Antibacterial properties of intestinal microbiota of the Japanese sea cucumber Apostichopus japonicus

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Abstract. The ability of 134 bacterial strains isolated from the digestive system of the Japanese sea cucumber Apostichopus japonicus to inhibit the growth of Vibrio alginolyticus, V. parahaemolyticus, V. splendidus, Staphylococcus aureus, Yersinia pseudotuberculosis, Pseudomonas aeruginosa and Escherichia coli was studied. 17 strains (13%) had different level of antimicrobial activity against investigated test-cultures, 8 (6%) of them were bacteria of the genus Bacillus. The highest antimicrobial activity was demonstrated by the strains Bacillus sp. K32, Arthrobacter sp. A16, Kocuria sp. A34, Bacillus megaterium K13 and Pseudoalteromonas sp. K59. The results indicate a high potential of symbiotic microflora of Apostichopus japonicus to confront pathogenic microorganisms.

1. Introduction

The Japanese sea cucumber Apostichopus japonicus (Selenka, 1867) is a marine bottom-dwelling invertebrate animal, belonging to the phylum Echinodermata, it is characterized by high nutritive and medicinal value. A rich variety of bioactive chemical compounds in the Japanese sea cucumber, which may act separately or as a complex, determine high pharmacological value of products, obtained from it [1].

Cited in literature, mass mortality of Apostichopus japonicus in conditions of artificial reproduction is most frequently a result of diseases, caused by such microorganisms, as Vibrio splendidus [2, 3] and Vibrio alginolyticus [4, 5]. Some infections of Apostichopus japonicus are mentioned, provoked by such infectious agents, as Vibrio parahaemolyticus [6, 7], Pseudoalteromonas sp., and Pseudoalteromonas tetraodonis [8].

Various antibiotics are routinely applied for elimination of outbreaks of infectious diseases, that results in a number of considerable negative consequences. Firstly, seafood, comprising chemicals, constitute a threat to human health. Secondly, when used in mariculture systematically, antibiotics lead to subsequent appearance of infectious disease agents, which become antibiotic-resistant. Thereby, the pressing task of the modern mariculture is the search of alternative practices for hydrobiont farming,
with minimum amount of chemicals, used in production. Application of probiotics-based food additives, which are able to inhibit the growth of pathogenic microbes selectively without injuring a host animal, appears to be one of the most promising methods to solve the problem [9].

At the present day there is quite a number of data in literary sources available about beneficial effects of particular bacteria species [3, 4, 10, 11], yeast species [2, 12], and bacteriophage species [5, 7], obtained from different source material, on Apostichopus japonicus reproduction. Benefits are gained through their ability to inhibit the growth of pathogenic microorganisms, and to increase the resistance of the hydrobiont to infections. Such approach, undoubtedly, is full of tremendous advantages, but also causes some concerns, so long as it is unknown, what kind of consequences a long-term and uncontrolled use of biologically active microorganisms, which are alien for an ecosystem, may bring.

Meanwhile, there are limited information available on composition, biochemical properties [13, 14, 15], and symbiotic microbiota role of Apostichopus japonicus for defense against pathogenic bacteria in literary sources [8, 16], although it is known, that any animal is characterized by the presence of normal microflora, which performs wide set of functions. Basic properties of normal microflora imply the following mechanisms: defense against pathogenic bacteria; participation in host digestion; enhancement of peristalsis of digestive system; neutralization of toxic substrates and metabolites; participation in training of local and general immunity; regulation of respiratory and water-salt metabolism. In this connection, the studies of antimicrobic potential of the holothurian symbiotic microflora are the subject of profound interest, both for improvement of theoretical knowledge and for search of promising probiotic microorganisms for mariculture.

The objective of the present research is to study ability of bacteria, isolated from digestive tract of the wild Apostichopus japonicus specimens, to inhibit the growth of pathogenic microorganisms.

2. Material and research methods

2.1. Objects of research

The collection of 134 cultured heterotrophic bacterial strains, obtained by us earlier, isolated from the digestive system of the sea cucumber Apostichopus japonicus from natural habitat, was used for the research [14]. The study of antagonistic properties of symbiotic microflora was carried out on Vibrio alginolyticus H1822, Vibrio parahaemolyticus H1845, and Vibrio splendidus H1802 strains, isolated from sea farm samples. In addition, the antimicrobial activity of the studied collection with respect to the type strains of Staphylococcus aureus 6538/206p, Yersinia pseudotuberculosis M-3515, Pseudomonas aeruginosa 2785 and Escherichia coli 26826 was checked, the strains were obtained from the museum of G.P. Somov Research Institute of Epidemiology and Microbiology in Vladivostok city.

2.2. Detection of the antimicrobial activity of symbiotic bacteria collection from the Japanese sea cucumber Apostichopus japonicus

Intermicrobial interactions were explored using the cross-streak method [17] on the solid modified marine broth (MMB) medium [18], appropriate for incubation of all studied types of microorganisms. In the beginning the bacterial strain – a potential producer of antimicrobial substances - was plated diametrically by streak on a Petri dish, containing the medium. The incubation was performed during 5 days at the 22°C temperature. Thereafter, the pathogenic bacterial test-culture was cross-streaked to the seeded material. The dishes with plate cultures were put into thermostat and incubated during 2 days at the temperature of 37°C. The size of growth inhibition zone for test-cultures near plated symbiotic bacteria, expressed in millimeters, gave grounds for determination of available antimicrobial activity. All experiments were repeated three times.
3. Results and discussion

Different levels of antimicrobial activity against studied test-cultures was shown by 17 strains (13%) of the whole quantity of 134 bacterial strains, isolated from the digestive tract of the wild sea cucumber Apostichopus japonicus, 8 strains of these (6%) were bacteria of the genus Bacillus (table 1). It should be noted, that specimens of the genus Bacillus comprised 27% of our collection [14].

Table 1. Antimicrobial activity of Apostichopus japonicus symbiotic microbiota.

| Strains producing antimicrobial substances | Growth inhibition zones for test-cultures, mm |
|-------------------------------------------|---------------------------------------------|
|                                           | V. alginolyticus H1822 | V. parahaemolyticus H1845 | V. splendidus H1802 | S. aureus 6538/206p | E. coli 26826 | Y. pseudotuberculosis M-3515 | P. aeruginosa 2785 |
| Bacillus megaterium A1                   | -                   | -                      | -                    | 5                  | -                   | -                      | 13                   |
| Bacillus pumilus A27                     | 6                   | -                      | -                    | -                  | -                   | -                      | 9                    |
| Micrococcus sp. K54                      | -                   | 6                      | -                    | -                  | -                   | -                      | 8                    |
| Bacillus sp. K33                         | 8                   | -                      | -                    | -                  | 6                   | -                      | 4                    |
| Micrococcus sp. A34                      | -                   | -                      | 5                    | -                  | -                   | -                      | 12                   |
| Bacillus sp. K29                         | 7                   | -                      | -                    | -                  | -                   | -                      | 14                   |
| Arthrobacter sp. A16                     | 14                  | -                      | 19                   | -                  | -                   | -                      | 19                   |
| Micrococcus sp. K55                      | -                   | -                      | -                    | 18                 | -                   | -                      | -                    |
| Kocuria sp. A34                          | 11                  | -                      | 18                   | -                  | 16                  | -                      | 13                   |
| Pseudoalteromonas sp. K59                | 10                  | -                      | 8                    | -                  | -                   | -                      | 8                    |
| Bacillus sp. A23                         | -                   | -                      | -                    | -                  | -                   | 24                     | 7                    |
| Bacillus megaterium K13                  | 11                  | -                      | 15                   | -                  | -                   | 16                     | 5                    |
| Aeromonas hydrophila A3                  | -                   | -                      | 12                   | -                  | -                   | 17                     | -                    |
| Bacillus sp. K32                         | 15                  | 5                      | 4                    | -                  | 6                   | -                      | 5                    |
| Halomonas sp. K49                        | -                   | -                      | -                    | -                  | -                   | 9                      | -                    |
| Bacillus pumilus K6                       | 7                   | -                      | -                    | -                  | -                   | -                      | -                    |
| Pseudomonas sp. K68                      | -                   | -                      | -                    | 7                  | -                   | -                      | -                    |

4 bacterial strains (3%) of the researched collection displayed antimicrobial activity against one test-culture, 7 bacterial strains (5%) developed antimicrobial activity against two test-cultures, 2 bacterial strains (1%) – against three cultures, 3 bacterial strains (2%) – against four cultures, 1
bacterial strain (less than 1%) – against five cultures. Thus, the most active strains inhibited two or more test-cultures, that may be probably dependent on a wide range of antimicrobial substances, produced by the strains. Nevertheless, none of the studied microorganisms inhibited the growth of as many as all pathogenic cultures.

9 strains of symbiotic bacteria (7%) demonstrated the ability to inhibit the growth of V. alginolyticus H1822 strain. Meanwhile, the largest growth inhibition zones for the strain of V. alginolyticus H1822 were on the dishes with the strains of Bacillus sp. K32 (15 mm) and Arthrobacter sp. A16 (14 mm) noticed. Concerning the other two pathogenic vibrios, only the insignificant suppression of its growth on the dishes with Bacillus sp. K32, Micrococcus sp. K54, Micrococcus sp. A34 was observed (table 1). On the basis of data received, the strain of Bacillus sp. K32 proved activity against all three pathogenic vibrios solely.

The growth of S. aureus 6538/206p was inhibited only by 5 strains (4%) from all collection of symbiotic bacteria. Zones of growth inhibition for Staphylococcus aureus extended over 8 to 19 mm. The maximum positive effect was in the dishes with the strains of Arthrobacter sp. A16 and Kocuria sp. A34 registered.

Interesting results were obtained while evaluating the antimicrobial activity of holothurian microflora against the specimens of the family Enterobacteriaceae. Though Y. pseudotuberculosis M-3515 and E. coli 26826 strains demonstrated the antibiotic susceptibility to the same antibiotics, but the ability to inhibit the growth of these microorganisms by different bacterial strains, isolated from the Apostichopus japonicus, was revealed. 9 strains (7%) inhibited the growth of Y. pseudotuberculosis M-3515 at various efficiency rate (zone of growth inhibition from 8 to 24 mm). The highest rate was for the strain of Bacillus sp. A23 found.

The growth of E. coli 26826 was only by 5 strains (4%) of holothurian symbiotic bacteria suppressed. The maximum efficiency rate was shown by the strain of Micrococcus sp. K55 (growth inhibition zone for test-culture covered 18 mm). It is significant to notice, that none of the strains in the collection demonstrated the antimicrobial activity against Y. pseudotuberculosis M-3515 and E. coli 26826 at the same time. It provably may be connected with different targets and action mechanisms of metabolites strains-producers.

In spite of Pseudomonas aeruginosa natural resistance to various antibiotics, during our researches the growth of P. aeruginosa 2785 was suppressed by 9 strains (7%), isolated from Apostichopus japonicus. The intensity of the process appeared in different ways, and the size of growth inhibition zone for test-culture varied within 5-16 mm range. The strain of Arthrobacter sp. A16 proved to be the most effective.

It is apparent from the data received, that the strongest antimicrobial activity against researched test-cultures was by the strains of Bacillus sp. K32, Arthrobacter sp. A16, Kocuria sp. A34, Bacillus megaterium K13, and Pseudoalteromonas sp. K59 demonstrated. According literary data, specimens of the pointed groups of microorganisms are quite often the producers of antimicrobial compounds.

Thus, among antibiotics, which are produced by bacilli, the Gramicidin S, generated by B. brevis, also Polymyxin M and Polymyxin B, generated by B. polymyxa, are used in the practice of medicine at the present time. Bacteria of the genus Bacillus are accepted as a powerful instrument for biocontrol of the quantity of phytopathogens due to the ability to produce a variety of secondary metabolites of different nature: cyclic lipopeptides, polypeptides, proteins, and non-peptide compounds. Besides, specimens of the genus Bacillus are often used as probiotics in commercial farming of Apostichopus japonicus for the purpose of prevention of hydrobionts immune diseases, as well as for increase of growth rate of animals [4, 10, 11].

Bacteria of the genus Arthrobacter are also highly biologically active. Thus, an Arthrobacter davidianelli-based vaccine was created and patented, which reduces risks of disease appearance for the atlantic salmon (Salmo salar) and the silver salmon (Oncorhynchus kisutch) species, which may be caused by such infectious agents as Renibacterium salmoninarum and Piscirickettsia salmonis [19]. As it follows from other data, bacteria of the genus Arthrobacter synthesize antimicrobial substances, that inhibit growth of Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus,
Salmonella enterica, Aeromonas salmonicida, Vibrio vulnificus, V. parahaemolyticus, V. harveyi, V. anguillarum, Yersinia enterolitica and Y. ruckeri [20].

The genus Kocuria is related phylogenetically to the genus Micrococcus, and it was comparatively recently defined as a separate taxon. There is not so much information on metabolic activity of these bacteria, but there are available data about Kocuria antimicrobial activity against Staphylococcus aureus and Vancomycin-resistant enterococcus due to cyclic peptide synthesis [21].

Bacteria of the genus Pseudoalteromonas are typical marine microorganisms, that are found often in association with marine eukaryotes, and they prove their antibacterial, antiviral, bacteriolytic, agarolytic, and algicidic activity. There are data, that some species of Pseudoalteromonas have ability to inhibit the growth of such pathogenic microorganisms, as Staphylococcus aureus [22], Vibrio sp. [23], Vibrio parahaemolyticus [24] and Vibrio anguillarum [25]. Synthesis of large quantities of bioactive substances by Pseudoalteromonas provided them with certain advantages under competition with other animals for nutrients and adhesive surfaces.

4. Conclusion

Thus, the results, obtained throughout the research, testify high potential capability of symbiotic microflora from the sea cucumber Apostichopus japonicus to resist impacts of pathogenic microorganisms. It is certain, that the research of chemical nature of synthesized antimicrobial substances, in order to understand mechanisms of positive influence, is a matter of strong interest. The obtained data may become useful both for exploring of competitive relationships in marine microbial communities, and for solving of applied tasks such as development of probiotics supplements as medicines with antimicrobial effect.

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