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RESEARCH ARTICLE

ENZYMATIC ACTIVITY OF BACTERIA ISOLATED FROM KHARAA RIVER

***,[1]Damdinsuren Narantuya, [2]Aruyna A. Radnagurueva, Elena V.Lavrentieva, [2]Bair B. Namsaraev, [3]Dorjdagva Sainjargal and [1]Davaasuren Tumenjargal**

[1]Department of Biology, School of Science, National University Mongolia, Ulaanbaatar 14201, Mongolia
[2]Institute of General and Experimental Biology of SB RAS, Ulan-Ude
[3]Central Joint Laboratory of the General Agency for Specialized Inspection, Ulaanbaatar, Mongolia

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ABSTRACT

The study of the extracellular enzymes activity, as protease, amylase and cellulase of bacteria isolated from the Kharaa River has been made. Proteases were also synthesized very intensively by bacterial strains isolated from the Kharaa River. Maximum amylase activity has been shown by the isolate from the sites with developed agriculture and livestock. The percentage of organisms having cellulytic activity is less in comparison with other enzymes; there are only few reports from bacterial strains. In the spring time, bacterial isolates have shown maximum cellulytic activity. Our study clearly revealed the potential of bacterial strains and their ability of extracellular enzyme production. Almost 92% of the strains have shown extracellular enzymatic activity for anyone of the enzyme screened.

INTRODUCTION

Microbial communities represent a fundamental part in aquatic ecosystems and are of great importance for the flux of matter and energy. Heterotrophic bacteria in particular play a decisive role in the destruction process of organic matter in river ecosystems which may be derived from allochthonous (primary production) as well as from autochthonous sources (e.g. surface run-off). Their contribution to self-purification, to processes of destruction as well as to the ecological integrity of rivers is of great interest within the scope of water quality assessment (Kavka *et al*., 1998). Most of the organic matters in aquatic ecosystems consist of compounds of a high molecular weight and polymeric structure, mainly proteins, starch, lipids, pectin, cellulose, chitin, nucleic acids, or lignin (Arnosti *et al*., 1998; Po Remba 1995; Unanue *et al*., 1999) For heterotrophic bacteria, those high molecular weight biopolymers constitute an important source of carbon, nitrogen, and energy used for biosynthesis or respiration (Brown *et al*., 1996; Patel *et al*., 2000). For this reason, according to Boetius (1995), Jackson *et al*. (1995), Mallet and Debroas (1999), enzyme assays can provide powerful tools for studying organic matter degradation and nutrient cycling in aquatic ecosystems. For heterotrophic bacteria, those compounds constitute a very important source of carbon, nitrogen, and energy, and are used for biosynthesis or respiration processes (Brown and Goulder 1996; Patel *et al.,* 2000). Because most of DOM and POM in aquatic ecosystems is polymeric in structure, it cannot be assimilated directly by bacteria because of the insufficient permeability of their membranes (Arnosti *et al.,* 1998; Hoppe *et al.,* 2002). The environmental benefits of enzymes that reduced chemical load, reduced water consumption, lower energy consumption, lower discharge of chemicals and wastewater and decreased handling of hazardous chemicals for textile workers (Enzyme Technical Association, 2001).

The Kharaa river catchment as the following problems effects significantly the quantity and quality of water resources: impact of global climate change, overexploitation of water resources, diffuse pollution, mining, water supply and waste water treatment in rural and urban areas, protection of ecological functions and nature conservation. Assuming that the production of constitutive enzymes by bacteria is a result of the adaptation of organic matter, the measured enzyme activities can be used as an indication for the quality of organic matter and its conversion in different beach regions. We investigated the potential capability of river water bacteria to decompose macromolecular organic compounds and to synthesize cellular enzymes.

Corresponding author: Damdinsuren Narantuya,
Department of Biology, School of Science, National University Mongolia, Ulaanbaatar 14201, Mongolia.

MATERIALS AND METHODS

Study area and sampling

The study area is situated in Tov and Selenge provinces, Northeastern Mongolia. Kharaa River, named downstream Khuin River, takes its source from Ar tolgoit Mountain. This river belongs to the Selenge basin, which is situated near Batsumber town after Darkhan city. The agriculture, mining, stockbreeding, railway, railway station and urbanization are developing in this region (Fig.1).

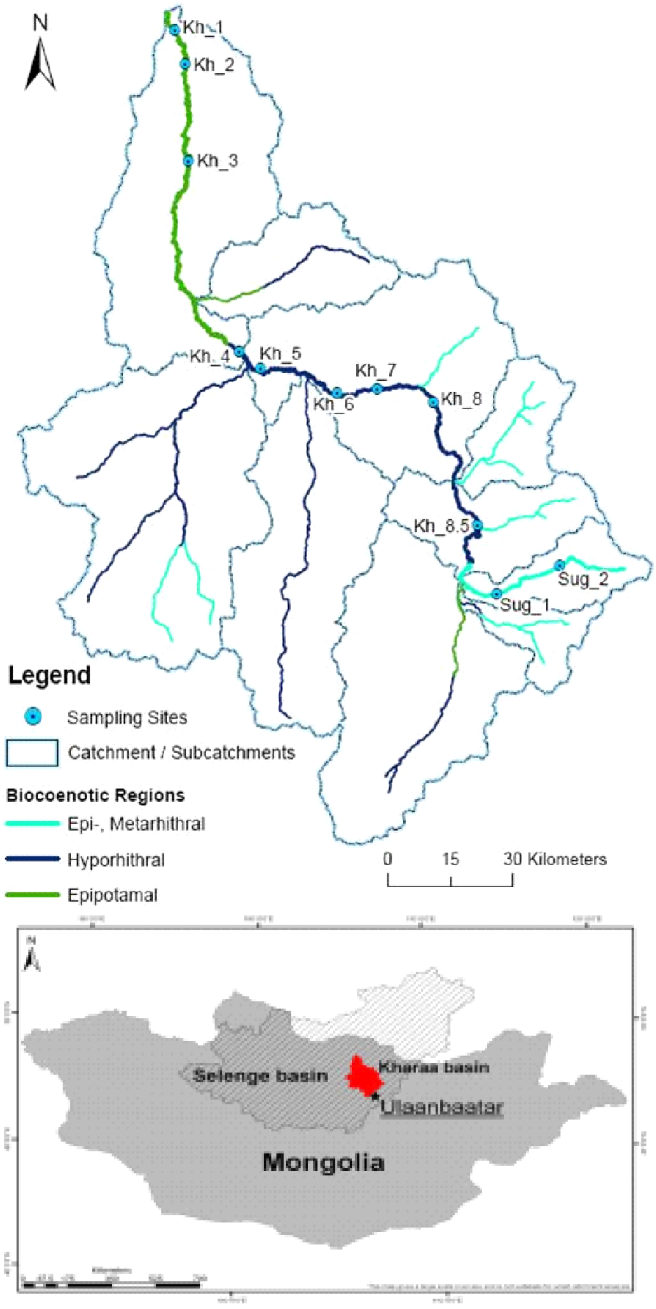

Fig. 1. Location of the sampling sites

The research area covered 281 km of the Kharaa River catchment (Munguntsesteg, 2006). Water samples were taken twice (in spring and autumn) in 2009, along longitudinal Kharaa River and five tributary rivers and assayed for bacterial activities of three enzymes. A total 17 samples were collected according to the guidelines in "Environment. Water quality. Part 6: Guidance on sampling of rivers and streams" (MNS ISO5667-6:2001). Samples were analyzed firstly in the field laboratory within 6 hr of collection and assayed for the enzymes in microbiological laboratory, General and Experimental Biology Institute, branch of Siberia, Russian Academy of Science, Ulan-Ude city, Republic of Buryatia, Russian Federation.

Isolation of bacterial strains

Plate techniques were used in order to isolate planktonic bacteria. Water samples were mixed, and then serial tenfold dilutions were prepared with distilled water to reach final concentrations ranging from 10^{-5} to 10^{-6}. Diluted samples for the proteolytic bacteria were inoculated by the spread method in two parallel replicates on meat peptone agar medium (MPA). The amylolytic bacteria isolated colonies were picked up from each plate containing pure culture and streaked in straight lines in starch agar plates with starch as the only carbon source. The total cellulose activity was determined using Whatman n.1 filter paper. Incubation was carried out at 20^0C and 37^0C for 3-7 days. Subsequently, from the whole surface of the plates or selected sectors, 36 bacterial colonies from each site and each water layer were picked out and transferred to a new MPA medium. The cultures maintained on this medium after purity control was kept at 4^0C and used for further analysis.

Measurement of the activity of bacterial extracellular enzymes

Altogether 36 strains were screened for extracellular enzymatic. Proteolyses assay was determined via modified Anson method using 2% casein as substrate (Anson, 1938. Cupp-Enyard, 2008) and protease activity was expressed as Anson units. One Anson unit is the amount of enzyme which, under the analytical specified conditions (2% casein as substrate, pH 7.0; for 15 min, at different temperatures analyzed) hydrolyzed the casein at a speed that facilitates release, in one minute, the hydrolysis products soluble in the trichloroacetic acid; this provides coloration equivalent, measured at OD_{280} nm, to 1 µmol of tyrosine, in the presence of the Folin-Ciocalteau reagent by using a tyrosine standard curve over the range 0.02-0.24 µmol/ml (Folin and Ciocalteau, 1929). Getchinson and Kleiton's media was used for inoculation of the aerobic cellulose-destroying bacteria. Getchinson medium with filter paper: K_2HPO_2 1.3g, $MgSO_2$ x 7 H_2O 0.3g, $CaCl_2$ x 6 H_2O 0.1g, $FeCl_2$ x 6 H_2O 0.01g, $NaNO_2$ 2.5g, distilled water 1000.0ml, pH 7.2 - 7.3. Cut filter paper into strips, sterilized by dry heat and immersed into the medium so that they did not completely go in the liquid medium. Amylase activity was determined in culture filtrates by measuring the amount of starch hydrolyzed in the reaction mixture by the iodine method (Manning and Campbell, 1961). One unit of enzyme activity has been defined as the amount of enzyme that hydrolyses 1 mg of starch/min under assay conditions (Manning and Campbell, 1961).

Effect of physical and chemical (factors) (condition) optima

The effects of physical and chemical optima were determined comparing to the enzyme activity meaning. The physical and chemical optima were determined using a portable water quality testing meters (WTW 340i - multi-parameter instrument) and PO_4, NO_2, NO_3, NH_4 were determined by the sealed-tube method. The commercial sealed-tube method is a Dr.Lange cuvette LCK 114.

Statistical analysis

Statistical analysis of data and simple linear regression was carried out using Excel, Minitab 16.0 and CCA.

RESULTS

General characteristics

Water samples from Kharaa River and five tributary rivers that ultimately feed into Orkhon River after Darkhan were studied. Over the river catchment have not a vacant part without anthropogenic influence and exceed the utilization. River water pH was slightly alkalinity (Table 1), best range for the growth of organism, as the Kharaa River catchment area is dominated by a stockbreeding and agriculture, which have little buffering capacity. Human activities should not change water temperatures beyond natural seasonal fluctuations. The temperature was lower in the spring than autumn, and mountain or spring fed streams that are normally cool. Total dissolved gas concentrations in river water were ranged 78.3% - 170.5% and oxygen level were ranged 4.11 – 14.5, which depended on temperature, season and pollution. Conductivity in water of Kharaa River fluctuated 40 – 571 µS/cm as the studied rivers that run through with granite bedrock and some part of the river with clay soils.

Measurement of the activity of bacterial extracellular enzymes

Bacteria isolated from the waters of Kharaa River and five tributary rivers varied in their ability to synthesize hydrolytic extracellular enzymes by seasons and sampling sites (Fig. 2, 3, 4).

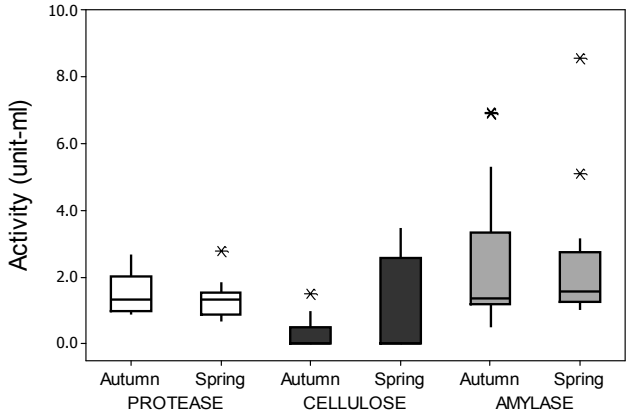

Fig. 2. Comparisons of three extracellular enzymes activities with 95% (default) confidence intervals for the median, by seasons

The highest rate enzyme activity was measured amylase in the autumn and spring (6.92 u/ml). The cellulose activity in the autumn was much lower (0.01 u/ml), and had increased in the spring season (3.5 u/ml). A similar trend was observed with protease activity in two seasons (ranged 0.67 – 2.79 u/ml).

In fact, extracellular enzyme activity was detected in all samples. As seen in Fig.3, activity fluctuated widely over the sampling period. In terms of relative activity, the sampling sites can be classified into several groups. The high activities of amylase group, six sampling sites, included Kh-5, Kh-7, Kh-9, Bayangol, Sugnugur and WWTP, and protease group, five sampling sites: Kh-1, Kh-2, Kh-3, Kh-6 and Zagdal, and cellulase group, two sampling sites: Kh-8 and Eroo.

Table 1. Water quality characteristics

№	Site	pH		T^0C		DO (mg/l and %)		G (µS/cm)	
		spring	autumn	spring	autumn	spring	autumn	spring	autumn
1	Kh - 1	8.2	8.32	8.4	18.9	10.69; 102.9%	6.65; 78.3%	385	369
2	Kh - 2	8.32	8.49	9	20.9	11.52; 108.3%	9.37; 115.1%	368	355
3	Kh - 3	8.27	8.28	12.4	19	11.93; 127.7%	9.04; 107.5%	364	348
4	Kh - 4	8.14	8.19	6.3	17.9	98.20%	9.49; 111.5%	375	331
5	Kh - 5	8.39	8.07	8.6	16.4	13.5; 126.2%	8.59; 96%	330	301
6	Kh - 6	8.53	7.66	8.4	16.5	12.58; 117.8%	7.51; 84.5%	253	250
7	Kh - 7	8.29	7.76	6.8	16.4	14.50; 138.3%	8.61; 98.5%	249	236
8	Kh - 8	8.26	7.85	7	17.3	12.7; 118.5%	9.35; 109.6%	230	280
9	Kh-8.5	7.59	7.42	4.7	12.1	11.66; 100.8%	9.7; 112,8%	231	116
10	Kh - 9	8.37	7.74	14	10.6	9.60; 108.6%	10.06; 116.1%	339	233
11	Bayangol	7.9	7.23	9.7	12	10.59; 104,7%	7.15; 83.2%	199	85
12	Sugnugur	7.54	6.85	5.5	9.2	11.58; 100.8%	9.48; 106.4%	59	40
13	Tunkhel	8.11	7.22	6.2	9.9	11.49; 104.4%	9.5; 105,8%	177	149
14	Zagdal	8.23	8.36	5.4	15.9	97,00%	9.43; 105.3%	476	460
15	Boroo	8.56	8.7	10.3	22	11.43; 115.3%	13.77; 170.5%	505	500
16	WWTP	8.8	7.22	11.3	20.5	12.2; 120.1%	4.11; 150.1%	571	516
17	Eroo	7.4	6.78	6.2	11.9	12.4; 110.4%	11.11; 108,4%	57	44

The lowest, but ubiquitous, cellulase activity group consisted of ten sampling sites: Kh-1, Kh-2, Kh-3, Kh-5, Kh-6, Kh-7, Kh-9, Bayangol, Sugnugur and Zagdal.

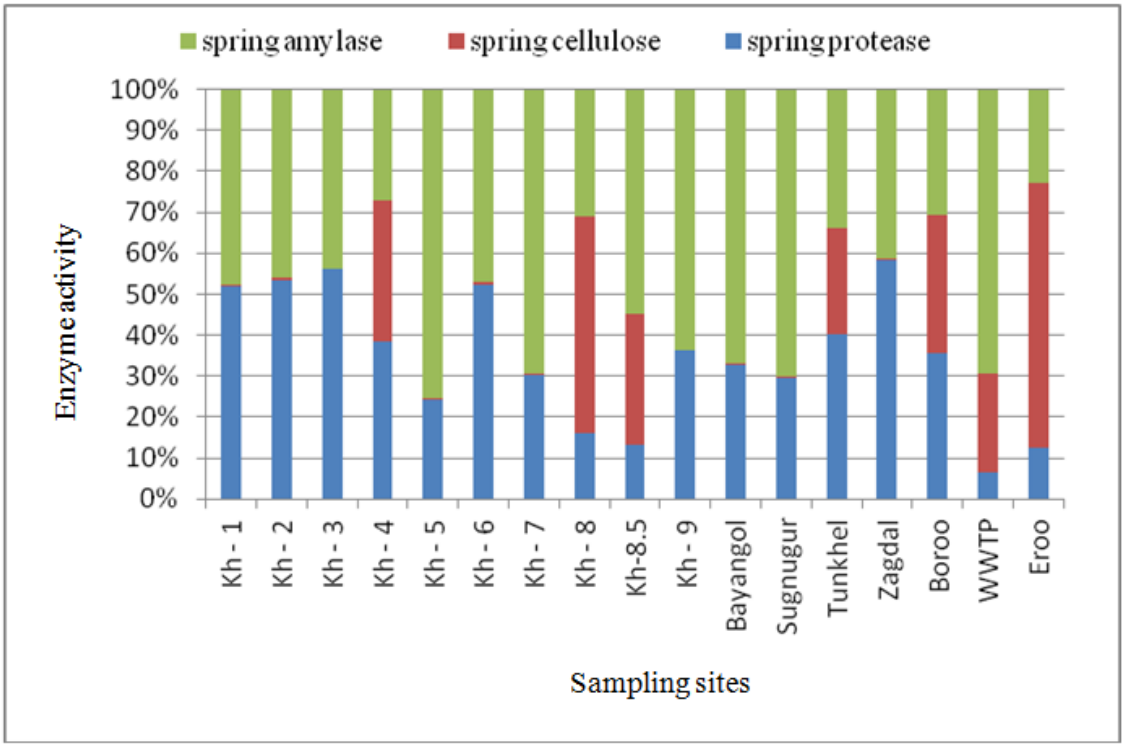

Fig. 3. Three enzymes activity of bacteria isolated from Kharaa River by sampling sites, in spring 2009

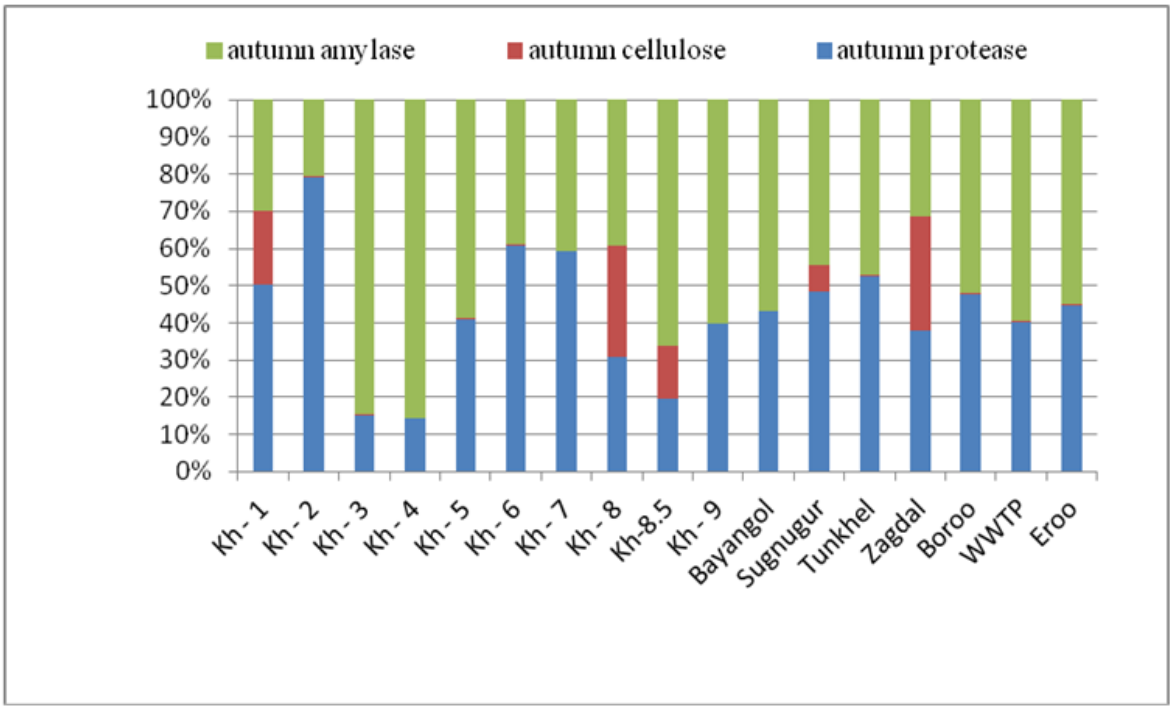

Fig. 4. Activity of three enzymes of bacteria isolated from Kharaa River by sampling sites in autumn season 2009

Data presented in Fig.4 shown bacterial enzymatic activity was through three tested extracellular enzymes. Bacterial strains isolated from Kh-3, Kh-4, Kh-5, Kh-8.5, Kh-9, Bayangol, WWTP and Eroo were higher activity of amylase in autumn. Bacteria isolated from Kh-1, Kh-2, Kh-6, Kh-7 and Tunkhel were higher activity than other sampling sites. Cellulose activity were lower the similar in spring.

The protease activities (Fig.5) presented variations along the investigated area, higher values of actual protease activity were found in sampling points Kh-5 - 1.436 unit/ml, Kh-7 - 1.348 unit/ml, Zagdal - 1.149 unit/ml, Eroo -1.281 unit/ml, positive protease activity was detected by the spectrophotometer (λ=280nm). These values may be attributed to additional contribution of organic substances provided by human activity.

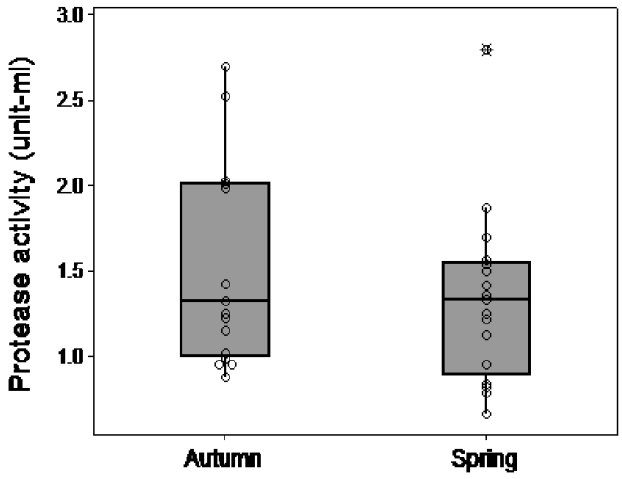

Fig.5. Boxplot analysis of protease by season

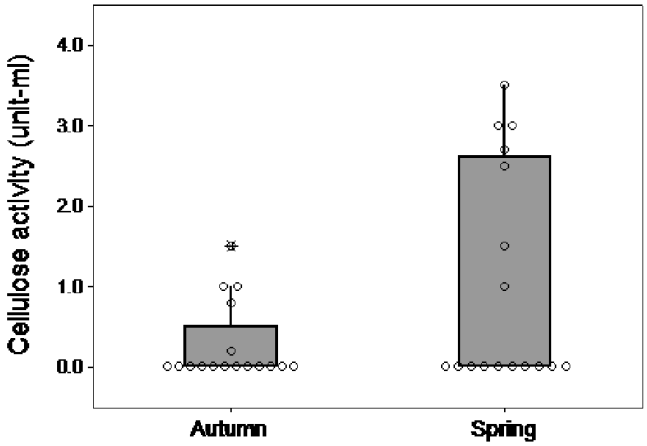

Fig.6. Boxplot analysis of cellulose by season

Results of the cellulose activity (Fig.6) have shown decomposed carbohydrates comprising starches, hemicelluloses and polyuronides in sample. The cellulose activities have large variations along the sampling points. The most intense cellulose activity can be found in sampling points Kh-4 - 3.0 mm, Kh-8 - 2.7 mm, Kh-8.5 - 3.0 mm, Tunkh - 1.0 mm, Boroo - 1.5 mm, Zagdal - 3.0 mm, Eroo - 3.5-4.0 mm were higher than other samples and in the other sampling points were not forming the zone on the plate with starch.

Amylase activity (Fig.7) does not show large variations in analyzed samples however the biggest value of this activity was found downstream of confluence Zagdal River and Kharaa River, which could indicate a fecal pollution due to the livestock present along Zagdal River. A clear zone developed around the colony indicated amylase activity and higher activity of isolated bacteria from as following sampling sites Kh-5 -0.194 unit/ml, Kh-8.5 -0.176 unit/ml, Kh-9 -0.111 unit/ml, Sug -0.170 unit/ml, Zagdal -0.361 unit/ml, Kh-3 - 0.186 unit/ml, Kh-4 - 0.274 unit/ml, Kh-8.5 - 0.272 unit/ml.

Our study clearly revealed the potential of bacterial strains and their ability of extracellular enzyme production. Almost 92% of the strains have shown extracellular enzymatic activity for anyone of the enzyme screened.

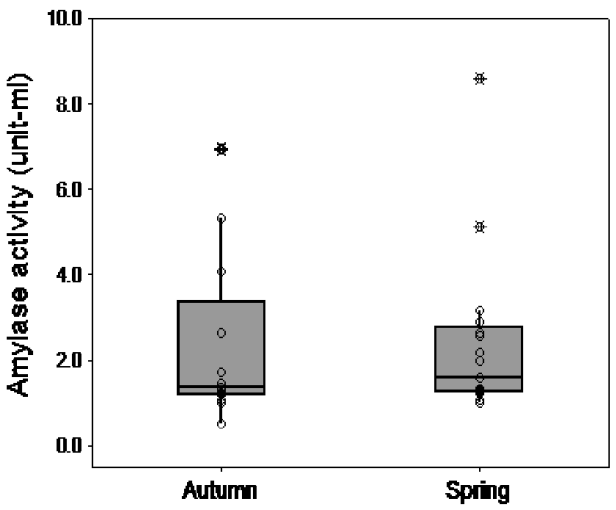

Fig.7. Season difference on amylase activity (unit-ml) in the Kharaa River in 2009 is presented by the box plot. Single dot shows the result from different study sites in the Kharaa River

Effect of physical and chemical optima

The results of the optimum physical and chemical parameters of the river water obtained in close relation with water quality and microbial communities, as well as with to three extracellular enzymes activities.

Fig.8. Effect of physical and chemical characteristics on enzyme activity of protease, cellulose and amylase by isolated bacteria from Kharaa River

The amylase activities of following sampling sites as Kh-9, Bayangol, Kh-7, Sugnugur, Kh-5 in spring depended on river water conductivity, which was compared with other physical and chemical optimum and sampling sites. The combined effects of dissolved oxygen and pH on cellulose and amylase were more related for Kh-8.5, WWTP in spring and Kh -8.5 in autumn. The temperature was influenced to the protease activity on samples from Kh-9, Kh-5, WWTP, Bayangol, Eroo and Boroo in autumn.

DISCUSSION

In running water ecosystems, a large part of the processes of the degradation and transformation of organic matter is mediated exclusively by heterotrophic bacteria using specific enzymatic systems (Liu *et al*., 2004; Wilczek *et al*., 2004). Several studies (Dellano *et al*., 2000; Foreman *et al*., 1998; Mallet *et al*., 1999) showed that the level of particular enzyme activity in water basins depends on the quantity and quality of organic compounds (Treitli *et al*., 2011) and other sources of pollution. Analysts determine water quality by testing for specific physical-chemical properties. Most often, the type of water being tested determines what *parameters*, or *analyses*, the analyst looks for. For example, dissolved oxygen, temperature, pH and conductivity are important parameters in natural water (*Quality Criteria for Water*, U.S. Environmental Protection Agency, 1986).

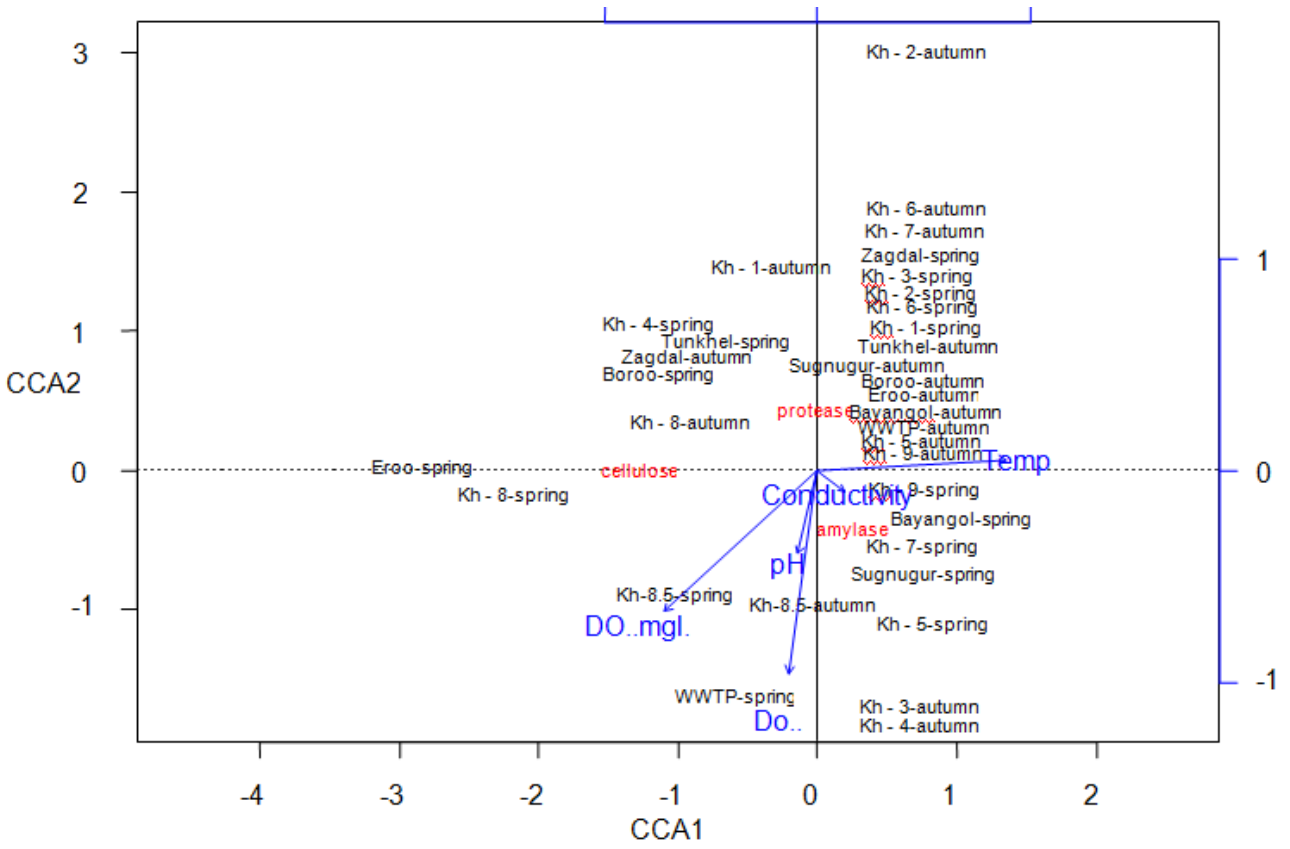

Fig.8. Effect of physical and chemical characteristics on enzyme activity of protease, cellulose and amylase by isolated bacteria from Kharaa River

A good level of dissolved oxygen is essential for aquatic life. Dissolved oxygen analysis measures the amount of gaseous oxygen (O2) dissolved in an aqueous solution. Oxygen gets into water by diffusion from the surrounding air, by aeration (rapid movement), and as a waste product of photosynthesis. (*Quality Criteria for Water*, U.S. Environmental Protection Agency, 1986.) The amount of Dissolved Oxygen (DO) in water is expressed as a concentration. A concentration is the amount of in weight of a particular substance per a given volume of liquid. The DO concentration in a stream is the mass of the oxygen gas present, in milligrams per liter of water. Usually streams with high dissolved oxygen concentrations (greater than 8 mg/L) are considered healthy streams. They are able to support a greater diversity of aquatic organisms. They are typified by cold, clear water, with enough riffles to provide sufficient mixing of atmospheric oxygen into the water. In general, DO levels less than 3 mg/L are stressful to most aquatic organisms. In Kharaa River, dissolved oxygen of almost all sampling sites were high concentrations (8.59 mg/l – 14.5 mg/l), and only at three sampling sites (Kh-1 – 6.65 mg/l, Kh-6 – 7.51 mg/l, WWTP – 4.11 mg/l) were less than 8 mg/l. It is to be noted that Kharaa River water qualities do not meet the minimum dissolved oxygen standard. In the main part of the river, DO concentration is above 8 mg/l, an indication of better quality of water. Since a healthy stream is considered to be 90-100 percent saturated (Streamkeeper's Field Guide: Watershed Inventory and Stream Monitoring Methods, 1991), our samples were indicated between 78.3% (Kh-1) and 170.5% (Boroo). Total dissolved gas concentrations in water should not exceed 110 percent. Concentrations above this level can be harmful to aquatic life. (*Quality Criteria for Water*, U.S. Environmental Protection Agency, 1986)

Water Temperature is a controlling factor for aquatic life: it controls the rate of metabolic activities, reproductive activities and therefore, life cycles. If, stream temperatures increase, decrease or fluctuate too widely, metabolic activities may speed up, slow down, malfunction, or stop altogether. (Streamkeeper's Field Guide: Watershed Inventory and Stream Monitoring Methods, 1991). High water temperatures stress aquatic ecosystems by reducing the ability of water to hold essential dissolved gasses like oxygen. Human activities should not change water temperatures beyond natural seasonal fluctuations (*Quality Criteria for Water*, U.S. Environmental Protection Agency, 1986).

In a warm water stream temperatures should not exceed 89^0F (31.6^0C). Cold water streams should not exceed 68^0F (20^0C). (Streamkeeper's Field Guide: Watershed Inventory and Stream Monitoring Methods, 1991) Kharaa River is cold water river, which temperature ranged between 4.7^0C (Kh-8.5) – 20.9^0C (Kh-2). pH is an important limiting chemical factor for aquatic life. If the water in a stream is too acidic or basic, the H+ or OH- ion activity may disrupt aquatic organisms' biochemical reactions by either harming or killing the stream organisms. Streams generally have a pH values ranging between 6 and 9, depending upon the presence of dissolved substances that come from bedrock, soils and other materials in the watershed.

(Streamkeeper's Field Guide: Watershed Inventory and Stream Monitoring Methods, 1991). Rainfall can lower pH in streams. The most significant environmental impact of pH involves synergetic effects. Synergy involves the combination of two or more substances which produce effects greater than their sum. This process is important in surface waters. Runoff from agricultural, domestic, and industrial areas may contain iron, aluminum, ammonia, mercury or other elements. The pH of the water determines the toxic effects, if any, of these substances. For example, 4 mg/l of iron would not present a toxic effect at a pH of 4.8. However, as little as 0.9 mg/l of iron at a pH of 5.5 can cause fish to die. Synergy has special significance when considering water and wastewater treatment. The steps involved in water and wastewater treatment require specific pH levels. In order for coagulation (a treatment process) to occur, pH and alkalinity must fall within a limited range (*Quality Criteria for Water*, U.S. Environmental Protection Agency, 1986).

In the Kharaa River measured pH was ranging from 6.78 (Eroo) to 8.8 (WWTP) in seventeen tests of a range locations. The average pH in the study was 7.73, a rate that is sufficient basic for bacteria, and other organisms. Conductivity in streams and rivers is affected primarily by the geology of the area through which the water flows, by temperature, and by the presence of inorganic dissolved solids. The conductivity of rivers in the United States generally ranges from 50 to 1500 µmhos/cm. Studies of inland fresh waters indicate that streams supporting good mixed fisheries have a range between 150 and 500 µmhos/cm. Conductivity outside this range could indicate that the water is not suitable for certain species of fish or macro invertebrates. Industrial waters can range as high as 10,000 µmhos/cm (*Quality Criteria for Water*, U.S. Environmental Protection Agency, 1986). The waters of the Kharaa basin had average conductivity between 40 µs/cm (Sugnugur) and 571 µs/cm (WWTP). Water of the Kharaa river basin is mainly composed by slightly leached and polluted by droppings, sewage. Consequently, the conductivity is lower in the low anthropogenic affect streams and river of the basin. At the more anthropogenic affected sites, conductivity increases significantly, as a result of polluted load.

Many heterotrophic bacteria are characterized by the ability to carry out the processes of depolymerization of macromolecular compounds to mono- or oligomers (Unanue *et al*., 1999, Martinez *et al*., 1996). The intensity of decomposition of organic macromolecules in water basins is usually determined not only by the number of bacteria capable of carrying out those processes, but also by the level of activity of their extracellular hydrolytic enzymes (Hoppe *et al*., 2002; Jackson *et al*., 1995). Several studies (Mallet *et al*. 1999, Dellano *et al*., 2000; Foreman *et al*., 1998; Lamy *et al*., 1999) have indicated that the level of activity of particular enzymes in water basins depends on the quantity and quality of organic compounds.

The present study is an effort to physiologically characterize river water isolates on the basis of extracellular enzymes. Results of many studies (Patel *et al*., 2000; Hoppe *et al*., 2002; Lamy *et al*., 1999) indicate that many heterotrophic bacteria in the aquatic environment are also capable of synthesizing proteases. Bacterial proteases are particularly important to the global carbon and nitrogen cycles in the recycling of proteins, and such activity tends to be regulated in by nutritional signals in these organisms. (Hooper, 2002) The net impact of nutritional regulation of protease activity among the thousands of species present in soil can be observed at the overall microbial community level as proteins are broken down in response to carbon, nitrogen, or sulfur limitation. (Puente *et al*. 2003)

Extracellular proteases hydrolyze proteins into mono- or oligomers, mainly peptides and amino acids (Pantoja *et al*. 1994). Those low molecular weight organic compounds are immediate precursors in the synthesis of proteins and participate in many pathways of microbial cell metabolism (Simon 1998; Mudryk *et al*., 2005). Proteases were also synthesized very intensively by bacterial strains isolated from the Kharaa River. Protease activity was found in all analyzed samples, which activity has values close to the potential protease activity indicating a higher physiological intensity. Isolates were recovered from different stations of river Kharaa, which are either surrounded by dense human population or by many agriculture and livestock that might be responsible for the existence of the proteolytic bacteria in river water. Amylases are enzymes that break down starch or glycogen and produced by a variety of living organisms, ranging from bacteria to plants and humans. Bacteria and fungi secrete amylases to the outside of their cells to carry out extra-cellular digestion (Cordeiro *et al*., 2003). When they have broken down the insoluble starch, the soluble end products such as (glucose or maltose) are absorbed into their cells (Rwarinda and Rangabhashiyam, 2013).

Amylases are industrially and ecologically valuable enzymes (Komaki 1956; Gaddad *et al*., 1986). Amylase activity by environmental isolates of river water were reported, therefore its detection in the water (also waste water) could be useful in determining the level of carbohydrates, which could be useful in sewage and industrial effluent treatment (Gaddad *et al*., 1986). Maximum amylase activity has been shown by the isolate from the sites with developed agriculture and livestock, such as Kh-5, Kh-8.5, Kh-9, Sugnugur, Zagdal, Kh-3, Kh-4. Lower values of amylase activity close to Darkhan city (Kh-2) can be caused by waste water treatment plant of Darkhan, here the remaining amount of H_2O_2 undecomposed was much higher than all other samples which indicate a lower number of microorganisms with a reduced enzymatic activity. Similar situations were reported in paper presented by Bodoczi and collaborators (Bodoczi *et al*., 2008).

Cellulosic waste-materials including agricultural, forestry, and municipal wastes are among the Earth's most abundant and available renewable resources (Gurdeer *et al*., 2009). By means of chemical or bioconversion methods, it is possible to transform this insoluble polymer into glucose, an excellent substrate for industrial fermentation. Mainly bacteria, fungi and actinomycetes achieve bioconversion of these materials (Immanuel *et al*., 2006). The percentages of organisms are having cellulytic activity less in comparison to other enzymes. In the spring time, bacterial isolates have shown maximum cellulytic activity.

Cellulolysis is the process of breaking down cellulose into smaller polysaccharides called cellodextrins or completely into glucose units; this is a hydrolysis reaction. Because cellulose molecules bind strongly to each other, cellulolysis is relatively difficult compared to the breakdown of other polysaccharides. (David *et al*., 2008) Processes do exist however for the breakdown of cellulose such as the Lyocell process (Lyocell, 2007). In the present study, three enzymes such as protease, amylase, and cellulose were identified for their ability of bacteria isolated from river water, to decompose against for the made polluted matters of the river water. The extracellular enzyme activity of the bacteria was studied by optimization at various environmental factors such as dissolved oxygen, temperature, pH, and conductivity. The amylase activity related to pH of the bacterial isolates from Kh-8.5 (autumn), Kh-5 (spring), Sugnugur (spring), Kh-7 (spring) were much higher than other sampling sites and conductivity influence were closed to the Kh-9 (spring), and Bayangol (spring). The dissolved oxygen relevance with amylase activity was down to the sampling sites as Kh-8.5 (spring), WWTP (spring). The optimum temperature of the Kharaa River was 10^0C, which more influenced to protease hydrolyzing activity of the bacterial isolates from Kh-5 (autumn), Kh-9 (autumn). The extracellular enzyme activities measured in our study provide a direct insight into the spatial distribution of microbial processes within the river water.

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REFERENCES

Arnosti, C., Jørgens en, B. B., Sagemann, J. and Tramdr up T. 1998. Temperature dependence of microbial degradation of organic matter in marine sediment: polysaccharide hydrolysis, oxygen consumption, and sulfate reduction – *Mar. Ecol. Prog. Ser.*, 165: 59–70.

Bodoczi, A. and Dragan-Bularda, M., 2008. The enzymatic activity from the sediments of the Aries River from upstream to downstream. Studia Universitatis Babes-Bolyai, Biologia, LIII, 2: 129-137.

Boetius, A. 1995. Microbial hydrolytic enzyme activities in deep-sea sediments, Hel. Meer. 49, 177

Brown, S. E. and Goulder, R. 1996. Extracellular - enzyme activity in trout –farm effluents and recipient river – *Aqua. Res.,* 27: 895–901.

Cordeiro, C.A.M., Martinas, M.L.L. and Lucaino, A. 2003. Production and Properties of alpha amylase from thermophylic *Bacillus specie. Braz. J.Microbiol.,* 33:1-3.

David, G. Barkalow, Roy L. Whistler, 2008."Cellulose", in Access Science, McGraw-Hill, doi:10.1036/1097-8542. 118200.

Dellano, A., Fabiano, M., Mei, L., Danovaro, M.R. 2000. Enzymatically hydrolyzed protein and carbohydrate pools in deep-sea sediments: estimates of the potentially bioavailable fraction and methodological considerations. Marine Ecology Progress Series, 196: 15-23

Enzymes: A primer on use and benefits today and tomorrow, 2001. Enzyme Technical Association, 1800 Massachusetts Avenue, N.W. Second Floor Washington, DC 20036

Foreman, C.M., Franchini, P. and Sinsabaugh, R.L. 1998. The tropic dynamics of riverine bacterioplankton: Relationships among substrate amiability, ectoenzyme kinetics, and growth. *Limnology and Oceanography*, 43: 1344-52.

Gaddad, S.M., Hosetti, H.B. and Yalamani, A.H. 1986. *Environmental Ecology*, 4, 465-468

Kavka, G., Kasimir, D.G., Farnleitner, A.H., 1998. "Microbiology and Hygiene" (EG-MH), At present members from 8 Danubian countries join the expert group.

Hooper, N.M., 2002. Proteases in Biology and Medicine. London: Portland Press, ISBN 1-85578-147-6.

Hoppe, H.G., Arnosti, C., Herndel, G.F. 2002. Ecological significance of bacterial enzymes in marine environment. In: RC. Burns, R.P. Dick (Eds). Microbial Enzymes in the Environment Activity, Ecology, and Applications Marcel Dekker, 73.

Immanuel, G., R. Dhanusha, R., P. Prema. P., A. Palavesam.A., 2006. Effect of different growth parameters on endoglucanase enzyme activity by bacteria isolated from coir retting effluents of estuarine environment, *International Journal of Enviornmental Science and Technology*, Vol. 3, No. 1, pp. 25-34

Jackson, C.R., Foreman, C.M. and Sinsabaugh R.L. 1995.Microbial enzyme activities as indicator of organic matter processing rates in a lake Erie coastal wetland. *Fresh Biol.*, 34, 329,

Komaki, T. 1956. Denpum Kogyo Gakkaishi 4, 291-320

Lamy, F., Bianchi, M., Van Wambeke, F., Sempere, R. and Talbot V. 1999.Use of data assimilation techniques to analyze the significance of ectoproteolytic activity measurements performed with the model substrate MCA-Leu. Mar. Ecol. Prog. Ser. 177, 27,

Liu, H., Dagg, M., Campell, L. and Urban-Rich, J. 2004. Picophytoplankton and bacterioplankton in the Mississippi River plume and its adjacent waters, Estuaries, 27: 147-56

Lyocell H., 2007. "*Cellulose" Issue* 41, pp 419

Mallet, C. and Debroas, D., 1999: Relations between organic matter and bacterial proteolytic activity in sediment surface layers of eutrophic lake (Lake Aydat, Puy de Dome, France). *Archiv fuer Hydrobiologie*, 145: 39-59.

Martinez, J., Smith, D.C., Steward, D.F. and Azam F. 1996.Variability in ectohydrolytic enzyme actives of pelagic marine bacteria and its significance for substrate processing in the sea. *Aqua. Microbial Ecol.,* 10.223.

Mudryk, Z. and Podgorska B. 2005. Spatial variability in the activity of hydrolytic enzymes in a marine beach (southern Baltic Sea). *Pol. J. Ecol.,* 53, 225,

Munguntsetseg, A. 2006. Hydrochemistry of Selenge River, Ulaanbaatar, p. 99.

Patel A.B., Fuk Ami K. and Nishijama T. 2000. Regulation of seasonal variability of aminopeptidase activities in surface and bottom waters of Uranouchi Inlet, Japan. *Aqua. Microbial. Ecol.,* 21, 139,

Poremba K. 1995. Hydrolytic enzymatic activity in deep-sea sediments. *FEMS Microbial. Ecol.,* 16, 213.

Puente, X.S., Sanchez, L.M., Overall, C.M. and Lopez-Otin C. 2003. Human and Mouse Proteases: a Comparative Genomic Approach. *Nat Rev Genet.,* 4:544-558

Quality Criteria for Water, 1986. U.S. Environmental Protection Agency, EPA#440/5-86-001

Rwarinda, U. Angelo and S. Rangabhashiyam, 2013. Amylase Production from Bacteria Isolated in the Soil Receiving Kitchen Wastes, *International Journal of Pharmaceutical and Chemical Sciences*, ISSN: 2277-5005 Vol. 2 (1)

Simon, M. 1998. Bacterioplankton dynamics in a large mesotrophic lake. II Concentration and turn-over of dissolved amino acids. *Arch. Hydrobiol.,* 144, 295,

Streamkeeper's Field Guide: Watershed Inventory and Stream Monitoring Methods, 1991.

Treitli, S., Filimon, M.N. and Petrucean, C. 2011. Evaluating the pollution from Mures River on Arad-Pecica sector based on enzymatic activities from sediments (Western Romania). Analele Universitatii din Oradea-Fascicula Biologie, Tom.XVIII. Issue:1. pp. 71-76

Unanue, M., Ayo, B., Agis, M., Slezak, D., Herndl, G. J. and Iriberrij, 1999. Ectoenzymatic activity and uptake of monomers in marine bacterioplankton described by a biphasic kinetic model. *Microbial. Ecol.,* 37, 36.

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