

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\*Corresponding author: E-mail address: Ulyanar@mail.ru

## Study on aggregation reaction of gold nanoparticles by some amino acid and human hormone

Arjnarong Mathaweesansurn<sup>1,2\*</sup>, Aurachat Lertitthiporon<sup>1,2\*</sup>, Tipachai Vatanavicharn<sup>2</sup>,  
Nathawut Choengchan<sup>1,2\*</sup>

<sup>1</sup>*Flow Innovation-Research for Science and Technology Laboratories (FIRST Labs),*

<sup>2</sup>*Applied Analytical Chemistry Research Unit, Faculty of Science,  
King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand*

### Abstract

In this work, the aggregation reactions of gold nanoparticles (AuNPs) by amino acid *i.e.*, 'cysteine' and by human hormone *i.e.*, 'parathyroid hormone' were studied. The AuNPs were synthesized by the modified 'Turkevich' method and were characterized by tunneling electron microscope (TEM). Results by TEM revealed that particle size distribution were ranging from 10 to 20 nm. Surface plasmon resonance band of the AuNPs was recorded by UV-visible spectrophotometer and the maximum absorption wavelength ( $\lambda_{\max}$ ) was located at 531 nm. The synthesized AuNPs was applied as the sensing probe for detection of cysteine and parathyroid hormone. Detection principle of cysteine is based on the aggregation reaction of AuNPs in the presence of cysteine. This leads to the color change of the AuNPs solution from red wine to purple. Effect of pH and the concentration of cysteine played the important roles on the degree of aggregation. Higher cysteine concentration resulted in higher degree of aggregation and the  $\lambda_{\max}$  was shifted from 531 to 650 nm. The appropriate pH was observed at pH 3.1. Precipitation was found at higher or lower pH value than the optimum pH. Linear correlation between the absorbance ratio (531:650 nm) of the AuNPs and the cysteine concentration from 1 to 500  $\mu\text{M}$  was achieved with good linearity ( $r^2 = 0.98x$ ). The second application is use of the AuNPs for sensing of parathyroid hormone (PTH). The synthesized AuNPs was labeled with anti PTH antibody. Then this solution was added with the PTH in phosphate buffer (pH 6.9). Aggregation reaction of the AuNPs by PTH was occurred and this brought about changing of the AuNPs solution color from red wine to purple. Red shift of the surface plasmon band was found (from 531 to 650 nm). We found that increasing in the PTH concentration resulted in higher degree of aggregation. Satisfied linearity between the absorbance ratio (531:650 nm) was observed ( $r^2 = 0.96x$ ). However, further studies on factors affecting aggregation of AuNPs by PTH were now under investigation.

**Keywords:** Gold nanoparticles; Aggregation; Cysteine; Parathyroid hormone.

### Acknowledgement:

Financial support from Faculty of Science, King Mongkut's Institute of Technology Ladkrabang (to NC) was gratefully acknowledged.

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\*Corresponding author: E-mail address: nchoengchan@gmail.com

## **Adaptive possibilities of assimilative structures of sheet *Rosa Canina* L. in different climatic terms**

Mikhina V.Y, Kuznetsova T. A.\*

*Peter the Great St. Petersburg Polytechnic University*

*The higher school of biotechnology and food technologies, St. Petersburg*

### **Abstract**

Currently, there are different mathematical approaches to the analysis of morpho-anatomical parameters characterizing the interaction of genotype and environment. The challenge was to adapt them to different stages of selection process for getting operational information about the morpho-anatomical responses to environmental factors, the potential assimilative capacity, the reactions reflecting the negative impact of limiting factors.

A comparative study of the structure of the leaves of the *Rosa canina* L., formed in different climatic conditions of the city of Belgorod and St. Petersburg. For the study we used fully developed leaves grown in good light, the phenological stage of full ripeness, given the morphological'.

With advancement to the North the thickness of lamina, number of layers of mesophyll, the coefficient of Palisades not significantly change. However, the shape and volume of the columnar cells of the mesophyll there is a substantial difference.

In the conditions of Belgorod from the studied species, the volume of the cells of the 1st layer of columnar mesophyll was significantly higher than in plants generated in St. Petersburg. In form these cells are more elongated, which is interpreted as the rate of increase of the intensity of photosynthesis under conditions of high solar radiation Belgorod. Surface area and volume of cells top layer is columnar mesophyll of leaves formed in the conditions of Belgorod significantly more, which perhaps reflects the storage function of these cells.

The intensity of photosynthesis involve gaseous exchange, which is also defined by the degree of openness of stomata, area of stomata and their number. But when unfavorable environmental factors (drought, high temperature) the stomata are closed, which is a protective mechanism and reduces the intensity of photosynthesis. But the lack of transpiration leaf may overheat.

In the conditions of Belgorod, the number of stomata per unit leaf surface was significantly higher, indicating a high potential in the intensity of gas exchange. The area of stomata and the degree of openness of the stomata higher in St. Petersburg, which indicates a greater throughput of gases with a single stoma, in comparison with the stomata of leaves formed in the conditions of Belgorod. Reduction in the degree of openness of stomata in plants in the conditions of Belgorod testifies to their protective reaction to adverse environmental conditions (drought). However, the capacity of stomata per unit leaf surface due to the greater number of them in conditions of the Belgorod significantly higher, suggesting a more intensive gas exchange.

Thus, the most informative signs indicating the greater photosynthetic activity of leaves in conditions of the Belgorod from *Rosa canina* is the shape and volume of the columnar cells of the mesophyll, as well as the bandwidth of stomata.

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\*Corresponding author: E-mail address: Tano\_lovely@mail.ru

## Адаптивные возможности ассимилирующих структур листа *Rosa Canina* L. в разных климатических условиях

Михина В.Ю. , Кузнецова Т.А.

Федеральное государственное автономное образовательное учреждение высшего образования «Санкт-Петербургский политехнический университет Петра Великого»,  
Высшей школы биотехнологии и пищевых технологий, Санкт-Петербург

В настоящее время существуют разные математические подходы анализа морфо-анатомических параметров, характеризующих взаимодействие генотипа и среды. Задача состоит в том, чтобы адаптировать их к разным этапам селекционного процесса для получения оперативной информации об морфо-анатомических ответных реакциях на факторы окружающей среды, о потенциале ассимилирующей способности, о реакциях отражающих угнетающее влияние лимитирующих факторов.

Проведено сравнительное изучение строения листьев шиповника собачьего (*Rosa canina* L.), сформированных в разных климатических условиях города Белгорода и Санкт-Петербурга. Для исследования использовались полностью сформированные листья, выросшие при хорошем освещении, в фазу полной спелости с учетом морфологического адреса.

С продвижением на север толщина листовых пластинок, число слоев мезофилла, коэффициент палисадности достоверно не меняются. Однако форма и объем клеток столбчатого мезофилла различаются существенно.

В условиях Белгорода у изучаемого вида объем клеток 1-го слоя столбчатого мезофилла достоверно выше, чем у растений сформированных в условиях Санкт-Петербурга. По форме эти клетки более удлиненные, что интерпретируется как показатель увеличения интенсивности фотосинтеза в условиях большей солнечной радиации Белгорода.

Площадь поверхности и объем клеток верхнего слоя столбчатого мезофилла листьев, сформированных в условиях Белгорода достоверно больше, что возможно отражает запасающую функцию этих клеток.

Интенсивность фотосинтеза сопряжена с газообменом, которая определяется в том числе степенью открытости устьиц (СОУ), площадью устьиц и их числом. Однако, при неблагоприятных факторах среды (засуха, высокая температура) устьица закрываются, что является защитным механизмом и снижает интенсивность фотосинтеза. Но при недостатке транспирации лист может перегреваться.

В условиях Белгорода число устьиц на единицу поверхности листа достоверно выше, что свидетельствует о большом потенциале в интенсивности газообмена. Площадь устьиц и СОУ выше в условиях Санкт-Петербурга, что свидетельствует о большей пропускной способности газов одним устьищем, по сравнению с устьищами листьев, сформированных в условиях Белгорода. Снижение СОУ у растений в условиях Белгорода свидетельствует об их защитной реакции на неблагоприятные условия окружающей среды (засуха). Однако, суммарная пропускная способность устьиц на единицу поверхности листа за счет большего их количества в условиях Белгорода существенно выше, что свидетельствует о более интенсивном газообмене.

Таким образом, наиболее информативными признаками, свидетельствующими о большей фотосинтетической активности листьев в условиях Белгорода у *Rosa canina* является форма и объем клеток столбчатого мезофилла, а также пропускная способность устьиц.

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\*Corresponding author: E-mail address: Tano\_lovely@mail.ru



**Biological properties of calcium-silver crosslinked hydroxyethylacryl chitosan/  
sodium alginate film as a potential wound dressing**

Pathavuth Monvisade\*, Pitchaya Treenate

*Polymer Synthesis and Functional Materials research Unit, Department of Chemistry, Faculty of  
Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand*

**Abstract**

In this study, biological properties of hydrogel films composed of hydroxyethylacryl chitosan (HC) and sodium alginate (SA) crosslinked by  $\text{Ca}^{2+}$  and  $\text{Zn}^{2+}$  were investigated. Firstly, HC was synthesized by following Michael addition reaction of chitosan (CS) and hydroxyethylacrylate. HC and SA in different proportions (75:25, 50:50, 25:75 and 0:100 w/w) were dissolved in distilled water and casted into the Petri dishes with later dried at 60 °C. The prepared film was soaked into 0.5 M calcium nitrate and subsequently soaked into 0.03 M silver nitrate and then dried to obtain calcium-silver crosslinked film. The crosslinking reaction of the hydrogel film was confirmed by fourier transform infrared spectrophotometer (FT-IR). The swelling behaviors of the hydrogel films in phosphate buffer solution (PBS), simulated wound exudate fluid, were investigated at 37 °C. The HC75SA25 hydrogel film exhibited the highest degree of swelling at about 20 times and later declined. The mechanical properties of the hydrogel films were measured in the fully hydrated state. The values of the tensile strength and elongation at break of the hydrogel films are up to 13.5 MPa and 61 %, respectively. The antibacterial activity of the hydrogel films was evaluated using agar well diffusion method. The calcium-silver crosslinked films evidenced antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. Cell viability of the hydrogel films was measured using MTT assay. The results showed that all of hydrogel films were not cytotoxic for Vero cells. The comprehensive results suggest their potential as a wound dressing.

**Keywords:** Hydroxyethylacryl chitosan; Sodium alginate; Wound dressing; Antibacterial property

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\*Corresponding author: E-mail address: kmpathav@gmail.com

## **Antibacterial activity of spice extracts against *Pseudomonas fluorescens*: application of clove and thyme extract in decontamination of raw chicken**

Suree Nanasombat<sup>\*</sup>, Gantharat Mahawiriyo, Chitlada Budsamongkon, Nudtapong Thummajinda

*Department of Biology, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand*

### **Abstract**

Antimicrobial activity of 6 spice methanolic extracts against *Pseudomonas fluorescens* using disc diffusion test and minimum inhibitory concentration (MIC) determination was studied. Clove (*Syzygium aromaticum*) extract was the most effective spice to inhibit the growth of *P. fluorescens* with the MIC of 3.2 mg/ml, followed by the extracts of thyme (*Thymus vulgaris*), star anise (*Illicium verum*), galanga (*Alpinia galangal*), caraway (*Ocimum gratissimum*) and licorice (*Glycyrrhiza glabra*). Clove extract also possessed the strongest antioxidant activity (3.63 mmol Fe (II)/ g extract by ferric reducing antioxidant power method), followed by the extracts of thyme, caraway, galanga, licorice and star anise. Therefore, clove and thyme extracts were selected for use to study their effect on reducing microbial load and delaying lipid oxidation in raw chicken thighs. Total viable counts and total *Pseudomonas* counts on raw chicken thigh surface after decontamination treatments by dipping in aqueous solution of 1-2% clove extract, 1-2% thyme extract or distilled water were compared. Addition of 2% cloves extract in the dipping solution was the most effective to reduce the microbial load and delay the lipid oxidation in the raw chicken thighs. The treatment of naturally contaminated chicken thighs (6.41 log CFU/ 25 cm<sup>2</sup>) by dipping in 2% clove extract solution resulted in 0.56 and 0.28 log CFU/ 25 cm<sup>2</sup> immediate reduction of total viable counts and total *Pseudomonas* counts, respectively. Moreover, the dipping treatment of 2% clove extract solution resulted in 0.53 log CFU/ 25 cm<sup>2</sup> reduction of total *Pseudomonas* counts, and delayed lipid oxidation in the raw chicken thighs after refrigerated storage for 7 days.

**Keywords:** Clove, thyme, psychrotrophic bacteria, lipid oxidation

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\*Corresponding author: E-mail address: snanasombat@gmail.com

## The optical trapping and manipulation for applications in biology

Worakarn Neeyakorn\*, Prathan Buranasiri, Weerachai Pornprasit, Sopa Jityasang

*Department of Physics, Faculty of Science, King Mongkut's Institute of Technology  
Ladkrabang, Bangkok, Thailand*

### Abstract

Optical trapping system is widely used to trap the microparticles and it has various applications in physics and biology fields<sup>1</sup>. However, in many applications, it has shown that it is important to be able to control the particles in real time<sup>1</sup>. The work describes the inexpensive setup of the optical trapping system with a single-beam gradient trap<sup>2</sup> which is used as “optical tweezers” for moving particles. We also focus on programming the XYZ translator integrated within the system using the Labview program. The result has shown that our Labview program can control the XYZ translator automatically within the micro scale. This system shows the potential that it can automatically control a biological cell<sup>2</sup> or bacteria in real time and it can be applied as a tool to study the mechanical of biological systems<sup>3</sup>.

Keywords: Optical tweezers, Optical trapping, cell micromanipulation

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\*Corresponding author: E-mail address: worakarn.ne@kmitl.ac.th

# Soybean-Biodiesel Upgrading by Partial Hydrogenation of Polyunsaturated Fatty Acid Methyl Esters over Supported Pd Catalysts

Natthida Numwong\*, Tawan Sooknoi

*Catalytic Chemistry Research Unit, Faculty of Science,*

*King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand*

## Abstract

Biodiesel or fatty acid methyl ester (FAME) is considered as an alternative for conventional diesel due to its biodegradability, higher flash point, lower sulfur content, lower exhausted emission as well as miscibility with petroleum-based diesel. However, properties of biodiesel are strongly influenced by the number of double bonds present in starting oil. Biodiesel produced from oil that contains higher unsaturated fatty acid (C18:3 and C18:2) composition performs the lower oxidative stability. The oxidation of unsaturated FAMEs produces peroxides, aldehydes, ketones, and acids that change biodiesel properties and combustion process. In contrast, the higher saturated fatty acid (C18:0) composition, the worse cold flow property becomes, where the solidified waxes cause the clogging of fuel filters or injectors in engines. Therefore, the optimization between oxidative stability and cold flow properties by partial hydrogenation of polyunsaturated FAMEs is a promising approach to solve this problem. This study focused on the improvement of soybean-biodiesel oxidative stability by partial hydrogenation of polyunsaturated FAMEs (C18:3 and C18:2). The partial hydrogenation reaction was operated in a batch reactor at 120°C, 0.4 MPa, 500 rpm stirring speed, and 50 mL/min hydrogen flow rate. The Pd supported on alumina (Pd/Al<sub>2</sub>O<sub>3</sub>) and Pd supported on silica (Pd/SiO<sub>2</sub>) prepared by incipient wetness impregnation (IWI), were used as catalysts. The results show that after 4.0 h of reaction using Pd/SiO<sub>2</sub>, C18:3 and C18:2 FAMEs are fully converted with a gradual decrease in monounsaturated FAME (C18:1) and an increase in saturated FAME (C18:0). This resulted in an improvement of the soybean-biodiesel oxidative stability from 2.0 to 50.5 h even without addition of anti-oxidant. Furthermore, the effect of support on Pd particle size and Pd dispersion as well as selective hydrogenation activity were also investigated. The result showed that Pd particle size and Pd dispersion had significant effect on hydrogenation selectivity of the catalyst. The Pd supported on higher surface area SiO<sub>2</sub> support exhibited higher selectivity towards C18:1 FAME due to smaller Pd particle size and higher Pd dispersion, which provided limited surface for the adsorption of C18:1 and hence low possibility in hydrogenation to C18:0. This suggests that hydrogenated biodiesel over Pd supported on high surface area SiO<sub>2</sub> shows better performance in terms of cold flow properties.

**Keywords:** Biodiesel, Oxidative stability, Cold flow properties, Partial hydrogenation, Pd catalyst

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\*Corresponding author: E-mail address: natthida.nu@kmitl.ac.th

# Optimization of Bacterial Cellulose Production from Wastewater of Noodles Processing by *Komagataeibacter* sp. PAP1

Duangjai Ochaikul\* and Tanyarat Akepatcha

*Department of Biology, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Chalongkrung Rd. Ladkrabang Bangkok, 10520, Thailand*

## Abstract

Bacterial cellulose (BC) production from Noodle Wastewater (NW) was fermented by *Komagataeibacter* sp. PAP1. In order to increase BC production, NW – based medium was prepared and optimized for the cultivation of this bacteria. The optimized NW - based medium was composed of 5% (w/v) mannitol, 0.1% (w/v) beef extract, 0.5% (v/v) ethanol, 1% (v/v) acetic acid, pH 7.0 and incubated at 30°C for 7 days. Under these conditions, BC yield was  $11.76 \pm 0.34$  g/L (4.40 fold) higher than in standard HS medium. The study on growth and BC production by *Komagataeibacter* sp. PAP1 fermented in optimized culture condition. This result showed that BC production by *Komagataeibacter* sp. PAP1 was growth – associated. Our results demonstrate that NW can be used as an alternative low – cost substrate for BC production.

**Keywords :** bacterial cellulose, Noodle Wastewater, optimization, *Komagataeibacter* sp. PAP1

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\*Corresponding author: E-mail address: daungjai.oc@kmitl.ac.th

## Bioactivities of *Bauhinia purpurea* crude extract

Rawissara Panya-ake, Luksanaporn Chuenjit, Waraporn Leelanut and  
Suttijit Sriwatcharakul\*

*Department of Biology, Faculty of Science*

*King Mongkut's Institute of Technology Ladkrabang, Bangkok, THAILAND*

### Abstract

The bioactivity studies of purple orchid tree; *Bauhinia purpurea* ethanolic crude extracts from leaf, seed and bark were analyzed for the growth inhibition of 5 bacterial species; *Pseudomonas aeruginosa* ATCC 27853, *Micrococcus luteus* TISTR 9341, *Salmonella typhimurium* TISTR 0562, *Clostridium sporogenes* TISTR 549, and *Streptococcus thermophilus*. At initial concentration crude extract of 50 mg/ml, the agar well diffusion results found that extracts inhibit all bacterial tested species. At 100 mg/ml, seed extract exhibited antibacterial activity was equivalent to antibiotics; gentamicin and ciprofloxacin significantly to inhibit *S. typhimurium* and *C. sporogenes*, respectively. While leaf extract gave the best result of growth inhibition against *P. aeruginosa*, *M. luteus* and *S. thermophilus*. Analyzed the efficacy of free radical scavenging by using DPPH radical scavenging assay with all crude extracts showed % reduction of leaf, seed and bark extracts at 0.625 mg/ml were 30.57, 78.08 and 13.43, respectively and seed extract had significant percentage of reduction as  $\alpha$ -tocopherol. The determination of total phenolic compounds in the crude extracts exhibited the highest phenolic content was 347.16 GAE/g dry weight in bark extract. Studied cytotoxicity of crude extracts on human lung cancer cell lines by MTT assay found that seed extract had the most cytotoxicity  $CC_{50}$  value, 4.00  $\mu$ g/ml. Antioxidant activity and cytotoxicity of crude extracts exhibited that the more increase of extract concentration, the more activities indicated. According to the bioactivities results, the seed crude extract of *Bauhinia purpurea* is the most interesting part for further work to search the beneficial of this plant.

**Keywords:** *Bauhinia purpurea*, antimicrobial, antioxidant activity, total phenolic compound, cytotoxicity test

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\*Corresponding author: E-mail address: [suttijit.sr@kmitl.ac.th](mailto:suttijit.sr@kmitl.ac.th)

## **Effect of substrates and supplements to the productivity and quality of straw mushroom**

Mongkol Phensaijai\* and Nuanphan Naranong

*King Mongkut's Institute of Technology Ladkrabang, Bangkok Thailand*

### **Abstract**

Straw mushroom (*Volvariella volvacea*) strains KM-Sc 001, KM-Sc 005 , KM-Sc 010 and KM-Sc 014 were cultivated on PDA for mycelium growth experiment . Then the fastest mycelial growth rate mushroom was cultivated on 12 different substrate formulas to evaluate productivity and quality of mushroom. Straw and coconut husk were used as choice of major substrate. Napier grass, narrowleaf cattail and water hyacinth were used as choice of minor substrate. While rice bran and wheat flour were used as choice of supplement.

Result showed that KM-Sc 010 had fastest mycelial growth rate on PDA (0.754 cm/day). Fruiting bodies obtained from substrate fomula no.3 (straw, water hyacinth and rice bran) displayed higher productivity than the other treatments (980.15g in 15 days). Nutrition values and mineral of mushroom from substrate formula no.3 had higher than the other formulas but not statistically different.

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\*Corresponding author: E-mail address: phensaijai.mongkol@yahoo.com

## **The use of materials based on chitosan as a fertilizer soil**

Politayeva N.A. \*, Taranovskaya E.A.

*Saint Petersburg State Polytechnic University, Graduate School of Biotechnology and Food Technology*

### **Abstract**

It is known that chitosan is biodegradable. In modern agriculture widely used products based on chitosan, in particular, to combat fungal infection. Materials based on chitosan have a stimulating effect on plant growth and resistance to adverse environmental factors. Studies E.V. Popova (1995), L.Y. Yudkina and S.A. Tarlakovskogo (1995), M.S. Yakubchika et al. (1995), A.P. Boyko (2000) found a positive effect of chitosan on the disease resistance of barley, wheat, tomato, cucumbers and potatoes. In recent years, in practice begin making use of chitin to soil for controlling nematodes and rots (Maksimov et al., 1992; Udalova et al., 1995; Dobrohotov, 2000). In this case, chitin induces growth of microorganisms sinteziruyuschih chitinase which inhibits pathogen growth. This influence induktora to protect plants is carried out through the ecosystem.

All of the above shows high prospects of the use of chitosan, its derivatives and chitosan in plant protection technologies.

We studied the use of sorption materials based on chitosan as a soil fertilizer. The experiment was conducted under artificial conditions identical for all experiments. Used fertile soil. The reaction of the soil environment was close to neutral (pH 6.4 - 6.8). The depth of planting of experimental samples of 3-5 cm. For the cultivation of legumes (kidney bean «Phaseolus vulgaris L») cultures were used flower pots with the addition of the soil as fertilizer waste materials based on chitosan, after their use in wastewater treatment from heavy metal ions. Phases of beans studied visually, when a 75% Plant symptoms onset phase of development. Measurement of plant growth on the main phases of development was made with a ruler. The experimental results showed that in the first stages of development of the beans were found differences in all experimental plots. The apparent difference in growth between the plants background plots (soil without fertilizer application) and control (with fertilizer) manifested itself in 10-12 days. The highest values of plant height was observed in areas with payment as fertilizer waste sorbents and chitosan compared with no use of fertilizers. It has been shown that the use of these fertilizers leads to a significant increase of 12% germination and growth of plants than without fertilization. Therefore, recommendations for the use of waste sorption materials based on chitosan as a rational fertilizer. It is necessary to take into account the dosage of their entering into the soil, to avoid secondary pollution in the accumulation of heavy metals. If we use sorption materials, after cleaning waste from the heavy metals, which are trace elements (copper, zinc), they may be used for growing food crops. After cleaning the waste water from the heavy metal ions non micronutrients (cadmium, lead), to prevent their bioaccumulation plants, recommended the introduction of "fertilizer" (waste sorbents) in the soil no more than once every five years and be used for industrial crops.

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\*Corresponding author: E-mail address: Conata07@list.ru



## Применение материалов на основе хитозана в качестве удобрения почв

Политаева Н.А., Тарановская Е.А.

Санкт-Петербургский политехнический университет Петра Великого

Высшая школа биотехнологии и пищевых технологий

Известно, что хитозан может подвергаться биологическому разложению. В современном сельском хозяйстве широко используются препараты на основе хитозана, в частности, для борьбы с грибковой инфекцией. Материалы на основе хитозана оказывают стимулирующее действие на рост и устойчивость растений к неблагоприятным факторам среды. Исследованиями Э. В. Поповой (1995), Л. Ю. Юджина и С. А. Тар-лаковского (1995), М. С. Якубчика и др. (1995), А.П. Бойко (2000) установлено положительное действие хитозана на болезнеустойчивость растений ячменя, пшеницы, томата, огурца и картофеля. В последние годы на практике начинают использовать внесение хитина в почву для борьбы с гнилями и нематодами (Максимов и др., 1992; Удалова и др., 1995; Доброхотов, 2000). В этом случае хитин индуцирует рост микроорганизмов, синтезирующих хитиназу, которая подавляет развитие патогена. Это влияние индуктора на защиту растений осуществляется через экосистему.

Все вышесказанное говорит о высокой перспективности применения хитозана, его производных и хитозановых технологий в защите растений.

Была изучена возможность использования сорбционных материалов на основе хитозана в качестве удобрения почв. Эксперимент проводили в искусственных условиях одинаковых для всех экспериментов. Использовался плодородный грунт. Реакция почвенной среды составила близкой к нейтральной (рН 6,4 - 6,8). Глубина посадки экспериментальных образцов составляет 3-5 см. Для выращивания бобовых (фасоль обыкновенная «*Phaseolus vulgaris L*») культур были использованы цветочные горшки с добавлением в грунт в качестве удобрений отработанные материалы на основе хитозана, после их использования в очистке стоков от ионов тяжелых металлов (ИТМ). Фазы развития фасоли изучали визуально, при появлении у 75 % растений признаков наступления фазы развития. Измерение роста растений по основным фазам развития производилось с помощью линейки. Результаты эксперимента показали, что на первых этапах развития фасоли не обнаружено различий на всех опытных делянках. Видимая разница в росте растений между делянками фона (почва без внесения удобрений) и контроля (при внесении удобрений) проявилась через 10-12 дней. Наибольшие значения высоты растений наблюдали на участках с внесением в качестве удобрений отработанных сорбентов и хитозана по сравнению без использования удобрений. Было показано, что использование данных удобрений приводит к существенному повышению всхожести на 12% и росту растений, чем без внесения удобрений. Следовательно, рекомендации по использованию отработанных сорбционных материалов на основе хитозана в качестве удобрений рациональны. При этом необходимо учитывать дозировку внесения их в почву, чтобы избежать вторичного загрязнения при накоплении солей тяжелых металлов. Если мы используем сорбционные материалы, после очистки стоков от ИТМ, которые являются микроэлементами (медь, цинк), то их, возможно, использовать для выращивания пищевых культур. После очистки стоков от ИТМ, не являющихся микроэлементами (кадмий, свинец), для предотвращения их биоаккумуляции растениями, рекомендуется производить внесение таких «удобрений» (отработанных сорбентов) в почву не чаще одного раза в пять лет и использовать только для технических культур.

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\*Corresponding author: E-mail address: Conata07@list.ru

## The dynamical model of dengue vertical transmission

Puntani Pongsumpun

*Department of Mathematics, Faculty of Science,*

*King Mongkut's Institute of Technology Ladkrabang,*

*Chalongkrung road, Ladkrabang, Bangkok 10520, Thailand*

### Abstract

Abstract— Dengue disease is usually found in many parts of the world, including Africa, Asia, South America and Australia. Dengue disease can pass from one individual to another by two distinct mechanisms such as horizontal transmission and vertical transmission. In horizontal transmission, susceptible individuals can be infected by direct or indirect contacts with infectious individuals who are stay at the same time. Vertical transmission means to direct transmission from infected parents to their offspring before or during birth. In this study, the dynamical model of dengue disease is formulated by considering the vertical transmission in *Aedes* mosquitoes. The analysis of our model is given. The results of this study should introduce the alternative ways to reduce the dengue outbreak.

**Keywords:** *Aedes* mosquitoes, dengue, dynamical model, vertical transmission

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\*Corresponding author: E-mail address: kppuntan@kmitl.ac.th

## **Stabilization of peptidyl-prolyl-cis/trans isomerase from *Pseudomonas fluorescens* using sodium alginate does not reduce its elicitor activity towards viral and fungal diseases of plants**

Popletaeva S.B.<sup>1</sup>, Arslanova L.R.<sup>1</sup>, Statsyuk N.V.<sup>1</sup>, Zernov A.L.<sup>2</sup>, Bonartseva G.A.<sup>2</sup>,  
Dzhavakhiya V.G.<sup>1</sup>

*1 All-Russian Research Institute of Phytopathology, Bolshie Vyazemy, Russia*

*2 Federal Research Centre "Fundamentals of Biotechnology" of the Russian Academy of Sciences, Moscow, Russia*

A possibility to stabilize peptidyl-prolyl cys/trans isomerase (PPIase) from *Pseudomonas fluorescens* using sodium alginate (Alg), a natural polysaccharide able to protect the target protein against proteolytic degradation on the surface and within plant tissues, has been studied. Complex microparticles containing 70% of Alg (carrier), 20% of bovine serum albumin (BSA, excipient), and 10% of PPIase (active component) were obtained by the spray drying and tested for their protecting activity against tobacco mosaic virus (TMV) and *Alternaria longipes*, a causal agent of tobacco brown spot, and also against *Stagonospora nodorum*, a major pathogen of wheat and related cereals. The experiments were arranged on detached leaves of tobacco and wheat, respectively; the results were compared with the protective activity of the Alg-BSA mix (75:25) and the activity of intact PPIase.

According to the obtained results, stabilized PPIase (1µg/mL) was able to prevent the development of *A. longipes* on tobacco leaves, and its activity was about the same as that of intact PPIase of the same concentration (disease suppression by 35±5% and 31±4%, respectively), and significantly exceeded the activity of the Alg-BSA mix (7±2%). In the case of TMV, no significant difference was revealed again between the stabilized and intact PPIase; in both cases, the average amount of necroses per a leaf decreased in 32-35 times as compared with the control. The Alg-BSA mix did not show any antiviral elicitor activity. The evaluation of the protective activity against *S. nodorum* revealed the positive effect for all three variants. The intact PPIase and Alg-BSA mix suppressed the pathogen development by 40±8 and 32±8%, respectively, whereas the same effect of stabilized PPIase was 88±3% indicating a synergism between Alg and PPIase.

Thus, the formation of a complex between PPIase and Alg does not result in the loss of the elicitor activity of the protein. The ability of stabilized PPIase to induce resistance of plants to fungal pathogens was confirmed for such phylogenetically distant species as tobacco and wheat; in addition, the same effect was demonstrated towards tobacco mosaic virus.

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\*Corresponding author: E-mail address: unavil@yandex.ru

**Комплексообразование с альгинатом натрия как способ дополнительной стабилизации пептидил-пролил-цис/трансизомеразы из *Pseudomonas fluorescens*, обеспечивающий сохранение ее элиситорной активности в отношении вирусных и грибных болезней растений**

Поплетаева С.Б.<sup>1</sup>, Арсланова Л.Р.<sup>1</sup>, Стацюк Н.В.<sup>1</sup>, Зернов А.Л.<sup>2</sup>, Бонарцева Г.А.<sup>2</sup>, Джавахия В.Г.<sup>1</sup>

1 ФГБНУ Всероссийский НИИ фитопатологии, Большие Вяземы, Россия

2 ФИЦ «Фундаментальные основы биотехнологии РАН», Москва, Россия

Исследована возможность создания комплексного препарата, представляющего собой продуцируемый бактерией *Pseudomonas fluorescens* элиситорный белок пептидил-пролил-цис/транс-изомеразу (ППИ-азу) в оболочке из природного полисахарида альгината натрия, способной обеспечить защиту целевого белка от действия протеаз на поверхности и внутри растительных тканей. Комплексные микрочастицы на основе альгината, загруженные ППИ-азой, были получены методом распылительного высушивания и состояли из альгината натрия (носитель белка, 70%); бычьего сывороточного альбумина (БСА, наполнитель, 20%) и ППИ-азы (целевой агент, 10%).

Защитная активность полученного комплекса была проверена на отделенных листьях табака в отношении возбудителя бурой сухой пятнистости *Alternaria longipes* и вируса табачной мозаики, а также на отделенных листьях пшеницы в отношении возбудителя септориоза пшеницы *Stagonospora nodorum*. Полученный результат сравнивали с активностью смеси, состоящей из 75% альгината натрия и 25% БСА, а также с препаратом интактной ППИ-азы.

Согласно полученным результатам, стабилизированная ППИ-аза в концентрации 1 мкг/мл сохраняла способность препятствовать развитию бурой сухой пятнистости табака, причем ее защитная активность была сравнимой с таковой у интактной ППИ-азы аналогичной концентрации (35±5% и 31±4%, соответственно), и достоверно превышала таковую для смеси альгината натрия и БСА (7±2%). Оценка активности полученного комплекса против вируса табачной мозаики в сравнении с интактной ППИ-азой также не выявила существенных различий между этими вариантами; в обоих случаях среднее количество некрозов на листьях снижалось в 32-35 раз. Для смеси альгината с БСА противовирусная элиситорная активность выявлена не была. Аналогичное исследование в отношении возбудителя септориоза пшеницы показало наличие эффекта подавления развития болезни как смесью БСА и альгината (32±8%), так и интактной ППИ-азой (40±8%); при этом аналогичный эффект от применения стабилизированной ППИ-азы составил 88±3%, что свидетельствует о синергическом характере взаимодействия альгината и ППИ-азы.

Таким образом, стабилизация ППИ-азы альгинатом натрия не приводит к потере белком элиситорной активности. На примере таких филогенетически отдаленных видов растений, как пшеница и табак, показано свойство полученного комплекса индуцировать устойчивость растений к грибным (а в случае табака – еще и к вирусным) патогенам.

# Development of lipase producing mixed whole-cell microorganisms as biodiesel by response surface methodology

Aree Rittiboon\*, Kitjasit Promsut, Marisa Jatupornpipat

*Department of Biology, Faculty of Science, King Mongkut's Institute of Technology  
Ladkrabang, Bangkok, Thailand 10520*

## Abstract

The strain KPB10, KPY9 and G47 were identified by compared 16S rRNA for bacteria and 26S rRNA for yeast with NCBI. It was found that KPB10 were identified as *Bacillus siamensis*, KPY9 were identified as *Pichia guilliermondii* and G47 were identified as *Candida orthopsilosis*. Subsequently, they were subjected to optimize as biocatalyst by response surface method. The optimal conditions for high lipase activity (0.36 U/mL) were 5.36 % of bran, 2.10 % of molasses with pH 7.32. This mixed-culture was biocatalyst selected for optimization of biodiesel production using response surface method. The optimal condition for high percentage of esters (96.67%) were oil to ethanol ratio of 3.78:1, 10.52% biocatalyst at 72.63 hr and 86.07% of biodiesel yield.

**Keywords:** lipase, mixed whole-cell, biodiesel, respond surface metrodology

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\*Corresponding author: E-mail address: rittiboonaer1@gmail.com

## Silver loaded alginate-guar gum hydrogel with antibacterial property

Chonlada Ritvirulh\*, Pathavuth Monvisade, Chanvit Limpaboon, Piyathida Hasitapong, Pusit Chantawut

*Polymer Synthesis and Functional Materials Research Unit, Faculty of Science,*

*King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand*

### Abstract

In this study, hydrogel from polysaccharides of alginate (Alg) and guar gum (GG) at selected weight ratio of 4:6 and 6:4 were prepared. Hydrogel network was formed using base sodium tetraborate (B) as a cross-linking agent with various concentrations of 0.2, 0.4 and 0.6 wt%. The addition of silver was achieved by immersing the hydrogel in a solution of silver nitrate and subsequently, sodium borohydride as a reducing agent. The structural characteristics of the hydrogel were examined by X-ray diffraction (XRD) and X-Ray Fluorescence (XRF). The results indicated that a presence of silver in hydrogel was observed in a range of 11-42 wt%. The higher weight ratio of alginate, the higher loading of silver was obtained. This is because a smaller molecule of alginate in comparison with guar gum leads to a higher diffusion rate of sodium borohydride for reducing silver nitrate to be silver. The percentage swelling behavior of hydrogel in distill water exhibited the maximum values at 60% for Alg4:GG6:B0.6. In addition, the anti-bacterial activity of the prepared hydrogels against *Bacillus subtilis* (gram-positive) and *Escherichia coli* (gram-negative) were investigated by agar diffusion method. It was found that *Bacillus subtilis* gave a higher value of inhibition zone diameter as compared to *Escherichia coli*. Moreover, it can be seen that an antibacterial activity of hydrogel against both bacteria was increased gradually with silver loading.

**Keywords:**Hydrogel, Alginate, Guar gum, Silver

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\*Corresponding author: E-mail address: chonlada.ri@kmitl.ac.th, krchonla@gmail.com

## Mangosteen shell extract/bacterial cellulose mat as potential antibacterial wound dressings

Suparat Rukchonlatee<sup>1\*</sup>, Duangjai Ochaikul<sup>2</sup>, Chuthamat Suankaew<sup>2</sup>, Wanida Janvikul<sup>3</sup>

<sup>1</sup>*Polymer Synthesis and Functional Materials Research Unit, Faculty of Science,  
King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand*

<sup>2</sup>*Department of Biology, Faculty of Science, King Mongkut's Institute of Technology  
Ladkrabang, Bangkok, Thailand*

<sup>3</sup>*National Metal and Materials Technology Center, Pathumtani, Thailand*

### Abstract

This study focused on property examination of bacterial cellulose mat prepared from *Acetobacter xylinum* TISTR 976 with mangosteen shell extract for antibacterial wound dressings. The mangosteen shell (*Garcinia mangostana* L.) extract was obtained using the maceration method with hexane, ethyl acetate and methanol. Its antibacterial activity by disc diffusion method was determined against *Escherichia coli* TISTR 887 and *Staphylococcus aureus* TISTR 118. Among the solvents used, the mangosteen shell extract with methanol gave the highest antibacterial activity. Therefore, it was used for further studies. To produce antibacterial wound dressings, the bacterial cellulose mat was immersed in the extract with various concentrations and then freeze-dried. The maximum concentration of extract which was non cytotoxic to L929 cells with agar diffusion method was 6.25 mg/ml. The inhibition zone of the dried bacterial cellulose mat incorporated with extract (6.00 mm in diameter) against *S. aureus* TISTR 118 was  $12.16 \pm 0.28$  mm; however, it did not show the growth inhibition of *E. coli* TISTR 887. In addition, SEM was used to determine the presence of extract in the bacterial cellulose mat. The SEM images illustrated that the extract particles were inserted between the cellulose fibers, resulting in increases in porosity and so providing rougher surface of the bacterial cellulose mat. As a consequence, the water vapor transmission rate and oxygen transmission rate of the dry wound dressing with the extract were increased. This would help better wound recovery. The water adsorption of the dry wound dressing with the extract was 4.44 times of its dry weight and was higher than that without the extract. Tensile strength, Young's modulus and elongation at break of dry wound dressings with the extract were  $13.3 \pm 1.9$  MPa,  $270 \pm 62$  MPa and  $2.8 \pm 0.7\%$ , respectively. The wet one gave elongation at break 3.74 times of the dry one. All the results supported that the mangosteen shell extract with methanol had good antibacterial activity and so potentially used for wound dressings.

**Keywords:** Wound dressings, Bacterial cellulose, Mangosteen shell extract, Antibacterial activity

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\*Corresponding author: E-mail address: suparat.ru@kmitl.ac.th, kjsupara@gmail.com

# **Anaerobic Co-digestion of Food Waste and Fat, Oil, Grease from Canteen Grease Trap for Biogas Production**

Saeng-aroon, W., Rodnoi, W., Sawangpatthana, W. and Junyapoon, S.\*

*Department of Chemistry, Faculty of Science,*

*King Mongkut's Institute of Technology Ladkrabang*

*1 Chalongkrung 1, Ladkrabang District, Bangkok, Thailand, 10520*

## **Abstract**

This study aims to investigate the biogas production potentials of food waste (FW) and fat, oil and grease (FOG) from canteen grease trap in a 4.2 m<sup>3</sup> anaerobic co-digestion tank under room temperature. A ratio of 19:1 of food waste to FOG was used as substrate. 30 kilograms of substrate and 60 liters of tap water were fed into the co-digestion tank each day between Monday and Friday with a retention period of 46.4 days. Biogas production and characteristics of substrate and effluent after digestion (i.e. pH, alkalinity, total solids (TS), volatile solids (VS), volatile fatty acids (VFA)) were examined. The experimental results showed that the substrate contained pH 3.57, alkalinity 880.42 mg/l as CaCO<sub>3</sub>, total solids 78,047.84 mg/l, volatile solids 70,980.17 mg/l, volatile fatty acids 1,320.63 mg/l as CH<sub>3</sub>COOH. The effluent after digestion contained pH 7.6, alkalinity 2,738.5 mg/l as CaCO<sub>3</sub>, total solids 19,154 mg/l, volatile solids 10,824.84 mg/l, volatile fatty acids 4,107.75 mg/l as CH<sub>3</sub>COOH. Removal efficiencies of TS and VS in the digestion tank were 75.46% and 84.75%, respectively. A cumulative biogas production was 2.5 m<sup>3</sup>/d. Biogas and methane yields were 461.77 ml biogas/g VS removed and 234.12 ml CH<sub>4</sub>/g VS removed, respectively. The biogas contained CH<sub>4</sub> 50.7%, CO<sub>2</sub> 34.2% and H<sub>2</sub>S 40 ppm.

**Keywords:** anaerobic co-digestion, biogas, food waste, fat, oil and grease (FOG)

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\*Corresponding author: E-mail address: sjunyapoon68@gmail.com



# Pretreatment and enzymatic hydrolysis of sugarcane bagasse for cultivation of *Clostridium acetobutylicum*

Vorapat Sanguanchaipaiwong

Department of Biology, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang,  
Chalongkrung Rd. Ladkrabang, Bangkok, THAILAND 10520

## Abstract

The purpose of this research was to study the possibility of using sugarcane bagasse as a carbon source for the production of organic solvents, acetone-butanol-ethanol (ABE), by *Clostridium acetobutylicum* DSM 792. The pretreatment was carried on using sulfuric acid, compared with sodium hydroxide. Consequently, the solid was neutralized. To investigate the lowest quantity of washing water, the volume of water used for washing was measured. The ratio of the sample to water was 5:100 (g/L). It has been found that the pretreatment with sulfuric acid generated more waste water than sodium hydroxide. The volume of water directly washing both acid and base on pretreated bagasse was 1100 mL, while the amount of washing water switching between acid and base pretreated bagasse decreased to 900 mL. Subsequently, various concentrations of acid and base (0.2-1.0M) were utilized to pretreat the samples. It has been found 1.0M NaOH solution yielded the highest level of reducing sugar of 1.09 g/g of sugar cane. For the appropriate ratio of enzyme investigation, the ratio of 0.8 mL enzyme per g of sugarcane bagasse produced sugar concentration of 1.13 g/g of sugarcane in 36 hours. The hydrolysate was cultured by *Clostridium acetobutylicum* DSM792 as comparison with the control of 50 g/L glucose at 37° C anaerobically. It was revealed that *Clostridium acetobutylicum* DSM792 efficiently utilized more sugar from bagasse hydrolysate to produce organic solvent than the use of glucose. Acetic acid and lactic acid are found with the concentration of 21.13 and 26.46 g/L at 36, and 120 hours, respectively. Organic solvent was found included acetone and ethanol with the concentration of 1.56 and 3.36 g/L. Compared to the controlled set, There were only 1.31 g/L acetic acid and 2.72 g/L ethanol at 84 h and 120 h. It might due to inhibitors from pretreatment stage.

**Keywords :** sugarcane bagasse, *Clostridium. acetobutylicum*, acetone-butanol-ethanol fermentation

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\*Corresponding author: E-mail address: vorapats@hotmail.com

## Recovery of vitamin A & E from bio-derived chemical feedstock

Pirulh Sehabua<sup>1</sup> and Tawan Sooknoi<sup>2,3\*</sup>

<sup>1</sup>*PTT Global Chemicals, 59, Ratniyom Road, Noenphra, Mueang Rayong, Rayong 21150  
Thailand*

<sup>2</sup>*Department of Chemistry, Faculty of Science,  
King Monkut's Institute of Technology Ladkrabang, Chalongkrung Road, Ladkrabang, Bangkok  
10520*

<sup>3</sup>*Catalytic Chemistry Research Unit, Faculty of Science,  
King Monkut's Institute of Technology Ladkrabang, Chalongkrung Road, Ladkrabang, Bangkok  
10520*

### Abstract

Various valuable chemicals are highly abundant in crude palm oil, a bio-derived chemical feedstock. Without appropriate recovery process, these will be lost during biodiesel and bio-derived chemical production. One of the most useful chemicals are tocopherol (vitamin E) and carotenoids, the precursor for vitamin A synthesis. Therefore, removal of these compounds does not only improve the combustion efficiency of the biodiesel or efficiency of other bio-derived chemical processes, but also provide natural-organic building blocks for pharmaceutical and food industries.

In this work, liquid phase adsorption of vitamin E and carotenoids-containing oil and the palm oil were investigated over non-microporous materials with high surface area. This is because the carotenoids are quite sensitive and relatively unstable to heat and light. Mesoporous MCM-48 was synthesized using surfactant-assisted sol-gel technique and used adsorbent. The liquid adsorption were tested in both batch and continuous process using 0.3-30% vitamin E in hexane and crude palm oil as feed. Desorption were also carried out in batch and continuous process using ethanol, propanol, acetone, ethyl acetate and butyl acetate as desorbing solvent. The adsorption isotherm shows that as high as 115-450 mg of vitamin E per 1 g of MCM-48 adsorbent, in pallet form, can be obtained. The vitamin E can be efficiently recovered using butyl acetate as solvent. Up to 97% recovery of vitamin E and carotenoids can be achieved at 40 °C. Kinetic study shows that the adsorption rate depends on the primary crystallite size of MCM-48. Desorption rate is relatively faster than that for adsorption, particularly at higher concentration.

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\*Corresponding author: E-mail address: kstawan@gmail.com

## Peculiarities of interaction of *Trichoderma longibrachiatum* with phytopathogenic micromycetes

Sidyakin A.I.

*Research and Production Association Biotechsoyuz, Moscow*

*Crimean Federal V.I. Vernadsky University, Simferopol*

The fungi of the genus *Trichoderma* are widely known as an agent that is used in various branches of industry. The representatives of this genus are known as producers of various glycosyl hydrolases (carbohydrase, glycosidase), as a source of antibacterial compounds, and are used in the production of biological preparations for the plant protection from the diseases and for the forcing of the growth and yield capacity of plants.

The most widely used species in various branches of industry are the following: the species of *Trichoderma longibrachiatum* Rifai are used in the production of glycosyl hydrolases for industrial purposes; in the agricultural biotechnology in the aspects of protecting plants from phytopathogens the following species are used: *Trichoderma harzianum* Rifai (synonym – *Trichoderma inhamatum* Veerkamp & W. Gams); *Trichoderma virens* (Miller, Giddens & Foster) Arx; *Trichoderma asperellum* Samuels, Liechfeldt & Nirenberg; *Trichoderma koningii* Oudem; *Trichoderma lignorum* Harz (synonyms – *Trichoderma glaucum* E.V. Abbott; *Trichoderma strictipile* Bissett; *Trichoderma viride* Persoon).

As a result of long-term scientific work in the research and production association Biotechsoyuz, a number of strains of *Trichoderma longibrachiatum* have been obtained. The strains have biocontrol properties in respect of a set of phytopathogenic micromycetes, which are pathogens of mycoses of agricultural crops. The strains of *Trichoderma longibrachiatum* GF 2/6, J230 Brz and Za 3/19 GF are the basis of the biological preparation ТрихоPlant (ТрихоПлант®).

In the scientific literature, a number of types of interaction of micromycetes of the genus *Trichoderma* with the phytopathogenic micromycetes are described. In reference with the foregoing, the purpose of this work is the study of the interaction peculiarities of strains of *Trichoderma longibrachiatum* GF 2/6, J2 30 Brz and Za 3/19 GF with various phytopathogenic micromycetes.

The following methods are used in the research: method of parallel strokes (reseeding) and its modifications. The phytopathogens and the strains, which were studied, were incubated in the of potato dextrose agar medium. The types of interaction of phytopathogens and trichoderma strains were evaluated according to the classification adopted in the works of Belyayev V.B. et al. (patent 1671684 SU). As test objects, the cultures of phytopathogens have been used. These phytopathogens are pathogens of mycoses of agricultural crops from the own collection of phytopathogenic micromycetes, which were isolated from plants with sings of various diseases. In the experiment, the following has been used: *Passalora fulva* (Cooke) U. Braun & Crous, (2003), *Fusarium avenaceum* (Fr.) Sacc., *Fusarium solani* (Mart.) Sacc., *Fusarium moniliforme* J. Sheld., *Fusarium sporotrichioides* Sherb., *Thanatephorus cucumeris* (A.B. Frank) Donk., *Phytophthora infestans* (Mont.) de Bary, *Alternaria alternate* (Fr.) Keissl., *Aspergillus fumigatus*. Fresen., *Aspergillus flavus* Link., *Penicillium aurantiogriseum* Dierckx.

The results of conducted studies have shown that *Trichoderma longibrachiatum* GF 2/6, J2 30 Brz and Za 3/19 GF exhibit biocontrol properties in regard to the studied phytopathogens to different extents. The interaction type “*Trichoderma*-pathogen” is determined by the species affiliation of the phytopathogen and/or by the strain *Trichoderma*. In most cases the following interactions types have been distinguished: fungistatic antibiotal and territorial antagonism – *Trichoderma* constrains and suppresses the growth of phytopathogens mycelium (distinguished for *F. avenaceum*, *F. solani*, *F. sporotrichioides*, *Fus. moniliforme*, *Pass. fulva*, *Ph. infestans*,

*Th. cucumeris* and *A. Alternate*, *Asp. fumigatus*, *Asp. flavus*, *Pen. aurantiogriseum*), or direct parasitism (fungistatic alimentary antagonism) in regard to *Ph. infestans*, *Th. cucumeris*, *Pass. fulva*, *F. sporotrichioides*, *F. solani*, *F. avenaceum*, and *A. alternata*.

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\*Corresponding author: E-mail address: acid2302@mail.ru

## Особенности взаимодействия *Trichoderma longibrachiatum* с фитопатогенными микромицетами.

Сидякин А.И.

НПО Биотехсоюз, г. Москва

Крымский федеральный университет им. В.И. Вернадского, г. Симферополь

Грибы рода *Trichoderma* широко известны как широко применяемый в различных отраслях промышленности агент. Представители данного рода известны как продуценты различных гликозил-гидролаз (карбогидразы, гликозидазы), как источник антибактериальных соединений, используются в производстве биопрепаратов для защиты растений от болезней и стимуляции их роста и урожайности. Наиболее широко используемыми видами в различных отраслях промышленности являются следующие виды: при производстве гликозил-гидролаз производственного назначения используются виды *Trichoderma longibrachiatum* Rifai, а в сельскохозяйственной биотехнологии в части защиты растений от фитопатогенов используют виды: *Trichoderma harzianum* Rifai (синоним – *Trichoderma inhamatum* Veerkamp & W. Gams); *Trichoderma virens* (Miller, Giddens & Foster) Arx; *Trichoderma asperellum* Samuels, Liechfeldt & Nirenberg; *Trichoderma koningii* Oudem; *Trichoderma lignorum* Harz (синонимы – *Trichoderma glaucum* E.V. Abbott; *Trichoderma strictipile* Bissett; *Trichoderma viride* Persoon).

В результате многолетней научно-исследовательской работы в НПО Биотехсоюз были получены несколько штаммов *Trichoderma longibrachiatum*, обладающие биоконтролирующими свойствами в отношении ряда фитопатогенных микромицетов – возбудителей микозов сельскохозяйственных культур. Штаммы *Trichoderma longibrachiatum* GF 2/6, J2 30 Brz и Za 3/19 GF являются основой биопрепарата ТрихоПлант®.

В научной литературе описано несколько типов взаимодействия микромицетов рода *Trichoderma* с фитопатогенными микромицетами. В связи с вышесказанным, целью настоящей работы является исследование особенностей взаимодействия штаммов *Trichoderma longibrachiatum* GF 2/6, J2 30 Brz и Za 3/19 GF с различными фитопатогенными микромицетами.

В работе использовали следующие методы: метод параллельных штрихов (подсева); и его модификации. Культивировали фитопатогены и исследуемые штаммы на среде КГА. Типы взаимодействия фитопатогенов и штаммов триходермы оценивали по классификации принятой в работе Беляева В. Б и соавторов (Пат. 1671684 SU). В качестве тест-объектов использовали культуры фитопатогенов – возбудителей микозов сельскохозяйственных культур из собственной коллекции фитопатогенных микромицетов, выделенных от растений с признаками тех или иных заболеваний. В эксперименте использовали: *Passalora fulva* (Cooke) U. Braun & Crous, (2003), *Fusarium avenaceum* (Fr.) Sacc., *Fusarium solani* (Mart.) Sacc., *Fus. sporotrichioides* Sherb., *Fus. moniliforme* J. Sheld., *Thanatephorus cucumeris* (A.B. Frank) Donk., *Phytophthora infestans* (Mont.) de Bary, *Alternaria alternate* (Fr.) Keissl., *Aspergillus fumigatus*. Fresen., *Aspergillus flavus* Link., *Penicillium aurantiogriseum* Dierckx.

В результате исследований показано, что *Trichoderma longibrachiatum* GF 2/6, J2 30 Brz и Za 3/19 GF в различной степени проявляют биоконтролирующие свойства в отношении исследуемых фитопатогенов. Тип взаимодействия «триходерма-патоген» определяется видовой принадлежностью фитопатогена и/или штаммом *Trichoderma*. В большинстве случаев отмечены следующие типы взаимодействий: фунгистатический антибиотический и территориальный антагонизм – *Trichoderma* ограничивает и подавляет разрастание мицелия фитопатогенов (отмечено для *F. avenaceum*, *F. solani*, *F. sporotrichioides*, *Fus. moniliforme*, *Pass. fulva*, *Ph. infestans*, *Th. cucumeris* и *A. Alternate*, *Asp. fumigatus*, *Asp. flavus*, *Pen. aurantiogriseum*), или прямой паразитизм (фунгистатический алиментарный антагонизм) в отношении *Ph. infestans*, *Th. cucumeris*, *Pass. fulva*, *F. sporotrichioides*, *F. solani*, *F. avenaceum*, и *A. alternata*.

## Facile synthesis of chitosan/CuO nanocomposites and potential use as biocontrol agents

Punnama Siriphannon\*, Jidapa Kunarak, Salinta Phoaon, Sukanya Promarak

*Department of Chemistry, Faculty of Science,*

*King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand*

### Abstract

Various biopolymer-metal oxide nanocomposites are particularly promising for biomedical and biotechnological applications because they possess a great potential to inhibit microbial growth and biocompatible [1-2]. From this point of view, the chitosan based nanocomposites containing copper (II) oxide (CuO) nanocrystals have been developed in this research since the CuO is one of the most effective biocontrol agents in killing a wide range of bacterial pathogens [3-4]. The chitosan/CuO nanocomposites were synthesized by facile absorption and hydrothermal reaction. Firstly, the chitosan was dissolved in dilute acetic acid and then ionically crosslinked with 0.5% w/w sodium tripolyphosphate (STPP). The crosslinked chitosan particles were soaked in an aqueous solution containing 0.001, 0.01 or 0.1 mol/L CuSO<sub>4</sub> for 24 hrs, in which the Cu<sup>2+</sup> ions were absorbed into the chitosan network. The higher CuSO<sub>4</sub> concentration, the higher Cu<sup>2+</sup> uptake was obtained. The chitosan/Cu<sup>2+</sup> precursors were hydrothermally reacted in two different basic solutions, i.e. NaOH and NH<sub>4</sub>OH. The concentration of basic solution was 0.5 mol/L, and the reaction temperature was maintained at 100°C. The crosslinked chitosan network acted as the host template for hydrothermal growth of CuO nanocrystals in both basic solutions. The quantity of CuO nanocrystals increased with the increase of Cu<sup>2+</sup> uptake in the chitosan/Cu<sup>2+</sup> precursors. The chitosan/CuO nanocomposites synthesized by using 0.01 and 0.1 mol/L CuSO<sub>4</sub> could inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* when testing by JIS L 1902: 1998 standard method, indicating their potential use as biocontrol agents.

**Keywords:** Chitosan, CuO, Nanocomposite, Hydrothermal, Antibacterial activity

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\*Corresponding author: E-mail address: punnama.si@kmitl.ac.th, kspunnam@gmail.com

## **Biogas Production from Taro Waste of Bakery Factory under Anaerobic Condition**

Pramote Sirirote\*, Paveenuch Butnil, Lalittaphat Phitchawong, Saowapak Phoompoung and  
Dusanee Thanaboripat

*Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520,  
Thailand*

### **Abstract**

Biogas production from taro waste of bakery factory under anaerobic batch condition by using laboratory scale reactor was investigated. The several inoculum concentrations (from the effluent of UASB reactor of waste water treatment plant, President Bakery Company) at 0, 20, 30, 60, 90 and 100 % (v/v) were studied with working volume of 6 liters and 27 days of fermentation period. The result showed that the highest cumulative biogas production was 22.2 liters when using inoculum size at 30 % and efficiency for the COD removal was 45.2 %. After that, this level of inoculum size was used for further investigation to find out the amount of taro waste for the highest cumulative biogas yield. Several volume of taro waste were used at the level of 0, 1, 3, 5, 7, 10, 15, 20, 25 and 30 % respectively. It was found that the highest cumulative biogas production of 45.5 liters from taro waste at 30 % after 18 days of fermentation period and the efficiency for COD removal was 93.0 %.

**Keyword:** Biogas, Anaerobic digestion, Taro waste, waste treatment.

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\*Corresponding author: E-mail address: fscipms@ku.ac.th

## The influence of mycelium *Stagonospora cirsii* physiological state on its pathogenicity

S.V.Sokornova\*, G.M.Frolova, A.O.Berestetskiy

All Russian Institute of Plant Protection, St. Petersburg-Pushkin, Russia

**Keywords:** physiological state of mycelium, *Stagonospora cirsii*, the structural polar lipids, phytotoxic second metabolites

The Phoma-like fungus *Stagonospora cirsii* is being evaluated as a potential bioherbicide for control of perennial weeds Canada thistle. It is known that mycelial type of *S. cirsii* inoculum fits better to the environment than conidial one (Berestetskiy et al., 2005).

**Objective:** The purpose of this work is to understand the influence of mycelium *S. cirsii* physiological state parameters on its pathogenicity.

**Methods:** The strain of *S. cirsii* 1.41 VIZR was used. The infection mycelium was incubated on liquid media (soyabean meal 15 g/l or NaNO<sub>3</sub> 3.5 g/l, sucrose 30 g/l, KH<sub>2</sub>PO<sub>4</sub> 1 g/l, MgSO<sub>4</sub> 0,5 g/l) in 250-mL Erlenmeyer flasks (50 ml liquid media pH 6.0) for 3, 6 and 9 days.

The discs cut off leaves Canada thistle (d=10mm) were inoculated with 20 µl mycelium suspension (40 mg/ml). Size of necrotic lesions was measured respective to whole leaf disc area in 48h after inoculation (Berestetskiy et al., 2007).

Total lipid content was determined by modified Folch method (Folch et al., 1957). Separation of lipid classes was carried out by solid phase extraction of the lipid extract (Fuchsa et al., 2011).

Ethyl acetate extract from culture filtrates and its fractions obtained with preparative thin-layer chromatography were analyzed by high performance liquid chromatography.

Phytotoxic activity of crude extract and fractions was analyzed by leaf disc-puncture bioassay (Berestetskiy et al., 2010).

**Results:** The effect of the nitrogen source (soybean meal and sodium nitrate) and cultivating time on the aggressiveness mycelium was shown. Highly aggressive mycelium that kills leaf segments of the host plants was produced in the middle of exponential phase of growth (3-day cultivation on the medium based on soybean meal). This mycelium was characterized by relatively high content of structural polar lipids (26 % phospholipids and 10% glycolipids). Phytotoxic exo-metabolites were not observed.

Another peak of mycelium aggressiveness was obtained at the beginning of a stationary phase of growth (6-day cultivation). This mycelium was characterized by decrease level of the structural polar lipids (19 % phospholipids and 7 % glycolipids). In culture filtrate a significant quantity of phytotoxic secondary metabolites (in particular, herbarumin I) was found.

Phytotoxic activity of 0.25% crude extracts produced from 6 and 9 day-old culture filtrates of *S.cirsii* 1.41 was shown (development of necrotic lesions 75±5% of the total square of leaf discs). The predominant metabolite isolated from the crude extract ( $\lambda$  max 201 nm) had high phytotoxic activity at the concentration of 0.025% (w/v). The 9 day-old mycelium showed lower virulence.

The physiological state of *Phoma*-like fungus influences on its pathogenicity. We suppose that aggressiveness of young mycelium is defined by the high level of structural lipids. Further colonization of plant tissues during development of the disease might grow due to production of phytotoxic secondary metabolites.

This work is performed with financial support of RSF, a project № 16-16- 00085 "Development of technologies of production and application of mycoherbicides for control of troublesome weeds".



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\*Corresponding author: E-mail address: svokornova@vizr.spb.ru

# The production of 3-hydroxypropionic acid from glycerol by a new isolated PN3

Somphit Sornyotha

Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520,  
Thailand

## Abstract

3-Hydroxypropionic acid (3-HP) is an industrially important platform chemical and that can be produced from renewable glycerol by microbial biosynthesis. Its two functionalities, hydroxyl- and carboxyl groups, make it a suitable precursor for synthesizing many optically active substances. It is used as crosslinking agent for polymer coatings, metal lubricants, and antistatic agents for textiles. In addition, 3-HP is expected in food industry, cosmetics and fertilizers. For biosynthesis of 3-HP, this work was isolated and selected microorganisms with potential to produce 3-HP from glycerol. One hundred and ninety eight natural source samples were collected, cultivated and screened for this purposes. There are 26 isolates were able to grow and produce the 3-HP when cultivated on the mineral medium containing glycerol as the carbon source. One of them, the isolate PN3 was selected and identified because it gave the 3-HP concentration at  $2.484 \pm 0.1$  g/l which higher than the previous report from the wild type producers. The selected isolate was identified by sequencing of the 16S rRNA gene and shown closely related to *Bacillus anthracis* with 97% sequence similarity. However, it has been demonstrated that similarity of 16S rRNA of the individual strain with a nearest neighbor below 97% represented the new species and the meaning of similarity scores of 97% was not as clear. Therefore, the isolate PN3 was probably represented as a new species or alternatively. Interestingly, it is the first time, when the fermentation glycerol to 3-HP by *Bacillus* sp. was reported. Furthermore, the effect of medium on 3-HP production was also examined. The result showed that the increase in 3-HP production, which changed to  $4.204 \pm 0.2$  g/l from  $2.484 \pm 0.1$  g/l when the isolate PN3 was cultivated in the rich medium containing glycerol as a carbon source.

**Keywords:** *Bacillus* sp., Biosynthesis, Glycerol, 3-Hydroxypropionic acid, The isolate PN3

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\*Corresponding author: E-mail address: somphit.so@kmitl.ac.th

**Chemical composition and antifungal activity of essential oil from  
*Limnophila aromatic* (Lamk.) Merr. against aflatoxigenic *Aspergillus flavus*  
and *Aspergillus parasiticus***

\*Sukcharoen, O., \*\*Sirirote, P., and \*\*Thanaboripat, D.

\*Department of Biotechnology, Faculty of Science,  
Ramkhamhaeng University, Bangkok 10240, Thailand

\*\* Department of Biology, Faculty of Science,  
King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand

**Abstract**

The essential oil of the aerial parts of *Limnophila aromatic* (Lamk.) Merr. was obtained by hydrodistillation using a Clevenger-type system for 3 hr and investigated by gas chromatography-mass spectrometry. The main compounds were pulegone (22.50%) and isolimonene (19.06%) followed cyclohexane, 1-methylene-3-(1-methylethenyl) (12.22%) and 1,3,6-heptatriene,2,5,6-trimethyl (10.72%). The *in vitro* antifungal activities of essential oil was done by broth microdilution method and the poisoned food technique against *Aspergillus parasiticus* IMI 283883 and *Aspergillus flavus* IMI 242684. Aflatoxin B1 was determined using the Enzyme-linked immunosorbent assay (ELISA). The results showed that the *Limnophila aromatic* (Lamk.) Merr. essential oil had a good inhibitory effect on mycelial growth, sporulation and aflatoxin B1 production. Thus, the essential oil from *Limnophila aromatic* (Lamk.) Merr. can be used as food preservation systems to inhibit the mycelial growth and retard subsequent aflatoxin production.

**Keywords :** *Limnophila aromatic* (Lamk.) Merr, essential oil, aflatoxin, *Aspergillus flavus*, *Aspergillus parasiticus*

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\*Corresponding author: Tel: 662-3194385 Fax: 662-3108239 E-mail address: osukcharoen@hotmail.com (Sukcharoen, O)

**Possibility of using lichen as biological index to monitor  
ambient air quality at Suvarnabhumi Airport**

Glinsukol Suwannarat\*, Bongkoch Submek, Chutamas Homchoam,  
Kansinee Wongwiwat, Pratamaporn Homyok

*Department of Chemistry, Faculty of Science, King Mongkut's Institute of Technology  
Ladkrabang, Bangkok, Thailand*

**Abstract**

According to the study on the ambient air quality at Suvarnabhumi airport by Comparison with using lichen as biological index. The survey was conducted with samples of the lichen species around Suvarnabhumi airport 5 points from January to March 2016. Air samples were collected for determination of sulfur dioxide (SO<sub>2</sub>) and nitrogen dioxide (NO<sub>2</sub>) in the ambient air by using passive sampling. Passive sampling tubes were hung and leaved for 24 hours. Ion chromatography was used for SO<sub>2</sub> and NO<sub>2</sub> analyzes. The results found a total of 6 lichen families, 9 genera, and 9 species *Graphis* sp., *Lecanora* sp., *Trypethlium eluteriae*, *Arthonia* sp., *Pyxine cocoes*, *Amandinea extunata*, *Rinaria* sp., *Physcia dimidiata* and *Anthracotheccium eluteriae*. The major types were *Anthracotheccium eluteriae* which were high durability lichen. In January, Febuary and March 2016, SO<sub>2</sub> concentration in each were range 13.17-19.93 ppbv. NO<sub>2</sub> concentration in each area range 38.21-47.20 ppbv. The statistical analysis of the ANOVA found that content in each area did not significantly different ( $p>0.05$ ). For conclusion, that the ambient air quality around Suvannabhumi airport was fair. But the average concentration of SO<sub>2</sub> for 24 hours and the concentration of NO<sub>2</sub> were on the threshold of the air quality standard, which did not affect human health.

**Keywords:** Sulfur Dioxide , Nitrogen Dioxide , Lichen , Passive Sampler

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\*Corresponding author: E-mail address: glinsukol@gmail.com, glinsukol.su@kmitl.ac.th

# Effect of zinc oxide nanoparticles on *Cercospora* spp. causing leaf spot of some lettuce from hydroponics

Alongkorn NA TAKUATHUNG<sup>1</sup>, Sakrin BOONLUM<sup>2</sup> and

Pornprapa KONGTRAGOUL<sup>1\*</sup>

<sup>1</sup> Program in Horticulture, Department of Agricultural of Technology, King Mongkut's Institute of Technology Ladkrabang Prince of Chumphon Campus, Chumphon, Thailand

<sup>2</sup> Scientific Instruments Center, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand

## Abstract

Leaf spot symptom on butter head, green cos, frillice iceberg, green oak, and red coral were collected from hydroponics farm in Chumphon province, Thailand. Six isolates of *Cercospora* spp. were studied the antifungal activity of zinc oxide nanoparticles (ZnO-NPs) with size of 50±25 nm. and concentrations of 0, 0.5, 1 and 2 g/l. Poisoned food technique and slide culture technique were used to study antifungal activities of ZnO-NPs and to characterize the changes in morphology of fungal hyphae treated with ZnO-NPs. The result showed that mycelial inhibition percentage showed significantly differences between ZnO-NPs concentrations. It revealed that ZnO-NPs at 2 g/l were the most effective treatment. However, all ZnO nanoparticles treatment caused deformation in fungal hyphae.

**Keywords:** *Cercospora* spp., hydroponics, leaf spot disease, zinc oxide nanoparticle

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\*Corresponding author: E-mail address: kkpornpr@kmitl.ac.th

## Antioxidant activity and antibacterial activity against food pathogenic and spoilage bacteria by *Hibicus sabdariffa* L. (roselle) extract

Montinee Teerarak<sup>1\*</sup>, Chamroon Laosinwattana<sup>1</sup>, Patchanee Charoenying<sup>2</sup>,  
Pussadee Tangwatcharin<sup>3</sup> and Komkhae Pilasombut<sup>3</sup>

<sup>1</sup> Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand

<sup>2</sup> Department of Chemistry, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand

<sup>3</sup> Department of Animal Production Technology and Fisheries, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand

### Abstract

Recently, natural ingredients from herb or herbal extract have received increased attention as sources of natural antioxidants and food additives. In this study, the antioxidant activities of an ethanolic extract of *Hibicus sabdariffa* L. (roselle) calyx were screened for 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity, reducing power, anti-lipid peroxidation ability and total phenolic content. The results showed that the half maximal inhibitory concentration (IC<sub>50</sub>) values of (roselle) calyx extract were 932.25 mg/L for DPPH radical-scavenging activity and 217.07 mg/L for anti-lipid peroxidation. Moreover, reducing power ability of the extract with half maximal effective concentration (EC<sub>50</sub>) value was 937.20 mg/L. The total phenolic content of the extracts in terms of gallic acid equivalent was 675.80 mg/100g dry weight. The *in vitro* antibacterial activity using agar well diffusion technique was performed. The results showed that roselle extract at the highest concentration of 50 mg/ml revealed all antibacterial activity against all 19 strains of both food pathogenic and spoilage bacteria. Antibacterial activity of the lowest concentration at 12.50 mg/ml inhibited *Salmonella* Typhimurium TISTR 292, *Staphylococcus aureus* TISTR 118, *Aeromonas hydrophila* TISTR 1321 and *Bacillus subtilis* JCM 1465. The results of the present study revealed that ethanolic extract of roselle calyx is a source of natural antioxidants which have free radical scavenging activity, reducing power and inhibition of lipid peroxidation and also inhibit some food pathogenic and spoilage bacteria.

**Keywords:** roselle extract, pathogenic bacteria, spoilage bacteria, antioxidant activity, phenolic content

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\*Corresponding author: E-mail address: montinee.te@kmitl.ac.th

## Inhibitory Effect of Six Essential Oils on Bacterial Skin Disease

Dusanee Thanaboripat<sup>1</sup> and Sittichai Charoensettasilp<sup>2\*</sup>

<sup>1</sup>*Department of Biology and* <sup>2</sup> *Department of Statistics, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand 10520*

### Abstract

The efficacy of six types of essential oils, i.e. Leech lime, lime, clove, sweet basil, cinnamon and lemongrass to inhibit bacterial dermatitis (*Staphylococcus aureus* ATCC 6538 and *Staphylococcus epidermidis* ATCC 12228) in Tryptic soy agar (TSA) was tested by the disc diffusion method. The result showed that cinnamon essential oil gave the best inhibitory effect on *S. aureus* ATCC 6538 and *S. epidermidis* ATCC 12228. The minimum inhibitory concentration (MIC) value for both bacteria was found to be 0.32 mg/ml and the minimum bactericidal concentration (MBC) value was 0.65 mg/ml. It can be concluded that cinnamon essential oil was the most effective oil for inhibiting bacterial skin disease.

**Keywords:** Essential oils, skin disease, *Staphylococcus aureus*, *Staphylococcus epidermidis*

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\*Corresponding author: E-mail address: kcsittic@yahoo.com

# Isolation and characterization of antibiotic producing Actinomycetes from the Anadaman Sea of Thailand

Chitti Thawai<sup>1,2</sup>

<sup>1</sup>Department of Biology and <sup>2</sup>Actinobacterial Research Unit, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand 10520

## Abstract

One hundred actinomycete strains were isolated from marine samples collected from the Anadaman Sea of Thailand. These strains were grouped using phenotypic, chemotaxonomic and genotypic properties into 7 groups. Phylogenetic position, chemotaxonomic analyses including some phenotypic characterization exhibited that the representative strains in each group belonged to the members of the genera *Streptomyces*, *Nocardia*, *Micromonospora*, *Salinispora* and *Actinomadura*. Furthermore, we found the strain ST01-07<sup>T</sup> showing morphological and chemotaxonomic characteristics typical of members of the genus *Nocardia* but which was genotypically and phenotypically distinguishable from all recognized *Nocardia* species. Therefore, this strain should be judged as a novel species of the genus *Nocardia*. Furthermore, the fermentation broths of these representative strains were extracted with ethyl acetate and were investigated for anti-microbial activity. The results showed that more than 63% of actinomycete strains exhibited the anti-microbial activity. In addition, the crude extracts of the selected actinomycete strains were analyzed for the primary determination of chemical constituents using <sup>1</sup>H-NMR spectroscopy and revealed that the crude extracts of the isolates AM2-22 and AM4-7 showed the interesting signal patterns of the <sup>1</sup>H-NMR spectra. Based on these results, it could be concluded that actinomycete diversity in the marine environment is very great and should be represented an excellent source for the discovery of bioactive compounds.

**Keywords:** Actinomycetes, *Nocardia*, Antibiotic, Anadaman Sea.

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\*Corresponding author: E-mail address: chitti.th@kmitl.ac.th



# Enhancing phase image of human cancer cells using modified transport of intensity equation

Jakkapol Visessamit and Prathan Buranasiri\*

*Department of Physics, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang  
1 Chalongkrung Rd., Ladkrabang, Bangkok 10520, Thailand*

## Abstract

In this work, the phase images of human cancer have been shown by using a transport of intensity equation<sup>1,2</sup> (TIE). The compressive sensing method<sup>3</sup> has, also, been applied to our proposed numerical model of TIE to improve the quality of the images. The contrast between the images of normal cells and those of tumor cells used by our numerical model have been explored. Our future research plan also has been discussed.

**Keywords:** transport of intensity equation, compressive sensing, cancer, phase image

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\*Corresponding author: E-mail address: prathan.bu@kmitl.ac.th

## Isolation of *Pseudomonas* lipase producing strain from natural source use as biocatalyst for biodiesel production

Worakrit Worananthakij<sup>1,2\*</sup>, Chotinan Junpradit<sup>1</sup>, Patsaraporn Thooppeng<sup>1</sup> and  
Ratchada Changchai<sup>1</sup>

<sup>1</sup>*Department of Biology, Faculty of Science, King Mongkut's Institute of Technology  
Ladkrabang, Chalongkrung Rd., Ladkrabang district, Bangkok 10520, Thailand*

<sup>2</sup>*Bioenergy Research Unit, Faculty of Science, King Mongkut's Institute of Technology  
Ladkrabang, Chalongkrung Rd., Ladkrabang district, Bangkok 10520, Thailand*

### Abstract

Lipase from bacteria is one of important that will be used as biocatalyst for biodiesel production. Thus, this research aims to isolate and screen *Pseudomonas* sp. potential lipase producing from waste and natural waters. A total of 90 isolates were obtained by spread plate technique on nutrient agar from 3 sampling areas including Khlong Prawet Buri Rom, Ladkrabang district, Bangkok; Klong Saen Saeb, Bangkok district, Bangkok and waste water area at the canteen, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok. The results showed 25 isolates able to lipase producing during primary screening on selective medium MP-3. Based on the morphological and biochemical characteristics, there are 8 isolates were identified as *Pseudomonas* sp. The lipase activity was detected by titrate with standard sodium hydroxide solution, a highest lipase activity of 4.71 unit/mL produced by isolate KS3005. Biodiesel production using lipase as a catalyst transesterification with palm oil and methanol with 1:4 ratio for 72 hours, the quality of biodiesel were analyzed by thin-layer chromatography (TLC) compared with the standard biodiesel. The results showed that lipase could be as catalyst for the biodiesel production.

**Keywords:** Biodiesel, lipase, *Pseudomonas*, transesterification

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\*Corresponding author: E-mail address: worakrit.wo@kmitl.ac.th

## Isolation and characterization of fungal metabolites *Phoma* sp. 22.2

S. Zakharenkova, A. Dalinova, A. Berestetskiy

All-Russian Research Institute of Plant Protection, Saint-Petersburg, Russia

For decades, searches of environmentally friendly methods of plant protection are conducted, which would reduce the intensity of chemical pesticides use. An alternative to synthetic active ingredients are nature-derived pesticides - products based on natural biologically active substances and their derivatives. One of the main sources of new bioactive compounds are micromycetes. It is known that they produce substances with antimicrobial, phytotoxic, insecticidal, cytotoxic and the other types of activity.

In this connection, the aim was to obtain an extract from the surface culture *Phoma* sp. 22.2, to isolate and characterize biologically active substances of fungus.

Strain *Phoma* sp. 22.2 was isolated from diseased leaves of *Chenopodium urbicum* in the Orel region in Russia. Liquid-phase surface cultivation on the environment DMG (yeast maltose - glucose medium) was used to cultivate the strain within 3 weeks.

Fungal metabolites were extracted from the culture filtrate by sequential extraction with hexane, methylene chloride, ethyl acetate at a pH of 6. The obtained extracts were combined and fractionated by column reversed-phase liquid chromatography. For the final purification of individual compounds, method of preparative HPLC was used. Individual compounds were tested for antimicrobial, phytotoxic, insecticidal activity.

Five compounds (F1, CD1, J2, B4, E2) were isolated from an extract of the fungus *Phoma* sp. 22.2 with yields 0.2 - 2.83 mg / l of culture medium respectively. Obtained substances were inactive against *Bacillus subtilis*. Three substances (F1, CD1, J2) showed the phytotoxic activity at a concentration of 2 mg / ml against sowthistle (*Sonchus arvensis*) and couch grass (*Elytrigia repens*). Four substances (CD1, E2, F1, J2) showed the insecticidal activity at the concentration 1 mg/ml against wheat aphids.

It is known that *Chenopodium* has a phytopathogenic fungus *Phoma chenopodiicola*, which is presumed to be related to the object of study *Phoma* sp. 22.2. From the culture filtrate of the fungus *Phoma chenopodiicola* were extracted the following substances: Chenopodolans A - D, Chenopodolins A and B and Chenisocumarin. The range of secondary metabolites of this fungus is relatively well studied.

Using the methods of NMR spectroscopy and mass spectrometry of high resolution two isolated compounds identified as chenopodolin A (F1) and chenopodolan C (B4). The absence of phytotoxic activity in chenopodolan C (B4) is correlated with the literature.

Molecular weight and the absorption maxima in the UV spectrum of the substance E2 are closed to chenopodolan D data. Secondary metabolites are often produced as family of related compounds, so that E2 may be a structural isomer of chenopodolans.

Other of the bioactive compounds (J2, E2 and CD1) are assumed to be new.

In future work, we plan to identify the species of *Phoma* sp. 22.2 which can be a new producer of biologically active substances. The work was supported by the RNF (projects 14-27-00067, 16-16-00085).

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\*Corresponding author: E-mail address: prickgirl@list.ru, toxbiotech@vizr.spb.ru

# Biological fungicidal product's influence on morphological characteristics of *Fusarium* spp. causing potato dry rot

Zhilinskaia Nadezhda<sup>1\*</sup>, Bazarnova Julia<sup>1</sup>, Shleikin Aleksandr<sup>2</sup>

*1 Peter the Great St. Petersburg Polytechnic University, St. Petersburg, Russia*

*2 ITMO University, St. Petersburg, Russia*

## Abstract

Safety of food and agricultural products during production and turnover is a keystone in development of food market. *Fusarium* dry rot is a second plant disease after late blight. *Fusarium* belongs to the imperfect fungi class. Sporulation is the main way of fungal reproduction by macro- or microconidium which are varied in shape, size and structure.

Our work is to evaluate the possibility of using the method of computer morphometry in study of biological fungicidal commercial product “Alirin - B” (Russia) containing active substance of *Bacillus subtilis* B-10 VIZR on *Fusarium* spp. cells causing potato dry rot effect.

Samples of potato tubers with dry rot symptoms were collected and then dissected to obtain damaged surfaces for investigation. The epicenter of contaminated plant tissue was studied. The fungal mycelium was taken in 5 % glucose solution. Two types of samples were researched: glucose solution with fungal mycelium (control) and glucose solution with fungal mycelium and “Alirin - B” (experiment). The dynamics of “Alirin-B” influence on *Fusarium* macro- and microconidium were studied in 2 hours, 48 hours (2 days), 144 hours (6 days) after “Alirin-B” adding to the 5 % glucose solution with fungal mycelium.

Samples of *Fusarium* spp. cells were stained by 1 % methylene blue solution. Morphological analysis of fixed preparations of microorganisms was made by microscope “Nicon Eclipse Ni”. The species identification of *Fusarium* according to the morphological characteristics of their macro- and microconidia wasn't studied. Macroconidium and microconidium morphometric characteristics of *Fusarium* spp. (square, length, width, number) were obtained by automatic image analyzer “Nicon” with software program «Image J» under magnification x 400 or x1000. The account was carried out in 20 randomly selected view fields per 100 cells in each. Statistic evaluation was made by GraphPad Prism 6.

The results of computer morphometric measurements of the *Fusarium* macroconidium cells in the control samples were following. In 2 hours: the area size was  $32.58 \pm 4.24 \mu\text{m}^2$ ; the cell length –  $16.02 \pm 1.47 \mu\text{m}$ ; the cell width –  $1.99 \pm 0.13 \mu\text{m}$ , the amount of sprouted macroconidium was 0 %. In 48 hours: the area size was  $49.56 \pm 5.23 \mu\text{m}^2$ ; the cell length –  $18.64 \pm 1.42 \mu\text{m}$ ; the cell width –  $2.42 \pm 0.14 \mu\text{m}$ , the amount of sprouted macroconidium – 40 %. In 144 hours: the area size was  $74.35 \pm 10.46 \mu\text{m}^2$ ; the cell length –  $21.19 \pm 1.44 \mu\text{m}$ ; the cell width –  $3.51 \pm 0.28 \mu\text{m}$ , the amount of sprouted macroconidium – 43 %. The microconidium cells area size ( $\mu\text{m}^2$ ) was unchanged:  $3.81 \pm 0.26$  (2 h),  $3.89 \pm 0.23$  (48 h) and  $3.75 \pm 0.15$  (144 h).

The results of computer morphometric measurements of the *Fusarium* cells macroconidium in the experimental samples were following. In 2 hours: the area size was  $35.22 \pm 11.30 \mu\text{m}^2$ ; the cell length –  $16.75 \pm 2.96 \mu\text{m}$ ; the cell width –  $2.29 \pm 0.28 \mu\text{m}$ , the amount of sprouted macroconidium – 0 %. In 48 hours: the area size was  $58.23 \pm 13.44 \mu\text{m}^2$ ; the cell length –  $21.99 \pm 4.23 \mu\text{m}$ ; the cell width –  $2.84 \pm 0.17 \mu\text{m}$ , the amount of sprouted macroconidium – 43 %. The

microconidium cells area size ( $\mu\text{m}^2$ ) was:  $3.78 \pm 0.19$  (2 h),  $3.52 \pm 0.26$  (48 h). In 144 hours macroconidium and microconidium were not found.

The quantifiable data showed that “Alirin-B” especially effects on *Fusarium* microconidium: their number was decreased from 40 % (2 h) to 25 % (48 h) and to 0 % (144 h). The number of macroconidium was increased at first time from 14 % (2 h) to 74 % (48 h) and then decreased to 0 % (144 h).

Conclusion: in our experiments was found that “Alirin-B” obliterates macro- and microconidium and thus affects on *Fusarium* spp. reproduction system. The method of computer morphometry can be recommended as a testing system in development of new plant's biofungicidal products.

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\*Corresponding author: E-mail address: jilinskie@mail.ru

## Seminar on Dereplication Techniques using Databases

According to 'The Free Dictionary', the biological definition of "dereplication" is the process of testing samples of mixtures which are *active* in a screening process, so as to recognize and eliminate from consideration those active substances already studied. In contrast, chemistry is focussing not on activity, but on the chemical structure. The chemical technique of dereplication applies a dragnet search, where data of the compounds in question are compared in a computer-aided search with *published* compound properties compiled in a database.

In this seminar on dereplication, strategies for the structure elucidation of microbial metabolites will be discussed using examples from our own research. Participants are encouraged to bring own data (presumably spectra) from fungal or bacterial metabolites for dereplication with the database AntiBase.

Hartmut LAATSCH (born 1946) studied chemistry from 1968-71 at the Georg-August University in Göttingen (Germany) and got his doctoral degree 1973 as the last student of Hans Brockmann. After his habilitation (1981), he joined the group of Ronald H. Thomson in Aberdeen (Scotland) as a Visiting Scientist. He was appointed as University Professor in 1986 and was a delegate of chair at the University of Clausthal (1991-93) and Guest Professor in Vienna (1993) and Qingdao (China, 1999). He obtained Visiting Professor Awards at the Swinburne University in Melbourne (Australia 2008) and the Northwest A&F University in Yangling (China, 2012). Together with nearly 50 PhD, > 50 master students and >120 guest students and guest scientists from 32 countries, he published about 320 papers, 10 patents and 2 books. He was (and is) member of the editorial board of several journals (e.g. Editor in Chief of Marine Drugs 2009-2013). Laatsch retired 2011 and continues since then project research with selected postdocs and guest scientists. Author and editor of AntiBase, A Data Base for Rapid Structure Determination of Microbial Natural Products.



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Руководитель лаборатории - кандидат биологических наук, Берестецкий Александр Олегович (контакт: [toxbiotech@vizr.spb.ru](mailto:toxbiotech@vizr.spb.ru)).

Проводим исследования на договорной основе для сторонних организаций, а также персональное обучение. Если предложенная работа будет нам интересна, исследования могут быть выполнены безвозмездно (то есть, даром).

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**DEPARTMENT OF PHYTOTOXICOLOGY AND BIOTECHNOLOGY** of All-Russian Institute for Plant Protection is responsible for creating of the scientific base for development of biorational pesticides, including biopesticides derived from phytotoxins, inductors of immunity, biochemical pesticides, synthetic derivatives and analogues of nature bioactive compounds. The works of laboratory are supported by RSF (Russian Scientific Fund) and RFBR (Russian Foundation for Basic Research). The head of Department is Dr. Alexander Berestetskiy (contact e-mail: [toxbiotech@vizr.spb.ru](mailto:toxbiotech@vizr.spb.ru)).

## **AUTHOR INDEX**

Agansonova N. 76  
 Akepatcha T. 100  
 Akhanaev Y. 26  
 Anisimov A. 10-11, 35, 72, 74  
 Arfan A. 9  
 Arslanova L. 106-107  
 Bachurina G. 14  
 Bazarnova J. 131  
 Belozerskaya T. 14  
 Belyakova N. 15  
 Berestetskiy A. 17, 22, 45, 86, 119, 130  
 Bonartseva G. 106-107  
 Boonlum S. 78, 124  
 Budsamongkon C. 97  
 Bunchak A. 18-19  
 Buranasiri P. 98, 128  
 Burova T. 79-81  
 Butnil P. 118  
 Cao G. 68  
 Chaiyaraksa C. 83  
 Chamniprasertkul A. 84  
 Changchai R. 129  
 Chantawut P. 109  
 Charoensettasilp S. 126  
 Charoenying P. 85, 125  
 Chebotar V. 20-21  
 Choengchan N. 93  
 Choochote W. 84  
 Chotsaeng N. 85  
 Chuenjit L. 101  
 Cirilli M. 49  
 Cristofori V. 49  
 Dalinova A. 22, 130  
 De Pace C. 49  
 Detsri E. 27  
 Dobrohotov S. 72  
 Donchenko D. 53  
 Dubovskiy I. 26  
 Duskaev G. 88  
 Dyadyuchenko L. 52  
 Dzhililov F. 31  
 Dzhavakhiya V. 23-24, 106-107  
 Efremenko E. 39-40  
 Elisaphenko E. 92  
 Filippovich S. 14  
 Frolova G. 119  
 Fursov K. 72  
 Gasich E. 86  
 Gessler N. 14  
 Glupov V. 26, 92  
 Grizanova E. 26  
 Groza N. 14  
 Hasitapong P. 109  
 He L.  
 Hefferon K. 29  
 Homchoam C. 123  
 Homyok P. 123  
 Hurshkainen T. 30  
 Ignatov A. 31  
 Imjai A. 83  
 Jaipong T. 83  
 Janprapa P. 84  
 Janvikul W. 110  
 Jatupornpipat M. 87, 108  
 Jityasang S. 98  
 Junpradit C. 129  
 Junyapoon S. 111  
 Kamhom K. 27  
 Karandashov V. 31  
 Karimov I. 88  
 Karlov A. 31  
 Kartashov M. 36  
 Kasaikina O. 32-33, 69  
 Kasem A.E.S. 35, 74  
 Khlopunova L. 86  
 Khodykina M. 31  
 Khrameeva N. 69  
 Khunprama A. 87  
 Kittiwongwattana C. 46  
 Klanbut K. 89  
 Knignitskaya L. 90  
 Kongtragoul P. 78, 124  
 Kor-arnan T. 91  
 Kotlyarov D. 53  
 Kotlyarov V. 53

Kozlova E. 74  
 Krukov V. 26, 92  
 Kryukova N. 26  
 Kuksova E. 47-48  
 Kulikova O. 36  
 Kunarak J. 117  
 Kurbatova E. 57  
 Kutchin A. 30  
 Kuznetsova T. 94-95  
 Laatsch H. 37, 133  
 Laosinwattana C. 85, 125  
 Leelanut W. 101  
 Lertitthiporon A. 93  
 Li Y. 68  
 Limpai boon C. 109  
 Liu B. 28, 65  
 Luangsa-ard J.J. 51  
 Lyagin I. 39-40  
 Mahawiriyo G. 97  
 Malfanova N. 20-21  
 Maltsev D. 36  
 Maneeruttanarungroj C. 38, 67  
 Maslova O. 39-40  
 Mathaweensansurn A. 93  
 Melnik I. 41-42  
 Mikhina V. 94-95  
 Mikityuk O. 23-24  
 Miroshnikov K. 31  
 Mongkolsamrit S. 51  
 Monvisade P. 96, 109  
 Muleo R. 49  
 Nanasombat S. 97  
 Naranong N. 102  
 Nazarova T. 23-24  
 Neeyakorn W. 98  
 Numwong N. 99  
 Ochaikul D. 100, 110  
 Overchenko M. 57-58  
 Pakdee P. 91  
 Panya-ake R. 101  
 Pavlyushin V. 44  
 Phensaijai M. 102  
 Phitchawong L. 118  
 Phoaon S. 117  
 Phoompoung S. 118  
 Pilasombut K. 125  
 Pisarenko L. 32-33  
 Piyaphanuwat R. 67  
 Pogorzshelskaya N. 47-48  
 Polenogova O. 26  
 Politayeva N. 103-104  
 Poluektova E. 45  
 Pongsumpun P. 105  
 Pooissarakul W. 51  
 Poonpipattanakul P. 91  
 Popletayeva S. 23, 106-107  
 Poplevina V. 53  
 Pornprasit W. 98  
 Prokophyeva D. 22  
 Promarak S. 117  
 Promsut K. 108  
 Rachevskaya O. 79-81  
 Raweekul W. 46  
 Rimareva L. 47-48, 57-58  
 Rittiboon A. 87, 108  
 Ritvirulh C. 109  
 Rodnoi W. 111  
 Rotskaya U. 92  
 Ruggiero B. 49  
 Rugini E. 49  
 Rukchonlatee S. 110  
 Rungjindamai N. 51  
 Saeng-aroon W. 111  
 Samasil K. 38  
 Sanguanchaipaiwong V. 112  
 Sartongyae R. 84  
 Sawangpatthana W. 111  
 Sedinina N. 53  
 Seehabua P. 113  
 Sendetsky V. 41-42, 55  
 Senko O. 39-40  
 Serba E. 57

Shcherbakov A. 20-21  
 Shcherbakova E. 20-21  
 Shcherbakova L. 23-24  
 Shebyakovsky S. 52  
 Shirshikov F. 31  
 Shleikin A. 131  
 Sidyakin A. 114-116  
 Silvestri C. 49  
 Siriphannon P. 117  
 Sirirote P. 59, 78, 118, 122  
 Sodchuen W. 46  
 Sokornova S. 119  
 Song J. 63  
 Sooknoi T. 99, 113  
 Sornyotha S. 121  
 Sriwatcharakul S. 101  
 Statsyuk N. 23, 106-107  
 Stepanov N. 39-40  
 Suankaew C. 110  
 Submek B. 123  
 Sudachan L. 91  
 Sukcharoen O. 59, 122  
 Sun X. 61  
 Sunnerhagen P. 65  
 Suwannarat G. 123  
 Takuathung A. 124  
 Tamnao P. 83  
 Tangwatcharin P. 125  
 Taranovskaya E. 103-104  
 Teerarak M. 125  
 Thamsathit W. 78  
 Thanaboripat D. 59, 78, 118, 122, 126  
 Thawai C. 127  
 Thooppeng P. 129  
 Thummajinda N. 97  
 Ting A. 60  
 Tomilova O. 26  
 Treenate P. 96  
 Treesaksri S. 62  
 Vaganova O. 52  
 Vatanavicharn T. 93  
 Visessamit J. 128  
 Voinov E. 20-21  
 Voinova T. 23-24  
 Volkova G. 47-48, 52, 57-58  
 Volosatova N. 22  
 Wang Z. 65  
 Wongwiwat K. 123  
 Worananthakij W. 129  
 Wuttitummaporn S. 46  
 Xiong ML 63  
 Yacout M.M.A.F. 64  
 Yamskov I. 36  
 Yamskova V. 36  
 Yang Q. 28, 61, 66, 68  
 Yang X. 65  
 Yaroslavtseva O. 26, 92  
 Yodsang P. 38, 67  
 Zakharenkova S. 130  
 Zaplatkin A. 20, 21  
 Zernov A. 106-107  
 Zhang L. 68  
 Zhilinskaya N. 131  
 Zinatullina K. 69  
 Zinoviev I. 32-33

