Extracellular enzyme activities of aquatic bacteria in polluted environment
2. Amylolytic activity

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Water samples were taken from Lake Drūkšiai tributaries (Ričanka Stream (Ričanka); Gulbinės Stream affected by urban rain sewerage from Visaginas (URS-2); Gulbinėlė Stream into which municipal sewage from Visaginas (MS) and industrial rain sewerage from the Ignalina Nuclear Power Plant (INPP) (IRS-1.2)) and their mouth, and Lake Dringis. Lake Dringis, in Aukštaitija National Park, was selected as an ecosystem pattern of a weak anthropogenic influence, while Lake Drūkšiai was chosen as a regularly polluted water body. Lake Drūkšiai, the cooling basin of the INPP, is being polluted with industrial and municipal sewage through its tributaries.

The amylolytic activity (AA) of heterotrophic aquatic bacteria was tested. The highest total mean AA of aquatic bacteria was calculated in Lake Dringis. Here, the results were significantly higher than in Lake Drūkšiai tributaries and their mouths, excepting the mouths of the Ričanka and MS. The lowest mean of AA in Lake Drūkšiai was characteristic of the IRS-1.2 tributary. A comparison of the mean AA of active isolates showed that certain bacterial strains from the sites of varying degrees of pollution could be noted for a relatively high level of enzymatic activity. Thus, anthropogenic pollution exerts a negative effect on the total mean AA, although certain strains of bacteria are able to adapt to the stressful environment and remain active.

Key words: aquatic bacteria, amylolytic activity, polluted environment

INTRODUCTION

The community of microorganisms in every water body plays a decisive role in the transformation of organic matter and the processes of metabolism (Chrost, 1990; Munster and Chrost, 1990). The bacteria belonging to various physiological groups decompose proteins, cellulose, starch, chitin, lignin, and other macromolecular organic matter (Billen, 1991; Chrost, Overbeck, 1990; Donderski, 1980; 1988; Hare et al., 1981; Vrba, 1992; Vrba et al., 1992). The intensity and dynamics of the process of decomposition are dependent on the ecological state of the water body (Chrost, 1991; Priest, 1984).

The purpose of the present study was to evaluate the amylolytic activity (AA) of heterotrophic aquatic bacteria from differently polluted test sites of Lake Drūkšiai and Lake Dringis.

MATERIALS AND METHODS

Water samples were drawn for microbiological testing during September, 1991–1995 and 1997. Water samples were taken from the depth of 0.5 m of Lake Drūkšiai tributaries (Ričanka Stream (Ričanka); Gulbinės Stream affected by urban rain sewerage from Visaginas (URS-2); Gulbinėlė Stream into which municipal sewage from Visaginas (MS) and industrial rain sewerage from INPP (IRS-1.2)) are released, and their mouths, and Lake Dringis. Lake Dringis, in Aukštaitija National Park, was chosen as an ecosystem of weak anthropogenic influence and Lake Drūkšiai as a regularly polluted water body. Lake Drūkšiai, the cooling basin of the INPP, is being polluted with industrial and municipal sewage through its tributaries. The characteristics of test sites are presented in (Arbačiauskienė, 2002).

Studies were carried out with aerobic and facultative anaerobic planktonic bacteria isolated in September (1991–1995 and 1997) from water samples. After preliminary studies, bacteria that decompose starch were used to study AA. Each isolate was incubated in a rotary shaker (100 rpm) at 22 °C in a liquid medium which contained: NH_4Cl – 9.0 g/l; K_2HPO_4 – 0.5 g/l; $MgSO_4 \cdot 7H_2O$ – 0.5 g/l; $CaCO_3$ – 3.0 g/l; glucose – 20.0 g/l; starch soluble – 10 g/l, and tap water (pH 7) (Asai et al., 1957). Extracellular enzyme activity was measured every 24 h for 5 days.

AA was determined by the GOST 20264.2–74 method (Препараты… 1981). A unit of AA used was the quantity of enzyme that catalyzed hydrolysis of 1 g of soluble starch under strictly determined conditions (temperature, pH, time of effect) to dextrins of various molecular weights. The quantity is expressed in terms of units per ml.

AA is presented as mean ± SD. The nonparametric Mann–Whitney *U* and Kolmogorov–Smirnov tests were used for comparison of enzymatic activity in the test sites.

RESULTS AND DISCUSSION

The results are presented in Figs. 1, 2 and Tables 1, 2. It has been determined that amylolytic activity is characteristic of 65% (39 of 60) of the bacteria isolated from the Lake Dringis cenosis of heterotrophic bacteria. According to a study of amylolytically active bacteria in Drūkšiai Lake tributaries and their mouths varying by their state of pollution, it was determined that the proportion of this group of bacteria was less in the tributaries than in their mouths. Amylolytically active bacteria comprised 10% to 25% in the tributaries, whereas in the mouths they comprised 25% to 45% of isolates. The greatest portion of such bacteria amongst the tributaries tested was found to be 25% (15 of 60) in the Ričanka. The lowest portion was 10% (6 of 60) in the URS-2. In the IRS-1.2 and MS, amylolytically active bacteria comprised 20% (12 of 60) of strains isolated in test sites. In tributary mouths the highest portion of amylolytic bacteria was found in the mouth of the Ričanka – 45% (27 of 60). In the mouths of the URS-2 and MS this group of bacteria reached 40% (24 of 60). The lowest portion of amylolytic bacteria – 25% (15 of 60) was found in the mouth of the IRS-1.2 (Fig. 1).

The total mean of AA of aquatic bacteria from sampling sites was not equal (Table 1). The total mean AA of aquatic bacteria from Lake Dringis reached 1.186 ± 1.360 units/ml and comprised the highest activity in comparison with the other sampling sites ($p < 0.01$), except those of the mouths of the Ričanka and MS (Table 2). The mean AA of bacteria isolated from Lake Drūkšiai tributaries of dissimilar levels of pollution was very low, comprising from 0.206 ± 0.653 to 0.559 ± 1.085 units/ml. The highest mean AA appeared in bacterial strains isolated from the Ričanka, whereas the lowest was found in strains from the IRS-1.2. The mean AA of bacterial strains taken from the URS-2 and MS differed little from the activity of bacteria in the IRS-1.2. The total mean AA of bacteria taken from all the mouths of tributaries ranged from 0.342 ± ± 0.812 to 1.092 ± 2.376 units/ml. The mean AA of bacteria taken from the mouth of the Ričanka was nearly two times higher in comparison with the bacterial activity in the tributary itself and was similar to the activity of strains in Lake Dringis. The total mean AA of bacterial strains taken from the mouth of the MS was three times higher (0.901 ± 1.559 units/ml), and in the mouth of the URS-2 it was two times higher (0.590 ± ± 1.126 units/ml) in comparison with the mean indices of the tributaries themselves. At the same time, the mean AA of bacterial strains taken from the mouth of the IRS-1.2 was slightly higher than in the tributary, reaching 0.342 ± 0.812 units/ml. Thus, the total mean AA of heterotrophic bacteria taken from the IRS-1.2 and its mouths was lower and significantly ($p < 0.01$ or $p < 0.05$) differed from the AA found in the mouths of the Ričanka and MS (Tables 1, 2).

By analysing the dynamics of the total mean AA over a five-day period, it was determined that the highest activity on the part of bacteria found in Lake Drūkšiai tributaries URS-2, MS and IRS-1.2 and Lake Dringis was reached on the third day of cultivation (Fig. 2a, b). The strains of bacteria taken from the mouths of the tributaries culminated on

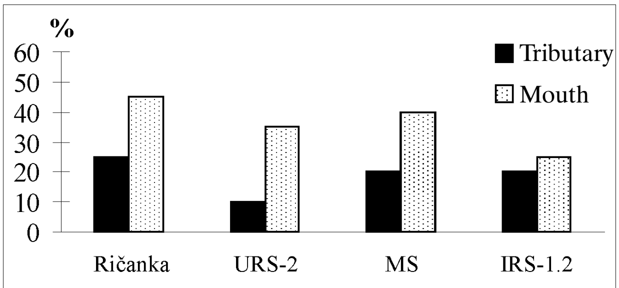

Fig. 1. Proportion of amylolytically active bacteria in Lake Drūkšiai tributaries (Ričanka, URS-2, MS and IRS-1.2) and their mouths

Table 1. **Total mean AA of heterotrophic aquatic bacteria over five days from Lake Drūkšiai tributaries (Ričanka, URS-2, MS and IRS-1.2) and their mouths and Lake Dringis (mean ±SD)**

Test sites	n	AA units/ml	Range of values units/ml
Lake Drūkšiai:			
Ričanka	60	0.559 ± 1.085	0.000–3.004
Ričanka mouth	60	1.092 ± 2.376	0.000–2.802
URS-2	60	0.263 ± 0.906	0.000–3.910
URS-2 mouth	60	0.590 ± 1.126	0.000–2.230
MS	60	0.306 ± 0.757	0.000–2.530
MS mouth	60	0.901 ± 1.559	0.000–3.246
IRS-1.2	60	0.206 ± 0.653	0.000–1.805
IRS-1.2 mouth	60	0.342 ± 0.812	0.000–1.830
Lake Dringis	60	1.186 ± 1.360	0.000–2.459

Table 2. **Tests of significance of difference in mean AA of heterotrophic aquatic bacteria over five days between sampling sites based on Mann–Whitney *U* (top-right) and Kolmogorov–Smirnov (bottom-left) tests. Number is p-level, in.d. – insignificant differences**

	Dringis	Ričanka	Ričanka mouth	MS	MS mouth	IRS-1.2	IRS-1.2 mouth	URS-2	URS-2 mouth
Dringis		<0.01	0.02	<0.01	0.21	<0.01	<0.01	<0.01	<0.01
Ričanka	<0.01		0.10	0.20	0.03	0.22	0.62	0.03	0.50
Ričanka mouth	in.d.	in.d.		<0.01	0.53	<0.01	0.02	<0.01	0.22
MS	<0.01	in.d.	0.05		<0.01	0.97	0.44	0.32	0.06
MS mouth	0.05	in.d.	0.05	0.05		<0.01	<0.01	<0.01	0.12
IRS-1.2	<0.01	in.d.	0.01	in.d.	0.01		0.42	0.26	0.05
IRS-1.2 mouth	<0.01	in.d.	0.05	in.d.	0.05	in.d.		0.07	0.24
URS-2	<0.01	in.d.	0.01	in.d.	0.01	in.d.	in.d.		<0.01
URS-2 mouth	0.01	in.d.	in.d.	in.d.	in.d.	in.d.	in.d.	in.d.	

the fourth day. Aquatic bacterial strains isolated from Lake Dringis reached the mean AA of 0.665 ± ± 0.878 units/ml on the second day. On the third day the activity became maximal, reaching 1.510 ± ± 1.591 units/ml, and on the fourth day it began to decrease, falling to 0.888 ± 1.167 units/ml by the fifth day. In the meantime, calculations of mean AA of tributaries Drūkšiai Lake were very low over the five-day period, ranging from 0.181 ± 0.520 to 0.665 ± 1.304 units/ml. Bacterial strains obtained from the IRS-1.2 showed the lowest degree of activity. On the second day of cultivation the numbers for mean AA reached a mere 0.181 ± ± 0.520 units/ml, then grew to 0.341 ± 0.967 units/ml on the third day, and on the fourth day fell to 0.096 ± 0.294 units/ml. No activity of bacterial strains was evident on the fifth day of cultivation. The activity of bacterial strains in the URS-2 and MS was somewhat higher, ranging from 0.147 ± ± 0.392 to 0.506 ± 1.060 units/ml. The highest activity amongst the tributaries tested was characteristic of bacterial strains isolated from the Ričanka. Their mean AA ranged from 0.328 ± 0.949 to 0.656 ± 1.304 units/ml over a five-day period. The highest mean AA was noted on the fourth day of cultivation (Fig. 2a).

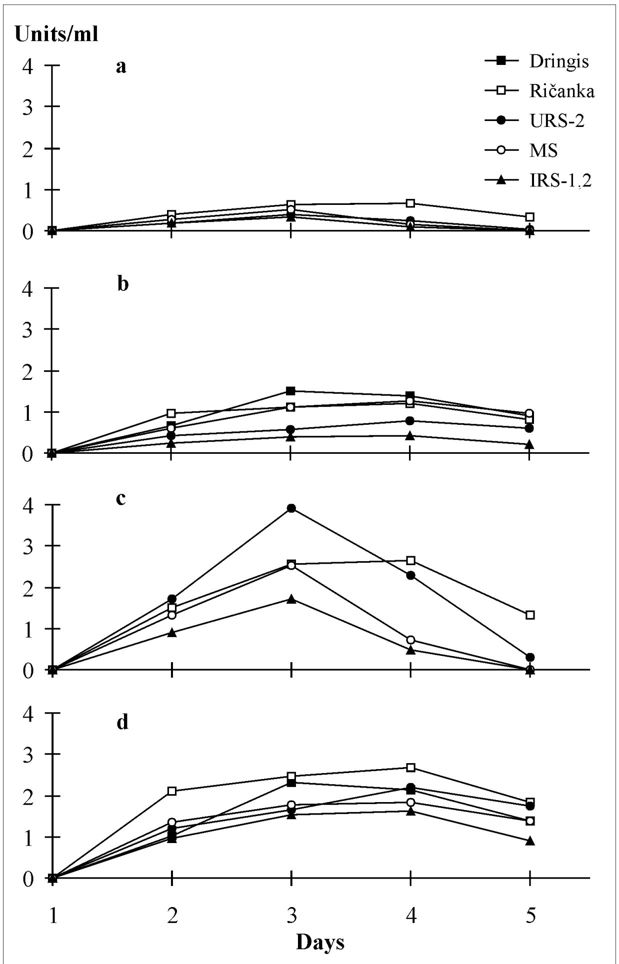

Fig. 2. Dynamics of total mean amylolytic activity (*a, b*) and mean activity of active isolates (*c, d*) over five days for aquatic bacteria from Lake Drūkšiai tributaries (Ričanka, URS-2, MS and IRS-1.2) (*a, c*) and their mouths, and Lake Dringis (*b, d*)

Testing of the mouths of Lake Drūkšiai tributaries showed that most active were the aquatic bacteria isolated from the mouth of the Ričanka. Their mean AA over a five-day period ranged from 0.821 ± 2.095 to 1.204 ± 2.436 units/ml. Strains of bacteria obtained from the mouth of the MS showed similar results (0.603 ± 1.326 to 1.275 ± ± 1.718 units/ml). The lowest was the mean AA over a five-day period, ranging between 0.223 ± 0.557 and 0.406 ± 0.837 units/ml, for aquatic bacteria taken from the mouth of the IRS-1.2. It virtually did not differ from the bacterial activity of the tributary itself (Fig. 2b).

The dynamics of the mean AA of active bacterial strains over a five-day period (Fig. 2c, d) indicated that the highest activity was reached during the third and fourth day of cultivation. Active aquatic bacterial strains from Lake Dringis ranged from 1.023 ± 0.908 to 2.323 ± 1.402 units/ml over a five-day period. The highest activity (2.323 ± ± 1.402 units/ml) was reached on the third day of cultivation. On the fourth day the activity decreased to 2.130 ± 1.203 units/ml. Active bacterial strains from Lake Drūkšiai reached their highest mean AA in the third day of cultivation. Just as Lake Dringis bacterial strains, the mean AA began to decrease during the fourth day, except the levels of activity noted in the Ričanka, where bacterial activity remained the same during the fourth and the third day. Active strains in the mouths of tributaries indicated their highest mean AA during the fourth day of cultivation.

Bacterial strains taken from the URS-2 showed the highest mean AA amongst active strains (3.910 ± ± 1.149 units/ml). Active bacterial strains from the IRS-1.2 and its mouths had the lowest mean AA (1.707 ± 1.677 units/ml) (Fig. 2c).

Not all aquatic bacteria react in an equally sensitive manner to changes in the environment. A number of works (Colwell, Walker, 1977; Colwell, Barkay, 1980; Klerks, Weis, 1987; Klug, Tiedje, 1993; Simidu et al., 1980) have shown that even in the presence of large-scale toxic compound pollution of hydroecosystems, certain taxonomic groups of bacteria are not only able to survive under conditions of increased stressor concentration, but are also able to actively participate in the processes of metabolism. In order to evaluate such biochemical activity amongst strains of bacteria, mean AA was determined for active strains. In our experiments, a comparison of mean AA among active strains showed that certain bacterial strains from the sites of varying degrees of pollution could be noted for a relatively high level of biochemical activity. The mean AA of all the active strains of aquatic bacteria found in Lake Drūkšiai tributaries and their mouths was significantly higher than the total mean AA. The data indicate the ability of certain strains to adapt to a polluted environment. The highest AA of active strains was found in the URS-2 on the third day of cultivation (3.910 units/ml) (Fig. 2c). It was higher than the activity of Lake Dringis aquatic bacteria.

In summary, the proportion of amylolytically active strains of heterotrophic bacteria was greater in Lake Dringis than in Lake Drūkšiai tributaries and their mouths. The highest total mean AA of all isolated heterotrophic bacteria was calculated for Lake Dringis. Here the results were significantly higher than for Lake Drūkšiai tributaries and their mouths, except the mouths of the Ričanka and MS. The lowest mean AA of activity in Lake Drūkšiai was characteristic of the IRS-1.2. The lowest mean AA for the mouths of tributaries was also found in the mouth of the IRS-1.2. A comparison of mean AA among active isolates showed that certain bacterial strains from the sites of varying degrees of pollution could be noted for a relatively high level of enzymatic activity. The mean AA of all active strains of aquatic bacteria found in Lake Drūkšiai tributaries and their mouths was significantly higher than the total mean AA. The data indicate the ability of certain strains to adapt to a polluted environment.

Thus, the results show that anthropogenic pollution exerts a negative effect on the total mean AA, although certain strains of bacteria are able to adapt to the stressful environment and remain active.

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VANDENS BAKTERIJŲ UŽLĄSTELINIŲ FERMENTŲ AKTYVUMAS UŽTERŠTOJE APLINKOJE 2. AMILOLITINIAI FERMENTAI

S a n t r a u k a

Vandens mėginiai buvo paimti iš Drūkšių ežero intakų (Ričankos upelio (Ričanka); Gulbinės upelio, kuriuo teka Visagino miesto lietaus kanalizacijos vandenys, (URS-2); Gulbinėlės upelio, į kurį patenka Visagino buitiniai nutekamieji vandenys, (MS); Ignalinos atominės elektrinės (IAE) pramoninės lietaus kanalizacijos vandenys, (IRS-1,2)) bei jų žiočių ir Dringio ežero. Dringio ežeras, esantis Aukštaitijos nacionaliniame parke, pasirinktas kaip silpnai žmogaus veikiamas ekosistemos etalonas, o Drūkšių ežeras – kaip lėtinės taršos vandens telkinys. Į Drūkšių ežerą – IAE aušinamąjį vandens baseiną – intakais (URS-2, MS, IRS-1,2) patenka pramoninės ir buitinės nuotekos.

Buvo tirtas heterotrofinių vandens bakterijų amilolitinis aktyvumas (AA). Didžiausias vandens bakterijų bendras vidutinis AA nustatytas Dringio ežere. Šis rezultatas buvo patikimai didesnis už Drūkšių ežero intakų ir jų žiočių bendrą vidutinį vandens bakterijų AA, išskyrus Ričankos ir MS žiotis. IRS-1,2 intako bakterijų vidutinis AA buvo mažiausias. Palyginus aktyvių kamienų vidutinį AA, nustatyta, kad ir iš užterštų vietų išskirti atskiri bakterijų kamienai pasižymėjo santykinai dideliu fermentiniu aktyvumu. Tyrimai parodė, kad antropogeniniai teršalai neigiamai veikia bendrą vidutinį AA, nors kai kurie bakterijų kamienai gali prisitaikyti prie stresinės aplinkos ir išlikti aktyvūs.

Raktažodžiai: vandens bakterijos, amilolitinis aktyvumas, užteršta aplinka