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BIOLOGICAL CHARACTERISTICS MYXOBACTERIA STRAINS ISOLATED FROM NATURAL SOURCES IN THE SOUTH-WEST OF UKRAINE

24 strains of Myxobacteria strains were isolated from samples of soil, seawater and tree cortex collected in the south-west of Ukraine. The isolated strains were identified as species *Myxococcus fulvus, Myxococcus xanthus, Myxococcus stipitatus, Archangium gephyra, Cystobacter fuscus, Polyangium vitellinum, Nannocystis exedens.* Biological characteristics of these strains were investigated. The obtained data show that the isolated strains have wild spectrum of lytic enzymes and can produce antagonistic substances.

Key words: Myxobacteria, identification, lytic activity, antagonistic activity.

Introduction

The Myxobacteria are phylogenetically a uniform group of gram negative bacteria. They have interesting cycle of development that is atypical for other bacteria and has morphologically mixed character at some stages. These properties are of interest for the investigators.

The main indicative feature of Myxobacteria is its capability of fruiting bodies formation. With the help of this ability we can attribute Myxobacteria to the order *Myxococcales* within the Gliding bacteria group [2], so the second main indicative feature of the bacteria are their gliding movement — i. e. the movement of cells on the substrate without some visible means of locomotion.

The peculiar feature of Myxobacteria is their ability for hydrolysis of some natural polymers such as polysaccharides, proteins, nucleic acids and other high molecular natural substances that Myxobacteria use as a source of carbon and energy. Depending on the type of hydrolyzed macromolecules Myxobacteria are distinguished in two metabolic groups: bacteriolytic and cellulolytic [4]. Members of this group are very promising in biotechnology processes [7].

Due to the mentioned above, the aim of this study was isolation of myxobacters from different ecological niches in the south-west of Ukraine and biological characteristics investigation of our region myxobacters.

Materials and methods

Myxobacteria were isolated from different sources of environment. The samples of soil (50 samples) and plants (20 samples) were taken in Botanical garden Odessa State University. The samples of see water (50 samples) were taken in Odessa region seaside. Small pieces of alive tree cortex (*Sambucus racemosa*, *Robinia pseudoacacia*), root plants,

homogenous soil samples (0,01 g) or water samples (0,01 ml) were placed on wet paper circle in Petri Dish with Manure agar [5]. After isolation and purification of myxobacters for cultivation we used Vy/2-agar [8]. The cultures were normally kept at 30 °C in dark incubator.

The isolated strains were identified by using of standard bacteriological methods described in Bergey's Manual [3] and by using the methods from original articles [6, 8].

The presence of flexirubin pigments was determined after treatment of the vegetative colony by 10% KOH solution as changing of colony color in red [8]. To determine metabolic activities the strains were grown on the basic medium [8], containing either fibrin, casein, collagen, lignin, chitin or skim milk at 1% concentration of powder; baker's yeast, Micrococcus luteus or Escherihia coli at 0.5% concentration of fresh cells. Appearance of a clear zone around the inoculum was interpreted as a positive result. Cellulose decomposition was determined in Petri Dishes with Stanier's mineral salts agar medium [8], covered by paper circle. Agar penetration was tested on Stanier's mineral salts agar medium without any paper circle. Starch hydrolysis was determined on the basic medium with soluble starch (1%). Gelatin liquefaction was determined on the basic medium with 15% gelatin.

The isolated strains antagonistic property was studied with the following test-strains: Escherichia coli, Staphylococcus enteritidis, Pseudomonas fluorescens, Micrococcus luteus. For this test we used agar block method [1].

Results and discussion

As a result of the research the samples of the cortex of alive tree, soil and seawater from the Ukrainian south-west have been selected and strains of myxobacteria have been isolated and investigated. Myxobacteria were found in 50% soil samples, 6% sea water samples and 10% samples of alive tree cortex. As purified culture we got 24 strains.

First, morphological characteristics of fruit bodies and vegetative cells of the isolated strains were studied to identify these strains. Vegetative colonies of these strains have very bright colour, but the colony edges are difficult to mark. All strains have gliding motility that is the main feature of this group. Majority of the strains doesn't form flexirubin. This does not correspond with tendency, which was found by Reichenbach and Dworkin [8]. Vegetative cells of all the strains have rod shape and negative reaction by Gram. It is very important that the cells arranged separately. All isolated strains have the ability to form fruiting bodies. Myxobacteria identification scheme was constructed by using morphological characteristics. But extreme instability of fruiting body formation in many species might make Myxobacteria identification difficult. The data showed in Table 1 were used for identification of the isolated strains.

The isolated strains were identified as species: *Myxococcus fulvus* (Cohn) Jahn 1921 (strains: 6, 11, 15, 49, 56, 59), *Myxococcus xanthus* Beebe 1941 (strains: 10, 14, 27, 55), *Myxococcus stipitatus* Thaxter 1897 (strain 34), *Archangium gephyra* Jahn 1924 (strains: 7, 8, 25, 32, 38, 45), *Cystobacter fuscus* Schroerer 1886 (strains: 22, 28, 42), *Polyangium vitellinum* Link 1809 (strains: 13, 39), *Nannocystis exedens* Reichenbach 1970 (strains 24, 35).

The greatest number of myxobacteria strains were isolated from soil samples — 19 strains, only 2 strains (*M. xanthus* — 14, *C. fuscus* — 42) — from alive tree cortex and 3 strains (*M. xanthus* — 55, *M. fulvus* — 56, *M. fulvus* — 59) — from seawater.

Table 1

Morphological characteristic of isolated strains

Strain	Cell	Fruit body		Myxospore		Colony				
	Size	Size	Shape	Size	Shape	Edge	Profile	Colour	Flexirubin	Kongo red
6	6,0 × 0,7	250	pear	1,4	ball	wavy	flat	orange	+	+
7	5,0 × 0,6	–	brain	2,1 × 2,0	oval	blade	hill	rose	–	+
8	6,0 × 0,5	–	brain	1,5	ball	wavy	flat	rose	+	+
10	5,0 × 0,5	300	hill	2,7	ball	blade	flat	yellow	–	+
11	4,8 × 0,4	400	pear	1,5	ball	cut	flat	orange	–	+
13	4,0 × 0,9	75	ball	3,5 × 0,9	rod	wavy	flat	cream	–	–
14	5,0 × 0,8	300	hill	2,7	ball	blade	flat	yellow	–	+
15	7,0 × 0,7	400	pear	1,5	ball	cut	flat	orange	+	+
22	1,0 × 0,7	60	oval	2,5 × 1,0	oval	even	flat	pink	+	+
24	2,2 × 1,1	30	seed	1,5 × 0,7	oval	cut	sink	yellow	–	–
25	6,0 × 0,6	–	brain	1,8 × 2,0	oval	blade	hill	rose	–	+
27	8,0 × 0,8	400	hill	2,5	ball	cut	flat	orange	+	+
28	3,0 × 0,7	60	oval	2,5 × 1,0	oval	blade	flat	pink	+	+
32	6,5 × 0,5	–	brain	2,0 × 1,8	oval	blade	flat	rose	–	+
34	5,5 × 0,5	200	ball	1,8	ball	wave	flat	yellow	–	+
35	2,5 × 0,4	30	seed	1,5 × 0,7	oval	blade	sink	yellow	–	–
38	6,0 × 0,7	–	brain	1,8 × 1,5	oval	even	flat	rose	–	+
39	4,0 × 0,9	75	oval	3,5 × 0,9	rod	cut	flat	cream	–	–
42	3,0 × 0,5	60	oval	2,5 × 1,0	oval	even	flat	pink	–	+
45	5,5 × 0,5	–	brain	2,1 × 2,0	oval	blade	hill	rose	–	–
49	2,5 × 0,4	400	pear	1,5	ball	cut	flat	orange	–	+
55	5,0 × 0,5	300	hill	2,8	ball	blade	flat	yellow	–	+
56	4,8 × 0,4	350	pear	1,4	ball	cut	flat	orange	–	+
59	5,0 × 0,4	400	pear	1,6	ball	cut	flat	orange	–	+

Note: "+" — presence of characteristic; "–" — absence of characteristic, all sizes are in micrometer.

In addition to morphological characteristics some physiological and biochemical characteristics were studied. It was found out that all strains are aerobes, mesophiles, prefer neutral and slightly alkaline pH and can grow within the wide range (0—10%) of NaCl concentration. This doesn't contradict with the published data [8].

All studied strains were catalase positive. Among the studied strains only the strains belonging to family *Archangiaceae* and species *P. vitellinum* and *M. stipitatus* were oxidase positive. All *Archangiaceae* strains don't reduce nitrate. The majority of other isolated strains can reduce nitrate in nitrogen (70%) and in nitrite (11%). With urease, b-galactosidase, ammonia formation and acetylmethylcarbinol tests the isolated strains identified as *Myxococcus fulvus, Myxococcus xanthus, Myxococcus stipitatus, Archan-*

gium gephyra, Cystobacter fuscus, Polyangium vitellinum, Nannocystis exedens reacted partially as negative and partially as positive.

No strain had decarboxilases of lysine and ornythine; degydrogenases of arginine; desaminases of phenilalanine. No strain could form indole and hydrogen sulphide.

It was interesting to estimate an ability of isolated myxobacters to utilise different carbon and energy sources. It was done for different sugars and multibase alcohols and for some natural polymers and microorganisms. We found out that only single strains can use either glucose, sucrose or arabinose. This is for myxobacters a typical attitude to sugars and multibases alcohols [3, 8]. In contrast to the mentioned above, the isolated myxobacteria have very strong lytic abilities with regard to natural organic polymers and microorganisms. The isolated strains can utilise collagen (96% of strains), starch (80%), *M. luteus* cells (80%), *E. coli* cells (70%), casein (68%), skimmed milk (68%), gelatine (56%), fibrin (56%), baker's yeast (56%), agar (44%), chitin (12%), cellulose (12%). The strains prefer mostly protein polymers because the majority of them belong to bacteriolitic Myxobacteria group.

The existing data testify that Myxobacteria play an active role in regulation of microbial population of biocenosis and in the processes of dead organic residue mineralisation. This idea also supported by the ability of the majority of the isolated strains (60%) to produce antagonistic substances with regard to test-strains E. coli, M. luteus, P. fluorescens. The growth of test-strain S. enteritidis was not suppressed by any of the isolated strains. This data confirm the wellknown information that Myxobacteria can produce wide spectrum of antibiotics [8]. Probably the antibiotic production helps them to play predator role in microbiocenoses.

So, the majority of Myxobacteria of different ecological niches of Ukrainian south-west were isolated from samples of soil (19 strains) and that confirm that the members of Myxobacteria group can be found in Ukraine like everywhere mostly in the soil [7]. We think that some myxobacters can get into seawater or on the tree cortex accidentally. It is confirmed by wide species composition isolated from Ukrainian south-west soil samples: *M. fulvus* (4 strain), *M. xanthus* (2 strain), *M. stipitatus* (1 strain), *A. gephyra* (6 strain), *C. fuscus* (2 strain), *P. vitellinum* (2 strain), *N. exedens* (2 strain). Seawater myxobacteria are presented by species *M. xanthus* (1 strain) and *M. fulvus* (2 strain). Alive tree cortex myxobacteria are presented by species *M. xanthus* (1 strain) and *C. fuscus* (1 strain).

References

1. *Егоров Н. С.* Микробы-антагонисты и биологические методы определения антибиотической активности. — М.: Высшая школа, 1985. — С. 48—49.
2. *Иваница В. А.* Скользящие бактерии порядков Myxobacterales и Cytophagales // Успехи микробиологии. — 1990. — Вып. 24. — С. 65—87.
3. *Bergey's* Manual of Systematic Bacteriology / Ed. F. F. Staby et al. — Baltimore: Vilkins and Villiams Co, 1989. — V. 3. — P. 2130—2140.
4. *Dworkin M.* Research on the myxobacteria: Rev. and Cell. Interact. — New York, 1984. — P. 221—245.
5. *Lecianova I.* Myxobacterie ve vodach // Prace a studie sesit. — 1987. — 128 p.
6. *McCurdy H. D.* Studies on the taxonomy of the *Myxobacterales* 1. Record of coneclian isolates and Survey of methods (isolation) // Canad. J. Microbiol. — 1969. — V.17, № 12. — P. 1453—1461.
7. *Reichenbach H.* The ecology of the myxobacteria // Environmental Microbiology. — 1999. — V. 1, № 1. — P. 15—21.
8. *Reichenbach H., Dworkin M.* Introduction to the gliding bacteria // Prokaryotes. — Berlin, 1981. — V. 1. — P. 315—327.

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БІОЛОГІЧНІ ВЛАСТИВОСТІ ШТАМІВ МІКСОБАКТЕРІЙ, ІЗОЛЬОВАНИХ З ПРИРОДНИХ ДЖЕРЕЛ ПІВДЕННО-ЗАХІДНОГО РЕГІОНУ УКРАЇНИ

Резюме

Із проб ґрунту, морської води і кори дерев, відібраних в південно-західному регіоні України ізольовані 24 штами міксобактерій. Ізольовані штами були ідентифіковані як види *Myxococcus fulvus, Myxococcus xanthus, Myxococcus stipitatus, Archangium gephyra, Cystobacter fuscus, Polyangium vitellinum, Nannocystis exedens*. З'ясовані їх біологічні властивості і показано, що штами мають широкий спектр літичних ферментів і здатні продукувати речовини з антимікробною дією.

Ключові слова: міксобактерії, ідентифікація, літична активність, антагоністична активність.

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БИОЛОГИЧЕСКИЕ СВОЙСТВА ШТАММОВ МИКСОБАКТЕРИЙ, ИЗОЛИРОВАННЫХ ИЗ ПРИРОДНЫХ ИСТОЧНИКОВ ЮГО-ЗАПАДА УКРАИНЫ

Резюме

Из проб почвы, морской воды и коры деревьев, отобранных на анализ в юго-западной части Украины изолированы 24 штамма миксобактерий. Изолированные штаммы идентифицированы как виды *Myxococcus fulvus, Myxococcus xanthus, Myxococcus stipitatus, Archangium gephyra, Cystobacter fuscus, Polyangium vitellinum, Nannocystis exedens.* Установлено, что штаммы имеют широкий спектр литических ферментов и способны продуцировать вещества с антимикробным действием.

Ключевые слова: миксобактерии, идентификация, литическая активность, антагонистическая активность.