Biofilm formation as a method of survival of *Escherichia coli* and *Pantoea spp* in the marine environment

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Abstract. The article shows the formation of biofilms of bacteria Escherichia and Pantoea, which were isolated from sea water, both in monoculture and in associations with marine heterotrophs. It studied the influence of the nutrient medium and temperature on the biofilm-forming properties of marine strains. The highest biofilm formation properties were found in monoculture in family enterobacteria compared to saprophytic marine bacteria, regardless of the medium and the culture temperature. In association with saprophytes, *Pantoea spp*. possess more pronounced biofilm-forming properties at 37 ° C compared to the control than at 22 ° C and 5 ° C irrespective of the culture medium. *Escherichia coli*, in association with saprophytes, have less pronounced biofilm formation properties than monoculture, regardless of the temperature and culture medium.

1. Introduction

The coastal zone of the sea, which is the place of the predominant concentration of human activity and taking a large amount of waste. Waste requires constant monitoring of the content of pollutants and changes in the main parameters of the environment caused by anthropogenic impacts. For coastal sea waters, actively used in the economic activity of the coastal regions, the highest pollution is observed simultaneously with different pollutants (oil hydrocarbons, phenols, heavy metals, etc.) [1,2]. The active use of coastal zones for recreational purposes causes the emergence of a large number of sanitary-indicatoryn microorganisms in the marine environment that affect not only marine microbial communities, but also pose a threat to human health. Microbiological monitoring in ecologically unfavorable areas is promising for assessing the state of the environment, as well as ecological mapping of pollution of coastal zones. It is known that the growth of bacteria in the form of biofilms is a form of response to a variety of stresses, forcing them to adapt to the negative effects of the environment (and all possible variants of this effect). Organic pollution of the marine environment by pathogenic microorganisms is an important environmental factor affecting the vital activity of biological systems at different levels of their organization [3]. But, the marine environment is not optimal for the life of opportunistic and pathogenic bacteria, although, according to the literature, a number of authors note their long existence under these conditions [4]. One of the possible mechanisms for survival of enterobacteria in sea water may be their ability to biofilm formation with marine saprophytic microorganisms [5-7]. Previously, it was shown that the joint incubation of such and enterobacteria as *Esherichia coli* [8-16]. Based on this, the purpose of our investigation was to

study the ability of *Esherichia coli and Pantoea spp.* to form biofilms with marine saprophytic bacteria.

2. Methods

Microorganisms were isolated from sea water b. Golden Horn (Primorsky Krai, Russian Federation), characterized high microbial contamination followed by identification with the RapiD 20 E test kit (Biomerieux) and PCR confirmation with appropriate primers. The formation of biofilms was studied using a "tablet method" [17] by growing strains in 150 μl. Manupalition was on beef-extract broth and marine microrganisms medium (in a 96-well polystyrene plate for 72 hours (biofilm maturation stage according to the bacterial growth indications) at 5°C, 22°C, 37°C. Removal From the wells of plankton cells, staining of biofilms with 1% crystal violet solution, rinsing the wells with distilled water, adding 96% of ethanol to them. It was measured the optical density (OD) of the supernatant at a wavelength (540 nm) using a Multiscan ascent photometer (Thermo Electron Co., China).

3. Results and discussion

In total, it was allocated 9 bacterial isolates from seawater that have been investigated for the formation of biofilms in monoculture at different temperatures in two kinds of culture media. It was shown experimentally that, properties of biofilms studied microorganisms isolated from sea water, depending on the species. High properties of biofilms was observed, 2 isolates belonging to family Enterobacteriaceae identified as a strain of 16 M *Escherichia coli* and 42M *Pantoea spp*. And 7 strains of other marine microorganisms (table 1). *Escherichia coli* is very stable in the environment for a long time are stored in the soil, water, feaces, can cause cholera, dizenteria disease and enterits. Pantoea can cause infections of the kidneys and urinary tract infections (acute pyelonephritis, acute exacerbation of chronic prostatitis), genitals, the respiratory system [18].

Table 1. Influence of temperature and culture medium on the biofilm-forming properties of bacteria isolated from sea water[a].

	Optical density (OD)					
	Beef-extract broth			Marine microrganisms medium		
Strains	5° C	22° C	37° C	5° C	22° C	37° C
Pantoea spp.	0.843±0.01	1.064±0.01	0.467±0.01	0.723±0.01	1.162±0.01	0.301±0.01
Escherichia coli	2.012±0.01	2.354±0.01	1.091±0.01	2.001±0.01	2.353±0.01	1.762±0.01
Rhodococcus spp.	0.623±0.01	0.838±0.01	0.701±0.01	0.655±0.01	0.923±0.01	0.776±0.01
Halomonas spp.	0.634±0.01	1.102±0.01	0.823±0.01	0.553±0.01	0.987±0.01	0.745±0.01
Micrococcus spp.	0.560±0.01	0.921±0.01	0.733±0.01	0.736±0.01	1.030±0.01	0.899±0.01
Vibrio spp.	0.455±0.01	0.853±0.01	0.800±0.01	0.544±0.01	0.765±0.01	0.664±0.01
Arthrobacter spp.	0.645±0.01	0.811±0.01	0.766±0.01	0.685±0.01	0.844±0.01	0.738±0.01
Pseudomonas spp.	0.789±0.01	0.923±0.01	0.856±0.01	0.663±0.01	0.976±0.01	0.765±0.01
Acinetobacter spp.	0.564±0.01	0.782±0.01	0.633±0.01	0.544±0.01	0.773±0.01	0.667±0.01
Medium(control)	0.10	0.10	0.10	0.10	0.10	0.10

[a]the indicators M ± m; The differences are statistically significant with respect to control ($p < 0.05$)

The highest values of optical density were obtained in the monoculture for enterobacteria in comparison with saprophytic marine bacteria, regardless of the medium and the culture temperature. By the degree of biofilm formation in monoculture at 22°C, microorganisms can be arranged in the following order: *Escherichia coli> Pantoea spp. > Halomonas spp. ≥ Micrococcus spp. ≥ Pseudomonas spp. ≥ Rhodococcus spp. ≥ Vibrio spp. ≥ Acinetobacter spp.* It is shown that, the factor influencing the biofilm formation in all the strains studied is the temperature, in contrast to the nutrient medium. The highest optical density values in monoculture were observed at 22°C, compared to other temperatures, for all strains under study, which is consistent with literature data [19]. Perhaps this is due to the fact that the strains studied were isolated from sea water, where the average temperature in

summer does not exceed 22°C-24°C. Enterobacteria possessing the most pronounced biofilm-forming properties in monoculture have been investigated for the possibility of biofilm formation in associations with bacteria isolated from sea water. It was shown that for bacteria Pantoea spp. The most optimal for biofilm formation in associations with marine microorganisms was the temperature of 37°C, regardless of the culture medium (table 2).

Table 2. Effect of temperature and culture medium on the biofilm-forming properties of *Pantoea spp.* in association with microorganisms isolated from sea water[a]

Pantoea spp and heterotrophs	Optical density (OD)					
	Beef-extract broth			Marine microrganisms medium		
	5° C	22° C	37° C	5° C	22° C	37° C
Rhodococcus spp.	0.622±0.01	0.728±0.01	0.689±0.01	0.876±0.01	1.123±0.01	1.100±0.01
Halomonas spp.	0.734±0.01	1.211±0.01	1.011±0.01	1.008±0.01	1.435±0.01	1.324±0.01
Micrococcus spp.	0.823±0.01	1.157±0.01	1.145±0.01	0.876±0.01	1.165±0.01	1.109±0.01
Vibrio spp.	0.945±0.01	1.157±0.01	1.099±0.01	0.768±0.01	1.020±0.01	0.896±0.01
Arthrobacter spp.	0.554±0.01	0.789±0.01	0.625±0.01	0.622±0.01	0.823±0.01	0.738±0.01
Pseudomonas spp.	0.574±0.01	1.183±0.01	0.442±0.01	1.461±0.01	0.763±0.01	0.564±0.01
Acinetobacter spp.	0.813±0.01	1.276±0.01	0.901±0.01	0.943±0.01	1.192±0.01	0.781±0.01
Pantoea spp. в (monoculture)	0.843±0.01	1.064±0.01	0.467±0.01	0.721±0.01	1.164±0.01	0.301±0.01

[a]the indicators M ± m; The differences are statistically significant with respect to control ($p < 0.05$)

Optical density in associations *Pantoea spp.* and marine bacteria is higher than in monoculture (except *Pseudomonas spp* at 37°C. There is no significant differences in the results on the studied media, both in monoculture and in associations at 22°C,. The same data were obtained at a temperature of 5°C (except for *Halomonas spp., Pseudomonas spp*, where optical density is higher in associations than in monoculture). In the case of the association of *Escherichia coli* with marine heterotrophs, it was shown that they were more active in the formation of biofilms in monoculture than in association with marine bacteria (table 3).

Table 3. Influence of temperature and culture medium on the biofilm-forming properties of *Escherichia coli* in association with microorganisms isolated from sea water.[a]

Escherichia coli and heterotrophs	Optical density (OD)					
	Beef-extract broth			Marine microrganisms medium		
	5° C	22° C	37° C	5° C	22° C	37° C
Rhodococcus spp.	0.328±0.01	1.016±0.01	1.154±0.01	1.103±0.01	1.431±0.01	1.231±0.01
Halomonas spp.	0.227±0.01	1.140±0.01	1.701±0.01	1.364±0.01	0.773±0.01	1.003±0.01
Micrococcus spp.	0.404±0.01	1.506±0.01	0.463±0.01	0.472±0.01	0.884±0.01	0.552±0.01
Vibrio spp.	0.276±0.01	0.678±0.01	0.601±0.01	0.652±0.01	0.491±0.01	0.333±0.01
Arthrobacter spp.	0.154±0.01	1.585±0.01	1.553±0.01	2.193±0.01	1.693±0.01	1.552±0.01
Pseudomonas spp.	1.008±0.01	1.096±0.01	1.090±0.01	0.435±0.01	0.626±0.01	0.634±0.01
Acinetobacter spp.	0.564±0.01	0.787±0.01	0.657±0.01	0.539±0.01	0.692±0.01	0.593±0.01
Pantoea spp.	0.878±0.01	1.185±0.01	1.034±0.01	1.077±0.01	1.218±0.01	1.118±0.01
Escherichia coli (monoculture)	2.012±0.01	2.354±0.01	1.094±0.01	2.001±0.01	2.351±0.01	1.763±0.01

[a]the indicators M ± m; The differences are statistically significant with respect to control ($p < 0.05$)

Thus, pathogenic and conditionally pathogenic bacteria are able to form biofilms both in monoculture and in association with other bacteria that are present in sea water, which allows them to remain permanently in an unfavorable environmental conditions and is one of the mechanisms of adaptation.

4. Conclusion

The main factor that influences biofilm formation of microorganisms isolated from the marine environment is temperature, but not the nutrient medium. The highest biofilm formation in monoculture shown at 22°C for all studied strains. It is shown that the biofilm forming properties of the microorganisms studied, isolated from sea water, depend on the species. Thus, the highest values were obtained in the monoculture for *Escherichia coli and Pantoea* spp in comparison with other marine bacteria irrespective of the medium and the culture temperature. By the degree of decrease in the capacity for biofilm formation, the species can be arranged in the following order: *Escherichia coli> Pantoea spp. > Halomonas spp ≥Micrococcus spp ≥Pseudomonas spp. ≥ Rhodococcus spp. ≥Vibrio spp. ≥ Acinetobacter spp.* In association with saprophytes, *Pantoea* spp. possess more pronounced biofilm-forming properties at 37°C compared to the control than at 22°C and 5°C irrespective of the culture medium. *Escherichia coli,* in association with saprophytes, have less pronounced biofilm formation properties than monoculture, regardless of the temperature and culture medium.

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