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STUDIES ON THE INTERDEPENDENCE OF THE SELECTED PSYCHROPHYLIC BACTERIA STRAINS AND THEIR ACTIVITY ON *SALMONELLA* AND *ESCHERICHIA* GROUPS

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Investigated, was an interdependence of the water microbes and their activity on Salmonella group. In some cases, the stimulating and bacteriostatic effects were noted. No interactivity had been noted in many cases.

An interactivity of the microbes may lead to a phenomenon of the growth inhibition (bacteriostasis), or its stimulation, or to no changes in growth at all.

By Waksman (1937), such phenomenon plays an important part in the quantitative formation of the microbes and in the specific formation of the bacterial population under the natural conditions.

A bacteriostatic activity of moulds and fungus on the microbes have well been known. Recently, many investigators had been working on a determination of the microbes antagonistic activity towards the yeasts, moulds and fungus. For more interesting, may be considered the results obtained by: Roth (1959), in selection of three strains Pseudomonas fluorescens restraining the growth of certain parasitic and saprophilic funguses: Lewis (1929), on antagonistic influence of Pseudomonas fluorescens on certain species of yeasts and moulds. Furthermore, Buck and Meyers (1963), Baam and co-authors (1966), in their analyses ascertained the yeasts-destructive activity on the bacteria of a sea origin.

The recently carried-out researches had been not limited to that problem only: the antagonistic activities of various groups and of the same taxanomic groups had also been investigated.

Landenberger (1952) ascertained the growth inhibition of Esch. coli and Staph. aureus caused by Pseudomonas fluorescens: Brunner (1957), called an attention to a bacteriostatic activity of Pseudomonas fluorescens against the strains of Aeromonas punctata.

W a l l h a u s e r, in his works published in 1951, proved strong bacteriostatic characteristics of Pseudomonas fluorescens and considerably great sensibility of the strain Flavobacterium.

The subject analyses proved that majority of bacteria possess the bacteriostatic or stimulating ability of action on the certain defined species of microbes.

An interactivity of the bacteria characteristic for a water and land-environment is still relatively little known: it may, however, play a certain part on the coastal waters, where the forms of a land origin are of the most frequent occurence.

Object of this work, was the qualification of an interactivity between the water strains and the microbes of Salmonella and Escherichia groups, and a recognition of the interactivity among the examined water microbes.

MATERIALS AND METHODS

For the investigations had been used 29 strains, out of which 22 were the bacterial psychrophilic strains and 7 strains of the mezophilic microbes. List of the strains applied is represented in Table 1.

The microbes were cultured on a meat-broth and on agar of 2% and 0.7%. The bacteria selected from a sea-environment were cultured on a medium prepared on sea-water. The pH of medium was 7.2. Incubation temperature amounted to 20°C.

An interactivity of the microbes had been tested by Fredericq-Levine method modified by Kjems, according to K l i n g e (1959). The 24-hr cultures of the meat-soup strains were innoculated on the plates with 2% agar. After the 48-hr incubation, they were killed with the chloroform vapours. The plates were, thenafter, aired and about 4 ml of agar, infected with the 24-hr culture of a defined strain, had been spilled on a surface of the first layer of agar.

RESULTS

The investigations of an interactivity of the Salmonella and Escherichia groups and of the psychrophilic forms, also an interactivity of the bacteria in psychrophilic group, comprised 841 combinations. Out of these, 148 revealed an antagonistic or stimulating activity, what amounts to ab. 17.7% of total combinations.

The results of investigations performed on the antagonistic activity among the psychrophilic microbes revealed that, the most sensitive is the strain of Flavobacterium sp. Nr. 244. Its growth had been inhibited by the 15 out of 24 strains used for tests, what amounts to ab. 62.5%.

Table 1

List of strains used for the investigations

Item	Name of strain	Denomination	Origin
1	Achromobacter	No 4	Strain selected from a sea-fish
2	Aeromonas hydrophila	NCMB No 72	Torry Research Station Aberdeen
3	Aeromonas punctata	ED No 95	Low Temperature Research Station Cambridge
4	Aeromonas punctata	ED No 112	Low Temperature Research Station Cambridge
5	Aeromonas punctata	ED No 116	Low Temperature Research Station Cambridge
6	Anitratum	No 5	Strain selected from a sea-fish
7	Escherichia coli	No 306	Institute of Hygiene Warsaw
8	Escherichia coli	No 308	Institute of Hygiene Warsaw
9	Flavobacterium sp.	NCMB No 244	Torry Research Station Aberdeen
10	Flavobacterium sp.	NCMB No 249	Torry Research Station Aberdeen
11	Flavobacterium sp.	NCMB No 251	Torry Research Station Aberdeen
12	Pseudomonas sp.	No 172	Institute of Hygiene Warsaw
13	Pseudomonas sp.	No 186	Institute of Hygiene Warsaw
14	Pseudomonas sp.	NCMB No 129	Torry Research Station Aberdeen
15	Pseudomonas sp.	NCMB No 406	Torry Research Station Aberdeen
16	Pseudomonas sp.	NCMB No 320	Torry Research Station Aberdeen
17	Pseudomonas sp.	NCMB No 224	Torry Research Station Aberdeen
18	Pseudomonas sp.	NCMB No 322	Torry Research Station Aberdeen
19	Pseudomonas sp.	NCMB No 114	Torry Research Station Aberdeen
20	Pseudomonas sp.	NCMB No 133	Torry Research Station Aberdeen
21	Vibrio sp.	No. 6	Strain selected from a sea-fish
22	Vibrio ichthyodermis	NCMB No 407	Torry Research Station
23	Vibrio sp.	NCMB No 844	Torry Research Station

cont'd tab.1

Item	Name of strain	Denomination	Origin
24	Xanthomonas	No.8	Strain selected from a fish
25	Salmonella enteritidis	No.29	Institute of Hygiene Warsaw
26	Salmonella enteritidis	No.47	Institute of Hygiene Warsaw
27	Salmonella typhi murium	No.156	Institute of Hygiene Warsaw
28	Salmonella typhi murium	No.157	Institute of Hygiene Warsaw
29	Salmonella typhi murium	No.697	Institute of Hygiene Warsaw

Slightly smaller sensitiveness revealed the strains of Flavobacterium sp. No.251 (bacteriostasis caused by 10 strains), and of Vibrio No.844 (under a bacteriostatic activity of 13 strains).

The strongest antagonistic activity had been revealed by the exponents of Pseudomonas and Aeromonas groups. No inhibiting activity had been noted at the 11 strains tested.

The stimulating activity, in this arrangement, had been ascertained in 46 cases for 576 possible, what amounts to 7.9%. The growth-stimulating characteristics had been revealed at 17 strains. High stimulating ability proved Aeromonas punctata No.112 and Pseudomonas sp.No.114. Each had been stimulating 6 strains out of 24 used for analysis. Pseudomonas sp.No.129 caused the distinct stimulating zones of 4-7 mm in 5 bacterial species. Out of the 19 active strains, only Pseudomonas sp.No.322 and Pseudomonas sp.No. 114 revealed an entire stimulating activity. The remaining 17 proved a bacteriostatic activity on the certain species and stimulating on the others.

No stimulating sensitiveness had been noted in 11 bacterial strains.

Analysing an activity of the psychrophilic microbes on a bacteria of Salmonella and Escherichia groups (Table 3) it may be ascertained that, an inhibition of growth (bacteriostasis) occured in 14 cases out of 154 possible, what amounts to about 8.1%.

The highest inhibiting effect on the exponents of Salmonella group resulted from Pseudomonas sp.No.129. It caused the restraining zones at the 2 out of 5 tested strains (Salmonella No.29 and No.47). Similiar effects proved, also, the Aeromonas punctata No.95 and Flavobacterium sp.No.249, though the inhibiting zones were somehow smaller.

The strains: Achromobacter No.5, Esch.coli No.306, Esch.coli No.308, Pseudomonas sp.No.320, Pseudomonas sp. No.322 and Vibrio No.844 caused an inhibition of growth at the singular exponents of Salmonella.

The highest sensitiveness was noted at Salmonella enteritidis No.29: its growth had been inhibited by 4 strains.

Table 2

Results of the microbes interactivity within the tested psychrophilic strains and two strains of Escherichia group

Item	Name of strain	Activity	
		Bacteriostatic	Stimulating
1	Achromobacter No.4	9b, 24c	-
2	Aeromonas hydrophila No.72	9c, 10a, 11a, 22c	24a
3	Aeromonas punctata No.95	8c, 9c, 11a, 15c, 23c	21c, 24a
4	Aeromonas punctata No.112	9c, 22c	3c, 11a, 12b, 21a, 23a
5	Aeromonas punctata No.116	9b, 14c	21a, 22a, 23a, 24a
6	Anitratum No.5	9b, 23c	-
7	Escherichia coli No.306	9c, 10a, 11b, 23c	22a
8	Escherichia coli No.308	10a, 23b	-
9	Flavobacterium sp.No.244	10b, 11b, 23a	22c
10	Flavobacterium sp.No.249	6c, 11b, 22c	-
11	Flavobacterium sp.No.251	3c, 10c	20b, 23b
12	Pseudomonas sp.No.172	9c, 14a, 23b	-
13	Pseudomonas sp.No.186	9c, 23c	-
14	Pseudomonas sp.No.129	9c, 23b	4a, 11a, 12b, 21b, 24a
15	Pseudomonas sp.No.406	8c, 9c, 11b, 23b	4b, 14c, 21c, 24a
16	Pseudomonas sp.No.320	1c, 9c, 11b, 23b	22c
17	Pseudomonas sp.No.224	6c	11a, 23a
18	Pseudomonas sp.No.322	-	11a, 12a, 23a, 24b
19	Pseudomonas sp.No.114	-	4a, 5a, 11a, 12a, 23a, 24a
20	Pseudomonas sp.No.133	8c, 9c, 11c, 23c	22c
21	Vibrio sp.No.6	9b, 11c, 23b	10b, 22b
22	Vibrio ichth.No.407	11b	9b, 21c
23	Vibrio sp.No.844	22c	-
24	Xanthomonas No.8	9b, 23c	10b, 22b

Legend to results:
a. - strong. (Inhibiting or stimulating zones of 5 mm and above).
b. - medium. (Inhibiting or stimulating zones within 3-4 mm).
c. - weak. (Inhibiting or stimulating zones within 1-2 mm).

digits - numbers of strains according to list. (Table 1)

Table 3

Results of the psychrophilic strains activity on microbes of Salmonella and Escherichia groups

Item	Name of strain	Activity	
		Bacteriostatic	Stimulating
1	Achromobacter No.4	25b	-
2	Aeromonas hydrophila No.72	-	25a
3	Aeromonas punctata No.95	8c, 26c, 28c	-
4	Aeromonas punctata No.112	-	26c
5	Aeromonas punctata No.116	-	-
6	Anitratum No.5	-	25a, 26a, 28a, 29b
7	Flavobacterium sp.No.244	-	-
8	Flavobacterium sp.No.249	26c, 28c	-
9	Flavobacterium sp.No.251	-	25b
10	Pseudomonas sp.No.172	-	25a
11	Pseudomonas sp.No.186	-	-
12	Pseudomonas sp.No.129	25b, 26c	-
13	Pseudomonas sp.No.406	8c	-
14	Pseudomonas sp.No.320	25b	-
15	Pseudomonas sp.No.224	-	-
16	Pseudomonas sp.No.322	29b	-
17	Pseudomonas sp.No.114	-	-
18	Pseudomonas sp.No.133	8c, 29b	-
19	Vibrio sp.No.6	25c	29a
20	Vibrio ichth. No.407	-	-
21	Vibrio sp.No.844	-	29b
22	Xanthomonas No.8	-	-

Legend to results:

a. - strong. (Inhibiting or stimulating zones of 5 mm and above).
b. - medium. (Inhibiting or stimulating zones within 3-4 mm).
c. - weak. (Inhibiting or stimulating zones within 1-2 mm).
digits - numbers of strains according to list. (Table 1)

The most resistant, proved to be the strain Salmonella typhi murium No.156, as no bacteriostatic activity had been revealed at any of the investigated species of microbes.

Considering an activity of the psychrophilic forms on the two strains of Escherichia group, the high resistance was noted at Escherichia coli No.306 (no antagonistic activity) and the bacteriostasis at Escherichia coli No.308 by two strains of Pseudomonas group.

A phenomenon of stimilation in this arrangement had been noted in 10 cases out of 154 possible, what represents 6.5%.

The most growth-stimulating was Anitratum No.5. It caused the stimulating zones (about 5 mm) at the 4 out of 5 investigated exponents of Salmonella group. Furthermore, out of the total 22 strains used for tests, 6 proved the stimulating effects on the singular Salmonella strains.

The Escherichia forms proved to be resistant to stimulating activity, as no growth stimulation was noted.

Table 4

Results of the Salmonella and Escherichia microbes activity on the psychrophilic forms

Item	Name of strain	Activity	
		Bacteriostatic	Stimulating
1	Escherichia coli No.306	9c, 10a, 11b, 23c	-
2	Escherichia coli No.308	10a, 23b	-
3	Salmonella typhi murium No.156	9c	-
4	Salmonella typhi murium No.157	9c, 23c	-
5	Salmonella typhi murium No.697	9b, 23b	-
6	Salmonella enteritidis No.29	9c, 23b	-
7	Salmonella enteritidis No.47	9c, 23c	-

Legend to results:
a. - strong. (Inhibiting or stimulating zones of 5 mm and above).
b. - medium. (Inhibiting or stimulating zones within 3-4 mm).
c. - weak. (Inhibiting or stimulating zones within 1-2 mm).
digits - numbers of strains according to list (Table 1)

To complete a picture of a phenomenon on the bacteriostasis and stimulation among the investigated microbes, additional analysis relating to an activity of Salmonella and Escherichia groups on the psychrophilic forms, had been performed. A phenomenon of stimulation was noted in one case only. High sensitiveness proved the strain Flavobacterium sp.No.244. Its growth had been inhibited by all used exponents of Salmonella group and one strain

Esch.coli. Furthermore, 4 strains of Salmonella group and 2 of Escherichia group proved distinctly a bacteriostatic activity on Vibrio No.844.

The results described above are represented in the Table 4.

DISCUSSION

For the investigations were used 29 bacterial strains. Not all the microbes were sensitive to the inhibiting or stimulating activity of a growth. The most active in relation to Salmonella group and towards the other strains used for tests, proved to be the microbes of Pseudomonas species. They caused the distinct zones of a growth inhibition (bacteriostasis).

An bacteriostatic activity of Pseudomonas strains had been already investigated many times. This is apparent from the works of R o t h (1959), L e w i s (1929), L a n d e n b e r g e r (1952), W a l l h a u s e r (1951).

The present-investigations proved that the sea forms are little sensitive to a bacteriostatic activity of the species of Salmonella group. The Flavobacterium strains, even though revealed a low degree of activity, proved high sensitiveness to an activity of the other bacteria species.

W a l l h a u s e r (1951) in his works obtained the similiar results. The effected investigations revealed an existance of the microbe species of Salmonella and Escherichia groups and of the psychrophilic forms, which are highly resistant to an bacteriostatic activity.

If the strain tested was growing in the presence of its own metabolites, no bacteriostatic or stimulating activity had been noted in the course of these investigations.

Not much data on stimulation is given in the accessible literature. The results obtained prove a high stimulating activity of Anitratum strain on the exponents of Salmonella group, but none of the Salmonella strains caused a growth-stimulation of the microbes used for the investigations.

The most sensitive to stimulation, appear to be the Vibrio and Flavobacterium forms. Some strains of Pseudomonas species and Aeromonas proved the highest growth stimulating activity.

In the investigations performed, an attempt had been made to indicate an interdependance of the microbes within the psychrophilic group, and an activity of the psychrophilic microbes and the species of Salmonella and Escherichia groups.

Purpose of the investigations was to determine, whether a phenomenon of bacteriostasis between the psychrophilies and Salmonella and Escherichia groups plays any part in the selfcleaning of waters, by eliminating certain species of microbes pathogenic for human and animals. An attempt had been made to pointout within what extent the microbes of Salmonella and Escherichia groups lose the pathogenic meaning due to antagonism towards the psychrophilic forms, which represent the normal water flora, or in this case sea-flora.

Such phenomenon could play an important part in a coastal zone, where the contamination with the Salmonella intestinal rods is largest.

It appears from the investigations that, the Salmonella strains were resistant to an activity of the psychrophilic forms in 91.9% of the combinations investigated. It may, thus be assumed that an antagonism plays relatively small part in the self-cleaning of waters from these strains, while the contamination with the Salmonella and Escherichia rods may constitute a pathogenic factor for human being and animals. Also, the contamination of fish, after catching, with these species does not elimi natetheir pathogenic importance.

CONCLUSION

The highest antagonistic activity proved the microbes of Pseudomonas species, whilst the highest sensitiveness revealed the Flavobacterium strains.

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BADANIA NAD WZAJEMNYM ODDZIAŁYWANIEM WYBRANYCH SZCZEPÓW BAKTERII PSYCHROFILNYCH ORAZ SALMONELLA I ESCHERICHIA

S t r e s z c z e n i e

W badaniach własnych stwierdzono oddziaływanie antagonistyczne i stymulujące jak również brak wzajemnego oddziaływania wśród szczepów należących do grupy Pseudomonas-Achromobacter.

Najsilniejsze oddziaływanie antagonistyczne wykazywały szczepy Pseudomonas sp., a działaniem stymulującym wyróżniał się Aeromonas punctata.

Działanie antagonistyczne pałeczek psychrofilnych na szczepy Salmonella i Escherichia stwierdzono tylko w 8.1% badanych przypadków, w 6.5% przypadków uzyskano działanie stymulujące.

ИЗУЧЕНИЕ ВЗАИМОДЕЙСТВИЯ ИЗБРАННЫХ ПАССАЖЕЙ ПСИХРОФИЛЬНЫХ БАКТЕРИЙ А ТАКЖЕ SALMONELLA И ESCHERICHIA

Р е з ю м е

Собственными исследованиями установлено антагонистическое и стимулирующее воздействие а также отсутствие взаимодействия среди пассажей принадлежащих к группе Pseudomonas-Achromobacter.

Самое сильное антагонистическое влияние показали пассажи Pseudomonas sp. а стимулирующим влиянием отличился Aeromonas punctata. Антагонистическое воздействие психрофильных палочек на пассажи Salmonella и Escherichia установлено только лишь в 8,1% изученных случаёв, в 6,5% случаёв получено стимулирующее влияние.

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