MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
ODESA MECNIKOV NATIONAL UNIVERSITY

International conference of young scientists

MODERN PROBLEMS OF MICROBIOLOGY AND BIOTECHNOLOGY

Odesa, Ukraine
June 20-24, 2017

Odesa
ONU
2017

UDC [579+60] (063)
M 78

M 78 **Modern** problems of Microbiology and Biotechnology: materials of young scientists international conference 20-24 june 2017. / Ed. board : M. B. Galkin, G. V. Yamborko, N. V. Limanska, O. Yu. Zinchenko – Odesa : «Odesa I. I. Mecnikov National University», 2017. – 101 p.
ISBN 978-689-218-2

This collection contains the materials of the young scientists international conference «Modern problems of Microbiology and Biotechnology».

The materials reflect the content of the conference reports, which outline the results and prospects of research in various fields of microbiology and biotechnology, in which young scientists, PhD students, and students.

The authors of the abstracts are responsible fo r the accuracy of the materials and text.

UDC [579+60] (063)

ISBN 978-689-218-2

UDC 579.841.1: 576.5: 57.017.64:551.464.626:57.014

Balko O.I., Yaroshenko L.V., Balko O.B., Avdeeva L.V.

Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, 154, Acad. Zabolotny St., Kyiv, 03143, Ukraine
E-mail: oleksandrbalko@gmail.com

INTERACTION OF *PSEUDOMONAS AERUGINOSA* UCM B-900 BIOFILM FORMATION COMPONENTS DURING MICROBIAL PROTECTION

The interaction of *Pseudomonas aeruginosa* UCM B-900 biofilm formation components under the influence of unfavourable conditions has been investigated. As a model of unfavourable factor it was chosen excess concentrations of glucose in the growth medium. It was shown that bacterial cells maintained the ability to grow and form biofilm in minimal salt medium with 0.1-20% of glucose. The increase of glucose concentration caused reduction of the sample area covered by biofilm and quantity of microorganisms in its composition, but didn't influence on the bacterium titer in planktonic form. Hence it was assumed that P. aeruginosa biofilm performs not only protective function, but also does as deposit formation, which maintains stable cell quantity in planktonic form.

Key words: *Pseudomonas aeruginosa*, biofilm formation, cells in biofilm and planktonic form, concentration of carbon source.

It's well known that in the environment, microorganisms exist predominately in attached state in biofilm [1]. The formation of this bacterial community optimizes functions of microorganisms, improves cell to cell communication, and also increases the resistance to unfavorable factors of environment, thus providing advantages of collective existence [2]. Biofilm formation can be considered as a result of interaction of three structures: biofilm, cells in biofilm and planktonic form. These structures exist simultaneously, are components of unitary system, but peculiarities of their interaction in bacterial population in nature stand over.

Thus, the aim of our work was the investigation of cooperation between *Pseudomonas aeruginosa* biofilm, cells in biofilm and planktonic form for protection of microbial population under unfavourable conditions of environment.

Materials and methods

The investigations were conducted using collection strain *Pseudomonas aeruginosa* UCM B-900 (ATCC 9027) – kept in Ukrainian collection of microorganisms (UCM, Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine). As a model of unfavourable factor it was chosen concentration of sole carbon and energy source. For this, *P.aeruginosa* UCM B-900 was grown in synthetic Kozer medium with minimal (0,1%), overstated (5%) and redundant (20%) concentrations of glucose. Biofilm formation was investigated in a stationary system on glass at 37ºC during 7 days. Every day the quantities of *P. aeruginosa* cells in biofilm and planktonic form, as well as percentage of sample area covered by biofilm, were measured in samples [3].

Results and discussion

During the cultivation of *P. aeruginosa* UCM B-900 in Kozer medium with 0.1% glucose the stepped increase of percentage of sample area covered by biofilm was observed (Fig. 1A). The index peak (of 54 mm2) was reached on 4th day of cultivation. On 5-7 days the tendency to stepped decrease of this index was observed. The quantity of viable cells in biofilm composition increased to 1.4×10^6 CFU mL^{-1} at the 1st day of cultivation and after this remained in dynamic balance in the range from 2.1×10^5 to 3.0×10^6 CFU mL^{-1}. The titer of microorganisms in planktonic form was characterized by more stable values, since it varied in range of 1.3 - 5.2×10^7 CFU mL^{-1} during all observation period.

During the cultivation of *P. aeruginosa* UCM B-900 in Kozer medium with 5% of glucose the sample area covered by biofilm increased gradually and reached 32 mm^2 at the 3rd day of observation (Fig. 1B). Then a slow decrease of this parameter to 21 mm^2 was observed at 5th day with its repeated accumulation to 37 mm^2 at 7th day of observation. Thus, covering of samples by biofilm was more moderate and stable in comparison with biofilm formation in medium with 0,1% of glucose. At the same time the quantity of microorganisms in biofilm form was subjected to essential changes over the range 1×10^4 to 3.6×10^7 CFU mL^{-1}. The analogous fluctuations were observed in the quantity of microorganisms in planktonic form: from 7.8×10^8 CFU mL^{-1} at the 2nd day to $1,3\times10^7$ CFU mL^{-1} at 4th day. The repeated but less intensive increase of microorganism quantity in planktonic form was observed at 5-7 days.

During the cultivation of *P. aeruginosa* UCM B-900 in Kozer medium with 20% of glucose the sample area covered by biofilm increased gradually and at the 3rd day reached maximum results – 18.8 mm^2, then diminished to 6.5 mm^2 at the 7th day of cultivation (Fig. 1C).

Thus, the intensity of biofilm formation during cultivation in medium with 20% of glucose was the lowest. In biofilm composition the microorganism quantity reached its maximum - 9.8×10^4 CFU mL^{-1}on the 2nd day. Subsequently their titer decreased gradually to 5.0×10^3 CFU mL^{-1} at 7th day. The microorganisms in planktonic form during all experiment were maintained at a stable high level - 5.4×10^7 CFU mL^{-1}.

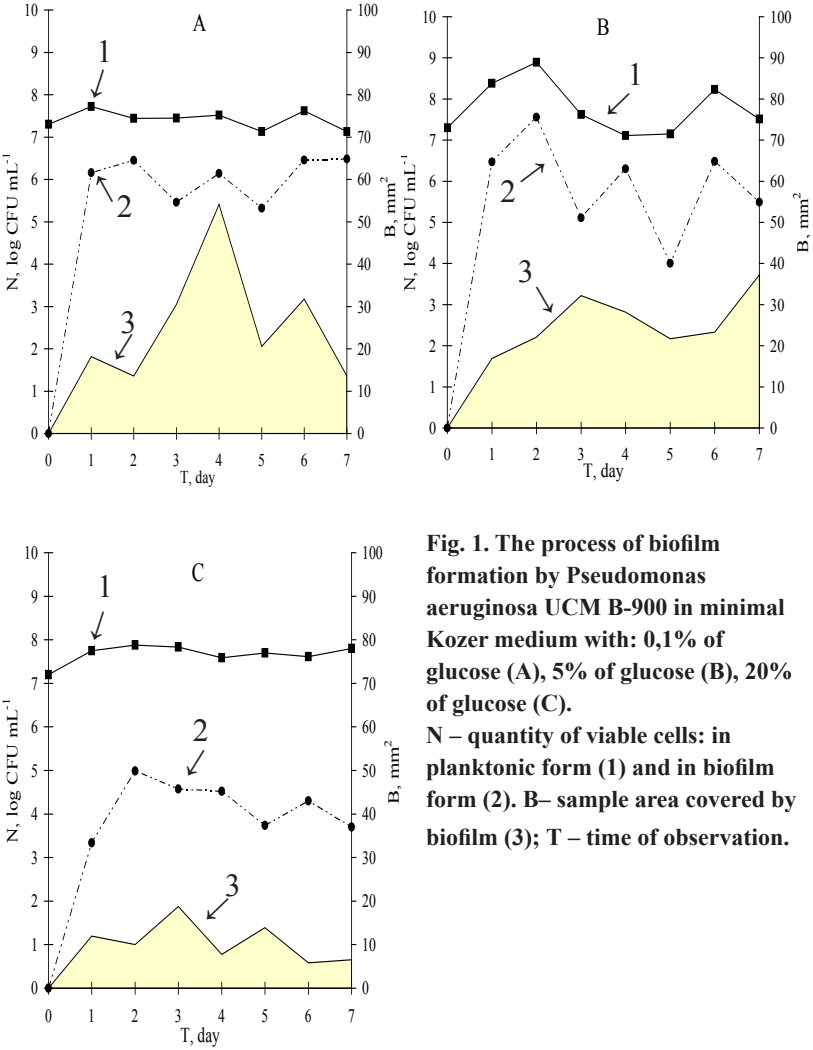

Fig. 1. The process of biofilm formation by Pseudomonas aeruginosa UCM B-900 in minimal Kozer medium with: 0,1% of glucose (A), 5% of glucose (B), 20% of glucose (C).
N – quantity of viable cells: in planktonic form (1) and in biofilm form (2). B– sample area covered by biofilm (3); T – time of observation.

The revealed regularities indicated that biofilm carried out protective function efficiently, since examined *P. aeruginosa* strain maintained the ability to grow even under the presence of 20% of glucose in the medium. It should be noted that with increase of glucose in medium the sample area covered by biofilm and the quantity of microorganisms in its composition decreased, whereas the titer of bacteria in planktonic form maintained at stable high level. Hence, it can be supposed that biofilm performs not only protective function, but also does as a deposit formation, creating pool of reserve cells. When it's necessary, microorganisms can pass from biofilm into planktonic form [4]. It could be supposed that existence of bacteria in planktonic form is more important for microbial population, since the maintenance of stable cell quantity in this form is realized even under unfavourable conditions.

Thus, the protective function of biofilm formation ensures the survival of investigated strain *P. aeruginosa* UCM B-900 in medium with 0,1-20% glucose. Under unfavourable conditions biofilm functions as a deposit formation. It stores cells and if it is necessary releases them to maintain the stable level of bacterial cells in planktonic form.

References

1. Nikolaev YA, Plakunov VK. Biofilm - "City of Microbes" or an analogue of multicellular organisms? Microbiol. 2007;(76):125–138.

2. López D, Vlamakis H, Kolter R. Biofilms. Cold Spring Harb Perspect Biol 2010;(2): a000398.

3. Pat. 89509 UA *MPK C12Q 1*/24. The method of estimation of biofilm forming intensity in microorganisms. Balko OI, Balko AB, Avdeeva LV. - Publ. 25.04.14, Byul. N 8. (In Ukrainian)

4. Vlamakis H, Aguilar C, Losick R, Kolter R. Control of cell fate by the formation of an architecturally complex bacterial community. Genes Dev. 2008;(22): 945–953.

UDC 632. 937

[1]Boiko M.V, [2]Vintskovs'ka Y.Y

[1]National University of Life and Environmental Sciences of Ukraine,
03041, Kyiv, 13, Heroi Oborony St., e-mail: maryaulina@gmail.com
[2]Institute of Horticulture, NAAS of Ukraine,
03027, Kyiv-27, 23, Sadova St., e-mail: yuliya_vinz@i.ua

ESTIMATION OF THE GENUS *BACILLUS* BACTERIAL STRAINS ANTAGONIST PROPERTIES

Abstract

The authors have investigated the antagonistic properties of the strains of the *g. Bacillus* (*B. thuringiensis* 87/3 – selected in vitro and *B. thuringiensis* 800– reference strain) concerning the test organism *Venturia* spp. (the apple scab causative agent). There are significant changes of the micromycetes morphogenesis under the influence of the strain *Bt 87/3*, in particular, expression of the lysis specific zones, changes of the density, thickness, the direction of the mycelium growth, and of the inhibition of the conidia germination within 86-93%. The inhibitory effect difference in the degree of the *B. thuringiensis* strains cultural liquid was shown on the test cultures *Fusarium spp.* ─ from 54.0 to 60.0%. The metabolites of spore-forming bacteria, besides the entomocidal activity against phytophages have appeared to be of considerable interest as for the microbial interaction and as the basis of the preparations of the multifunctional actions. Those metabolites present selective activity and do not brake the natural biocoenosis.

Key words: antagonistic activity, *Bacillus thuringiensis*, cultural liquid, microbial preparations, phytopathogenic organisms.

Introduction

Microbiological method of protecting plants using biotic origin components and metabolic preparations based on living cultures of microorganisms are important scientific and practical dimension of biotechnology in agricultural science. So finding and comprehensive studies of strains-producers as the basic components of multifunctional action biological preparations is extremely urgent issue today [8].

Each selected production strain of the genus *Bacillus* bacteria is distinguished by uniqueness and diversity of biochemical processes that occur in severity of their antimicrobial activity, in particularly

has expressed antagonistic properties on many pathogenic and opportunistic pathogenic microorganisms [2]. In addition, they are able to competitively colonize the rhizosphere and occupy the same ecological niche in plants that form phytopathogenic microorganisms. Thus, this bacteria is perspective agent in biocontrol of phytopathogens [1].

The most widespread and investigated representatives of the rhizosphere bacteria from g. *Bacillus (B. subtilis, B. mycoides* i *B. sphaericus)* show high antagonistic activity against microorganisms g. *Fusarium spp., Erwinia spp., Pseudomonas spp.* and others due to exometabolites different chemical nature [10]. Having a selective action entomopathogens *B. thuringiensis* ensure the active participation of other natural regulators to control the number of phytophages and causative agents of diseases and weeds, including *S. marcescens, P. fluorescens* and others [6,3]. Therefore, studying the g. *Bacillus* new strains multifunctional properties relevant to expanding the effective using of bio-agents in agricultural technologies.

Materials and methods

In this work we have used new entomopathogenic bacteria strain *B. thuringiensis var. thuringiensis (Bt H1) №87/3* selected in vitro, isolated from the larvae of leaf-eating insects natural populations of *Leptinotarsa decemlineata* Say. Bacterial strains and test cultures were cultivated in common nutrient media: peptone-glucose agar, Luria Bertani (LB), Czapek's agar [2,4]. Antifungal activity of *B. thuringiensis 87/3* strain was determined by using conventional diffusion methods (hole, blocks) [2,4], where as test culture were used micromycetes g. *Venturia ssp., Fusarium spp.,* isolated from infected apple varieties Ruddy mountain climber (IH plantations, NAAS of Ukraine). Inhibitory effect of bacterial culture metabolites was calculated using the formula (1):

$\frac{D_c - D_{exp}}{D_c}$ x100% (1), where D_c and D_{exp} – diameter of test culture accordingly in the control and experimental variant, cm.

Results and discussion

It is known that some *Bacillus* species produce in medium lytic enzymes of protease, chitinase types, which are able to lysis the micromycetes-pathogens cell walls. Scientists have been linked the antibiotic activity of *B. thuringiensis* with the crystal δ-endotoxin, produced by bacteria and conceive that this effect is associated with separation of target objects oxidative phosphorylation and respiration [9].

Laboratory model experiments showed that under the influence of *B. thuringiensis 87/3* strain spore-crystal complex were

significant morphogenesis test culture changes in all variants, especially the lysis specific zones expression, changing the density, thickness and direction of mycelium growth, and the inhibition degree of conidia germination within 86.0 - 93.0%. In the control (without introducing *B. thuringiensis* culture bacterial metabolites) average micromycetes diameter for 10 days was 2.1 cm compared to research variants, which observed mycelium growth less than 0.4 - 0.7 cm. The *B. thuringiensis 87/3* strain showed different technological effect and accumulation of biomass cultures in different nutrient media and titer of viable spores and crystals ─ from 2.0 to 3.6 billion spores / ml. Besides, synchronous sporogenesis observed in the yeast-polysaccharide medium. It was accompanied by the high output of crystal endotoxin than in conventional common media (2,0-2,4 billion / ml).

The inhibitory effect difference in the degree of the *B. thuringiensis* strains cultural liquid was shown on the test cultures *Fusarium spp.* ─ from 54.0 to 60.0%, with appropriate darkening of the mycelium. In control the average diameter of the test *Fusarium spp.* at 10 day was equal to 4.5 cm compared with experimental variants of the strains, which experienced growth of mycelium less than 1.8 cm. The combination of these experimental data shows a selective effect of bacteria *B. thuringiensis* metabolic complex as a multifunctional agent with the potential activity of enzymatic components.

Conclusions

Evaluation of antifungal properties of *B. thuringiensis 87/3* selected entomopathogenic strain in model experiments on pathogenic micromycetes showed that it can be successfully used in plant pathogens system control, in particular against pathogens of stone fruit crops scab and mixed infections.

References

1. Гадзало Я.М. Агробиология ризосферы растений: монография / [Я.М. Гадзало, Н.В. Патыка, А.С. Заришняк]. ─ К. : Аграрна наука, 2015. ─ 386 с.

2. Егоров Н.С. Основы учения об антибиотиках / Н.С. Егоров. — М.: Высш. шк., 1995. – 240 с.

3. Каленич Ф.С. Агроекологічні основи інтегрованого захисту яблуні від парші та інших хвороб / Ф.С. Каленич. – К.: Аграрна наука, 2005. – 244 с.

4. Лабинская С.А. Микробиология с техникой микробиологических исследований / С.А. Лабинская. – М. : Медицина, 1978. – 269 с.

5. Методы экспериментальной микологии /Под ред. В.И. Билай. – К. : Наукова думка, 1982. – 550 с.

6. Кандыбин Н.В. Микробиоконтроль численности насекомых и его доминанта *Bacillus thuringiensis*: монография / Н.В. Кандыбин, Т.И.Патыка, В.П. Ермолова, В.Ф. Патыка. – СПб, Пушкин: Научное издание «Инновационный центр защиты растений», 2009. – 252 с.

7. Смирнов В.В. Бактерии рода *Bacillus* – перспективный источник биологически активных веществ / В.В. Смирнов //Мікробіологічний журнал. – 2001. – Т. 63, № 1. – С. 72-79.

8. Chandler D. The development, regulation and use of biopesticides for integrated pest management / D. Chandler, Al.S. Bailey, G. M. Tatchell et al.// Phil. Trans. R. Soc. B. – 2011. – 366. – P. 1987-1998 (doi:10.1098/rstb.2010.0390).

9. Elkahoui S. Mass spectrometry identification of antifungal lipopeptides from *Bacillus* sp. BCLRB2 against *Rhizoctonia solani* and *Sclerotinia sclerotiorum* /S. Elkahoui, N. Djébalin, I. Karkouch et al.// Appl. Biochem. Microbiol. –2014. – 50(2). – P. 161–165 (doi: 10.1134/S0003683814020082).

10. Ennouri K. Improvement of the production of entomopathogenic proteases of *Bacillus thuringiensis* /K. Ennouri, R. Ben Ayed, H. Ben Hassen, H. Azzouz, M.A. Triki // Tunisian Journal of Plant Protection. – 2015. – 10. – P. 95-103.

УДК 57.576.08

Buziashvili A.Yu., Yemets A.I.

Institute of Food Biotechnology and Genomics NAS of Ukraine,
Ukraine, 04123, Kyiv, Osipovskogo str., 2A.
e-mail: buziashvili.an@gmail.com

ESTABLISHMENT *IN VITRO* CULTURE AND PLANTLET REGENERATION OF TOMATO CULTIVAR PERLYNA

In this work the optimal nutrition medium composition for cultivation *in vitro* of Ukrainian tomato variety Perlyna was determined. Also, the influence of different combinations of plant growth regulators in media on cotyledons, hypocotyls and internodes was identified to determine the most suitable conditions for high level of direct plant regeneration from these explants. It was found that the favourable nutrition medium for *in vitro* cultivation and for the obtaining the highest frequency (100%) of direct shoot regeneration on internodes was MS medium without addition of B5 vitamin and plant growth regulators.

Key words: *in vitro* culture, plant growth regulators, direct regeneration.

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important vegetable crops throughout the world. In Ukraine, tomato is one of the leading vegetable crops in terms of the volume of production. One of the most favourite amateur Ukrainian tomato varieties is Perlyna. However, this variety is susceptible to fungal and bacterial plant pathogens. Therefore, it is necessary to improve the resistance of this variety against pathogenic microorganisms, which could be achieved with the use of methods of genetic engineering only. The first step for genetical improvement is an elaboration the method for efficient in vitro cultivation and plant regeneration of Perlyna. Thus, the goal of this work was the study of influence of various nutrient compounds and phytohormones to identify the most suitable ones for micropropagation and direct plant regeneration from different Perlyna explants.

Materials and methods

Seed sterilisation was carried out for 2-3 min in 70% ethanol and for 15 min in 5% sodium hypochlorite (NaOCl). For seed germination and micropropagation of plants Murashige & Skoog (MS) medium

(4.3 g/l of micro- and macrosalts, 30 g/l of sucrose, 7 g/l of agar, pH 5.7) [1] and MS-based MST medium [2] supplemented with B5 [3] vitamins and 2 mg/l of glycine were used. To determine the optimal conditions for direct regeneration, 3 types of explants (cotyledons, hypocotyls and internodes) were cultivated on different types of nutrition media, which composition is listed in Table 1 [1, 4-6]. Effects of the influence of different combinations of plant growth regulators on the explants were observed during 6 weeks. The frequency of the effects was calculated as a ratio of the whole explant number towards the number of explants which showed some type of morphogenetical reaction.

Table 1

Composition of nutrition media used for determination of optimal conditions for direct plant regeneration from explants of tomato cultivar Perlyna

MST0	MS + B5 + glycine 2 mg/l
MST1	MST0 + Zt 1 mg/l+ IAA 1mg/l
MST2	MST0 + Zt 1 mg/l+ IAA 0.1 mg/l
MST3	MST0 + Zt 2 mg/l+ IAA 0.1 mg/l
MST4	MST0 + BAP 2 mg/l+ IAA 0.5 mg/l
MST5	MST0 + BAP 3 mg/l+ IAA 0.1 mg/l
MST6	MST0 + BAP 0.5 mg/l+ IAA 0.5 mg/l
MST7	MS + BAP 3 mg/l+ NAA 1 mg/l
MST8	MS + BAP 2 mg/l+ NAA 1.5 mg/l
MST9	MS + BAP 0.5 mg/l+ 2.4-D 4 mg/l
MST10	MS + BAP 2 mg/l+ NAA 0.2 mg/l

Results and discussion

Aseptic tomato seeds were germinated on MS and MST0 media. After 10 days, seedlings cultivated on MS medium were characterized by well developed bright green cotyledons (Fig. 1, e), while cotyledons of seedlings cultivated on MST0 medium were pale green and considerably smaller (Fig. 1, f). Micropropagation of tomato plants was also carried out on MS та MST0 media. Thus, on MST0 medium the shoots of plants were short, thin and fragile, leaves were also thin (Fig. 1, g); whereas the shoots of plants cultivated on MS medium were higher, leaves were bright green and well developed (Fig. 1, h).

Another Ukrainian tomato variety (Lahidnyi) was characterized by formation of viable plantlets and shoots on MST0 medium [2].

Optimal composition of nutrition medium for direct regeneration

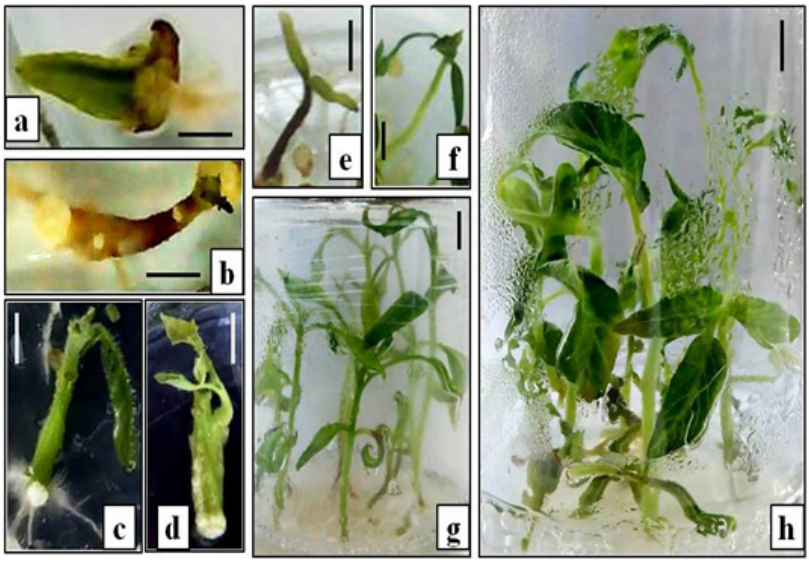

Fig. 1. Influence of different media composition on explants, plantlets and shoots of tomato cultivar Perlyna

a – cotyledon with callus and root cultivated for 4 weeks on MST8 medium; b – hypocotyl with callus, root and somatic embryo cultivated for 4 weeks on MST3 medium; c – internode with callus, roots and a shoot, directly regenerated from axillary bud, cultivated for 2 weeks on MS medium; d – internode with callus with shoot cultivated for 2 weeks on MST10 medium; e – 10 days old seedling on MST0 medium; f – 10 days old seedling on MS medium; g – plants cultivated on MST0 medium for 30 days; h – plants cultivated on MS medium for 30 days. Scale bar: 0.5 cm.

of tomato shoots was determined for 3 types of explants – cotyledons, hypocotyls and internodes. In many studies high frequency of tomato shoot regeneration *in vitro* (60-100%) is obtained using cotyledons as explants [2, 4-6]. In this work most of used cotyledons, cultivated on MST1-MST9 media, in four week of cultivation became brown and finally died (Table 2). Formation of callus was observed during 1-3 weeks of cultivation (Table 2).

Moreover, during 2-4 weeks of cultivation root formation (rhizogenesis) was observed on cotyledon explants (Table 2).

In other studies in order to induce rhizogenesis ½ MS medium without addition of growth regulators [6] was used, because endogenic auxins are enough for successful rooting of tomato plants *in vitro.*

Table 2

Frequency of effects of different combinations of plant growth regulators in composition of nutrition media on cotyledons of tomato cultivar Perlyna

Survival rate of cotyledons (%) on culture media of different composition											
cutl. time	**MST0**	**MST1**	**MST2**	**MST3**	**MST4**	**MST5**	**MST6**	**MS**	**MST7**	**MST8**	**MST9**
1 week	94.12	54.19	78.26	74.04	84.56	84.21	89.43	100	88.97	89.15	98.12
2 weeks	92.03	32.33	34.67	33.63	35.57	46.85	37.4	100	80.23	77.37	79.84
3 weeks	90.21	20.07	25.09	26.64	34.35	39.13	35.13	100	57.47	66.28	46.63
4 weeks	89.65	7.63	16.54	20.61	15.17	23.38	16.08	100	35.52	48.56	17.95
5 weeks	87.57	0.3	0.7	1.3	1.2	0.8	0.7	98.01	13.19	25.46	1.6
6 weeks	75.15	0	0	0.5	0.3	0	0	97.68	0.3	1.2	0
Frequency of root formation (%) on cotyledons											
cutl. time	**MST0**	**MST1**	**MST2**	**MST3**	**MST4**	**MST5**	**MST6**	**MS**	**MST7**	**MST8**	**MST9**
2 weeks	25.16	0	1.98	0	2.01	1.5	2.43	35.95	23.89	0	0
3 weeks	58.78	0	2.89	0	4.13	9.09	14.7	75.88	24.16	1.4	0
4 weeks	72.06	0	3.54	0	6.2	11.18	21.32	100	24.16	13.5	0

Continued table 2

Frequency of callus induction (%) on cotyledons											
cutl. time	**MST0**	**MST1**	**MST2**	**MST3**	**MST4**	**MST5**	**MST6**	**MS**	**MST7**	**MST8**	**MST9**
1 week	0	3	5.07	8.39	9.39	5.26	5.3	9.78	12.65	16.5	26.89
2 weeks	2.04	3.05	5.64	18.58	11.72	10.98	6.62	25.15	45.12	42.08	27.02
3 weeks	5.88	3.05	5.64	18.58	13.74	11.45	8.3	30.05	45.12	47.17	27.02

Table 3

Frequency of effects of different combinations of plant growth regulators in nutrition media on hypocotyls of tomato cultivar Perlyna in 6 weeks

Frequency of effects (%) of cultivation hypocotyls on different media											
	MST0	**MST1**	**MST2**	**MST3**	**MST4**	**MST5**	**MST6**	**MS**	**MST7**	**MST8**	**MST9**
white callus	30.95	48.27	71.67	77.78	56.89	47.37	74.57	35.85	38.16	51.72	33.14
brown callus	33.33	70.69	81.67	85.18	94.82	82.45	88.13	18.67	45.19	62.07	47.31
necrosis	0	15.52	11.67	1.85	0	0	0	0	3.35	0	6.78
embryogenesis	0	6.89	1.67	3.7	1.72	0	0	0	0	0	0
root formation	96.13	0	1.67	0	25.86	1.75	90.47	100	0	15.52	0

High level of regeneration *in vitro* in other studies was shown not only for cotyledons, but also for hypocotyls [1]. In this work somatic embryogenesis, which frequency on different culture media is noted in Table 3, was observed on hypocotyls in 6 weeks of cultivation (Fig. 1, b). Also, callus formation of much higher intensity than on cotyledons and slight level of explant death was noted (Table 2, Table 3). High level of rhizogenesis, which frequency was of similar values as on cotyledons, was observed on hypocotyls cultivated on MS, MST0 and MST6 media (Table 2, Table 3).

Furthermore, callus induction on hypocotyls was more intensive than on cotyledones (Table 2, Table 3). In different studies the values of callus induction intensity and morphogenetic potential of hypocotyls and cotyledons differs significantly [1,6].

Among all types of explants used in this research, only internodes showed direct regeneration (Рис. 1, c, d). The frequencies of regeneration, rhizogenesis and calligenesis on internodes of tomato Perlyna cultivar are listed in Table 4. There is only a few data on the use of internodes for direct regeneration of tomato *in vitro*. Thus, in [6] the 100% level of regeneration frequency was obtained on a medium of the similar composition to MST10.

Table 4

Frequency of effects of different combinations of plant growth regulators in media on internodes of tomato cultivar Perlyna

Regeneration frequency (%)			
cult. time	MS	MST	MST10
1 week	40	36	0
2 weeks	70	45	30
3 weeks	100	63	40
Callus induction frequency (%)			
cult. time	MS	MST	MST10
1 week	0	0	0
2 weeks	60	100	100
3 weeks	80	100	100
Rhizogenesis frequency (%)			
cult. time	MS	MST	MST10
1 week	0	0	0
2 weeks	80	0	0
3 weeks	80	63	0

Conclusions

In this work the optimum composition of nutrition medium for in vitro cultivation, micropropagation and direct regeneration of shoots of tomato Perlyna variety was identified. This medium is MS without addition of B5 vitamins [3] and plant growth regulators, since on this medium viable plantlets and shoots are formed, and direct regeneration frequency of internodes cultivated on this medium is up to 100%.

References

1. Murashige T., Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures // Physiol. Plant. – 1962 – V. 15. – P. 473–497.

2. Buziashvili A. Yu., Yemets A.I. The analysis of influence of different phytohormone concentrations on plantlets regeneration *in vitro* of valuable *Lycopersicon esculentum* varieties // Fact. of Exp. Evol. of the Org. – 2016. – Vol.19. – P. 88–91

3. Gamborg OL, Miller RA, Ojima K. Nutrient requirements of suspension cultures of soybean root cells // Exp. Cell Res. – 1968. – Vol.50. – P.151–158.

4. Lutfun N., Nasar A., Zinnah K. *In vitro* growth media effect for regeneration of tomato *(Lycopersicon esculentum)* and evaluationof the salt tolerance activity of callus // J. of Agricult. and Sust. – 2013. – Vol.3. – №2. – P.132–143.

5. Sohail A., Sabir S., Shaukat A., Ghulam M. The effect of plant growth regulators on callus induction and somatic embryogenesis of hybrid tomato // Pak. J. Bot. – 2015. – Vol.47. – №.5. – P.1671–1677.

6. Sherkar H., Chavan A. Studies on callus induction and shoot regeneration in tomato // Sci. Res. Rep. – 2014. – Vol.4. – №.1. – P.89–93.

UDC 57.08(075.8)

Nataliia Vasylieva, Gurgenidze Alexandra

Odessa I.I. Mechnikov National University,
2, Dvorianska str., Odesa, 65082, Ukraine,
e-mail: gyrgenidzealeksandra@gmail.com

PHYLOGENETIC ANALYSIS OF OUTER MEMBRANE PROTEIN FAPA PRESENT IN DIFFERENT BACTERIA

The microorganisms, fungi and plants have evolved strategies to scavenge and absorb iron from soil, fresh and marine water, and living organisms (plants and animals). One of the most common strategies for iron accumulation is siderophore production. Siderophores are low-molecular-weight compounds (500–1500 daltons) possessing a high affinity and selectivity for iron(III). The biosynthesis of siderophores is typically regulated by the iron levels of the environment where the organism is located.

A large proportion of bacterial siderophore uptake studies have been centered on enteric bacteria, as typified by *Escherichia coli* and *Salmonella typhimurium*.

Key words: siderophores, protein FepA, phylogenetic analysis.

The aim of the research was to compare the results of phylogenetic analysis of amino acid sequence of the outer membrane protein FapA in different bacteria. As is known from the publication "Chemistry and biology of siderophores" by Robert C. Hider and Xiaole Kong [1], the outer membrane proteins, FecA, FepA, FhuA and FpvA *(Pseudomonas sp.)* have been characterized by X-ray crystallography and shown to be homologous structures with molecular weights in the region of 80 000. It is also known different proteins are selective for particular iron complexes, for instance, FepA (ferric–enterobactin permease) is selective for iron–enterobactin. FepA is an 81,000-dalton *E. coli* outer membrane protein that functions in the initial step of iron uptake by binding ferrienterochelin. It is also known that this protein is involved in the transport of pyoverdine, *P. putida* W619 [2].

In this connection it was interesting to see the presence of this protein in other bacteria and to distinguish the phylogenetic connections between them.

Materials and methods

We used annotated in Uniprot database [http://www.uniprot.org/] to obtain amino acid sequences of the protein FepA [3]. There were analyzed 80 sequences. The sample was formed by ignoring repeated releases of the sequences and avoiding excessive inclusion of the same microorganisms.

For phylogenetic analysis, consistently used local programs ClustalW and MEGA6. Clustal X is a windows interface for the ClustalW multiple sequence alignment program. It provides an integrated environment for performing multiple sequence and profile alignments and analysing the results. MEGA6 automatically infers the evolutionary tree by the NeighborJoining (NJ) algorithm that uses a matrix of pairwise distances estimated under the Jones–Thornton–Taylor (JTT) model for amino acid sequences [http://www.megasoftware.net/].

Results

As can be seen from Figure 1, 80 selected protein sequences can be grouped into several clusters, each of which is highly likely to form an internal node.

Cluster 1 have been combined *gammaProteobacteria* represented by *Enterobacteriaceae* family. Basically, this are gram-negative bacteria related to the genera *Citrobacter* and *Salmonella.*

In the Cluster 2 were also grouped *gammaProteobacteria* represented by *Enterobacteriaceae* family. However, in this case, most of the microorganisms in this cluster were representatives of the *Shigella* and *Escherichia* genera.

In the Cluster 3 have been combined gram-negative bacteria related to the genera *Enterobacter* and *Klebsiella.*

The most diverse in composition was Cluster 4, which we conditionally divided into 4 subclusters (Fig. 1). The probability of forming an internal node was 82%, which indicates a correct topology of the winds and nodes of the phylogenetic tree. Within the cluster, the probability for internal nodes was smaller and ranged from 50 to 12 %% (Fig, 1).

The combination of microorganisms in Cluster 4 are interesting. So in subcluster 4.1, have been combined according to the results of phylogenetic reconstruction, representatives of genera *Agrobacterim, Candidatus, Pseudoalteromonas, Pseudomonas, Kangiella, Arenibacter, Janthinobacterium.*

Subcluster 4.2 was mainly composed of representatives of genera *Bordetella, Xanthomonas, Pectobacteriom.*

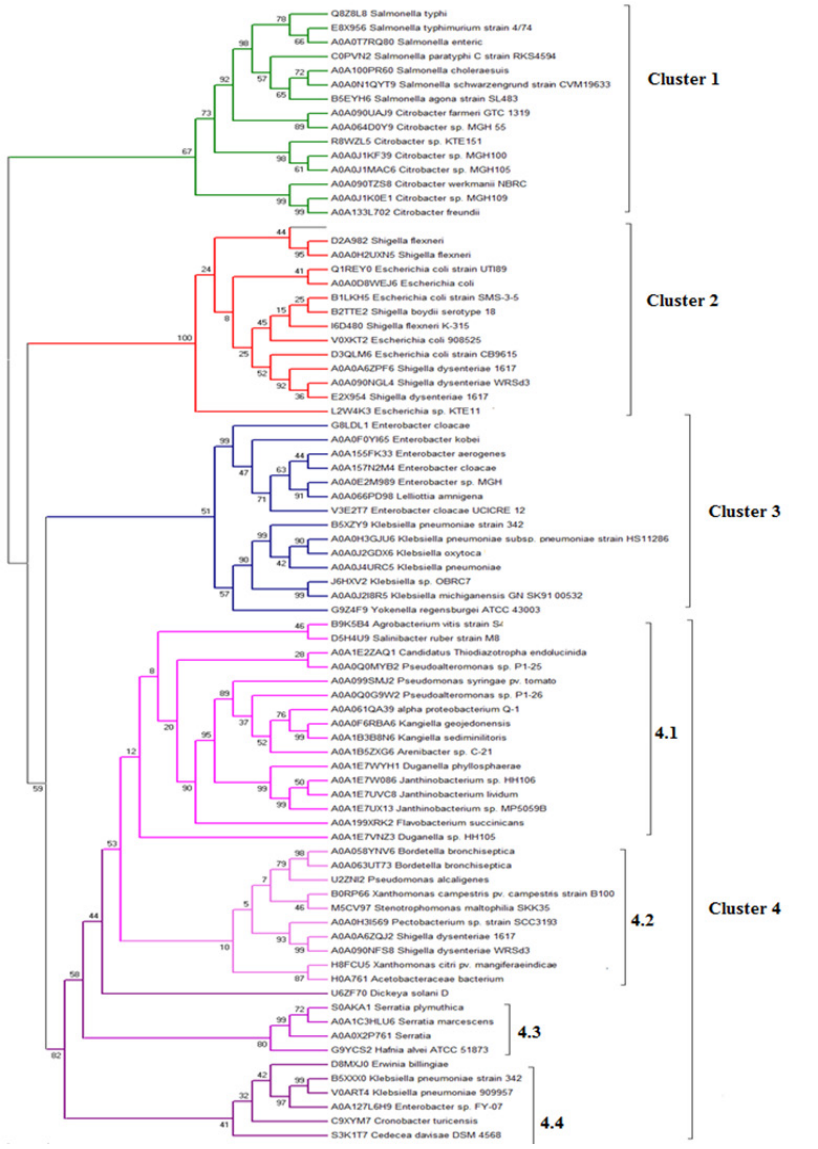

Fig. 1. Phylogenetic tree of the 80 amino acid sequence of protein FepA that been received from Uniprot database. The tree shown was obtained by NJ using Jones–Thornton–Taylor (JTT) model. Symbols on branches show bootstrap values with both the NJ (1,000 replicates)

Rarely mentioned genus *Serratia* and *Hafnia* was assigned to subcluster 4.3. They both belong to the *Enterobacteriaceae* family, and are gram-negative, facultatively anaerobic rod-shaped bacteria.

The biggest doubt was caused by subcluster 4.4, which include representatives of the genera *Enterobacter* and *Klebsiella* on a par with the representative of the genus *Cronobacter.* Previously, most of the amino acid sequences of the protein FepA defined for representatives of these species were assigned to Cluster 3, with the probability of forming an internal node of 53 % (Figure 1).

Based on the results of the study, it can be said that the amino acid sequence of the FepA protein for most microorganisms is indeed highly homologous. The presence of this protein is most famously characteristic of microorganisms belonging to the *Enterobacteriaceae* family.

References

1. *Robert C. Hide, Xiaole Kong* Chemistry and biology of siderophores // Natural Product Reports. – 2010. – Vol. 27. – P. 637– 657.

2. *Isabelle J. Schalk,, Pavel Kyslik, Danielle Prom.* Copurification of the FpvA Ferric Pyoverdin Receptor of *Pseudomonas aeruginosa* with Its Iron-Free Ligand: Implications for Siderophore-Mediated Iron Transport//Biochemistry. – 1999. – Vol. 38, No. 29. – P. 5639 – 9365.

3. Uniprot database [Електронний ресурс]. – Режим доступу: http://www.uniprot.org/

UDK 579.26:635.1/8-035.2

[1]Yamborko G.V., [1]Marinova I.V., [1]Vatipko R.A., [1]Babynina M.O., [2]Pylypenko L.N., [2]Pylypenko I.V., [2]Closhka N.V.

[1]Odessa I. I. Mechnikov National University,
Dvoryanskaya st., 2, Odesa, 65082, Ukraine, e-mail: jamborkoann@ukr.net
[2]Odessa National Academy of Food Technologies,
Kanatnaya st., 112, Odesa, 65039, Ukraine

ENTEROTOXIN PRODUCTION ABILITY OF *BACILLUS CEREUS* STRAINS FROM SOUTH UKRAINIAN REGION

The objective of this study was to identify and detect emetic toxin- and enterotoxin-producting bacteria among 42 *Bacillus cereus* strains, isolated from Ukrainian food plant raw materials and products. The detection rate of nheA, hblD and cytK enterotoxin genes among investigated *B. cereus* strains was 100, 90,0 and 61,9%, respectively. The ces gene encoding emetic toxin was detected in 9,5 % of strains. Our finding revealed that nhe and hbl enterotoxins encoded by nhe and hbl genes were the major toxins among *B. cereus* tested in this study and enterotoxic type of *B. cereus* was predominant in South Ukrainian region.

Key words: emetic toxin- and enterotoxin-producting *Bacillus cereus.*

Food poisoning caused by the presence of *Bacillus cereus* in foodstuffs is recorded in almost all countries. *Bacillus cereus*, a rod shapes, gram-positive, spore-forming food pathogen, play an important role as the causative agent of diarrheal and emetic types of food poisoning [1]. The diarrheal type of food poisoning is caused by heat-labile enterotoxins such as hemolysin BL (hbl), nonhemolytic entrotoxin (nhe) and cytotoxin K (cyt K). The hbl- and nhe-complex both consist of three proteins (tripatite toxins). Cytotoxin K is a pore forming toxin cause necrotic enterotitis.

The diarrheal syndrome, including abdominal pain and diarrheal symptoms, appears 8 to 16 h after ingestion of contaminated food. The emetic syndrome, which is characterized by nausea and vomiting within 1 to 5 h after ingestion of contaminated food, is causes by emetic toxin cereulide, a depsipeptide structurally related to potassium ionophore valinomycin, which is produced by a nonribosomal peptide synthetase (NRPS) and coded ces gene [2].

The objective of this study was to identify and detect enterotoxin-producting bacteria among *Bacillus cereus* strains, isolated from Ukrainian food plant raw materials and products.

Materials and Methods

The widespread and industrially grown kinds of vegetables, fruits, berries, in particular, green peas, beetroot, tomatoes, carrots, apples, pears, plums, peaches, dill, spinach, parsley, strawberry, a number of canned and dried products, and also spices have been investigated [3]. Samples of tested materials were selected according to standardized selection rules for the average sample [4, 5].

The reference strain *B. cereus* ATCC 11778 and 42 bacilli strains isolated from food plant raw materials and products, and according to the results of previous studies, identified as *B. cereus* by studying their physiological and biochemical characteristics and fatty acid composition of cells [6].

Multiplex PCR was performed using specific primers to bacilli sequences according to Zhang et al. [7]. DNA was isolated from the samples using the SureFast® PREP Bacteria F1021 (CONGEN, Germany). The following 4 pairs of specific oligonucleotide primers for the toxicity genes were used (Table 1).

Table 1

PCR primers used in the study

Target toxin gene	Sequence (5′–3′)	Amplicon size (bp)
nheA	GTTAGGATCACAATCACCGC	617
	ACGAATGTAATTTGAGTCGC	
hblD	ACCGGTAACACTATTCATGC	465
	GAGTCCATATGCTTAGATGC	
cytK	GTAACTTTCATTGATGATCC	800
	GAATACTAAATAATTGGTTTCC	
cesB	ACCCATCTTGCGTCATT	154
	CAGCCAAGTGAAGAATACC	

PCR cycles are are primary denaturation at 95° C for 10 min, 38 cycles of denaturation at 95 ° C for 1 min, annealing at 5,2° C for 1 min, elongation at 72° C for 1 min, final elongation at 72° C for 10 min (Thermal cycler with BioRad software, USA). Primers were chosen on the basis of literature data [7, 8] and synthesized by SPC «Simesta VAAL» (Odessa, Ukraine).

As a negative PCR control, deionized water was used to control the purity of the reagents. A visual evaluation of the size of the formed amplicons was carried out using molecular weight markers.

Results and discussion

The detection rate of nheA, hblD and cytK enterotoxin genes among investigated *B. cereus* strains was 100, 83,3 and 61,9%, respectively. The ces gene encoding emetic toxin was detected in 9,5 % of strains (Table 2).

Table 2

Distribution of enterotoxin genes in Bacillus cereus strains from different sources of south Ukrainian region

Toxin gene	Bacillus cereus strains with enterotoxin genes (n=42)				
	Vegetables, n=14	Fruits, n=8	Canned products, n=8	Dried products, n=6	Total,%
nheA	14	8	8	6	100
hblD	12	7	8	8	83,3
cytK	12	4	9	1	61,9
cesB	3	1	-	-	9,5

The results suggest that the examined canned and dried products were free of the emetic toxin but not free of enterotoxins and the distribution of enterotoxigenic genes was significantly different among the B. cereus isolates from various sources.

All investigated strains of *B. cereus* were divided into 5 groups according to the presence or absence of enterotoxic genes (Table 3).

Table 3

Enterotoxin genes profiles in Bacillus cereus strains from different sources of south Ukrainian region

Group	nheA	hblD	cytK	cesB	No, (%) of strains (n=42)
I	+	+	+	+	2 (4,7%)
II	+	+	+	-	7 (16,6%)
III	+	+	-	-	9 (21,4%)
IV	+	-	+	-	8 (19,0%)
V	+	-	-	-	16 (38,1%)

Only 2 strains from group I (4,7%) have to ability to cause both diarrheal and emetic type of food poisoning. Group II (7 strains, 16,6%) contained the nheA, hblD and cytK enterotoxin genes, but no cesB encoded emetic toxin.

Group V was the major patterns and represented 38,1% strains. The reference strain *B. cereus* ATCC 11778 has all the tested genes of toxicity.

These finding revealed that nhe and hbl enterotoxins encoded by nheA and hblD genes were the major toxins among *B. cereus* investigated in this study and enterotoxic type of *B. cereus* was predominant in South Ukrainian region.

Our research of contamination of enterotoxin-producting strains *Bacillus cereus* raw materials from Ukrainian region are original, although these results are good agreement with food products investigation from Mexican, Dutch and Korean regions [1, 2, 8].

References

1. *Biesta-Peters E.G., Dissel S., Reij M.W., Zwietering M. H., Paul H.* Characterization and Exposure Assessment of Emetic *Bacillus cereus* and Cereulide Production in Food Products on the Dutch Market // Journal of Food Protection. –2016. – V. 79. – № 2. – P. 230-238.

2. *Flores-Urbá K., Natividad-Bonifaci I., Vázquez-Quiñone, C.,Vázquez-Sali, C., Quiñones-Ramíre E.* Detection of Toxigenic *Bacillus cereus* Strains Isolated from Vegetables in Mexico City // Journal of Food Protection. – 2014. – V. 77. – Issue 12. – P. 21-44.

3. *Пилипенко І.В., Пауліна Я.Б., Пилипенко Л.М., Ямборко Г.В.* Склад мікробних контамінантів овочевої сировини // Мікробіологія і біотехнологія. – 2015. - № 3 (31). – С. 83 – 95.

4. *Пилипенко Ю.Д., Мазуренко І.К., Пилипенко І.В., Пилипенко Л.М.* Державні нормативні документи на сировину, напівфабрикати, матеріали та консервовану продукцію. Показники безпечності та якості (Методичні вказівки. Видання офіційне). – К.: Мінагрополітики, 2009. – 114 с.

5. *Harley J.P., Prescott L.M.* Laboratory Exercises in Microbiology, Fifth ed. – N.Y.: The McGraw Hill Companies, 2002. – 466 p.

6. *Ямборко Г. В., Остапчук А. М., Сергєєва Ж. Ю., Пилипенко Л. М., Пилипенко І. В.* Хемотаксономічні особливості та плазмідні профілі аеробних та факультативно-анаеробних спороутворювальних бактерій з овочевої продукції // Мікробіологія і біотехнологія. - 2017. - № 1 (37). - С. 56-72.

7. *Zhang Z., Feng L., Xu H., Liu C. et al.* Detection of viable enterotoxin-producing *Bacillus cereus* and analysis of toxigenecity from ready-to-eat foods and infant formula milk powder by multiplex PCR // J. Dairy Sci.- 2015. – V. 99.- P. 1-9.

8. *Kim J.B., Kim J.M., Kim S.H. et al.* Emetic toxin producing B. cereus Korean isolates contains genes encoding diarrhea related enterotoxins // International Journal of Food Microbiol.- 2010. V. 144. – P. 182-185.

UDC 579.262:616.6-008.87-053.2

Vodianyk, A.A.[1], Grechukha, Y.O.[1], Druzenko, M.O.[1], Kornijko, Y.Y.[1], Ponyatovskiy, V.A.[1],Gniloskurenko, A.V.[1], Lavrenchuk, O.V.[2], Bagdasarova, I.V.[2], Rudenko, A.V.[3], Mitiuriaeva-Kornijko, I.O.[1]

[1]Bogomolets National Medical University,
13 Tarasa Shevchenka Blvd, Kyiv 01601, Ukraine. E-mail: nmu@ nmu.edu.ua
[2]SI Institute of Nephrology, National Academy of Medical Sciences of Ukraine,
17-B Dehtiarivska Str., Kyiv 04050, Ukraine. E-mail: director@inephrology.kiev.ua
[3]SI Institute of Urology, National Academy of Medical Sciences of Ukraine,
9-A Vynnychenka Str., Kyiv 04053, Ukraine. E-mail: amnurol@ukr.net

BACTERIA IN THE CROSSHAIRS: BIOFILM FORMATION, ADHESION AND MOTILITY OF ISOLATED PATHOGENS IN CHILDREN WITH URINARY TRACT INFECTION

Abstract

It is the purpose of this research to determine the intensity of biofilm formation, adhesion and motility of bacteria depending on different types of children's urinary tract infections.

The paper contains the research of adhesion, motility and biofilm formation properties of 44 clinical isolates of bacteria from children with lower urinary tract infections, acute and chronic pyelonephritis.

The ability of bacteria to form biofilms was tested using the method of microtiter plates. The ability of bacteria to adhere was investigated according to Brilis, through the use of formalinized erythrocytes.

Differences were found in the ability to form biofilm between strains of bacteria that cause infections of the lower urinary tract (optical density 0.40 ± 0.06) and chronic pyelonephritis (optical density 0.48 ±0.07) ($p<0.05$). Furthermore, a correlation was established between adhesion and biofilm formation abilities of bacteria ($r = 0.529$ ($p<0.01$)).

The bacteria isolated from children with acute and chronic pyelonephritis showed higher ability to adhere and form biofilm than the bacteria isolated from children with lower urinary tract infection.

High ability to form biofilm and adhere of the isolated bacteria can be considered an adverse predictive factor in the course of urinary tract infection.

Key words: Biofilm, adhesion, urinary tract infection.

Introduction

In recent years, results from scientific studies have indicated the role biofilms play in the occurrence and development of up to 65% of nosocomial infectious diseases [4] and over 80% of all the infections of bacterial origin [5]. Urinary tract infections (UTI) are among the most common infections that annually affect 150 million people worldwide [2]. UTI rank second among all pediatric infections, being outranked only by respiratory tract diseases.

Currently there are a limited number of publications on interrelation between different types of motility, biofilm formation and adhesive activity of bacteria, and the impact caused by these pathogenicity factors on the course of UTI.

As there are differences in severity degree of adhesion and biofilm formation factors between children and adults [6], the purpose of this research was to determine the intensity of biofilm formation, adhesion and motility of bacteria depending on different types of UTI in children.

Materials and Methods

The research was held at the Department of Pediatrics No. 4 and the Department of Microbiology, Virology and Immunology of Bogomolets National Medical University. In the course of the research, the analysis was conducted of the biological properties of 44 strains of bacteria isolated from 56 patients that were under medical treatment in children's clinical hospitals No. 6 and No. 7 of the city of Kyiv within the period from September 2016 until January 2017 with such diagnosis as lower urinary tract infection (19 strains), acute pyelonephritis (12 strains), chronic pyelonephritis (13 strains).

A bacteriologic study with an extended antibiogram of the urine samples of all patients was conducted at the Laboratory of Microbiology, Virology and Mycology of the State Institution "Institute of Urology of National Academy of Medical Sciences of Ukraine".

Biofilm Formation Study

The ability of bacteria to form biofilms was tested using the method of 96-well microtiter plates [3]. A biofilm was considered formed if optical density index was higher than the optical density of negative control plus 3 standard deviations (Std. dev.) of negative control. Thus:

1. No biofilm: OD < OD of negative control (0.34) + Std. dev. of negative control (0.02); OD < 0.4;
2. Weak biofilm 0.4 < OD < 0.46;
3. Medium biofilm 0.46 < OD < 0.52;
4. Strong biofilm OD > 0.52.

Determination of Adhesion

Adhesion of bacteria was tested through the use of formalinized erythrocytes [1]. We used formalinized erythrocyte concentrate of a healthy donor with third rhesus-positive blood group (B(III)Rh+).

The microorganisms were considered nonadhesive if microorganism adhesion index was less than two, moderately adhesive at IAM value from 2 to 4, and highly adhesive at IAM higher than 4.

Determination of Motility

Definition of different types of motility was carried out by standard methods [7].

Analysis of the results was performed using the SPSS program version 12. The results were considered statistically significant at $p<0.05$.

Results

Out of 29 patients of the first group, bacteriuria of more than 103 CFU/ml was found in 12 (41%) and 19 clinical strains of bacteria were isolated. Out of 14 patients of the 2 group bacteriuria of more than 10^5 CFU/ml was found in 12 (86%) and 8 strains of bacteria were isolated. In all the patients of the 3 group bacteriuria of more than 10^4 - 10^5 was found and 13 strains of bacteria were isolated.

Out of the isolated bacteria E.coli prevailed - 57% (group 1 - 52%; group 2 - 75%; group 3 - 54%), S.epidermidis - 18% (group 1 - 19%; group 2 - 25%; group 3 - 12%), E. faecalis - 11% (group 1 - 13%, group 2- 0%, 3 - 8%). In group 1, 16% of isolates were represented by microorganism associations (E.coli, S.epidermidis, E.faecalis, E.aerogenes).

Among the selected pathogens only 32% did not form biofilm, mainly group 1 strains (group 1 - 48%, group 2 - 13%, group 3 - 15%); 43% of bacteria formed weak biofilm (group 1 - 47%, group 2 - 25%, group 3 - 23%); 14 % of strains formed medium biofilm (group 1 - 4%, group 2 - 38%, group 3 - 38%); 11 % of bacteria formed strong biofilm (group 1 - 7%, group 2 - 25%, group 3 - 23%).

Group 1 bacteria formed biofilm at the level of 0.40 ± 0.06 OD, which was significantly less than group 2 bacteria formed – 0.45 ± 0.05 OD ($p<0,05$) and group 3 – 0.48 ±0.07 OD ($p<0,05$) (see fig. No. 3). Biofilm formation of the first group patients' strains did not significantly differ and equaled as follows: E.coli (n=9) 0.41 ± 0.07 OD, S.epidermidis (n=4) 0.38±0.03 OD, E.faecalis (n=4) 0.5 ± 0.1 OD. In the second group, biofilm formation index of E.coli (n=9) equaled 0.43 ± 0.03 OD, which is less than of S.epidermidis (n=3) 0.52 ± 0.1 OD ($p<0.05$). Biofilm formation index of E.coli (n=9) of the third group was at the level of 0.47 ± 0.05 OD.

The indices of biofilm formation by bacterial associations under co-cultivation of three strains equaled 0.42 ± 0.03 OD. When passaging on a solid nutrient medium with a subsequent counting the colonies, it was established that within the associations (E.coli, S.epidermidis, E.faecalis та E.coli, S.epidermidis, E.aerogenes) over 90% of colonies were formed with E.coli. The level of biofilm formation by the members of the association separately did not significantly differ from the level of biofilm formed in the course of their joint cultivation.

27 % of the investigated strains did not possess adhesive properties (group 1 - 36%, group 2 - 0%, group 3 - 25%), 39% of bacteria possessed low adhesive ability (group 1 - 48%, group 2 - 25%, group 3 - 25%), 22% of isolates showed medium adhesive ability (group 1 - 14%, group 2 - 50%, group 3 - 12,5%), 12% of bacteria possessed high adhesive ability (group 1 - 0%, group 2 - 25%, group 3 - 37,5%).

Correlation analysis carried out between the film formation and adhesion ability of bacteria showed a direct credible link $r = 0.529$ ($p<0.01$).

Out of 27 (61%) investigated strains able to perform different types of motility (E.coli, M.morganii, P.mirabilis) 15 % of the strains possessed the ability of twitching (group 1 - 14%, group 2 - 20%, group 3 - 14,5%), 37% had swarming ability (group 1 - 21%, group 2 - 60%, group 3 - 43%), 22 % - swimming ability (group 1 - 7%, group 2 - 40%, group 3 - 43%), not a single one of the studied bacteria had the ability of catheter swarming.

A credible direct correlation was established between swimming and swarming ability of the bacteria $r= 0.706$ ($p<0.01$) and swimming and twitching ability $r=0.55$ ($p<0.01$). It was observed that increased motility ability of the bacteria reduces the force applied by this strain to form biofilm.

Conclusions

Diverse adhesion and biofilm formation ability of bacteria was established depending on the type of UTI from which they were isolated.

The bacteria isolated from children with acute and chronic pyelonephritis possess higher level of adhesion and biofilm formation ability than the bacteria isolated from children with lower urinary tract infection.

Adhesive ability of the bacteria correlates with their biofilm formation ability, whereas we have established no dependencies between microorganism motility and biofilm formation ability or UTI type.

High level of biofilm formation and adhesion ability of the isolated bacteria can be considered an adverse predictive factor in the course of UTI.

References

1.Brilis V.I., Brilene T.A., Lenzner H.P. et all Metodika izuchenia adhesivnoho procesa microorganizmov // Lab.delo. - 1986. - № 4. - P. 210–212.

2.Flores-Mireles A. L., Walker J.N., Caparon M., Hultgren S.J. Urinary tract infections: epidemiology, mechanisms of infection and treatment options // NatRevMicrobiol. – 2015.–N 13(5). – P. 269–284.

3. Merritt J. H., Kadouri D.E., O'Toole G.A. Growing and Analyzing Static Biofilms //CurrProtoc in Microbiol. Authormanuscript– 2005.– Chapter 1. - Unit - 1B.1.

4. Romlingand U., Balsalobre C. Biofilm infections, their resilience to therapy and innovative treatment strategies // Journal of Internal Medicine – 2012. - Vol. 272, N 6. – P.541–561.

5. Stowe S. D., Richards J. J., Tucker A. T., Thompson R., Melander C., Cavanagh J. Anti-biofilm compounds derived from marine sponges // Marine Drugs – 2011. - Vol. 9, N 10.- P. 2010–2035.

6. Vollmerhausen T. L., Katouli M. Molecular characterisation of Escherichia coli isolated from hospitalised children and adults with urinary tract infection//Eur J ClinMicrobiol Infect Dis. – 2014. – N 33(6). – P. 975-982.

7. Villemur. R., Comeau Y., Déziel E. Initiation of biofilm formation by Pseudomonas aeruginosa 57RP correlates with emergence of hyperpiliated and highly adherent phenotypic variants deficient in swimming, swarming, and twitching motilities // Journal of Bacteriology.- 2001. - N 183(4). - P. 1195-1204.

UDC 531/534/: [57+61]

Gorshkova O.G., Voliuvach O.V., Bebyk V.V., Kolomiets O.G., Lagovskaya L.S., Khadzhy V.D.

Odessa I.I. Mechnikov National University,
Dvoryanskaya str., 2, Odessa, 65082, Ukraine; E-mail: tgudzenko@ukr.net

BIODESTRUCTION OF PHENOL BY NON-PATHOGENIC BACTERIA OF THE GENUS *PSEUDOMONAS*

Abstract

It has been experimentally established that two strains of bacteria genus *Pseudomonas*, isolated from the marine environment and identified by the fatty acid composition of their cellular lipids as *P. fluorescens* ONU328, *P. maltophilia* ONU329, are not pathogenic and have a high phenol-oxidizing ability. Bacteria are cultured for two days at a temperature of 30 °C on MPA, further bacterial cells are suspended in the mineral medium M-9, and in the optimum amount is introduced into the contaminated water containing up to 300 mg/l phenol. It was established that the strain of *P. maltophilia* possessed a greater phenol-oxidizing activity than the strain *P. fluorescens*. When water was treated with strain *P. maltophilia* with a culture concentration of 3.5×10^4 CFU/ml, deep purification from phenol was observed on 34 day, and with an increase in the culture concentration to 7.5×10^5 CFU /ml on 18 day, t=30 °C.

Key words: biodegradation, phenol, bacteria of the genus *Pseudomonas*

Introduction

The modern development of chemical, medical and pharmaceutical production causes a powerful release into the environment of phenolic compounds (PC) that have a toxic effect. Energy dependence and high cost of physical and chemical technologies for neutralizing phenol-containing wastewater stimulates the need for widespread introduction of effective, ecosafety and non-volatile biological methods in the purification of such effluents. A method has been developed for the oxidation of PC using tyrosinase of *Agaricus bisporus* fungi [1].There are data on the complete mineralization of PC in the process of their biodegradation by pathogenic microorganisms - *Aspergillus niger* [2], the Indian strain *Staphylococcus aureus* isolated from Amla Khadi, Ankleshwar [3], halophilic fungi *(Aspergillus, Pencillium , Fusarium)*, isolated from sediments along the Gulf of Suez and the sediments of the Red Sea [4].

The aim of the work is to investigate the destruction of phenol by nonpathogenic bacteria of the genus *Pseudomonas.*

Materials and methods

Biochemically active strains of bacteria of the genus *Pseudomonas* spp. The strains were previously isolated from the marine environment and, in the aggregate of morphological, cultural and physiological-biochemical features determined using classical bacteriological methods and the API 50 CHB Medium test system (bioMerieux, France), are classified as *P. fluorescens* ONU328 and *P. maltophilia* ONU329. Additionally, the fatty acid composition, whose spectra were obtained on an Agilent 7890 gas chromatograph and deciphered using the RTSBA6 6.21 library of the MIDI Sherlock program, investigated strains with a high similarity index (Sim Index $\geq$ 0.72) were identified as *P. fluorescens* ONU328, *P. maltophilia* ONU329. They are not pathogenic and are currently stored in the collection of microorganisms of the Department of microbiology, virology and biotechnology of the Odessa I.I. Mechnikov National University.

To carry out the microbiological method of water purification from phenol, the bacteria were cultivated at a temperature of 28±1°C, in a nutrient medium of the composition (g/l): KH_2PO_4 – 1.5; Na_2HPO_4 – 3.0; NaCl – 5.0; NH_4Cl – 1.0; peptone – 10.0; glucose – 2.0; yeast extract – 5.0 (pH = 7). Biomass build-up was carried out for 48 hours before reaching a culture density of at least 5 g/l in dry biomass. The concentration of phenol in water samples was determined by a photometric method based on the formation of stained phenol compounds with 4-aminoantipyrine in the presence of potassium hexacyanoferrate (III) at pH 10.0±0.1 [5]. The measurements were made on a photoelectric colorimeter at a wavelength of 540 nm. The experiments were performed in five replicates. Statistical processing of the results of the studies was carried out using standard methods of variation statistics using the program «Microsoft Office Excel 2003» with the definition of Student's t-test. The difference was statistically significant for $p<0.05$.

Results and discussions

Results on the biodegradation of phenol in the presence of marine microorganisms *P. fluorescens* ONU328 and *P. maltophilia* ONU329, obtained at different temperatures, are shown in Fig. 1. It has been experimentally established that the strains of microorganisms used have a high phenol-oxidizing ability at temperatures of 18 and 30 °C. The strain of *P. maltophilia* ONU329 as compared to strain *P. fluorescens* ONU328 possessed a greater biochemical activity with respect to phenol with a concentration of 300 mg/l.

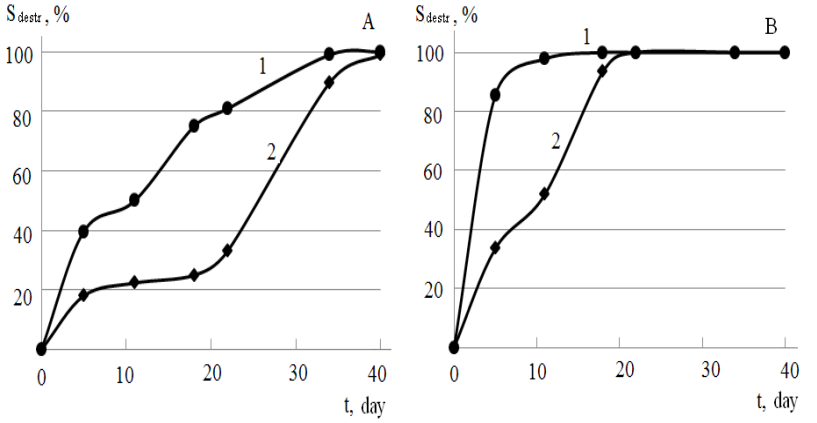

Fig. 1. Kinetic curve of the degree of destruction of phenol* (Sdestr,%) in the presence of P. maltophilia ONU329 (curve 1), P. fluorescens ONU328 (curve 2) in an amount of 7.5×105 CFU/ml at a temperature: A –18 °C; B – 30 °C.

Note: * The initial concentration of phenol in water is 300 mg/l.

When water was treated with strain *P. maltophilia* ONU329 with a culture concentration of 3.5×10^4 CFU/ml, deep purification from phenol was observed on day 34, and with an increase in the culture concentration to 7.5×10^5 CFU/ml on day 18 at a temperature of 30 ° C.

Conclusions

It has been experimentally established that the following conditions are optimal for the deep purification of water from phenol by a microbiological method using the phenol of Pseudomonas genus as the destructors: 30 ° C; culture concentration - 7.5×10^5 CFU/ml; The contact time of microorganisms with phenol-containing water is from 18 to 22 days using the strain *P. maltophilia* ONU329 and *P. fluorescens* ONU328, respectively.

References

1. Севастьянов О.В. (2012). Исследование удаления анилина и фенольных соединений путем соокисления с помощью тирозиназы agaricus bisporus. Вісн. Одес. нац. ун. Сер.: Хімія., 17(4):55-60.

2. Mrs. Ch. supriya.(2014). Biodegradation of phenol by *Aspergillus niger.* J. of Pharmacy, 4(7):11-17.

3. Butani Naresh, and other (2012). Biodegradation of Phenol by a Bacterial Strain Isolated From a Phenol Contaminated Site in India. I Research Journal of Environment Sciences, 1(1):46-49.

4. Aida M. Farag, and other (2014). Degradation of Phenol by A New-Degradable Marine Halophilic Fungus Fennellia Flavipes isolated from Mangrove Sediments. Life Science Journal,11(9):836-845.

5. Лурье Ю.Ю. Аналитическая химия промышленных сточных вод. - М.: Химия, 1984. – 448 с.

6. Gorshkova O.G., Gudzenko T.V., Voliuvach O.V., Beljaeva T.O., Konup I.P. (2017). Water treatment from highly toxic metal ions using non-pathogenic bacteria of the genus *Pseudomonas.* Materials XV International Scientific and Practical Conference «Special Metallurgy: Yesterday, Today, Tomorrow» - April 11, 2017, Kiev, NTUU «KPI Igor Sikorsky.» - P. 331-339.

UDC 579.22:579.64

Gorshkova O.G., Voliuvach O.V., Kolomiets O.G., Bebyk V.V., Khadzhy V.D., Lagovskaya L.S.

Odessa I.I. Mechnikov National University,
Dvoryanskaya str., 2, Odessa, 65082, Ukraine; E-mail: tgudzenko@ukr.net

BIOMARKER VALUES OF NONPATHENOUS MICROORGANISMS WITH POLYFUNCTIONAL BIOTECHNOLOGICAL PROPERTIES

Abstract

Isolated from the oil-contaminated soil of Zmeiniy island and the marine environment are four non-pathogenic biochemically active strains of microorganisms identified by the fatty acid composition of their cell lipids as *Microbacterium barkeri* OZ-3, *Bacillus megaterium* OZ-5 (isolated from soil), *Pseudomonas fluorescens* ONU-328, *Pseudomonas maltophilia* ONU-329 (separated from the marine environment). The strains possess polyfunctional biotechnological properties: sorption-accumulating ability relative to heavy metal ions, oxidizing activity with respect to petroleum products, anionic surfactants and phenolic compounds. Biomarker values for distinguishing the strains of microorganisms studied (*Microbacterium barkeri* OZ-3, *Bacillus megaterium* OZ-5, *Pseudomonas fluorescens* ONU-328, *Pseudomonas maltophilia* ONU-329) are proposed at the species level.

Key words: biomarker values, microorganisms, non-pathogenic, polyfunctional.

Introduction

In most countries in the world, biological methods are preferred from known methods for purifying water and soil from pollutants of different nature. They are more effective, universal and ecologically safe than non-pathogenic microorganisms in comparison with physico-chemical methods, they do not cause secondary pollution.Therefore, today, biotechnologists are faced with the pressing need to produce new generation biologics intended for cleaning the environment from a wide range of toxic pollutants - heavy metal ions, petroleum products, synthetic surfactants, phenolic compounds. The search and identification of new biochemically active microorganisms, which are non-pathogenic, and have polyfunctional biotechnological properties, continues. The aim of the work is to isolate from non-polluting sources non-pathogenic biochemically

active microorganisms with polyfunctional biotechnological properties and to analyze the fatty acid spectra of cellular lipids to offer biomarker values for their differentiation at a species level.

Materials and methods

The subjects of the study were four biochemically active strains of microorganisms isolated from the oil-polluted soil of Zmeiniy island and from a polluted marine environment. Fatty acid analysis was carried out on an Agilent Technologies 7890 gas chromatograph using an automatic microorganism identification system.

Results and discussions

Specially selected biochemically active microorganisms from the obtained fatty acid composition (the spectra of which are deciphered using the RTSBA6 6.2 library database of the Sherlock MIDI program with a high index of similarity of Sim Index ≥0.7) are assigned to *Microbacterium barkeri* OZ-3, *Bacillus megaterium* OZ-5 (isolated from oil contaminated soil of Zmeiniy island) and *Pseudomonas fluorescens* ONU328, *Pseudomonas maltophilia* ONU329 (isolated from the marine environment). According to the decoding of chromatographic spectra of common cell lipids of the investigated ground and sea microorganisms, fatty acids C15:0 anteiso and C15:0 iso were found to prevail among the available fatty acids of the branched structure (in total they constituted the largest percentage of the total of the total areas of the peaks). To distinguish microorganisms at the generic level, we suggest considering the ratio of branched fatty acids as biomarker values (Table 1).

Table 1

Biomarker values of some marine and soil strains of microorganisms of broad biotechnological use

Biomarker values	Strains of soil microorganisms		Strains of marine microorganisms	
	M.barkeri O3-3	B.megaterium O3-5	P.maltophilia ONU-329	P.fluorescens ONU-328
[C_{15}:0 anteiso/ C_{15}:0 iso]	8,4	1,2	0,54	----
[C_{17}:0 anteiso/ C_{17}:0 iso]	16,2	1,4	----	----
[C_{15}:0 anteiso/ C_{17}:0 anteiso]	1,7	14,7	----	----

In the fatty acid profiles of soil strains *M. barkeri* OZ-3 and *B. megaterium* OZ-5, in contrast to the fatty acid profiles of marine strains *P. maltophilia* ONU329 and *P. fluorescens* ONU328 were present from branched fatty acids 15-methylhexadecanoic (C17:0 iso) and 14 -methylhexadecanoic acid (C17:0 anteiso), which allowed them to calculate the ratios [C17:0 anteiso / C17:0 iso], [C15:0 anteiso / C17: 0 anteiso].

The calculated data obtained in the analysis and comparison of the fatty acid profiles of soil strains *M. barkeri* OZ-3 and *B. megaterium* OZ-5 showed that the value of [C17:0 anteiso/C17:0 iso] is an order of magnitude higher for strain *M. barkeri* OZ-3; and vice versa, the biomarker value [C15:0 anteiso / C17:0 anteiso] during the transition from strain *B. megaterium* OZ-5 to strain *M. barkeri* OZ-3 decreases by an order of magnitude from 14.7 to 1.7.

In the fatty acid spectrum, the saturated fatty acids of the strain *M. barkeri* OZ-3 lacked saturated cycloalkanoic acids, and from the saturated fatty acids of the normal structure were not fixed, as in the composition of the cell lipids of strains *B. megaterium* OZ-5, *P. maltophilia* ONU-329, *P. fluorescens* ONU328 [2], decanoic C10:0, dodecanoic C12:0 and pentadecanoic acid C15:0. Dodecanoic acid was observed only in the fatty acid profile of *P. fluorescens* ONU328 strain. The fatty acid composition of the *M. barkeri* OZ-3 strain compared to *B. megaterium* OZ-5, P. maltophilia ONU-329, *P. fluorescens* ONU-328 strains is, That in its fatty acid pool, although in a small amount biomarker heptadecanoic acid is fixed. In the study, strains of microorganisms differ in the quantitative content in their fatty acid profile of hydroxy acids [1, 2]. The metal-resistant ability of microorganisms to highly toxic heavy metal ions [Pb (II), Zn (II), Cr (VI) [3-5] has been experimentally established and the ability to decompose oil: the degree of biodegradation of oil with a concentration of 10 mg/10 ml of bacterial suspension for 20 days of exposure was 40-45% when using soil strains of microorganisms [1] and 70-75% when using marine strains of microorganisms. A good growth of microorganisms on «hungry» agar with 1% sodium dodecyl sulfate was noted, which allows them to be proposed for use in biotechnologies for cleaning the environment from organic compounds. When compiling a new biopreparation on the basis of the association of the strains of microorganisms under study, we established their synergistic adsorption action with respect to Cr (VI) [5] and oxidizing activity with respect to petroleum hydrocarbons and phenolic compounds.

Conclusions

Proposed biomarker values for distinguishing at a species level non-pathogenic strains of *M. barkeri* OZ-3, *B. megaterium* OZ-5, *P. fluorescens* ONU-328, *P. maltophilia* ONU-329. They possess a sorption-accumulating ability with respect to heavy metal ions, oxidizing activity with respect to petroleum products, anionic surfactants and phenolic compounds, do not inhibit each other, and therefore can be successfully used for the preparation of a bacterial association for a wide biotechnological purpose.

References

1. *Іваниця В.О. та ін.* Склад жирних кислот ліпідів штаму *Bacillus* sp. ОЗ-5, виділеного із забрудненого нафтою грунту о. Зміїний // Мікробіологія і біотехнологія – 2015. - № 4. – С. 28-35.

2. *Гудзенко Т.В. та ін.* Склад жирних кислот ліпідів нафтоокиснювальних штамів бактерій роду *Pseudomonas* // Мікробіологія і біотехнологія. – 2014. – № 3(27). – С. 31–40.

3. Патент України № 90119. Спосіб мікробіологічного очищення води від іонів цинку/ *Іваниця В.О. та ін.* Опубл.: 12.05.14., Бюл. № 9.

4. Патент України №106378. Спосіб мікробіологічної очистки розчинів від іонів свинцю/ *Іваниця В.О., Гудзенко Т.В. та ін.* Опубл. 25.04.2016, Бюл. № 8.

5. Патент України № 102265. Спосіб очищення води від хрому (VI) з використанням мікроорганізмів / *Іваниця В.О., Гудзенко Т.В. та ін.* Опубл.: 26.10.2015., Бюл. № 20.

UDC 531/534/: [57+61]

Poltavchenko I.V., Elinskaya N.A

Odessa I.I. Mechnikov National University,
Dvoryanskaya str., 2, Odessa, 65082, Ukraine; E-mail: tgudzenko@ukr.net

THE TAXONOMICAL COMPOSITION AND SENSIBILITY TO ANTIBIOTICS OF BACTERIA – CAUSATIVE AGENTS OF INFECTIONS OF THE UPPER RESPIRATORY TRACT

Abstract

The purpose of this work was to identify, study the biological features, identify and determine the sensitivity of antibiotics actions of dominant microorganisms isolated in patients with inflammatory diseases of the upper respiratory tract.

Key words: inflammatory diseases of upper respiratory tract, *Staphylococcus*, *Streptococcus*, mono infections, mixed infections, sensitivity to antibiotics

Introduction

Upper respiratory tract occupy one of the leading inflammatory diseases [2]. Often acute process becomes chronic, there is a significant percentage of severe disease, there is a high mortality

In this regard, the need to constantly improve specific prevention study pathogens circulation upper respiratory tract infections in the population in terms of mass immunization [1].

The constant increase in bacterial resistance to antibiotics complicates the process of effective treatment of patients with inflammatory diseases of the upper respiratory tract bacterial etiology [2]. At the same time knowledge of the etiology of inflammation and sensitivity to antibiotics of infectious agents facilitates adequate therapy improves prognosis and reduces the risk of a sharp transition process in chronic[5].

Materials and methods

The material for the study of the allocation of the throat, nose and phlegm. All tests were performed according to [3]. In 2016 - 2017 years as a result of bacteriological examination of sputum, secretions of the nose and throat, in 897 patients with upper respiratory tract infection - residents.

The experimental part of the work was performed in the clinical diagnostic laboratory of the State Scientific and Technical Engineering Center for Problems of Water Purification and Water Conservation of the STI «Vodoobrobka» Physico-Chemical Institute. A.V. Bogatsky National Academy of Sciences of Ukraine.

From sputum, secretions from the nose and throat was isolated 1490 strains of microorganisms patients with abnormalities of ENT organs.

Material collected in compliance with the rules of aseptic. From collecting dry sterile cotton swab is introduced into the interior cavity of the nose, throat phlegm . Morning phlegm that stand out in the attack of coughing, collected in a sterile bank. .In this research Drill test material using the following nutrient media: Yolk-salt agar, Endo Agar, Saburo Agar, Blood agar..

Determination of the sensitivity of microorganisms to antibiotics was carried disco diffusion method according to [4]. Research on sensitivity to antibiotic drugs subject to pure cultures of microorganisms or colony isolated from dense nutrient mediums initial seeding of clinical material. Disks with antibiotics used to determine antibiotic susceptibility of infectious agents isolated from patients with pathological material

Results and discussions

Isolated strains were identified as the bacterium of 11 genera *Staphylococcus, Streptococcus, Klebsiella, Escherichia, Enterobacter, Proteus, Pseudomonas, Candida, Bacillus,* representing 12 species dominated by representatives of the genera *Staphylococcus* and *Streptococcus*(Fig. 1).

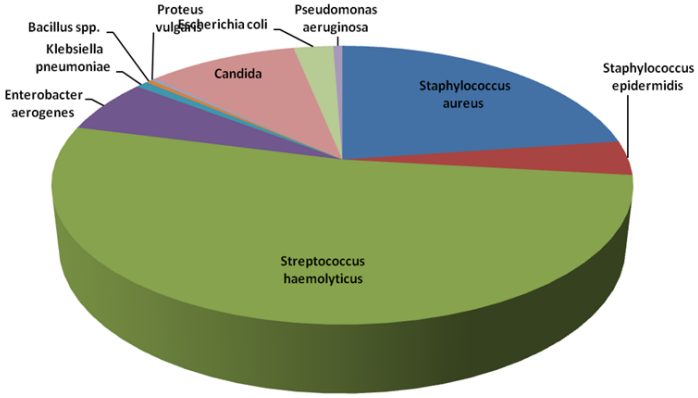

Fig. 1 Frequency allocation microorganisms of different taxonomic groups of patients with inflammatory diseases of the upper respiratory tract.

In patients with inflammatory diseases of the upper respiratory tract, infections were more often caused by a monoinfects.(Fig.2)

The amount of examined	mono- infection		mixed infection	
	abs.	%	abs.	%
897	522	58,2	375	41,8

Fig.2 The frequency of mono- and mixed infections in the patients with inflammatory diseases of the upper respiratory tract

The most common mixed infection is caused by *Staphylococcus aureus* and *Streptococcus haemoliticus*, which is found in 39.2 % of patients.

A study of the sensitivity of isolated strains to antibiotics showed that the bacteria of the genera *Staphylococcus* and *Streptococcus* were resistant to the action of amoxicillin and doxycycline, and are sensitive to ofloxacin, gatifloxacin ceftriaxone, cefoperazone. The bacteria of the *Enterobacteriaceae* family were most sensitive to ofloxacin, cefoperazone, gatifloxacin. And resistant to doxycycline, cefazolin, amoxicillin.

Conclusions

Our data indicate that in recent years has been an increasing resistance of microorganisms, including bacteria genera *Staphylococcus* and *Streptococcus* to antibacterial drugs used in clinical practice.

References

1. *Жуковицкий В. Г.* Обоснование рациональной антибактериальной терапии в оториноларингологии с позиции бактериолога. // Consilium medicum. – 2001. – Т. 3, № 8. – С. 362 – 368

2. *Крюков А.И.* Гнойно–воспалительные заболевания уха, горла, носа и верхних дыхательных путей: актуальность проблемы и пути // Вестник Оториноларингологии. – 2004. – №1 – С. 3–13.

3. *Наказ* № 535. Про уніфікацію мікробіологічних (Бактеріологічних) методів дослідження, застосовуваних в клініко-діагностичних лабораторіях лікувально-профілактичних закладів. – М.: МЗ СССР, 1985. – 126 с.

4. *Наказ* МОЗ України № 167 Визначення чутливості мікроорганізмів до антибактеріальних препаратів, 2007. – 63 с

5. *Zwart S., Ruijs G.J., Sachs A.P.* Beta–haemolytic streptococci isolated from acute sore–throat patients: cause or coincidence A case–control study in general practice // Scandinavian Journal of Infectious Diseases – 2000.–Vol. 32, № 4. – P. 377 – 384

UDC 579.64:633.11

Zhunko I.D., Merlich A.G., Limanska N.V.

Odessa National I.I. Mechnikov University,
2, Dvorianska str., Odessa, 65082, Ukraine,
tel.:+38 (0482) 68 79 64; e-mail: zhunkinn@gmail.com

EFFECT OF *LACTOBACILLUS PLANTARUM* AND *BACILLUS ATROPHAEUS* ON GROWTH OF WHEAT SEEDLINGS

Abstract

The effect of bacteria of the strains *Lactobacillus plantarum* ONU 12, ONU 311 and *Bacillus atrophaeus* ONU 528 on growth characteristics of wheat seedlings have been studied. Inoculation of surface sterilized seeds with the diluted cultures was conducted. The inoculated seeds were brought into wet-chambers, and after germination the measurements of seedling growth characteristics were done. The treatment by *L. plantarum* ONU 311 resulted in increase of the length of seedlings roots in 31% as compared with the control and stimulation effect on the height of seedlings was 48%. The mixture of the strains *L. plantarum* ONU 12 and ONU 311 increased the length of roots and height of seedlings in 41,1% and 52,4% accordingly. The treatment of wheat seeds by bacteria *B. atrophaeus* ONU 528 resulted in increase of mean length of roots in 39,7% and height of seedlings in 45% as compared with the control. Bacteria *L. plantarum, B. atrophaeus* and their mixtures had the stimulation effect both on roots and height of wheat seedlings.

Key words: *Lactobacillus plantarum, Bacillus atrophaeus,* stimulation activity, wheat.

Introduction

Efficiency of the use of various microorganisms including rhizosphere bacteria in the agriculture is well known. Nitrogen-fixing and phosphate-mobilizing bacteria can stimulate plant growth and development. From this point of view, a diverse group of lactic acid bacteria (LAB) that also inhabit plant rhizosphere is less studied [3].

It is known that plant growth stimulation can be achieved indirectly by increasing the absorption of minerals and nutrients or directly by regulation of plant hormones such as indole-3-acetic acid (IAA), cytokinins and ethylene [4].

The aim was to study the effect of *Lactobacillus plantarum* and *Bacillus atrophaeus* on seed germination and growth characteristics of wheat seedlings.

Materials and methods

Strains of *Lactobacillus plantarum* ONU 12, ONU 311 (isolated from plant material) and *Bacillus atrophaeus* ONU 528 (isolated from soil) were used in experiments. The LAB were cultivated in MRS liquid medium at 37 °C [1], *B. atrophaeus* – in NB liquid medium at 28 °C [2].

Several variants of seed treatment were used in experiments: bacterial culture of *L. plantarum* ONU 12, *L. plantarum* ONU 311, *B. atrophaeus* ONU 528, mix of bacteria cultures *L. plantarum* ONU 12 + *L. plantarum* ONU 311, *L. plantarum* ONU 12 + *B. atrophaeus* ONU 528. MRS 1% and tap water were used as controls.

Wheat seeds were surface sterilized in 25% H_2O_2 for 1 minute. Seeds were washed three times in sterile tap water from the residual H_2O_2. Seeds were soaked in prepared bacterial suspensions for one hour and then were brought into sterile Petri dishes with filter paper disks. The bottom of the chambers was moistened with 15 ml of tap water. Seeds germinated in a greenhouse at 25 °C, wet-chambers were moistened as far as drying. The measurement of seedling growth characteristics was done after 5 days of germination. Statistical analysis was performed using Microsoft Office Excel.

Results and discussion

L. plantarum ONU 12 (1,78x10^7 CFU/mL) showed no significant effect on plant growth. The treatment by *L. plantarum* ONU 311 (1,47x10^7 CFU/mL) resulted in increase of the mean length of seedlings roots (5,7 ± 0,4 cm) in 31% as compared with the control (4,4 ± 0,4 cm) and stimulation effect on the height of seedlings was 48% (mean height - 7,3 ± 0,3 cm / control – 5,0 ± 0,4 cm) (fig. 1, 2).

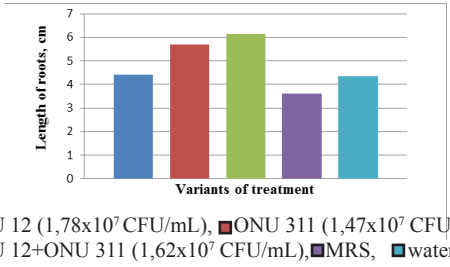

Fig. 1. Effect of bacteria of *L. plantarum* strains on growth of wheat roots

*Note: * – significant different from the control (water) (p≤0,05)*

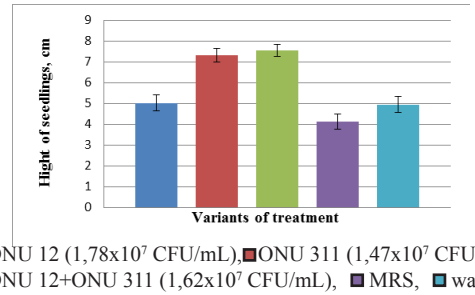

Fig. 2. Effect of bacteria of *L. plantarum* strains on wheat hight

Note: * – significant different from the control (water) ($p \leq 0,05$)

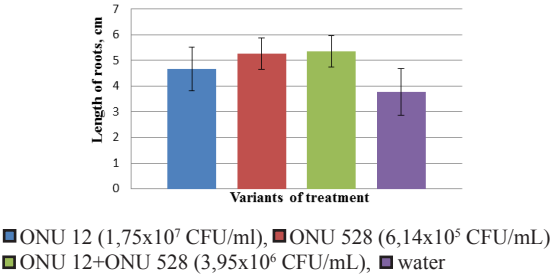

Fig. 3. Effect of the treatment with bacteria of *B. atrophaeus* ONU528 and *L. plantarum* ONU12 on mean length of wheat roots

Note: * – significant different from the control (water) ($p \leq 0,05$)

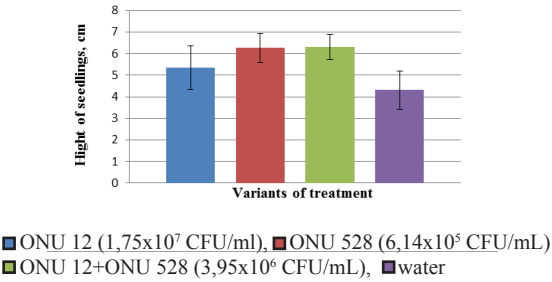

Fig. 4. Effect of the treatment with bacteria of *B. atrophaeus* ONU528 and *L. plantarum* ONU12 on mean height of wheat seedlings

Note: * – significant different from the control (water) ($p \leq 0,05$)

The mixture of the strains *L. plantarum* ONU 12 and ONU 311 ($1{,}62 \times 10^7$ CFU/mL) increased the length of roots (6,2±0,4 cm) and height of seedlings (7,5±0,3 cm) in 41,1% and 52,4% accordingly (fig. 1, 2).

The treatment of wheat seeds by bacteria *B. atrophaeus* ONU 528 ($6{,}14 \times 10^5$ CFU/mL) resulted in increase of mean length of roots (5,3±0,6 cm) in 39,7% and height of seedlings (6,3±0,7 cm) in 45% as compared with the control (3,8±0,9 cm and 4,3±0,9 cm, accordingly) (fig. 3, 4).

L. plantarum ONU 12 ($1{,}75 \times 10^7$ CFU/mL) showed no significant effect on plant growth. The treatment of wheat seeds by mixture of the strains *L. plantarum* ONU 12 та *B. atrophaeus* ONU 528 resulted in increase of mean length of roots (5,4±0,6 cm) in 42,3% and height of seedlings (6,3±0,6 cm) in 46,4%.

Conclusions

Bacteria *L. plantarum, B. atrophaeus* and their mixtures had the stimulation effect both on roots and height of wheat seedlings. The different LAB strains in similar concentrations of bacterial cells had a different effect on the growth of wheat seedlings. These data suggest that the stimulating properties of bacteria are strain-specific.

References

1. *Abdel-Aziz S.M., Moustafa Y.A.,* and *Hamed H.A.* Lactic acid bacteria in the green biocontrol against some phytopathogenic fungi: treatment of tomato seeds // Journal of Basic and Applied Scientific Research. – 2014. – V. 4 (12). – P. 1–9.

2. *Dragovoz I.V., Pasichnyk L.A., Zhukova D.A., Lapa S.V., Kriuchkova L.O., Avdeeva L.V.* Antagonistic activity of strains of *Bacillus amyloliquefaciens* – perspective agents of biocontrol of grain crops / / Mikrobiologichny zhurnal. – 2014. – № 5, T. 76. – C. 15–19 (In Ukrainian).

3. *Rzhevskaia V.S., Oturina I.P., Teplickaia L.M.* Study of biological properties of strains of lactic acid bacteria // Scientific Notes of Taurida National V.I. Vernadsky University, Series Biology and Chemistry. – 2014. – №1, T. 27 (66). – C. 145–160 (In Russian).

4. *Shrestha A, Kim B.S., Park D.H.* Biological control of bacterial spot disease and plant growth-promoting effects of lactic acid bacteria on pepper // Biocontrol Science and Technology. – 2014. – V. 24, № 7. – P. 763–779.

UDC 579.64:633.11

Kladnitskii V. Y., Galkin M. B.

Odessa I.I. Mechnikov National University,
Dvoryanskaya str., 2, Odessa, 65082, Ukraine; E-mail: quarzonia@gmail.com

AMIXIN EFFECT ON DIFFERENT STRAINS OF *PSEUDOMONAS AERUGINOSA* WHICH HAVE DIFFERENT ABILITIES FOR BIOFILM FORMATION

Abstract

The study of how tilorone in different concentrations interact with collection strains of *Pseudomonas aeruginosa,* which have different ability to form the biofilm was performed. For the experiment were prepared five dilutions of tilorone 25, 50, 100, 250 and 500 mcg/ml. To determine the effect of tilorone on the biofilm formation used method for cultivation of the biofilms in the plates for cell culture, using the medium LB. The presence of the biological activity of the compounds judged by the presence of optical density difference between the experimental and control samples. The results showed that the different concentrations of test compound act differently on growth and biofilm formation of test strains *Pseudomonas aeruginosa,* causing stimulation or inhibition, or nothing changes at all.

Introduction

Today, epidemics of many diseases are less common now; infections caused by conditionally pathogenic microorganisms are becoming more widespread. In the twentieth century, antimicrobial agents were created which almost immediately entered clinical practice and produced high results.

However, through the widespread use of antibiotics, science and medicine have faced a rather complex problem of resistance to various antimicrobial drugs.

Particular attention should be paid to conditionally pathogenic bacteria that very quickly acquire polyresistance to a wide range of antimicrobial agents; in particular, *P. aeruginosa* is a microorganism that very often causes nosocomial infections. An additional factor of danger is that it is very resistant to disinfectants. This makes it possible to reproduce on medical instruments, which must be sterile.

Thus, the development and introduction of the medical practice of new approaches for combating opportunistic pathogenic microorganisms are relevant.

Materials and methods

The experiment was conducted on a several strains of *Pseudomonas aeruginosa* PA01 (wild type) and its derivatives *P. aeruginosa wsp*F1 carrying a mutation in the gene *wsp*F1 and has a high ability to form biofilms, and *P. aeruginosa* pJN2133 carrying plasmid pJN2133 and has reduced ability to form biofilms.

In work used the method of culturing microorganisms in static conditions using sterile 96-well polystyrene flat-bottomed plates. To determine the intensity of biofilm formation was used the method of staining by crystal violet with some modifications. Formation of biofilm determine spectrophotometrically [1].

Results and discussions

The influence of tilorone on the formation of biofilms by *P. aeruginosa* PA01, *P. aeruginosa wsp*F1, and *P. aeruginosa* pJN2133 was performed after 24 hours of incubation with different concentrations of test compound. Comparison of control culture and culture with Tilorone showed that stimulation of biofilm formation at all concentrations was observed only in the strain *P. aeruginosa* PA01 (Figure 1).

There was inhibition of biofilm formation at all concentrations only in strain of *P. aeruginosa wsp*F1 (Figure 2), the highest inhibition effect found at tilorone concentration of 100 mcg/ml and was 31, 29% compared with the control.

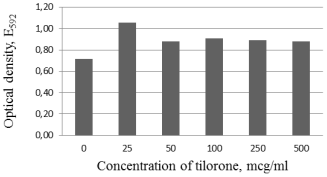

Fig. 1. Indicators of biofilm formation by *P. aeruginosa* PA01 in the presence of tilorone

Note: * - significant difference compared with control

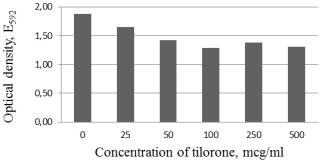

Fig. 2. Indicators of biofilm formation by *P. aeruginosa wsp*F1 in the presence of tilorone

Note: * - significant difference compared with control

Different concentrations of test compound affected differently as inhibiting or stimulating the formation of biofilm only for strain of P. aeruginosa pJN2133, but mainly observed stimulation and maximum gain was 162, 81% at concentration of tilorone 500 mcg/ml (Figure 3).

The results showed that the different concentrations of test compound act differently on growth and biofilm formation of test strains

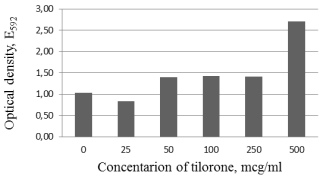

Fig. 3. Indicators of biofilm formation by *P. aeruginosa* pJN2133 in the presence of tilorone

Note: * - significant difference compared with control

Pseudomonas aeruginosa, causing stimulation or inhibition, or nothing changes at all.

But, at certain concentrations in specific strains experienced a pronounced stimulatory and/or inhibition effect on the intensity growth and formation of biofilm of microorganism. In this regard *P. aeruginosa* strain *wsp*F1 was chosen as the model of inhibition effect of tilorone on ability to form biofilm, and strain *P. aeruginosa* pJN2133 as model of stimulation.

Conclusions

After comparison with other synthetic low-molecular inducers of interferon, for example, a derivative of acridine acetic acid, which have a pronounced antibacterial activity and suppress a number of opportunistic microorganisms in vitro, we can speak of the effectiveness of tilorone as an antibacterial drug; however, this efficiency depends on its concentration and microorganism characteristics. The conducted study of the antimicrobial properties of this compound may be the foundation for further study of its mechanisms of action on a bacterial cell or influence on the processes associated with the formation of biofilms.

References

1. *Christensen G. D.* Adherence of coagulase-negative Staphylococci to plastic tissue culture plates: a quantitative model for the adherence of Staphylococci to medical devices // J. clin. microbiol. – 1985. –V. 22. – № 6. – P. 996-1006.

UDC 579.64:633.11

Kladnitskii V. Y., Galkin N. B.

Odessa I.I. Mechnikov National University,
Dvoryanskaya str., 2, Odessa, 65082, Ukraine; E-mail: quarzonia@gmail.com

TILORONE EFFECT ON *SALMONELLA ENTERITIDIS* BIOFILM FORMATION

Abstract

The study of how tilorone in different concentrations effect on biofilm formation ability of collection strains of Salmonella enteritidis, was performed. For the experiment were prepared five dilutions of tilorone 25, 50, 100, 250 and 500 mcg/ml. To determine the effect of tilorone on the biofilm formation used method for cultivation of the biofilms in the plates for cell culture, using the medium LB. The presence of the biological activity of the compounds judged by the presence of optical density difference between the experimental and control samples. The results showed that the different concentrations of test compound act differently on growth and biofilm formation of test strains *S. enteritidis*, causing stimulation or inhibition, or nothing changes at all.

Introduction

Today, epidemics of many diseases are less common now; infections caused by conditionally pathogenic microorganisms are becoming more widespread.

In the twentieth century, antimicrobial agents were created which almost immediately entered clinical practice and produced high results.

However, through the widespread use of antibiotics, science and medicine have faced a rather complex problem of resistance to various antimicrobial drugs.

Particular attention should be paid to conditionally pathogenic bacteria that very quickly acquire polyresistance to a wide range of antimicrobial agents; in particular, S. enteritidis is a microorganism that very often causes acute intestinal infections. An additional factor of danger is that it is very common bacteria, which very often present in raw food. Thus, the development and introduction of the medical practice of new approaches for combating opportunistic pathogenic microorganisms are relevant.

Materials and methods

The experiment was conducted on a several strains of *Salmonella enteritidis* ONU 266, ONU 465 and ONU466.

In work used the method of culturing microorganisms in static conditions using sterile 96-well polystyrene flat-bottomed plates. To determine the intensity of biofilm formation was used the method of staining by crystal violet with some modifications. Formation of biofilm determine spectrophotometrically [1].

Results and discussions

The influence of tilorone on the formation of biofilms by *S. enteritidis* ONU 266, *S. enteritidis* ONU 465 and *S. enteritidis* ONU 466 was performed after 24 hours of incubation with different concentrations of test compound. Comparison of control culture and culture with tilorone showed that stimulation of biofilm formation at all concentrations was observed only in the strain *S. enteritidis* ONU 466 and maximum gain was 85, 4%, compared with the control, at concentration of tilorone 100 mcg/ml (Figure 1).

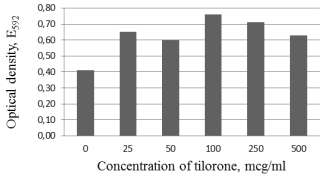

Fig. 1. Indicators of biofilm formation by *S. enteritidis* ONU 466 in the presence of tilorone

Note: * - significant difference compared with control

Mainly observed stimulation effect of compound at strain *S. enteritidis* ONU 465 (Figure 2). Different concentrations of test compound affected differently, inhibiting and stimulating the formation of biofilm only at the strain of *S. enteritidis* ONU 266, but this strain was shown highest inhibition effect, about 57, 47%, compared with the control, at concentration of tilorone 50 mcg/ml (Figure 3).

The results showed that the different concentrations of test compound act differently on growth and biofilm formation of test strains *Salmonella enteritidis*, causing stimulation or inhibition, or any changes at all. But, at certain concentrations in specific strains experienced a pronounced stimulatory and/or inhibition effect on the intensity growth and formation of biofilm of microorganism. In this regard *S. enteritidis* ONU 266 was chosen as the model of inhibition effect of tilorone on ability to form biofilm, and strain *S. enteritidis* ONU 466 as model of stimulation.

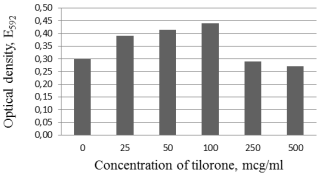

Fig. 2. Indicators of biofilm formation by *S. enteritidis* ONU 465 in the presence of tilorone

Note: * - significant difference compared with control

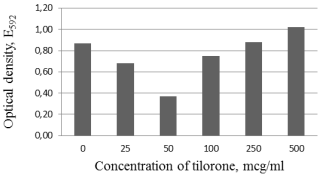

Fig.3. Indicators of biofilm formation by*S. enteritidis* ONU 266 in the presence of tilorone

Note: * - significant difference compared with control

Conclusions

After comparison with other synthetic low-molecular inducers of interferon, for example, a derivative of acridine acetic acid, which have a pronounced antibacterial activity and suppress a number of opportunistic microorganisms in vitro, we can speak of the effectiveness of tilorone as an antibacterial drug; however, this efficiency depends on its concentration and microorganism characteristics. The conducted study of the antimicrobial properties of this compound may be the foundation for further study of its mechanisms of action on a bacterial cell or influence on the processes associated with the formation of biofilms.

References

1. *Christensen G. D.* Adherence of coagulase-negative Staphylococci to plastic tissue culture plates: a quantitative model for the adherence of Staphylococci to medical devices // J. clin. microbiol. – 1985. –V. 22. – № 6. – P. 996-1006.

UDC 579.62

M.S. Kovtalo, O.Yu. Zinchenko, D.O. Khaydukova, Yu.V. Karavanskiy

Odessa I.I. Mechnikov National University,
Dvoryanskaya str., Odessa, 65058, farmikr@ukr.net

ANTIBIOTIC SUSCEPTIBILITY OF WOUND INFECTION CAUSATIVE AGENTS OF *POTAMOTRYGON LEOPOLDI* (CASTEX & CASTELLO, 1970)

Skin ulcer microbiota of stingray *Potamotrygon leopoldi* was identified and its antibiotic susceptibility was detected. Isolated strains belonged to *Shewanella algae* and *Citrobacter freundii* and showed high resistance to 25 of 34 used antimicrobials.

Key words: stingray, wound infection, antibiotic susceptibility.

Introduction

Potamotrygon leopoldi is an endemic freshwater stingray restricted to the Xingu River Basin in Brazil. It is seldom taken for food but juveniles enter the ornamental fish trade due to the attractive colour pattern. The species faces persecution in some areas due to fear of sting injuries [2]. Although most cases are related to the riverside population in the natural environment of stingrays growing popularity of this fish as an aquarium inhabitant increases the risk of accidents at home. The local injury caused by these stingrays is due to mechanical penetration of the sting into the tissue and subsequent release of venom leading to the development of local edema, necrosis, intense local pain and cases of secondary infection [3].

To date, normal microbiota of *Potamotrygon leopoldi* has not been studied. Data about possible causative agents of infectious diseases of this species is also absent. However, any representative of stingray microbiota can cause severe tissue infection of human. In addition, there are no known means for treatment of any kind of infection in these animals themselves. Taking into account their high cost as ornamental fish information about effective antimicrobials can be very valuable for aquarists.

Objective

The objective of this study was to detect antibiotic susceptibility of two bacterial strains isolated from skin ulcer of *Potamotrygon leopoldi* female kept in aquarium.

Materials and methods

Sterile swabs were used for isolation of microorganisms from damaged stingray's skin. Petri plates with nutrient agar were then inoculated with obtained material and incubated overnight at 28 ℃ in the dark and during next 48 h in the light at room temperature. Isolated strains were identified by classical bacteriological methods and using API systems. Antibiotic susceptibility of cultures was detected by disk-diffusion method.

Results and discussion

Two different cultures of motile rod like Gram negative bacteria were isolated from skin ulcer on *Potamotrygon's* tail. Isolated strains were identified as *Shewanella* algae and *Citrobacter froundii* on the basis of their morphological, cultural, physiological and biochemical properties. Both species are rarely associated with human diseases. However, some strains of *C. freundii*, which is a part of normal gut microbiome, have been associated with opportunisitic nosocomial infections of blood, respiratory and urinary tract in immunocompromised patients [5]. Also, reports of *Shewanella* infections have been increasing [1, 4].

Taking into account mixed nature of skin infection we performed drug susceptibility test for isolated consortium of bacteria to estimate their mutual influence (table 1).

It was found that isolated microorganisms possessed high level resistance to 25 of 34 used antibiotics. Only levomycetin, levofloxacin, tetracycline, cefoperazone, ceftriaxone and cefixime were able to inhibit the growth of bacteria effectively.

The activity of meropenem, nalidixic acid and cefotaxime was estimated as moderate (table 1).

Conclusion

1. *Potamotrygon leopoldi* kept as an ornamental aquarium fish can be a potential origin of human infections caused by multi-resistant bacteria.

2. Cefalosporins, fluoroquinolones, tetracyclines and levomycetin can be considered potential chemotherapeutical means for treatment of skin ulcers of *Potamotrygon leopoldi* in aquarium conditions and also of human infections caused by contact with this species.

Table 1

Antibiotic sensitivity of isolated consortium of bacteria

№	Antibiotic	Result (growth inhibition zone. mm)
1	Azlocillin	R
2	Amoxycillin/clavulanate	R
3	Ampicillin	R
4	Ampicillin/sulbactam	R
5	Amphotericin B 40 μg	R
6	Amphotericin B 100 μg	R
7	Benzilpenicillin	R
8	Vancomycin	R
9	Carbenicillin	R
10	Clarithromycin	R
11	Clindamycin	R
12	Ketoconazole	R
13	Clotrimazole	R
14	Levomycetin	S (28)
15	Levofloxacin	S (16)
16	Lincomycin	R
17	Meropenem	RS (12)
18	Nalidixic acid	RS (10)
19	Nistatin	R
20	Oxacyllin	R
21	Penicillin	R
22	Rifampicin	R
23	Tetracycline	S (16)
24	Teicoplanin	R
25	Fluconazole	R
26	Ceftazidime	R
27	Cefotaxime	RS (11)
28	Cefoperazone	S (28)
29	Cefuroxime	R
30	Cefepime	R
31	Ceftriaxone	S (18)
32	Cefazoline	R
33	Cefixime	S (17)

Note: R – resistant, I – intermediate (moderately sensitive), S – sensitive

References

1. *Bulut C., Ertem G.T., Gokcek C., Tulek N., Bayar M.A., Karakoc E.* A rare cause of wound infection: *Shewanella putrefaciens* // Scand J Infect Dis. – 2004. – Vol. 36. – P. 692-694.

2. *Garrone Neto, D., Haddad Jr., V., Vilela, M.J.A., Uieda, V.S.* Registro de ocorrência de duas espécies de *Potamotrígonídeos* na região do Alto do Rio Paraná e algumas considerações sobre biologia // Biota Neotrop. – 2007. – Vol. 7. – P. 205–208.

3. *Potamotrygon leopoldi* http://dx.doi.org/10.2305/IUCN.UK.2009-2.RLTS.T39403A10226180.en

4. *Sharma K.K., Kalawat U.* Emerging Infections: *Shewanella*– A Series of Five Cases // J Lab Physicians. – 2010. – Vol. 2(2). – P. 61–65.

5. *Whalen J.G., Mully T.W., English J.C.* Spontaneous *Citrobacter freundii* Infection in an Immunocompetent Patient // Archives of Dermatology. – 2007. – Vol. 143 (1). – P. 124-125.

UDC 579.6:632.3

Kononiuk Yu.V., Adarma N.Yu., Melnikovich D.B., Limanska N.V.

Odessa National I.I. Mechnikov University, Dvorianska str., 2, Odessa 65082
e-mail: limanska@gmail.com

STIMULATION AND PLANT PROTECTION PROPERTIES OF *LACTOBACILLUS PLANTARUM*

Antagonistic and plant stimulation activities of *Lactobacillus plantarum* strains were studied. 50% of the studied strains-antagonists caused the complete inhibition of *Rhizobium radiobacter* C58 on a model of carrot explants *Daucus carrota* L. If crown gall appeared, the manifestation of symptoms was less as compared with the control. Stimulation activity was studied on a model of seedlings of *Quercus robur* L. Treatment with *L. plantarum* suspensions didn't improve the germination but positively effected the growth of seedlings (7,1-11,0% increase in plant height).

Key words: *Lactobacillus plantarum,* seedlings, crown gall, stimulation.

Introduction

Lactic acid bacteria are well known for their high antagonistic activities including the activity against plant pathogen [5]. But the studies of the effect of lactobacilli on crown gall agent *Rhizobium radiobacter,* by former nomenclature - *Agrobacterium tumefaciens* and *Agrobacterium vitis (Rhizobium vitis)* have been just recently started and need more attention from scientists.

The conception of Effective Microorganisms started in 1980s includes a wide use of lactic acid bacteria in fertilization and stimulation of plant growth [3].

The **aim** of the investigation was to study the possibility of some strains to inhibit the phytopathogen *Rhizobium radiobacter* C58 and to stimulate the growth of plants.

Materials and Methods

The effect of *Lactobacillus plantarum* strains on crown gall pathogenesis was studied on carrot roots (*Daucus carota* subsp. *sativus L.*). For test-object treatments, overnight culture of rhizobias in

LB-broth [1] (at concentration 10^8 CFU/ml) and experimental mixture of lactobacilli were mixed in a ratio of 1:1. Mixture of rhizobias overnight culture with sterile distillated water in a ratio of 1:1 was used as a positive control. The negative control was sterile distillated water. Lactobacilli were grown overnight in MRS medium [2].

Carrot roots were thoroughly washed in chlorine-containing detergent, rinsed in running water, dipped in ethanol and flamed, peeled from the external tissues, and then cut into discs with the thickness of 0,5 cm. Disks were placed in sterile Petri dishes with watered filter paper. On the surface of fresh cut discs (cambial ring) 100µl of rhizobial culture mixed with lactobacilli were applied. Positive and negative controls were applied the same way.

Disks were placed in sterile Petri dishes at 25 º C for 21 days, and after the results were evaluated by the following scale: «+ + + +» - 100% cambial ring covered with tumours, «+ + +» - 75% of cambial ring have tumours, «+ +» - 50 % cambial ring covered with tumours, «+» - less than 25% of cambial ring covered with tumours, «-» - no tumours.

To study the stimulation activity, a model of oak *(Quercus robur L.)* was used. 1% suspension of overnight *Lactobacillus plantarum* ONU 12 culture was applied to surfaces of acorns, and after incubation for 1 hour the suspensions was poured to the soil where the acorns were planted. Commercial soil with abundance of peat (Polisski Universalnii) was used. Germination and subsequent growth occurred under greenhouse conditions (20-22°C).

After germination, seedlings were poured with 1% suspensions of lactobacilli every week. After 2 months of cultivation, the heights of seedlings were measured.

Results and Discussion

The studied strains of lactobacilli prevented the crown gall infection in the majority of cases (Table 1).

50% of the studied strains-antagonists caused the complete inhibition of *Rhizobium radiobacter* C58. Other strains inhibited infection in 60% of cases (Table 1).

Only one strain - *L. plantarum* ONU 365 - was the less effective - it inhibited gall formation only in 30% of samples.

It can bee seen that the studied strains of lactobacilli are the effective antagonists against crown gall agent on carrot discs. The further experiments should include more plant species used as test models.

Literature data show perspective results of treating agriculturally important plants with lactic acid bacteria to stimulate their growth [3, 4].

We were interested in applying lactobacilli to a plant well studied in forestry but not from the view of treatment with lactic acid bacteria - to oak *Quercus robur L.* We found out that applying *L. plantarum* suspensions didn't improve the germination: it reached 83,3% both in a control just treated with water and in experimental acorns inoculated with lactobacilli. But the mean height of seedlings was 7,1-11,0% increased in plant treated with *L. plantarum* ONU 12 suspension. Next experiments with other concentrations of overnight bacterial suspensions will be carried out.

Table 1

Formation of gall on carrot discs by *Rhizobium radiobacter* C58 in presence of antagonists

Antagonistic strains	Results of three independent experiments		
L. plantarum 011	0	0	0
L. plantarum ONU 365	0	+	+
L. plantarum ONU 337	0	0	0
L. plantarum ONU 471	0	0	++
L. plantarum ONU 356	0	0	0
L. plantarum ONU 355	0	0	0
L. plantarum ONU 475	0	0	++
L. plantarum 06	0	0	+
L. plantarum ONU 474	0	0	0
L. plantarum ONU MFL	+	0	0
-(control)	++	+++	++

Conclusions

Strains *L. plantarum* 011, *L. plantarum* ONU 337, *L. plantarum* ONU 356 , *L. plantarum* ONU 355, *L. plantarum* ONU 474 are the active antagonists against the crown gall agent. *L. plantarum* ONU 12 can be used as plant growth stimulator.

Literature

1. Bertani G. Studies on lysogenesis. I. The mode of phage liberation by lysogenic *Escherichia coli* // Journal of Bacteriology. – 1951. – Vol. 62. – P. 293-300.

2. de Man J.C., Rogosa M., Sharpe M. E. A medium for the cultivation of lactobacilli // Journal of Applied Bacteriology. – 1960. – № 23. – P. 130-135.

3. Higa T., Kinjo S. Effect of lactic acid fermentation bacteria on plant growth and soil humus formation // In: Proceedings of 1st International Conference on Kyusei Nature Farming. - 1989. – P. 140-147.

4. Merlich A.G., Limanska N.V., Zhunko I.D., Babenko D.O. Effect of *Lactobacillus plantarum* and *Bacillus atrophaeus* on germination and growth of wheat seedlings // Microbiology and Biotechnology. - 2017. - №1. - P. 36 - 47.

5. Visser R., Holzapfel W. Lactic acid bacteria in the control of plant pathogens // The Lactic acid Bacteria. - Elsevier Science Publishers. - 1992. – P. 193 – 194.

UDC 579.6:606

Matkovska A.I., Strashnova I. V., Jamborko G. V., Ischenko T. A.

Odessa I.I. Mechnikov National University,
Dvoryanskaya str., 2, Odessa, 65082, Ukraine;
E-mail:nastya.mmm96@gmail.com

THE ANTAGONISTIC PROPERTIES OF LACTOBACILLI ISOLATED IN DIFFERENT REGIONS

Abstract

Antagonistic activity of Lactobacillus has been tested for 34 strains isolated from different ecological niches and five collection strains. Agar-well diffusion method was used to test the antagonistic effect. Test cultures were C. albicans, E.coli, B. subtilis, P. aeruginosa та S. aureus.

Among all investigated strains of Lactobacillus 79,5 % showed antagonistic activity of at least one of the test cultures.The most antagonistic active were strains of Lactobacillus isolated from pickled eggplants (Odessa region).

Key words: antagonistic effect, Lactobacillus spp., agar-well diffusion method

Introduction

Search for new strains of bacteria of the genus *Lactobacillus*, which exhibit high antagonistic activity is an important and promising area of microbiology. Lactobacilli inherent antagonistic properties, so that they inhibit the growth of unwanted microbiota during production and the finished product, and adjust dysbiotic violation.

There are different mechanisms for control and inhibition of other microbes, e.g. nutrient competition, production of inhibitory compounds, immunostimulation and competition for binding sites. Among these activities, the production of organic acids (such as lactic acid), which results in lowered pH, is the most important. Additionally, certain strains are also capable of producing bioactive molecules, such as ethanol, formic acid, fatty acids, hydrogen peroxide and bacteriocins, that have antimicrobial activity [2, 4]. The aim of this study was to determine the antagonistic activity bacteria *Lactobacillus* isolated from the different habitats and different regions and to determine the extent of their antagonistic activity.

Materials and methods

Research materials were 34 strains of bacteria of the genus *Lactobacillus,* isolated from different habitats: from neonatal gastrointestinal (Odessa region): *Lactobacillus spp.* 13, 20, 87, 146, 175, 275, 291, 892, 921, 12λ; from raw meat (Odessa region): *Lactobacillus spp.*M1, M2, M3, M4, M5, M6; from pickled eggplants (Odessa region): *Lactobacillus spp.*B1, B3, B4, B5, B6; from pickled vegetables (Vietnam): *Lactobacillus spp.*5, 6, 8, 13, 14b, 24b, 29b, 31b, 32, 54m; from pickled cucumbers (Sweden): *Lactobacillus spp.*O1, O5, O6 (tab. 1) and 5 collection strains: *L. buchneri* ATCC 4005, *L. acidophilus* ATCC 32200, *L. plantarum* VTCC 0921, L. plantarum UCM B 11/16 and *L. plantarum* UCM B 2209.

The antagonistic properties of the isolated strains against test cultures: *Candida albicans* UCM У 2501^{T}, *Escherichia coli* UCM B 906, *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa* were determined.

Agar-well diffusion method was used to determine the inhibitory effect [1]. To determine antagonistic activity previously prepared daily culture of lactic acid bacteria and test cultures. Then spent preparing microbial suspension test cultures of microorganisms daily bouillon cultures for cell concentration of 1,5 × 10^{8} CFU/cm^{3}. Then 0.1 cm^{3} bacterial suspension test cultures bring in the prepared of the agar plates. Wells, 8 mm in diameter, were punched in the agar plates and 0,2 cm^{3} of daily bouillon native culture lactobacilli were added to the wells. After incubation overnight at temperature conditions indicator test cultures, the antimicrobial activity was expressed as the diameter of the zones lack of growth (mm) around the wells [1].

The extent of antagonist activity was determined by the following criteria:

- lack of growth zone diameter 1 - 15 mm - low antagonistic activity;
- lack of growth zone diameter of 16 - 25 mm - middle antagonistic activity;
- lack of growth zone diameter of 25 mm or more - high antagonistic activity [1].

Results and discussions

Antimicrobial activity is a very important criterion for selection of starter and probiotic culture as natural antagonists of potentially harmful bacteria. Antagonistic activity of *Lactobacillus* has been tested for 34 strains isolated from different ecological niches and five collection strains.

Nearly all strains of lactic acid bacteria isolated from the

gastrointestinal tract of infants (except *Lactobacillus sp.* 87, *Lactobacillus sp.* 921) antagonists were quite active against gram-negative bacteria *E. coli* and *P. aeruginosa*, as well as gram-positive microorganisms *S. aureus*. *Lactobacillus sp.* 175 showed the highest antagonistic activity against these test cultures diameter zones lack of growth varied within the following limits: *E. coli* – 28,33 ± 1,73 mm, *P. aeruginosa* – 25,66 ± 2,36 mm, *S. aureus* – 23,0 ± 1,13 mm.

Among the bacteria of the genus *Lactobacillus*, isolated from raw meat *Lactobacillus spp.* M1, M2, M3 and M6 showed themselves as active antagonists against all prokaryotic test cultures.

The most active antagonist of *Lactobacillus* strains Odessa region was isolated from pickled eggplant. The most active antagonist for test cultures *E. coli, B. subtilis* and *P. aeruginosa* was strain B1, lack growth areas were 37,66 ± 1,73 mm, 31,0 ± 1,13 mm and 33 0 ± 2,26 mm respectively.

Lactobacilli isolated from pickled vegetables from other regions characterized by antagonism less pronounced compared to the strains of the Odessa region.

The lowest activity showed strains of bacteria of the genus *Lactobacillus*, isolated from Vietnamese pickled vegetables. We can assume that there is a relationship presence of antagonistic activity of *Lactobacillus* region of origin. Less antagonistic activity of these strains may be associated with the transportation device isolates and strains to other conditions exist [3, 5].

It is interesting to note that among all tested lactobacilli isolated from different regions of only one strain inhibited the growth of *C. albicans* – *Lactobacillus sp.*54m isolated from pickled vegetables (Vietnam Origin).

Collection strains bacteria genus Lactobacillus showed less antagonist activity.

Results determination the extent of antagonistic activity of lactic acid bacteria, are shown in Fig. 1.

The nearly half of the investigated lactobacilli demonstrated a high extent of antagonism against gram-negative microorganisms. The third of strains was characterized of middle antagonistic activity against gram-positive test cultures.

The low extent of antagonist activity was inherent in a small number of bacteria of the genus *Lactobacillus.*

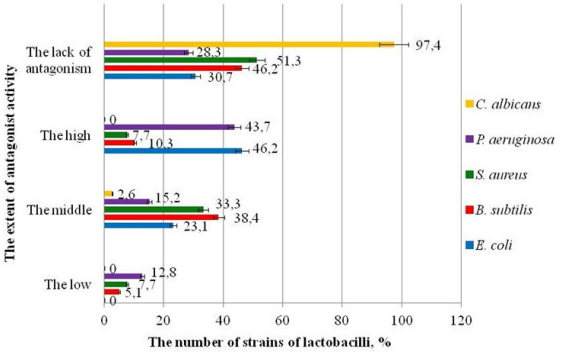

Fig. 1. Average the extent antagonistic activity of *Lactobacillus sp.* in relation to the test cultures

Conclusions

So during this study were able to identify 39 inherency antagonistic properties of *Lactobacillus* strains, of which 79.5% lactobacilli showed antagonistic activity against at least one of the test cultures. Five strains (*Lactobacillus spp.* B1, B3, B4, B5 and B6), which is isolated from pickled eggplant (Odessa region) are the most active antagonist for prokaryotic microorganisms.

References

1. *Иркитова А. И., Качан Я. Р., Соколова Г. Г.* Сравнительный анализ этапов определения антагонистической активности молочнокислых бактерий // Известия Алтайского государственного университета. – 2012. – Т. 3, № 1. – С. 41 – 44.

2. *Соловьева И .В., Точилина А. Г., Новикова Н. А. и др.* Изучение биологических свойств новых штаммов рода *Lactobacillus* // Вестник Нижегородского университета им. Н. И. Лобачевского – 2010. – № 2 – С. 462 – 468.

3. *Al-Madboly L. A., Abdullah A. K.* Potent antagonistic activity of Egyptian *L. plantarum* against multiresistant and virulent food-associated pathogens // Front. Microbiol. – 2015. – Vol. 6. – Art. 347.

4. *De Vuyst L. Leroy F.* Bacteriocins from lactic acid bacteria: production, purification, and food applications. J Mol Microbiol Biotechnol. 2007;13(4):194–199.

5. *Georgieva R., Yocheva L., Tserovska L.* Antimicrobial activity and antibiotic susceptibility of *Lactobacillus* and *Bifidobacterium* spp intended for use as starter and probiotic cultures // Biotechnology, Biotechnological equipment. – 2015. – Vol. 29. – P. 84 – 91.

UDC 579.6:606

Andrii Merlich

Microbiology, Virology and Biotechnology Chair, Biology Department, Odesa National I.I. Mechnykov University, 65000 Dvorianska str., 2, Odesa, Ukraine
andriymerlich@gmail.com

PURIFICATION OF BACTERIOCIN FROM *ENTEROCOCCUS ITALICUS* ONU547 BY REVERSED PHASE-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Abstract

Pre-purified fractions of bacteriocin from *E. italicus* ONU547 were subjected to further purification by RP-HPLC with various methods. Two types of analytical columns (RP C_8 and $C1_8$), gradient and step elution methods were used for experiment. After using of both columns with gradient elution method on chromatograms two peaks were observed, which fraction exhibited antagonistic activity against *L. sakei* that could indicate the two component nature of the studied bacteriocin. The most effective was the RP-HPLC with C^8 column performed by step elution method resulted in bacteriocin activity given the zone of inhibition with diameter 1,75 sm. The purified fraction can be used for further study and characterization of bacteriocin.

Key words: bacteriocin, purification, *Enterococcus italicus,* reversed phase-high performance liquid chromatography

Introduction

Bacteriocins are ribosomally synthesized antimicrobial peptides produced by variety of bacterial species including lactic acid bacteria (LAB) [3]. The bacteriocins were suggested as biopreservatives in foods due to antagonistic activity against food-borne pathogens [4] and their anti-infective effect is a promising alternative to antibiotics [1]. Purification of bacteriocin is an important procedure needed for its study and characterization [7].

The aim of this study was to purify bacteriocin from *Enterococcus italicus* ONU547 by reversed phase-high performance liquid chromatography by various methods.

Material and methods

In the experiment were used the fractions of bacteriocin pre-purified before by ammonium sulfate precipitation, cation-exchange

and hydrophobic chromatographies according to Hwanhlem et al. (2013) [6] with some modifications. For performing of reversed phase-high performance liquid chromatography (RP-HPLC) the two types of analytical columns were used – C_8 (Symmetry C_8, 3,5 µm, Ireland) and C_{18} (Symmetry C_{18}, 3,5 µm, Ireland). 100 µl of bacteriocin active fraction were injected into the analytical columns that were equilibrated with solvent A (98% MilliQ water / 2% acetonitrile / 0,05% TFA). The elution was performed by two methods: with the linear gradient of solvent B (20% MilliQ water / 80% acetonitrile / 0,04% TFA) and step elution by different concentrations of acetonitrile. The detected peaks were manually collected and the acetonitrile was evaporated by Speed-Vac concentrator, the pH was neutralized and activity of bacteriocin tested by agar well diffusion assay against indicator *Lactobacillus sakei* [5].

Results and discussions

First method of elution was the gradient elution performed by solvent B. After using of C_8 as well as $C1_8$ analytical columns two peaks were observed on chromatograms with retention time of 8-9 minutes. The fractions of two peaks were collected together and when tested by agar well diffusion assay against *L. sakei* showed inhibitory activity. It could indicate the two component nature of the studied bacteriocin. The diameter of inhibitory zone of pre-purified fractions before RP-HPLC was 1,7 sm and after using of C_8 column the diameter of inhibition zone was 0,9 sm and those of C_{18} – 1,3 sm. It indicates the decrease of bacteriocin activity after purification with using of this method. The decreasing of total activity of bacteriocin after procedures of purification is in agreement with literature data [2].

The next purification procedure was performed by RP-HPLC with step elution using different concentration of acetonitrile. In result, on obtained chromatogram several peaks were observed (Fig. 1) fractions of which were collected and activity was tested against *L. sakei.*

In case of C_8 column only first peak eluted by 100% acetonitrile showed antagonistic activity against target strain *L. sakei* (Fig. 2) with diameter of zone of growth inhibition of 1,75 sm. The fractions of all other peaks did not exhibit activity of bacteriocin that indicates the nature of contaminant which could be presented by non-bacteriocin proteins.

The activity of eluted fraction by this method was less in the case of C_{18} analytical column and diameter of inhibition zone was only 1 sm that can be explained by higher hydrophobicity of column matrix and irreversible binding of bacteriocin to it resulted in decreasing of activity.

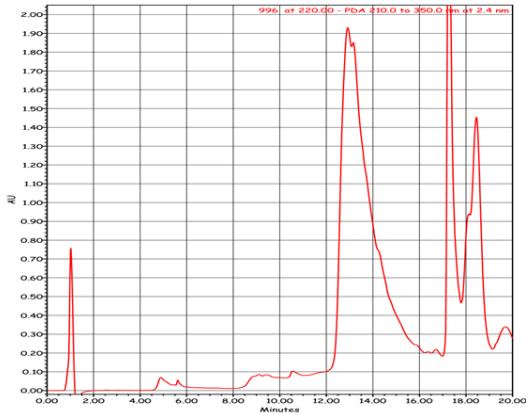

Fig. 1. Chromatogram of purified bacteriocin by RP-HPLC with C_8 column performed by step elution with different concentration of acetonitrile: 1-20%, 2-30%, 3-40%, 4-5-100%

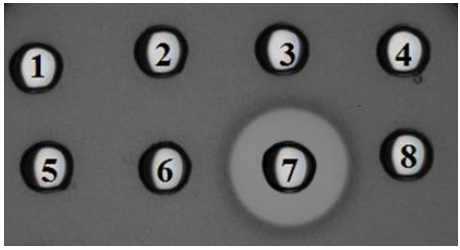

Fig. 2. Antagonistic activity of fractions after RP-HPLC with C8 column tested by agar well diffusion assay against indicator *L. sakei* eluted by different concentration of acetonitrile: 1 – 20%, 2 – 3 – 30%, 4 – 6– 40%, 7 – 100% 1 peak, 8 – 100% 2 peak

Conclusion

After using of C8 as well as $C1^8$ analytical columns with gradient elution method two peaks were seen with retention time of 8-9 minutes and the fractions from these two peaks showed antagonistic activity against indictor *L. sakei* when tested by agar well diffusion assay. The most effective method of bacteriocin purification was RP-HPLC with C8 column performed by step elution method resulted in bacteriocin activity given the zone of inhibition with diameter 1,75 sm. The purified fraction can be used for further study and characterization of bacteriocin.

Literature

1. *Alvarez-Sieiro P., Montalbán-López M., Mu D., Kuipers O. P.* Bacteriocins of lactic acid bacteria: extending the family // Appl. Microbial Biotechnol. – 2016. – V 100 (7). – P. 2939 – 2951.

2. *Casaus P., Nilsen T., Cintas L. M., Nes I. F., Hernández P. E.* and *Holo H.* Enterocin B, a new bacteriocin from *Enterococcus faecium* T136 which can act synergistically with enterocin A // Microbiology. – 1997. – V. 143. – P. 2287 – 2294

3. *Elayaraja S., Annamalai N., Mayavu P., Balasubramanian T.* Production, purification and characterization of bacteriocin from *Lactobacillus murinus* AU06 and its broad antibacterial spectrum // Asian Pacific Journal of Tropical Biomedicine. – 2014. – 4 (Suppl 1). – P. 305 – 311.

4. *Franz Ch. M. A. P., Grube A., Herrmann A., Abriouel H., Stärke J., Lombardi A., Tauscher B., and Holzapfel W. H.* Biochemical and genetic characterization of the two-peptide bacteriocin enterocin 1071 produced by *Enterococcus faecalis* FAIR-E 309 // Applied and Environmental Microbiology. – 2002. – V. 68, №5. – P. 2550 – 2554.

5. *H–Kittikun A., Biscola V., El–Ghaish Sh., Jaffrès E., Dousset X., Pillot G., Haertlé T., Chobert J-M, Hwanhlem N.* Bacteriocin-producing *Enterococcus faecalis* KT2W2G isolated from mangrove forests in southern Thailand: Purification, characterization and safety evaluation // Food Control. – 2015. – V. 54. – P. 126 – 134.

6. *Hwanhlem N., Biscola V., El-Ghaish Sh., Jaffrès E., Dousset X., Haertlé T., H-Kittikun A., Chobert J. M.* Bacteriocin-producing lactic acid bacteria isolated from mangrove forests in southern Thailand as potential bio-control agents: purification and characterization of bacteriocin produced by *Lactococcus lactis* subsp. *lactis* KT2W2L // Probiotics & Antimicro. Prot. – 2013. – V. 5. – P. 264 – 278.

7. *Perez R. H., Zendo T., Sonomoto K.* Novel bacteriocins from lactic acid bacteria (LAB): various structures and applications // Microbial Cell Factories. - 2014. - 13 (Suppl 1). - P. 1 - 8.

UDC 633.11:631.085:581

Pykalo Serhii

The V.M. Remeslo Myronivka Institute of Wheat NAAS of Ukraine
08853, Ukraine, v. Tsentralne, Myronivka district, Kyiv region
pykserg@ukr.net

TOLERANCE OF CALLUS LINES OF TRITICALE TO OSMOTIC AND SALT STRESSES

Triticale (×*Triticosecale* spp. Wittmack ex A. Camus 1927) is an artificial grain obtained through the hybridization of wheat (*Triticum durum* Desf. or *Triticum aestivum* L.) and rye (*Secale cereale* L.). Advantages of triticale are the high yielding capacity and good quality grains intended for feed. However, deterioration of ecological situation causes insistent necessity to improve triticale resistance to some abiotic factors. The level of cross-tolerance of winter triticale callus lines obtained both to salt and osmotic stress was analyzed. Forms of three-species hexaploid winter triticale bred at Myronivka Line 38/1296 and variety Obrii Myronivskyi were studied. Tissue culture methods with application of selective systems based on mannitol and sodium chloride were used. It was established that the stability of performance of cross-tolerance to salt and osmotic stress in callus lines obtained was sufficiently high – of 50 to 76% calli survived to the end of the sixth passage. It was shown that in the presence of sublethal concentrations of the stress-factors in selective medium tolerant calli actively continued to accumulate biomass. Estimation of tolerance to abiotic stressors demonstrated high enough level of cross-tolerance of Triticale callus lines obtained for both salt and osmotic stresses.

Key words: Triticale, salt stress, osmotic stress, tolerance, callus lines

Introduction

Being important for triticale breeding improvement, tolerance to abiotic stressful environmental factors, including drought and soil salinization will encourage expanding the crop areas in regions with unfavorable climatic conditions [1, 2]. Solving the problem of stress tolerance requires the development of new biotechnological approaches. One of them is cell selection, i.e. selection of genotypes with desirable new hereditary characters at *in vitro* cultured cells level in specific conditions [3, 4]. The formation of complex plant tolerance to abiotic stresses (salt,

osmotic, temperature) at the cellular and tissue level has some similar mechanisms [5]. The results give evidence that cell adaptation to osmotic stress can be applied to select salt-tolerant lines or the reverse, and such studies are of interest. The purpose of the research – to analyze the level of cross-tolerance of salt- and drought-tolerant callus lines of winter triticale both to osmotic and salt stresses.

Material and methods

Carrying the genomes of three species hexaploid winter triticale forms bred at Myronivka, namely Line 38/1296 and cultivar Obrii Myronivskyi that are characterized by high agronomic traits were studied. Callus lines of these genotypes were obtained in our previous studies [6] by cell selection for tolerance to salt and osmotic stresses. Analysis of cross-tolerance of triticale osmotolerant callus lines to salinity was carried out according to the scheme: selective medium with 1.2% NaCl (2 passages) → Murashige and Skoog (MS) medium [7] (2 passages) → selective medium with 1.2% NaCl (2 passages). Analysis of cross-tolerance to salt-tolerant callus lines osmotic stress was conducted along similar lines: selective medium with 0.6 M mannitol (2 passages) → MS medium (2 passages) → selective medium with 0.6 M mannitol (2 passages). Tolerance of callus cultures to ionic and osmotic stresses was assessed by their survival and wet weight growth. The experimental data obtained were processed with the methods of statistical analysis [8].

Results and discussion

It is established that stability of expression of cross-tolerance to salt stress lines derived was high enough – to the end of the sixth passage of 51 to 75% calli survived. When determining the crude weight increment, resistant callus were revealed to continue growth despite the presence of sublethal concentrations of NaCl in selective medium. In general, tolerant cell lines 3L/os and 5L/ derived from the Line 38/1296, as well as lines 1C/os and 4C/os derived from the variety Obrii Myronivskyi should be identified, whereas they were characterized by the highest proportion of alive calli and kept the ability to increase biomass in selective conditions. Cell lines with cross-tolerance were distinguished with following morphological characteristics: compact yellow callus of globular structure.

Based on the existence of both specific and general system of tolerance [5], we also analyzed cross-tolerance of salt-tolerant callus lines to osmotic stress. As in previous studies, tolerance of cell cultures to osmotic stress was assessed by survival and growth of wet weight. During the research we found that the stability of expression of cross-tolerance to

osmotic stress in cell lines obtained was at high enough level – by the end of the sixth passage of 50 to 76 % calli survived. It was also shown that, even in the presence of sublethal concentrations of mannitol in selective medium resistant calli actively continued to increase biomass.

Thus, as a result of consistent work we have found cross-tolerance of triticale callus lines obtained as to salt and osmotic stresses. Earlier T.V. Chugunkova [9] by methods of cell selection on selective media added by sodium sulphate and sodium chloride has obtained callus line of sugar and fodder beet resistant to both the individual and the complex stress factors, including toxin of bacteriosis pathogen, low positive temperature as well as one type of salinity –chloride or sulfate. Test for stability of tolerance to the complex of stress factors proved the tolerance of most beet callus lines and regenerated plant derived from them. In selective system with PEG salt-tolerant rice clones were obtained [10]. Among corn plants regenerated from mannitol tolerant callus the forms with increased tolerance not only to water deficit, but also to salinity, low positive and negative temperatures were selected [11]. The authors explain it to the fact that due to general non-specific mechanisms of tolerance the resistance to alone adverse factor may lead to increase to the other one.

Conclusions

Test of tolerance to abiotic stressors showed high enough level of cross-tolerance of triticale callus lines obtained as to salt and to osmotic stresses. It is shown that in the presence of sublethal concentrations of the stress-factor in selective medium tolerant calli kept accumulating biomass actively. In prospect it is necessary to test maintenance of tolerance in regenerated plants and their seed generations.

References

1. Blum A. The abiotic stress response and adaptation of triticale – a review. Cereal Res. Commun. 2014; 42(3): 359-375.

2. Avdeev YuI, Slashcheva LA. Stability of winter Triticale to extreme abiotic factors of the environment in the arid zone of cultivation. Astrakhanskiy Vestnik Ekologicheskogo Obrazovaniya – Astrakhan Bulletin of Ecological Education. 2014: 29(3): 84-87.

3. Dubrovna OV, Morgun BV. Cellular selection of wheat for resistance to stress factors of environment. Fiziologiia i Biokhimiia Kul'turnykh Rastrenii – Physiology and Biochemistry of Cultivated Plants. 2009; 41(6): 463-475.

4. Reshetnikov VN, Spiridovich EV, Nosov AM. Plant biotechnology and perspectives of its development. Fiziologiya Rastenii i Genetika – Plant Physiology and Genetics. 2014; 46(1): 3-18.

5. Shakirova FM. Non-specific Plant Resistance to Stress Factors and its Regulation. Ufa: Gilem; 2001. 160 p.

6. Pykalo SV, Zinchenko MO, Voloshchuk SI, Dubrovna OV. In vitro selection of winter triticale for the resistance to water deficit. Biotechnologia Acta. 2015; 8(2): 69-77.

7. Murashige T, Skoog F. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant. 1962; 15(3): 473-497.

8. Lakin GF. Biometry. Moscow: Vysshaya Shkola; 1990. 352 p.

9. Chugunkova TV. The using of cell selection for creation of resistant forms of beet. Fiziologiia i Biokhimiia Kul'turnykh Rastrenii – Physiology and Biochemistry of Cultivated Plants. 2009; 41(6): 509-515.

10. Belyanskaya SL, Shamina ZB. Obtaining and characteristics of rice clones resistant to stress factors. Fiziologiya Rasteniy – Russian Journal of Plant Physiology. 1993; 40(4): 681-685.

11. Al-Kholani KhAM. Obtaining stress-tolerant maize plants by cellular selection. Thesis Abstract for Candidate of Science (Biology): 03.01.05. Moscow; 2010. 24 p.

UDK 582.282.23.045

Ovchinnikov S., Vasyil'yev M., Rusakova M.

Biotechnological Research and Training Center Odessa National I.I. Mechnikov University
Dvoryanska str. 2, 65082 Odessa, Ukraine
ovchinnikovseryi@gmail.com

THE ANTI-CANDIDA ACTIVITY OF NEW N-METHYL-QUINOLINYL-PORPHYRINS *IN VITRO*

The activity of new N-methyl-quinolinyl-porphyrins against different morphological forms of *Candida albicans* (unattached, free-dividing cells, as well as structures formed in the process of biofilm maturation) was characterized. Modification of porphyrin molecules by N-methyl-6-quinolinyl increases the fungicidal effect on the yeast cells, in contrast to N-methyl-7-quinolinyl derivatives that are effective against hyphal structures. *Candida albicans* biofilm cells are more resistant to low concentrations of the studied compounds.

Key words: *Candida albicans*, biofilm, planktonic cells, porphyrin.

Porphyrins are macroheterocyclic compounds, containing conjugated system, which is based on four pyrrole rings linked by four methine groups in α-positions. Two classes of porphyrins, natural and synthetic, can be distinguished on the basis of their origin and structure. More than 50 different metals can be introduced into the porphyrin ring to create a wide variety of metalloporphyrins in both classes [1].

The *Candida albicans* fungus commonly colonizes the epithelial surfaces of human organism. Impairment of innate and adaptive host defenses, perturbation of normal microbiota or underlying disease can contribute to fungal overgrowth and candidiasis progress. In the course of this infection *C. albicans* forms a massive biofilm on the large surface of affected tissues and organs. *C. albicans* also grows as a biofilm on prosthetic devices, contributing to the failure of antifungal therapy and recurrent infection.

Treatment of superficial *C. albicans* infections with photosensitizing agents and light, termed photodynamic therapy, offers an alternative to conventional treatments. In the presence of molecular oxygen, the irradiation of photosensitizers with the appropriate wavelength of light results in the local production of singlet oxygen, which rapidly oxidizes

cellular macromolecules found nearby, leading to cell damage and death [4].

The *Candida albicans* ATCC 18804 strain obtained from the Microbiology, Virology and Biotechnology Department culture collection was used as the test object. For the experiment 18-24-hrs *C. albicans* cultures that were grown on Nutrient Agar at 37 ^{0}C were used. The working microbial suspension contained 10^6 CFU/ml.

The studies were carried out for a synthetic *meso*-N-methyl-quinolinyl-porphyrins -substituted free porphyrin bases.

The *C. albicans* cultivation in the presence of the studied porphyrins was carried out in sterile polystyrene plate in Sabouraud nutrient medium. The porphyrin concentrations in the culture medium corresponded to the values presented in the following work [2].

The following parameters were noted: planktonic biomass (cells that have developed in the suspension) and the biofilm formation (cells that developed on «solid surface (the well bottoms) – liquid (culture medium)» phase edge). The obtained data were calculated with a spectrophotometer BioTek «μQuant» (at 540 nm and 592 nm, respectively) [3].

The studied culture was sensitive to the porphyrin action.

After 24 hrs the most active compounds was N-methyl-6-quinolinyl porphyrin that inhibited growth by 80 % in comparison with the control value. On the 2nd cultivation day the cell number in suspension did not exceed 60 % of the control.

Formation of *C. albicans* biofilm was subjected to influence of the studied porphyrins, as on the 1st and the 2nd day. The greatest decreasing of the biofilm formation intensity takes place in the presence of the substance N-methyl-7-quinolinyl porphyrin (24–48 hrs) and substances N-methyl-6-quinolinyl porphyrin (48 hrs). However, the most of the results was above the reference values, in some cases up to 2 times higher than that.

So, therapy with porphyrins has the potential to evolve into a useful treatment for difficult to eradicate fungal infections of accessible regions of the body. For example, the prospect of eradicating oral thrush in an AIDS patient, or denture stomatitis in an elderly nursing home resident in a single session, or a once-off curative treatment for *Candida* mycosis, is a scenario that would be attractive to both patients and health service providers [3]. However, this will not become a clinical reality until pharmaceutical companies and grant-awarding bodies devote considerable resources to the development of both photosensitisers specifically designed for antifungal treatment and drug delivery systems that allow such agents to be efficiently delivered to their sites of action.

References:

1. Soman R., Raghav D., Sujatha S., Rathinasamy K., Arunkumar C. RSC Advances, 2015, 5 (75), 61103-61117.

2. Kato I.T., Prates R.A., Sabino C.P. , Fuchs B.B. , Tegos G.P. Antimicrobial agents and chemotherapy, 2013, 57 (1), 445-451.

3. Junior J.C., Sabino C.P., Tan X., Junqueir J.C., Wang Y. BMC microbiology, 2013, 13 (1), 217.

4. Mizuno K., Zhiyentayev T., Huang L., Khalil S., Nasim F., Tegos G.P., Gali H. Journal of nanomedicine & nanotechnology, 2011, 2 (2), 1.

UDK 582.282.23.045

Palamarchuk A., Rusakova M.

Department of Microbiology, Virology and Biotechnology, Odessa National I. I. Mechnikov University
Dvoryanska str. 2, 65082 Odessa, Ukraine
E-mail: anastasia.pdl.09@gmail.com

THE RHIZOSPHERE MICROBIOTA INFLUENCE ON THE *FUSARIUM* FUNGI

The influence of bacterial cultures *(B. megaterium* ONU 500, *B. subtilis* ONU 410, *P. fluorescens* ONU 303, *P. chlororaphis* ONU 305) on the *Fusarium (F. oxysporum, F. graminearum)* growth was determined by the methods of agar block and well co-cultivation. The most expressed anti-fusarium effect was characterized for *P. chlororaphis* ONU 304.
Key words: *Pseudomonas* sp., *Bacillus* sp., *Fusarium* sp., rhizosphere.

The rhizosphere is the zone of soil surrounding a plant root where the biology and chemistry of the soil are influenced by the root. This zone is about 1 mm wide, but has no distinct edge. Rather, it is an area of intense biological and chemical activity influenced by compounds exuded by the root and by microorganisms feeding on the compounds [1]. Bacteria, actinomycetes, fungi, protozoa, slime moulds, algae, nematodes, earthworms, millipedes, centipedes, insects, mites, snails, small animals and soil viruses compete constantly for water, food and space. Soil chemistry and pH can influence the species mix and functions of microbes in the rhizosphere [2].

In the natural environment, microbial root colonization leads to multiple types of physical and chemical interactions between microorganisms and plants. These interactions can vary from neutral to beneficial on the one side, and deleterious on the other side when plant pathogenic microorganisms are involved To complicate matters, microorganisms can transition between pathogenic and symbiotic states depending on environmental conditions [1]. Many non-pathogenic soil bacteria have the ability to promote the growth of plants and, therefore, are often designated as plant growth-promoting rhizobacteria.

Soil suppressiveness is the phenomenon that in spite of the presence of a virulent pathogen and a susceptible host plant, disease does not occur [4].

Specific suppression of plant pathogens has been found for representatives of a wide variety of bacterial genera, including *Agrobacterium, Alcaligenes, Arthrobacter, Bacillus, Enterobacter, Erwinia, Pseudomonas, Rhizobium, Serratia, Streptomyces* and *Xanthomonas.* Efficient root colonization and establishment of biocontrol bacteria is of key importance for effective suppression of deleterious organisms [3].

The work was carried out on the basis of Biotechnological Scientific Educational Center of I. I. Mechnikov Odessa National University. The studied strains were representatives of the genera *Bacillus (B. megaterium* ONU 500, *B. subtilis* ONU 410), *Pseudomonas (P. fluorescens* ONU 303, *P. chlororaphis* ONU 305) and *Fusarium (F. oxysporum* BSEC I, *F. graminearum* BSEC I). The influence of bacterial cultures on the fungal growth was determined by the co-cultivation of microorganisms on Nutrient Agar surface by the methods of agar blocks and wells.

The pre-cultivation of *Bacillus* strains was on Nutrient Agar, *Pseudomonas* ones – on King B agar. *Fusarium* spp. were cultured on Potato Dextrose Agar for 5 days. The applied media were containing all the necessary nutrients for microbial growth.

The plates with the studied co-cultivated microorganisms were incubated at 22 °C for 8 days. Every 24 hrs the fungal growth inhibition zone diameter (in mm) around the bacterial strains (wells or blocks) was measured. The antifungal zone diameter was calculated as the average of 3 replications of randomly chosen diameter measurements.

The mutual microbial influence studying by the agar block method detected the presence or absence of common nutrient needs and also takes into account the microbial growth rate that influence on colonization speed of ecological niches, including rhizosphere. It was determined that the antagonism of the studied bacterial species to fungi have species and strain specific characteristics (fig. 2). The maximum antifungal effect of the most strains increased during the first two days. But *F. graminearum* was more stable than *F. oxysporum* to *P. chlororaphis* and *P. fluorescens* influence.

During the research by means of the second method the wells in the dense medium allowed to distinguish exometabolite contribution to the microorganism interaction development. It was possible to take into account the different nutritional needs of the consortium members by means of two media: liquid – in holes and dense – plate layer.

The most expressed anti-fusarium effect was characterized for *P. chlororaphis* ONU 304 on the fourth day of incubation. The determination

of the sensitivity of fungal cells to *P. chlororaphis* showed that the largest area of the growth delay was noted for *F. oxysporum* and was 25.0 mm on the eighth day of cultivation. As for the genus *Bacillus*, the maximum antifungal effect was observed for *B. megaterium* ONU 500 also to F. oxysporum.

Thus, the study of the microbial group functioning, including the rhizosphere microbiota, will improve the understanding of their formation and to develop approaches to create new therapeutic and prophylactic biological products.

References

1. Newton A.C., Fitt B.D.L., Atkins S.D., Walters D.R., Daniell T.J. Pathogenesis, parasitism and mutualism in the trophic space of microbe-plant interactions // Trends Microbiol. – 2010. – Vol. 18. – P. 365–373.

2. Raaijmakers J.M., Paulitz T.C., Steinberg C., Alabouvette C., Moënne L.Y. The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms // Plant Soil. – 2009. – Vol. 321. – P. 341–361.

3. Weller D.M., Raaijmakers J.M., McSpadden G.B.B., Thomashow L.S. Microbial populations responsible for specific soil suppressiveness to plant pathogens // Annu. Rev. Phytopathol. – 2002. – Vol. 40. – P. 309–348.

4. Weller D.M. *Pseudomonas* biocontrol agents of soilborne pathogens: looking back over 30 years // Phytopathology. – 2007. – Vol. 97. – P. 250–256.

UDK 582.282.23.045

Rushchak O., Savitskaya K., Shpak A., Rusakova M.

Biotechnological Research and Training Center Odessa National I.I. Mechnikov University
Dvoryanska str. 2, 65082 Odessa, Ukraine
ruschak.olya@gmail.com

THE *CANDIDA ALBICANS* AND *PSEUDOMONAS AERUGINOSA* INTERACTION CHARACTERISTICS DURING THE MULTISPECIES BIOFILM FORMATION

The *Candida albicans* and *Pseudomonas aeruginosa* interaction characteristics during the multispecies biofilm formation were studied. At all formation stages the cultures of a microbial multispecies association influenced each other, manifesting an antagonistic form of interactions. The antagonism degree of the cultured microorganisms was not the same in different media.

Key words: *Candida albicans, Pseudomonas aeruginosa,* multispecies biofilm, antagonism.

Historically, interspecies interactions have focused on growth-inhibitory interactions, yet a variety of phenotypic outcomes other than antibiosis are possible, including alterations in developmental processes such as sporulation and biofilm formation or production of secondary metabolites.

Detecting phenotypic or developmental biomodulation between two organisms can indicate when they are communicating via small molecules, and thus can denote the presence of overlooked compounds. In other cases, signaling has been shown to occur via “repurposed” compounds – known molecules that are functioning in an unexpected manner. One exciting potential result of interspecies interactions is the induction of novel secondary metabolite production by the responding organism. Thus, examination of microbial relationships can lead to the discovery of new molecules – in some cases as the small molecule mediating the interaction, and in others as the consequent result of two microbes interacting [3].

The aim of the work was the study of the *Candida albicans* and *Pseudomonas aeruginosa* interaction characteristics during the multispecies biofilm formation.

The work was carried out at Biotechnological Research and Training Center. The studied microorganisms were pre-cultivated in the Sabouraud (for *C. albicans* ATCC 18804) and King B (for *P. aeruginosa* ATCC 15692) media. The interaction of microorganisms during biofilm formation was determined during the strain co-cultivation in polystyrene plates by means of the described method [1].

In the work the gradual formation of monobiofilms in both nutrient media was noted. This process consisted of some stages that corresponded with data [2]. In stage I, planktonic bacteria initiate attachment to an abiotic surface, which becomes irreversible in stage II. Stage III corresponds to microcolony formation. Stage IV corresponds to biofilm maturation and growth of the three-dimensional community. So, the largest number of biofilm cells was observed on the 3rd cultivation day. It is well known that in the case of *C. albicans* biofilm formation the consortium morphology depends on the nutrient composition but in the Sabouraud and King media only the yeast cells were detected.

To determine the microorganism interaction form during the multispecies biofilm creation co-cultivation of *C. albicans* and *P.aeruginosa* was carried out and then obtained results were compared with the sum of monospecies ones that was 100 %.

It was found that at all formation stages the cultures of a microbial multispecies association influenced each other, manifesting an antagonistic form of interactions.

The antagonism degree of the cultured microorganisms in the studied media was not the same. In King B nutrient medium for the first day the microbial interaction level caused decreasing in the biofilm cell number in almost 2 times compared with monocultures and remained unchanged with increasing the cultivation period. In Sabouraud medium the level of antagonism increased over time, reaching a maximum value on 72 hrs after the cultivation start.

Candida albicans is commonly found in mixed infections with *Pseudomonas aeruginosa,* especially in the lungs of cystic fibrosis patients. Both of these opportunistic pathogens are able to form resistant biofilms and frequently infect immunocompromised individuals. The interaction between these two pathogens, which includes physical interaction as well as secreted factors, is mainly antagonistic.

References

1. *Costerton J. W., Cheng K. J., Geesey G. G.* Bacterial biofilms in nature and disease // Annu Rev Microbiol. – 2007. – Vol. 41. – P. 435 – 464.

2. *Rasamiravaka T., Labtani Q., Duez P.* The Formation of Biofilms by *Pseudomonas aeruginosa:* A Review of the Natural and Synthetic Compounds Interfering with Control Mechanisms // BioMed Research International. – Vol. 2015 – P. 17.

3. *Ryan R. P., Dow J. M.* Diffusible signals and interspecies communication in bacteria // Microbiology. – Vol.154 – 2008 – P. 1845 –1858.

UDK 582.282.23.045

Semenets A.S., Lisova O.O., Filipova T.O.

Department of Microbiology, Virology and Biotechnology,
Odessa I. I. Mechnikov National University, Dvoryanska str. 2,
65082 Odessa, Ukraine
E-mail: assems@ukr.net

SENSITIVITY TO CIPROFLOXACIN OF *PSEUDOMONAS AERUGINOSA* STRAINS WITH DIFFERENT LEVELS OF C-DI-GMP BIOSYNTHESIS

Abstract

It was investigated the antibiotics sensitivity of *Pseudomonas aeruginosa* strains with different levels of c-di-GMP biosynthesis: PA01 Δ 100 wspF1 (increased biosynthesis level) and PA01 pJN2133 (low level of biosynthesis) in comparison with the collection *P. aeruginosa* strains: PA01 and ATCC 27853. Not established significant differences between the strains sensitivity to ciprofloxacin. It is shown that the minimum concentration of ciprofloxacin that inhibit biofilm formation is significantly lower than minimum inhibitory concentrations of P. *aeruginosa* growth. It is established that subinhibitory concentrations of ciprofloxacin violates the biofilms morphology.

Key words: biofilm, *Pseudomonas aeruginosa*, ciprofloxacin, antibiotic sensitivity, minimum inhibitory concentration.

Relatively new methods in treatment become fruitless and require correction or development of completely new approaches [2,3]. The study of new possibilities of antibiotics is developing. IV generation of cephalosporins are highly active against *Pseudomonas spp.,* and they should also be included in the study.

Ciprofloxacin is an antibacterial agent of the second fluoroquinolones generation. Suppresses DNA-gyase, disrupts biosynthesis of DNA, growth and division of bacteria; causes pronounced morphological changes [1].

Pseudomonas aeruginosa strains with different levels of c-di-GMP biosynthesis were used in the work as test-microorganisms: PA01 Δ 100 wspF1 (elevated biosynthesis level) and PA01 pJN2133 (reduced biosynthesis level). *Pseudomonas aeruginosa* ATCC 27853 [5] and PA01 were control strains.

Biofilm incubation was performed according to the Swift technique [6] with addition of the 20 µl different ciprofloxacin concentrations: together with bacteria (for biofilm formation effect) and after 24 h of biofilm incubation (in case of mature biofilm). Accounting results were after 24 h in the system biofilm-plankton. For biofilm staining crystal violet was used («µQuant» BioTek, λ=592 nm).

Ciprofloxacin sub-inhibitory concentration (0. 1 µg / ml), or did not affect the formation of biofilm shown for strains *P. aeruginosa* PA01 and PA01 Δ100 wspF1, or reduced its biomass by 2.4 times in case of the strain ATCC 27853 and at 1, 4 times for strain PA01 pJN2133 (Fig. 1).

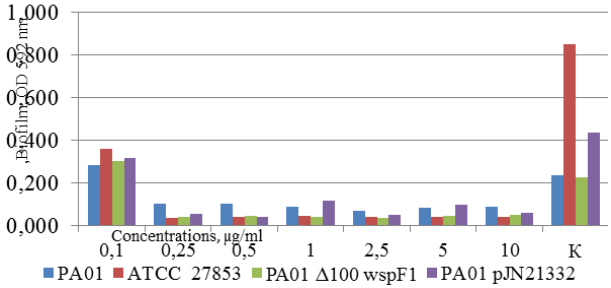

Fig. 1. Ciprofloxacin concentrations influence on *P. aeruginosa* biofilm formation.

The biofilms of the strains uniformly covers the surface and contains a significant amount of microcolonies submerged in the extracellular matrix.

To determine the antibiotics effect on mature biofilm, from plates, in which biofilms were cultivated during 24 h., plankton was removed. The biofilm on the bottom of the wells was washed with a sterile 0.9% NaCl, fresh nutrient medium with antibiotics was added into the wells for 24 hours. The influence of antibiotic was evaluated by the number of cells leaving the biofilm in the liquid medium and the biomass of the biofilm. The results are shown in Fig. 2-3.

According to the data shown on Fig. 2, ciprofloxacin expressly inhibits the release of cells from mature biofilms. At the lowest concentration of antibiotic (0.1 µg/ml) the number of planktonic cells was lower compared with control by 35-55%. With an increase in the content of ciprofloxacin, the inhibitory effect increases and in the range of concentrations of 1-10 µg/ml the amount of planktonic cells was only

7-10% of control in the case of strains *P. aeruginosa* ATCC 27853, PA01 Δ100 wspF1 and PA01 pJN2133. For *P. aeruginosa* PA01, these results were higher (20-30%).

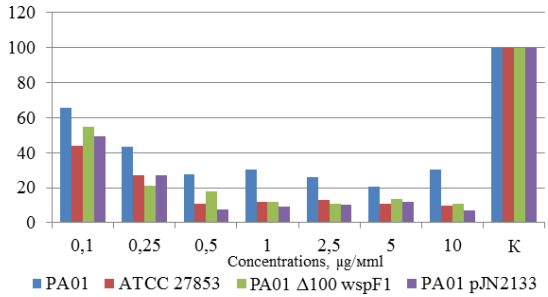

Fig. 2. Ciprofloxacin influence on the number of *P. aeruginosa* planktonic cells.

Ciprofloxacin does not cause a significant reduction in the biomass of the strains mature biofilm, with the exception of *P. aeruginosa* PA01 pJN2133, which has a very low content of c-di-GMP. The biofilm biomass decreasing was dependent on the concentration of antibiotic and was: 18% with its content of 0.1 μg/ml; 37% at 0.25 μg/ml; 40% at 0.5-2.5 μg/ml and 50-55% at 5 and 10 μg/ml, respectively (Fig. 3).

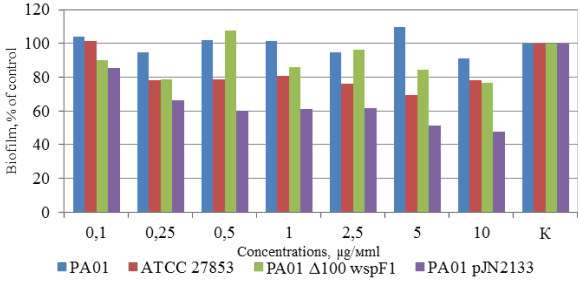

Fig. 3. Effect of various ciprofloxacin concentrations on mature biofilm.

For ciprofloxacin, an increase in this concentration is estimated at about 70%, which corresponds to data on a higher level of bacterial resistance to antibiotics in biofilms [2, 3, 4].

Obtained results showed that all strains mature biofilms are resistant to ciprofloxacin. Under antibiotic influence in no case was the complete destruction of the biofilm. The maximum decrease in the biofilm mass by 55% occurred only in the case of *P. aeruginosa* PA01 pJN2133 in the presence of 10 μg/ml of ciprofloxacin.

References

1. *Козлов Р. С., Голуб А. В.* Ципрофлоксацин в современной клинической практике // Клиническая микробиология и антимикробная химиотерапия. – 2010. – Т. 12. – №. 2. – С. 154 – 162.

2. *Bjorkman J., Andersson D. I.* The cost of antibiotic resistance from a bacterial perspective // Drug Resist Updat. – 2000. – V. 3. – P. 237 – 245.

3. *Brown M., Simith, A.* Antimicrobial Agents and Biofilms // Med. Impl. of Biofilms. – 2010. – V.2. – P. 36 – 38.

4. *Macia M. D, Perez J. L, Molin S, Oliver A.* Dynamics of mutator and antibiotic-resistant populations in a pharmacokinetic/ pharmacodynamic model of *Pseudomonas aeruginosa* biofilm treatment // Antimicrob Agents Chemother. – 2011. – V. 11. – P. 52 – 57.

5. *Methods* for the determination of susceptibility of bacteria to antimicrobial agents. EUCAST Definitive document // J. Clin Microbiol Infect. – 1998. – V 4. – P.497 – 507.

6. *Swift S., Downie J.A., Whitehead N.A., Barnard A.M.L., Salmond G.P.C., Williams P.* Quorum sensing as a population-density-dependent determinant of bacterial physiology // Adv. Microb. Physiol. – 2001. – Vol. 45. – P. 199–270.

UDC 579.6:632.3

Tverdokhlib V.S., Sokolova N.V., Sudak A.A., Limanska N.V.

Odessa National I.I. Mechnikov University

EFFECT OF *LACTOBACILLUS PLANTARUM* ON GERMINATION AND SOME GROWTH CHARACTERISTICS OF WHEAT

L. plantarum ONU 12 and *L. plantarum* ONU 311 positively effected on morphological characteristics of wheat seedlings germinated from seeds in a gel Aquasave S. Treatments of seeds with 1% of overnight cultures of these strains increased mean lengths of roots of seedlings in 72,2 - 75,0% and mean heights of the plants in 44-48,8%.

Key words: stimulation activity, plant growth, lactobacilli

Bacteria of *Lactobacillus plantarum* species, common in fermented foods, inhabit plant surfaces as a part of plant microbiota. Possessing one of the largest genomes among lactic acid bacteria, it presents many characteristics potentially applicable in different fields. Recently, the stimulating effect of lactobacilli on plant growth has been reported [Goffin et al., 2010].

The aim of our work was to study the effect of bacterial suspensions of *Lactobacillus plantarum* strains as well as their mixture on germination and morphological characteristics of wheat cv Odesska ozyma.

Materials and Methods

Overnight cultures of lactobacilli with concentration 10^8 cells/ml were used for preparation of 1% suspensions. Seed surface was sterilized by 25% of hydrogen peroxide for 1 min. Seeds were treated with the suspensions for 1 hour. Strains of *Lactobacillus plantarum* ONU 12 and ONU 313 used in this experiment were initially isolated from grape must. Control seeds were soaked in sterile distillated water instead of the bacterial suspensions. Germination was carried out under greenhouse conditions in a gel Aquasave S. Totally, 100 seeds of each variant were brought to three independent experiments.

Results and Discussion

We did not see any statistically significant differences in germination of wheat seeds soaked in lactobacilli or water (Fig. 1). But after germination, strong stimulation of morphological characteristics of seedlings was observed. Mean height of the seedlings treated with *L. plantarum* ONU 12 increased in 44%, treated with *L. plantarum* ONU 311 and with the mixture *L. plantarum* ONU 12 + *L. plantarum* ONU 311 – in 48,8% (Fig. 2).

Mean root lengths were also highest in case of *L. plantarum* ONU 311: it increased in 75% as compared to the control seedlings from seeds soaked in water.

If seeds were treated with *L. plantarum* ONU 12 and the mixture, almost the same increase was observed - 72,2% and 70,2% (Fig. 3).

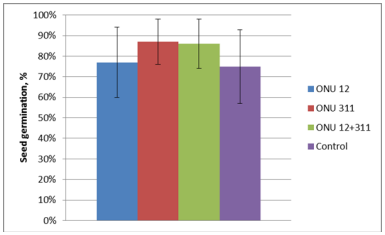

Fig 1. Effect of *Lactobacillus plantarum* on seed germination

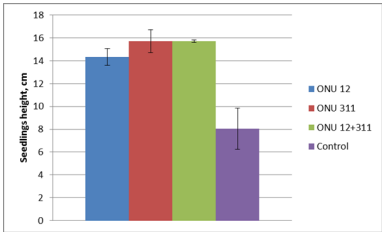

Fig. 2. Effect of *Lactobacillus plantarum* on seedlings height

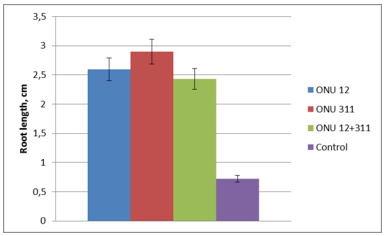

Fig. 3. Effect of *Lactobacillus plantarum* on root length

High percentages of increase in morphological characteristics of wheat seedlings indicate the stimulation potential of the strains. However, it should be taken into account that our experiments were carried out in gel and not in soil yet where the effect of soil microbiota could decrease the stimulation ability of lactobacilli.

Thus, in order to prove the positive effect of lactobacilli not only under laboratory conditions but also under natural field conditions, further experiments with germination of seeds in soil will be carried out.

Conclusion

Plant stimulation activity of the strains *L. plantarum* ONU 12 and *L. plantarum* ONU 311 make them the perspective microorganisms for biopreparation development.

Literature

1. *Goffin P., de Bunt B., Giovane M., Leveaue J.H.J., Hoppener-Ogawa S., Teusink B., Hugenholtz J.* Understanding the physiology of *Lactobacillus plantarum* at zero growth // Molecular Systems Biology. – 2010. – Vol. 6, № 431. doi: 10.1038/msb.2010.67.

UDC 60:- 634.8: -579.64

A.A. Klachun, V. B. Barabash, N.I.Tesliuk

Odesa I. I. Mechnykov National University, 2, Dvoryanska st., Odesa, 65082, Ukraine, tel.: +38 (0482) 68 79 64, e-mail: alannat@rambler.ru

USING CULTURE *IN VITRO* IN VITICULTURE

Summary

The influence of various jelling components, the composition of the nutrient medium on the survival, growth and development of initial grape explants in the primary stages of clonal micropropagation of grapes was investigated. The optimal nutrient medium Murasige and Skoog with the addition of a modified corn starch was developed and implemented to accelerate the breeding of grape variety Kobzar in vitro culture. The cost of microclon on the primary stages of clonal micro propagation has been reduced. The developed methods are tested in practice.

Keywords: in vitro culture, initial explants, clonal micropropagation, microclones, nutrient medium.

The main idea of biotechnology in agriculture is using biotechnology processes, systems and organisms in agriculture. All these make agriculture highly and competitive culture. The main goal is the improvementing old and make new highly various of grapes. Biotechnology in agriculture makes easier the new technology that improving efficiency agriculture [1]. There is the method of culture or genetic ingeneering the most popular in another countries. They make highly and invulnerable (for pests and hubrids) various of grapes. They make the technique for plants with more healthy plants excluding infections. It is important for plants which propagation vegetative.

The plant's cells are using isolation cells culture is propagation and recovery planting mathematical. This method name is clonal micro propagation. If we using this method, we will get able from one plants (donor) thousand explants. The propagation is microclonal only then microclones are the identical donor's plant. This process will be good if we will use axillary buds. The axillary bugs is genetically stable without bacterial.

The clonal propagation adventaged is:

1- the high rate of plants propagation

2- the works possibility throughout all year.

3 - the saving greenhouse area.

4 - we have genetically uniform planting material.

5 - easier plant's propagation.

6 - propagation plants which have a long life cycle (the trees).

7 - the distriction of viral infection.

The rate of clonal micro propagation depends on genotype donor plants, physical condition, size of initial explants and nutrient medium. The plant's quality depends on nutrient medium, cultivation conditionally and rooting. We can confidently say that culture in vitro the most productive and actuality. But this culture has some problems and questions. For general use in practice we must upgrade something stage.

The purpose of the work - the perfection culture in vitro for accelerate grape's propagation.

To achieve we must:

1 - determine the optimal consist of nutrient medium and cultivation's conditions.

2 - search a new jelly components and increase efficiency clonal micropropagation the various of grape «Kobzar» to culture in vitro.

Research materials and methods

For solving problems we carrying research «using cornstarch and some various of nutrient medium for accelerate and cost-effective plants propagation in vitro. For research we used the various of grape « Kobzar «. Harvesting vines vas conduct in grapes plants (we checked their absence of disease). The vines derminated to stage «green sprout» after then we selected axillary buds and using their in culture in vitro. For cultivation we using nutrient medium: Gamborga, Nitsch and Nitsch, MC (modified)[2]. For jelly property we used: potato and corn starch, agar. For culture in vitro nutrient medium must be optimally thick, provides vertical position explants. Experimentally was determined an optimal concentration of starch- 70g/l. This nutrient medium was milky-white color, has flat, thick surface, jelly consistency. We sterilized nutrient medium 20 min(autoclave). After that we entered explanrs in culture in vitro in laminar box. Cultivation initial explants conducted in the cultural box at 25-27 corners C. We determine the survival rate in 10-20th day from the starch of cultivation, after that we can see a begins proliferation and roots formation. After that we will transplant explants in medium 2 phase. We conduct this propagation by technology of Tairov NRC for viticulture and vine [3, 4]. After 2cond place plants transplant in greenhouse.

Results

Using new jelly substances in micropropagation grape's plants in vitro.

The basic of method culture «in vitro» is induction organogenesis from initial buds to condition cultural box. Good effect due to correct selection nutrient medium. Quantitative and qualitative composition affect the environments to survival and begins proliferation and rizogenezis. Usually in culture in vitro we using nutrient medium with agar. Made in abroad (USA, Russia). It is so expensive. The Ukrainian agaroid differed from abroad agar. Be can't use agaroid in culture in vitro.We conducted search a new jelly components. Was evaluated replement agar to effective process clonal micropropagation [4]. In results we have approbation some various of starch in different concentration. As first,we investigated influence different jelly substances in nutrient medium(MC). When we comparing the survival rate we can see,that the best results vas in nutrient medium when was modified agar. When we have some conditions of cultivation survival initial explants in this nutrient medium was on 12-20% more than with agar.

The axillary buds proliferation with starch begins in 3-4 day, then usually it's 3.7-5 day. In nutrient medium with agar proliferation begins in 5-7 day, then usually 6.3-7 days. After long observation we can say that plants grow and develop so good. After observing we can say that plants who lives in potato starch was died in 7-8 day. We understand that next observation potato starch is mining-less.The nutrient medium with potato starch split apart and it's so bad. In next observation we can't use potato starch.

Survival initial explants

For testing and implementation results We conducted a series of studies using cornstarch and different nutrient mediums for accelerate propagation implants various of grape «Kobzar» in vitro. We using nutrient mediums: MC, Nisch and Nisch, Gamborga (table 1).

Table 1

Survival initial explants various of grape «Kobzar» in different nutrient mediums

Nutrient mediums	Average value
Murasige and Skoog+agar	73.75
Murasige and Skoog(modified)+corn starch	91.25
Nisch and Nisch+agar	65.00
Nisch and Nisch(modified)+corn starch	73.75
Gamborga+agar	47.50
Gamborga(modified)+corn starch	60.00

We understand that cornstarch make a positive effect to survival initial explants this variety of grape «Kobzar» (fig 1). Thanks corn starch content we have seen an increase survival initial explants. But when jelly consisted with agar, we observed a bed results. And we have to understand that nutrient medium Murasige and Skoog(modified)+corn starch was the best.

Fig. 1. The development of initial explants on the nutrient medium

Proliferation

Proliferation has the main role in plant's development. After speed proliferation plants development so good. In process research we conducted observation our explants development and learn proliferation process. We understand that propagation developed on variety nutrient medium. When we study nutrient mediums and their proliferation's influence, we must say that proliferation in nutrient medium Gamborga was so bad and slowly (table 2). If we use Nisch and Nisch we will speed proliferation for 1-2 day. But different jelly components give for us different results. The analysis of the impact nutrient medium to beginning proliferation. We can see that MC was the best nutrient medium.In this nutrient medium proliferation begins in 3-4 day. And I must to say that MC(modified)+ corn starch was better than MC+agar.

Rizogenezis microclones in different nutrient mediums

In next part of this work we analyzed root formation process for explants various of grape «Kobzar» in vitro. We know that acceleration rizogenezis has a positive effect for plant's growth and power (table 3). We found that Gamborga isn't conductive to the information of roots.

For max acceleration roots formation we used MC(modified)+corn starch. In nutrient medium MC(with agar) rizogenezis begins in 9-11,

but in MC(modified)+corn starch roots formation begins in 7 day. It accelerate roots formation to 2.5 day.

Table 2

Proliferation initial explants variety of grape «Kobzar»

*average value, less mentioned

Nutrient mediums	Average value
Murasige and Skoog+agar	5.70
Murasige and Skoog(modified)+corn starch	2.90
Nisch and Nisch+agar	7.50
Nisch and Nisch(modified)+corn starch	5.80
Gamborga+agar	9.25
Gamborga(modified)+corn starch	8.30
Average value+agar	7.48
Average value+corn starch	5.68

Table 3

Begins roots formation initial explants various of grape «Kobzar» in vitro in different nutrient mediums

*average value,less mentioned

Nutrient mediums	Average value
Murasige and Skoog+agar	9.60
Murasige and Skoog(modified)+corn starch	7.10
Nisch and Nisch+agar	10.7
Nisch and Nisch(modified)+corn starch	9.45
Gamborga+agar	13.00
Gamborga(modified)+corn starch	10.90
Average value+agar	11.10
Average value+corn starch	9.15

Economic efficiency

The main arguments in favor using corn starch is a price. It cost 20-30 grn/kg, but cost agar is 1500-1700 grn/kg. Economic efficiency using Murasige and Skoog(modified)+corn starch was achieved though reducing the cost of using agar. The increase survival initial explants and reduction timing for 5 days. The cost of planting was 11.9 grn,but if we will use our technology,the cost of planting will be 9.0 grn. Additionally income is 2,5 grn.

Conclusions

The influence of various jelling components, the composition of the nutrient medium on the survival, growth and development of initial

grape explants in the primary stages of clonal micropropagation of grapes was investigated. The optimal nutrient medium Murasige and Skoog with the addition of a modified corn starch was developed and implemented to accelerate the breeding of grape variety Kobzar in vitro culture. The cost of microclon on the primary stages of clonal micro propagation has been reduced. The developed methods are tested in practice.

Literature

1. Kushnir V.V., Sarnadska. Klonal micropropagation plants. Scientific opinion. 2012.- p.215

2. A patient for an incantation: pat. Bul. Numb 7. Numb 47417 Ukraine 7 A 01 G 31/00,17/00 printed 15. 07. 2002. Nutrient medium for planted grapes microclones. / Stitsko S.A., MilkusB.N. ,Teslyuk N.I.

3. Zelenanska N.N. The technology propagation grape with using culture in vitro. / N.N. Zelenanska, DgaburiaL.V. ,Teslyuk N.I. //. VitiCulture. - 2009.- numb 3 - p. 50-53

4. Teslyuk N.I. Using new jelly components for grapes propagation in culture in vitro. / Teslyuk N.I. // Black see agriculture journal: biology and agriculture science. -2005.- numb 29. - p. 141-143.

UDC: 616.314. – 089.843:57.083.3

Mariia Faustova[1], Olexandr Nazarchuk[2], Maiia Ananieva[1]

[1]Higher State Educational Establishment of Ukraine
"Ukrainian medical stomatological academy"
[2]National Pirogov Memorial Medical University
36011, Shevchenko st., 23, Poltava, Ukraine,
email – mashafaustova@ukr.net

THE COMPARATIVE EVALUATION OF NEUTROPHIL ACTIVITY DEPENDING ON TIME OF PERI-IMPLANTITIS DEVELOPMENT

Abstract

Peri-implantitis belongs to the number of the most common complications of dental implant placement that can occur during the procedure as well as in the postoperative period. A key role in the immune defence of oral cavity belongs to nonspecific factors that act as a powerful barrier and are the first to respond to foreign agents entering the mouth

The **aim** of this work was an exploring the neutrophil bactericidal activity depending on time of peri-implantitis development by using NBT tes.

For success of this aim samples of capillary blood of 50 patients were checked up by NBT tes. Among the 100 cells we counted the share of active neutrophils. Period of peri-implantitis development after dental implant placement correlates with the changes of the share of active neutrophils in patients' blood.

Key words: implantation, neutrophil activity, peri-implantitis, complications of dental implantation.

Introduction

Nowadays, popularity of dental implantation is rising on the background of dental disorders prevalence [1]. More than 2 million implants are screwed every year worldwide [2]. That is why new protocols of operation, modern implantation systems are created, what sometimes leads to the decreasing of quality procedure [3]. According the 11th European Workshop on Periodontology peri-implantitis belongs to the number of the most common complications of dental implant placement that can occur during the procedure as well as in the postoperative period (within year and even more) [1]. Age of a patient, his immunity and oral microflora cause the key role on development of complications. Therefore,

investigations of some immune marks are important for treatment of peri-implantitis as well as diagnostic of them [4].

This study was **aimed** at exploring the neutrophil bactericidal activity depending on time of peri-implantitis development by using NBT tes.

Materials and methods

A total of 50 patients of average age group by WHO (46-59 years) whom were detected peri-implantitis were subjected to the comprehensive check-up. The first group enrolled 25 people with peri-implantitis before 3 months after implant placement. 25 people of the same age with peri-implantitis in a long term after implantation (around 1 year) made up the second group. Samples of capillary blood were taken into sterile vials containing heparin and then delivered to the laboratory. The functional activity of neutrophils was evaluated by nitroblue tetrazolium (NBT) reduction test (spontaneous NBT by Wixman M. E., Mayansky A.N.) [5]. Among the 100 cells we counted the share of active neutrophils (AN), containing dark-violet formazane (diformazane) granules and presented their percentage. Neutrophils containing clearly visible diformazane deposits were regarded as active cells, and netrophils with residual granules were considered as inactive. Statistical data processing was performed using Microsoft Excel 2010; statistical significance was determined by Student's test. Data were statistically significant at $p < 0.05$.

Results and their discussion

According to our data the share of active neutrophils in blood of the patients of the first group was increased compared with their normal evaluation. It reached 60,6±1,18 % that corresponds to the active stage of inflammation caused by surgical intervention and acute process around the implant.

However, the share of active neutrophils in blood of the patients with peri-implantitis which developed in remote period was significantly lower (28,3±0,98%) then normal evaluation. It can be explained by the displacement of acute process to chronic that promotes inhibition of some chains of immunity.

Conclusions

Thus, period of peri-implantitis development after dental implant placement correlates with the changes of the share of active neutrophils in patients' blood. Peri-implantitis that is developed exactly after surgery causes increasing of neutrophil activity and remote complications cause decreasing of this mark.

References

1. Derks J, Tomasi C. Peri-implant health and disease. A systematic review of current epidemiology. J Clin Periodontol. 2015;42 Suppl 16:158–71.

2. Froum, S. J. (Ed.). (2015). Dental implant complications: etiology, prevention, and treatment. John Wiley & Sons.

3. Oshida, Y., Tuna, E. B., Aktören, O., & Gençay, K. (2010). Dental implant systems. International journal of molecular sciences, 11(4), 1580-1678.

4. Yugay Y. V., Tolmachev V. E., Markelova E. V., Golitsyina A. A. Otsenka tsitokinovogo profilya u patsientov do i posle dentalnoy implantatsii // TMZh. 2013. #1 (51) S.31-33. Russian

5. Viksman M.E., Mayanskiy A.N. Sposob otsenki funktsionalnoy aktivnosti neytrofilov cheloveka po reaktsii vosstanovleniya nitrosinego tetrazoliya: Metod. rekomendatsii. — Kazan: Kazanskiy NIIEM, 1979. — 11. Russian

UDC 579.6:632.3

Filonenko G.[1], Talalaiev O1., Kovalenko N.[1], Kyryk D.[2], Skorohod I.[1], Salamanina A.[1]

[1] Ukrainian Children's Cardiac Center, 24, Melnikova Str., Kyiv, Ukraine, 04050
[2]Shupyk National Medical Academy of Postgraduate Education, 9, Dorohozhytska Str., Kyiv, Ukraine, 04112, Baklabccc@ukr.net

DETECTION OF ESBL IN *ENTEROBACTERIACEAE*: A CHALLENGE FOR DIAGNOSTIC MICROBIOLOGICAL LABORATORIES

Objective

To study ESBL phenotypes, as well as to identify individual genotypes of β-lactamases and to study their prevalence among clinical strains of *Enterobacteriaceae* collected from children with congenital heart diseases.

Methods

Clinical strains of *Enterobacteriaceae* were studied in order to determine their susceptibility to antibiotics, using an automated system, before genotyping resistance determinants using multiplex PCR.

Results

During the period of the study, 10.9% of ESBL-positive isolates were found among clinical strains of *Enterobacteriaceae.* Most ESBL-producing strains (33.3%) were *Klebsiella pneumoniae.* Most strains producing extended-spectrum β-lactamases were isolated from the respiratory tract and accounted for 83.3%.

Conclusions

An experimental study provided new data as regards the prevalence of ESBL-producing genetic resistance determinants within the *Enterobacteriaceae* family, and their role in the development of complications. The study showed diagnostic value of molecular biological methods in identifying resistance determinants of microorganisms.

Key words: ESBL, β-lactam antibiotics, *Enterobacteriacea,* antibiotic resistance, PCR, genotyping

Introduction

Resistance against antibacterials in clinically relevant bacteria is one of the most imminent threats to public health and especially to our most vulnerable patient populations. The World Health Organization has

recognized antimicrobial resistance (AMR) as "a global health security threat that requires action across government sectors and society as a whole."[1] The Centers for Disease Control and Prevention (CDC) has estimated the excess direct healthcare costs associated with AMR to be as high as $20 billion, and additional costs to society for lost productivity as high as $35 billion a year in the United States alone. Reliable global estimates are needed for the proportion of total infections that are caused by multidrug resistant (MDR) bacteria, and the proportion for each bacterial species isolated from clinical samples that displays an MDR phenotype [2].

Enterobacteriaceae are a family of bacteria that encompass many bacteria that are commonly isolated from clinical cultures, including *Klebsiella pneumoniae, Escherichia coli, and Enterobacter spp. From the perspective of antimicrobial resistance, Enterobacteriaceae* are especially important as they are a common cause of community-associated as well as healthcare-associated infections [3,4].

Multiple molecular typing methods, including PCR-based assays, have been developed for detection and identification of the growing number of bla_{TEM}, bla_{SHV} and bla_{CTX-M} genes [3,4,5]. Though, there is still limited scientific data on epidemiology of ESBL spread in the Ukraine. To the best of our knowledge, this is the first study on the molecular epidemiology and antimicrobial susceptibilities of ESBL-producing *Enterobacteriaceae* among surgical patients in Ukraine.

The purpose of this study was to explore feasibility of simultaneous identification of bla_{TEM}, bla_{SHV} and bla_{CTX-M} genes by multiplex PCR detection in a series of clinical isolates of Enterobacteriaceae with previously characterized ESBL phenotype, to implement routing monitoring of ESBL-producing *Enterobacteriaceae* strains at the Ukrainian Children's Cardiac Center (Kyiv, Ukraine), to determine predominant genotypes among ESBL-producing *Enterobacteriaceae* strains.

Materials and methods

All study strains were selected based on the screening tests for the detection of ESBL-type enzymes. The isolates were collected from clinical specimens of patients hospitalized at the Ukrainian Children's Cardiac Center (Kyiv, Ukraine) during the period from January to December, 2015. The isolates were recovered from various clinical specimens, mostly tracheal discharges, throat swabs, wounds, urine and blood. The majority of the collected strains were obtained during cardiac surgery in 704 patients representing different regions of Ukraine.

Multiplex PCR-based genotyping tests were conducted on thirty ESBL-positive isolates including strains of *Enterobacteriaceae (Klebsiella pneumoniae, Enterobacter cloacae, Serratia marcescens and Klebsiella oxytoca)*.

Results and discussion

During the study period, total of 30 (10.9%) ESBL-producing *Enterobacteriaceae* were isolated. Among the isolates, *K. pneumoniae* (33.3 %, n = 22) was prevalent ESBL-producing bacteria, ESBL-phenotype of *E. coli* accounted for 13.3 % (n = 4), and other ESBL-producing *Enterobacteriaceae* accounted for 13.3 % (n = 4). These pathogens were isolated from a variety of clinical samples, respiratory system – 83.3 % (n = 25), blood – 6.6 % (n = 2), and others – 10.0 % (n = 3).

Among *K. pneumonia* strains expressing ESBL activity, high levels of resistance to ampicillin (100%), cefazolin (100%), ceftriaxone (100%), cefuroxime (100%), and cefepime (95.7%) were observed. Tested strains of *K. pneumoniae* were susceptible to imipenem (69.6%) and meropenem (69.6%). All *E. coli* ESBL-positive strains were resistant to ampicillin (intrinsic resistance), cefazolin, cefuroxime, ceftriaxone, and cefepime. Furthermore, ESBL-positive E. coli strains were highly susceptible to meropenem (100%) and imipenem (100%). Additionally, other *Enterobacteriaceae* ESBL-positive strains were completely resistant to ampicillin, cefazolin, and cefuroxime. High activity against other *Enterobacteriaceae* was shown by ceftriaxone (50%), cefepime (50%), imipenem (100%), and meropenem (100%).

The ESBL-producing pathogens identified by phenotypic methods were also analyzed by using PCR methods. Of 30 ESBL isolates, 6.6 % harbored multiple *bla* genes simultaneously and the prevalence of bla_{TEM} was as high as 70.0 %, followed by bla_{SHV} at 46.6%, and $bla_{CTX\text{-}M}$ at 46.6%. The majority of the TEM-positive isolates were *K. pneumoniae* (81.8%), *E. coli* (50.0%), and other *Enterobacteriaceae,* (33.1%); however, all CTX-M and SHV positive isolates were *K. pneumoniae* (50.0% and 59.0% respectively). Furthermore, all three bla genes (TEM, SHV, and CTX-M) were detected in only 9.0 % of *K. pneumoniae* isolates, while two genes (SHV/CTX-M) were present in 9.0 % of K. pneumoniae, with TEM/CTX-M being present in 22.5 % of *K. pneumoniae* and TEM/SHV being detected in 45.5% of *K. pneumoniae*, and 33.3 % in other *Enterobacteriaceae* isolates.

Conclusions

Compared with previous data our study shows relatively low

prevalence (10.9 %) of ESBL-producing *Enterobacteriaceae*. Notably, majority of isolates were multi-drug resistant and belonged to TEM plasmid-type. Additionally, antimicrobial control and early detection by active surveillance in combination with effective infection monitoring programs and methods are key steps for reducing or controlling the spread of ESBL-positive hospital-acquired infections in Ukraine.

References

1. Organization WH. Antimicrobial Resistance: Global Report on Surveillance. 2014; http://wwwwhoint/drugre sistance/documents/surveillancereport/en/

2. Centers for Disease Control and Prevention. ANTIBIOTIC RESISTANCE THREATS in the United States, 2013. 2013; http://wwwcdcgov/drugresistance/threat-report-2013/

3. D. Ojdana, P. Sacha, P. Wieczorek et al. The Occurrence of blaCTX-M, blaSHV, and blaTEM genes in extended-spectrum β-lactamase-positive strains of *Klebsiella pneumoniae, Escherichia coli,* and *Proteus mirabilis* in Poland. Internat. J. of Antibiotics 2014, 7 pages.

4. Sid Ahmed et al. Antimicrobial susceptibility and molecular epidemiology of extended-spectrum betalactamase-producing Enterobacteriaceae from intensive care units at Hamad Medical Corporation, Qatar. Antimicrobial Resistance and Infection Control. 2016; 5:4.

5. Monstein H.J, Ostholm-Balkhed A, Nilsson M.V. et al. Multiplex PCR amplification assay for the detection of bla_{SHV}, bla_{TEM} and bla_{CTX-M} genes in *Enterobacteriaceae.* APMIS 2007 Dec; 115(12): 1400-8.

Наукове видання

Сучасні проблеми мікробіології і біотехнології

Матеріали
Міжнародної наукової конференції молодих учених
20-24 червня 2017 року

За редакцією авторів

Підп. до друку 16.06.2017. Формат 60x84/16.
Ум.-друк. арк. 11,45. Тираж 130. Зам. № 1611.
Видавець і виготовлювач
Одеський національний університет імені І. І. Мечникова Свідоцтво суб'єкта видавничої справи ДК № 4215 від 22.11.2011 р. Україна, 65082, м. Одеса, вул. Єлісаветинська, 12
Тел. (048) 723-28-39. E-mail: druk@onu.edu.ua

Scientific publication

MODERN PROBLEMS OF MICROBIOLOGY AND BIOTECHNOLOGY

Materials
International conference of young scientists
June 20-24, 2017

In Author's Edition

Signed in Print 16.06.2017. Format 60´84/16. Conv. pr. Sheets 11,45.
Circulation of 130 copies. Order № 1611.
Publisher and editor
Odesa I. I. Mechnikov National University
Certificate of state registration of the print media DK № 4215 dated November, 22, 2011
Ukraine, 65082, Odesa, Elisavetyns'ka str. 12
Tel. (048) 723-28-39. E-mail: druk@onu.edu.ua