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*Original Research*

# Enzymatic Activity of Bacterial Strains Isolated from Marine Beach Sediments

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**Abstract**

Potential capability of heterotrophic bacteria for extracellular enzyme synthesis and their activity were determined in a transect from dunes to a water depth of 1 m in a sandy beach near Sopot on the southern Baltic coast. Among studied enzymes, alkaline phosphatase, esterase lipase, and leucine arylaminase were synthesized in a higher degree, whereas α-fucosidase, βglucouronidase and α-galactosidase had only low levels. No clear horizontal gradients were observed in the transect from dune to water. The enzyme activities of bacteria isolated from the surface and subsurface did not differ in their height and composition. Bacteria isolated form the sand of studied beach in different seasons, as a rule, synthesized the tested hydrolytic enzymes with similar intensity.

**Keywords:** Baltic Sea, marine beach, sand, bacteria, enzymatic activity

## Introduction

Sandy beaches are important and widespread marine coastal ecosystems characterized by uncontrollable dynamic nature whose structure is determined by wind, sand and water in a state of constant motion [1]. According to Brown and McLachlan [2], sandy beaches can be regarded as gigantic filters through which large amounts ($10 – 70\ m^3 \cdot m^{-1} \cdot d^{-1}$) of water are filtered [3, 4]. During water permeation great amounts of organic matter are adsorbed on the sand grain surfaces in the form of particulate (POM) and dissolved (DOM) organic matter. Such concentrations of organic matter on the sand grains allow its further utilization by interstitial organisms so that in most beaches the interstitial system functions as a biological filter that mineralizes organic matter and thus cleanses the surface water [5-7]. Bacteria play a key role in the processes of decomposition of organic matter accumulated in marine beaches [8]. According to Koop and Griffiths [9] these organisms can mineralize about 70% of the organic matter reaching sea beaches.

Most of the organic matter in marine ecosystems consist of compounds of a high molecular weight and polymeric structure, mainly proteins, starch, lipids, pectin, cellulose, chitin, nucleic acids, or lignin [10, 11, 12]. For heterotrophic bacteria, those high molecular weight biopolymers constitute an important source of carbon, nitrogen, and energy used for biosynthesis or respiration [13, 14]. As polymeric molecules are too large to be directly incorporated into bacterial cells [15], they have to be decomposed by extracellular enzymes into simple compounds [12] that can easily diffuse into the periplasmic space [16]. Many heterotrophic bacteria are known to carry genetic and metabolic potentials to synthesise and control extracellular enzymes, which can degrade and modify a large variety of natural polymers in water basins [17, 18]. For this reason, according to Boetius [19], Jackson et al. [20], Mallet and Debroas [16], enzyme assays can provide powerful tools for studying organic matter degradation and nutrient cycling in aquatic ecosystems.

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The beach is strongly influenced by human activities (industry, tourism). 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.

## Material and Methods

### Study Area and Sampling

The study was carried out on a non-tidal sandy beach near Sopot (54°27`N, 18°33`E), Poland, on the southern Baltic coast (Fig. 1). The studied beach is localized in the Gulf of Gdańsk – one of the Baltic's open bays characterized by high levels of pollution and eutrophication [7]. There is a lot of industry in this area and heavy discharge of pollutants (the River Vistula and the Tri-City urban complex) occurs here [21]. The beach represents a dissipative beach type with longshore bars and troughs; it has a slope of 7° and is 46 m wide. In general, fine and medium-grained sand (350 – 700 μm) predominate [7, 8]. The salinity of the overlying water ranges from 0.8 to 3.6 PSU. The organic content of the sand varied from 0.20 to 0.57% dry weight with lower values recorded in the middle of the beach, and higher ones towards both the dune and the waterline [8]. The Sopot beach with its surf zone is ideal for tourists and a suitable area for recreational activities. It is frequented by tourists whose density in summer reaches 30 persons per 100 $m^2$; about 3,000 people can pass daily [22].

Sand samples were taken once per season in February, May and June 1999. A transect was marked along a profile perpendicular to the shoreline; four sampling sites were located along this transect (Fig. 1). Site 1 was located approximately 1-1.5 m from the waterline into the water, at a depth of about 1 m; site 2 was situated at the waterline, site 3 lay halfway up the beach, at a 30 m distance from the shore, and site 4 lay in a sheltered place among the dunes, 60 m away from the shore. Core samples were taken with a 30 x 15cm core scoop. They were immediately divided into two sections: 0-1 cm (surface layer) and 5-10 cm (subsurface layer), and placed on ice in sterile glass boxes which were transported to the laboratory; analysis commenced within 2-3 h.

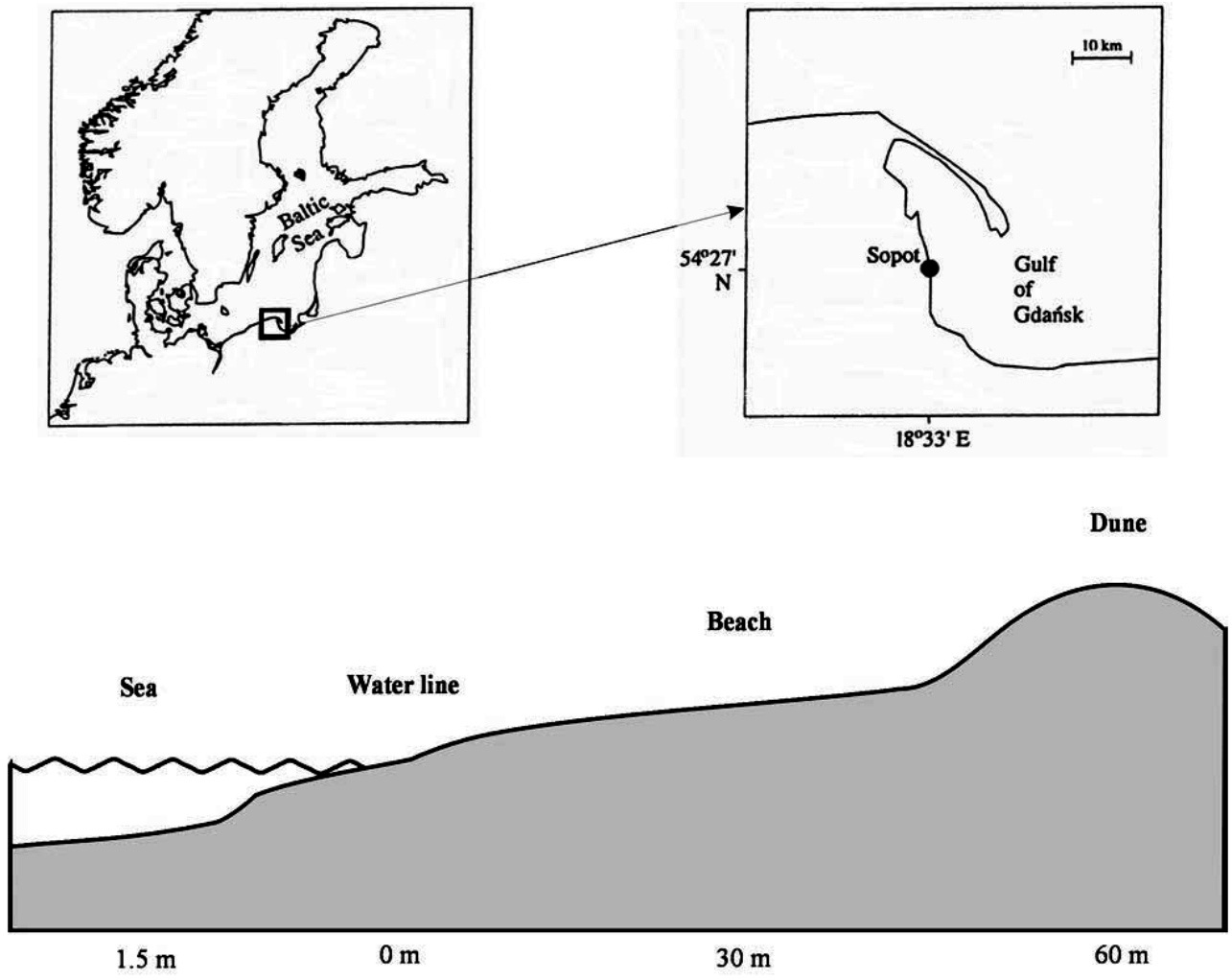


Fig.1 Location of the beach studied at the Baltic Sea and position of the sampling sites along the sandy beach.

## Isolation of Bacteria

Each of the 10.0 g sand samples was weighed aseptically and transferred to 100 $cm^3$ of sterile seawater for subsequent homogenization (5 minutes at 23,000 rpm in a NPW120 homogenizer). The supernatant was serially diluted with sterile seawater and plated by the spread method onto ZoBell 2216 agar medium (ZB) [23] prepared with old brackish water salinity 8 PSU. Triplicate plates from each tenfold dilution were incubated for 14 days at 20°C. Afterwards, 30 bacterial colonies from each sampling site and each core section were collected at random and transferred to semisolid ZB medium. After purity control, bacteria were stored at 4°C, with inoculation on fresh medium carried out every 3 months, and used for further studies of their enzymatic activity.

## Measurement of the Activity of Bacterial Extracellular Enzymes

To characterize the complete potential organic matter degradation, a broad spectrum of enzyme activities was tested [36]. The activity of constitutive bacterial enzymes was determined with the use of the semiquantitative API Zym (API bioMerieux Ltd.) micromethod [24]. Nineteen tests for the presence of the following enzymes were carried out: alkaline phosphatase (Bph), esterase (Est), esterase lipase (Esl), lipase (Lip), leucine arylamidase (Leu), valine arylamidase (Val), cysteine arylamidase (Cys), trypsin (Try), chymotrypsin (Chy), acid phosphatase (Aph), naphtol-AS-Bi-phosphopydrase (Nap), α-galactosidase (αGa), β-galactosidase (βGa), β-glucuronidase (βGl), α-glucosidase (αGs), β-glucosidase (βGs), N-acetyl-β-glucosaminidase (Nac), α-mannosidase (αMa), and α-fucosidase αFu (Table 1). Bacteria were multiplied on agar slants (ZB) for 72h at 20°C. Afterwards, they were rinsed from the slants with 5 $cm^3$ of liquid ZB medium, and adjusted to the turbidity of 4 MacFarland standard corresponding to $10^9$ bacterial cells per 1 $cm^3$. According to the manufacturer's instructions, 65 µl of this suspension was inoculated on a plastic strip to cupules containing different substrates. All strips were incubated at 20°C for 24 h; afterwards API reagents ZYM 1 and ZYM 2 were applied. The obtained results were compared with the colour chart provided by the kit manufacturer; enzyme activities were expressed in nanomoles of hydrolyzed substrates.

Table 1. Enzymes and the substrates degraded by these enzymes.

| No. | ENZYME ASSAYED | SUBSTRATE |
|---|---|---|
| 1 | Alkaline phosphatase | 2-naphthyl phosphate |
| 2 | Esterase (C 4) | 2-naphthyl butyrate |
| 3 | Esterase Lipase (C 8) | 2-naphthyl caprylate |
| 4 | Lipase (C 14) | 2-naphthyl myristate |
| 5 | Leucine arylamidase | L-leucyl-2-naphthylamide |
| 6 | Valine arylamidase | L-valyl-2-naphthylamide |
| 7 | Cystine arylamidase | L-cystyl-2-naphthylamide |
| 8 | Trypsin | N-benzoyl-DL-arginine-2-naphthylamide |
| 9 | α-chymotrypsin | N-glutaryl-phenylalanine-2-naphthylamide |
| 10 | Acid phosphatase | 2-naphthyl phosphate |
| 11 | Naphthol-AS-BI-phospnohydrolase | Naphtol-AS-BI-phosphate |
| 12 | α-galactosidase | 6-Br-2-naphthyl-αD-galactopyranoside |
| 13 | β-galactosidase | 2-naphthyl-βD-galactopyranoside |
| 14 | β-glucuronidase | Naphthol-AS-BI-βD-glucuronide |
| 15 | α-glucosidase | 2-naphthyl-αD-glucopyranoside |
| 16 | β-glucosidase | 6-Br-2-naphthyl-βD-glucopyranoside |
| 17 | N-acetyl-β-glucosaminidase | 1-naphthyl-N-acetyl-βD-glucosaminide |
| 18 | α-mannosidase | 6-Br-2-naphthyl-αD-mannopyranoside |
| 19 | α-fucosidase | 2-naphthyl-αL-fucopyranoside |

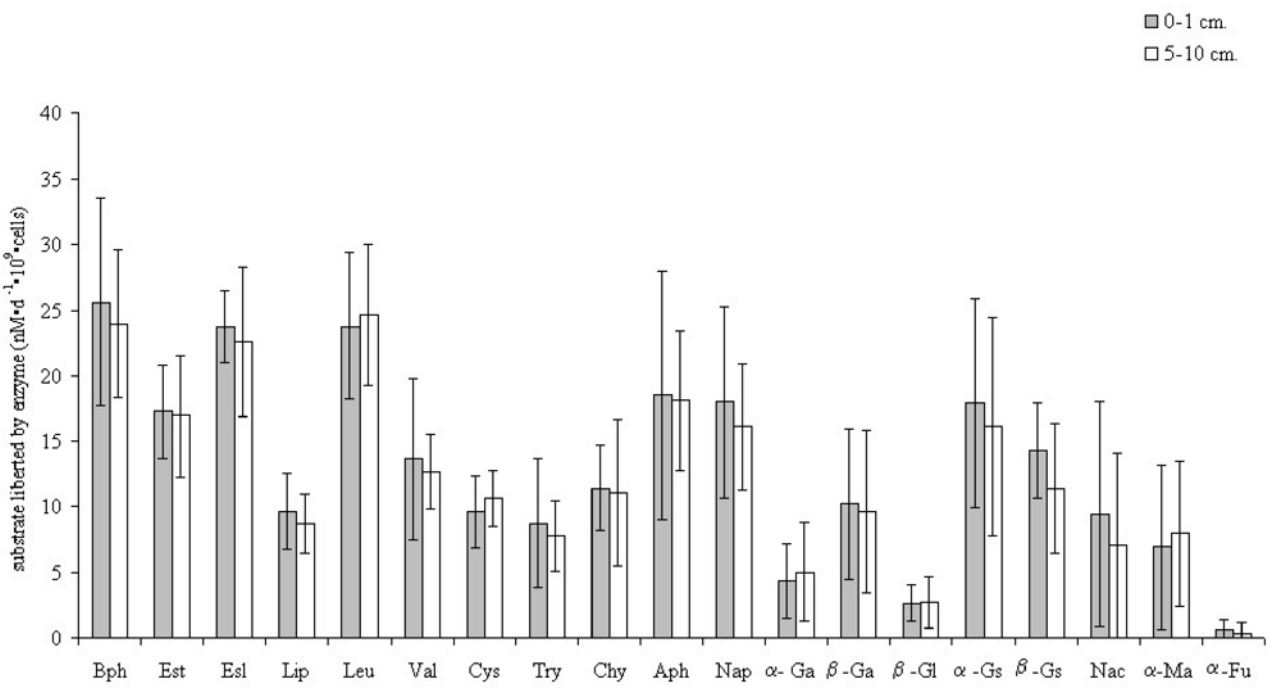


Fig.2. Activities of constitutive enzymes synthesized by bacteria isolated from surface (0-1cm) and subsurface (5-10 cm) sand layers of studied beach. Bars are based on mean of all sites. Vertical lines show standard deviation (± SD) (n = 120). Explanations: alkaline phosphatase (Bph), esterase (Est), esterase lipase (Esl), lipase (Lip), leucine arylamidase (Leu), valine arylamidase (Val), cysteine arylamidase (Cys), trypsin (Try), chymotrypsin (Chy), acid phosphatase (Aph), naphtol-AS-Bi-phosphopydrase (Nap), α-galactosidase (αGa), β-galactosidase (βGa), β-glucuronidase (βGl), α-glucosidase (αGs), β-glucosidase (βGs), N-acetyl-β-glucosaminidase (Nac), α-mannosidase (αMa), and αfucosidase αFu.

Table 2. Seasonal difference of level bacterial enzymatic activity (nM · $d^{-1}$ ·$10^9$ cells). Data represented mean of surface and subsurface sand layers.

| Enzymes | Season | | | average |
|---|---|---|---|---|
| | winter | spring | summer | |
| alkaline phosphatase (Bph) | 26.9 | 22.9 | 24.4 | 24.7 |
| esterase (Est) | 14.6 | 20.9 | 15.6 | 17.0 |
| esterase lipase (Esl) | 22.1 | 23.2 | 24.1 | 23.1 |
| lipase (Lip) | 7.5 | 10.7 | 9.2 | 9.1 |
| leucine arylamidase (Leu) | 22.5 | 27.7 | 22.4 | 24.2 |
| valine arylamidase (Val) | 11.3 | 17.1 | 10.9 | 13.1 |
| cysteine arylamidase (Cys) | 9.0 | 10.8 | 10.4 | 10.1 |
| trypsin (Try) | 8.7 | 8.1 | 7.8 | 8.2 |
| chymotrypsin (Chy) | 9.2 | 10.9 | 13.4 | 11.2 |
| acid phosphatase (Aph) | 24.6 | 15.1 | 15.2 | 18.3 |
| naphtol-AS-Bi-phosphopydrase (Nap) | 21.1 | 12.4 | 17.6 | 17.0 |
| α-galactosidase (αGa) | 6.7 | 3.3 | 3.9 | 4.6 |
| β-galactosidase (βGa) | 11.1 | 7.9 | 10.6 | 9.9 |
| β-glucuronidase (βGl) | 3.1 | 1.9 | 2.9 | 2.6 |
| α-glucosidase (αGs) | 21.2 | 13.7 | 16.1 | 17.0 |
| β-glucosidase (βGs) | 15.0 | 10.3 | 13.0 | 12.8 |
| N-acetyl-β-glucosaminidase (Nac) | 11.5 | 6.4 | 6.6 | 8.2 |
| α-mannosidase (αMa) | 7.9 | 7.8 | 6.4 | 7.4 |
| α-fucosidase (αFu) | 0.2 | 0.4 | 0.7 | 0.4 |

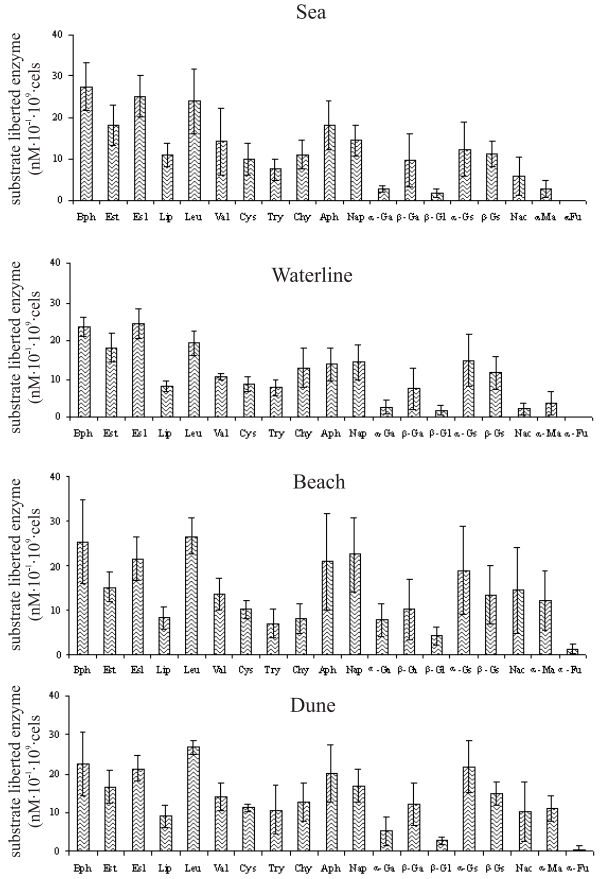


Fig.3. Horizontal variations of potential bacterial enzymatic activity in studied beach. Each bar shows mean enzymatic activity in surface and subsurface sand layers. Vertical lines represented ± SD (n = 60). Explanations as in Fig. 2.

## Results

Bacteria isolated from the sand of Sopot beach were capable of synthesizing a wide spectrum of hydrolytic enzymes(Fig.2).Alkalinephosphatase(Bph)(24.7 $nM \cdot d^{-1} \cdot 10^{-9}$ cells), leucine arylamidase (Leu) (24.2 $nM \cdot d^{-1} \cdot 10^{-9}$ cells) and esterase lipase (Esl) (23.1$nM \cdot d^{-1} \cdot 10^{9}$cells) were synthesized most actively while α-fucosidase (αFu) (0.4 $nM \cdot d^{-1} \cdot 10^{9}$ cells), β-glucouronidase (βGl) (2.6 $nM \cdot d^{-1} \cdot 10^{9}$ cells) and α-galactosidase (αGa) (4.6 $nM \cdot d^{-1} \cdot 10^{9}$cells) were synthetised least actively, both by bacteria isolated from the surface (0-1 cm) and subsurface sand layers (5-10 cm). The level of activity of the tested enzymes synthesized by bacteria inhabiting both sand layers was similar. Fig. 3 presents the variability in the level of activity of bacterial enzymes at the sampling sites in Sopot beach. As a rule, bacteria inhabiting the whole horizontal profile of the beach synthesized enzymes with similar intensity. Only the strains isolated from the middle part of the beach and the dune synthesized N-acetyl-β-glucosaminidase (Nac) and α-mannosidase (αMa) more actively than the strains from the other sampling sites.

Data presented in Table 1 show that bacteria isolated in different seasons form the sand of the studied beach synthesized the tested hydrolytic enzymes with similar intensity. Only acid phosphatase, naphtol-AS-Bi-phosphopydrase, α-glucosidase and N-acetyl-β-glucosaminidase were synthesized more actively in winter while an increased activity of esterase, leucine arylamidase and cysteine arylamidase was observed in spring.

Individual isolates were capable of synthesizing a wide spectrum of extracellular enzymes (Fig. 4). The

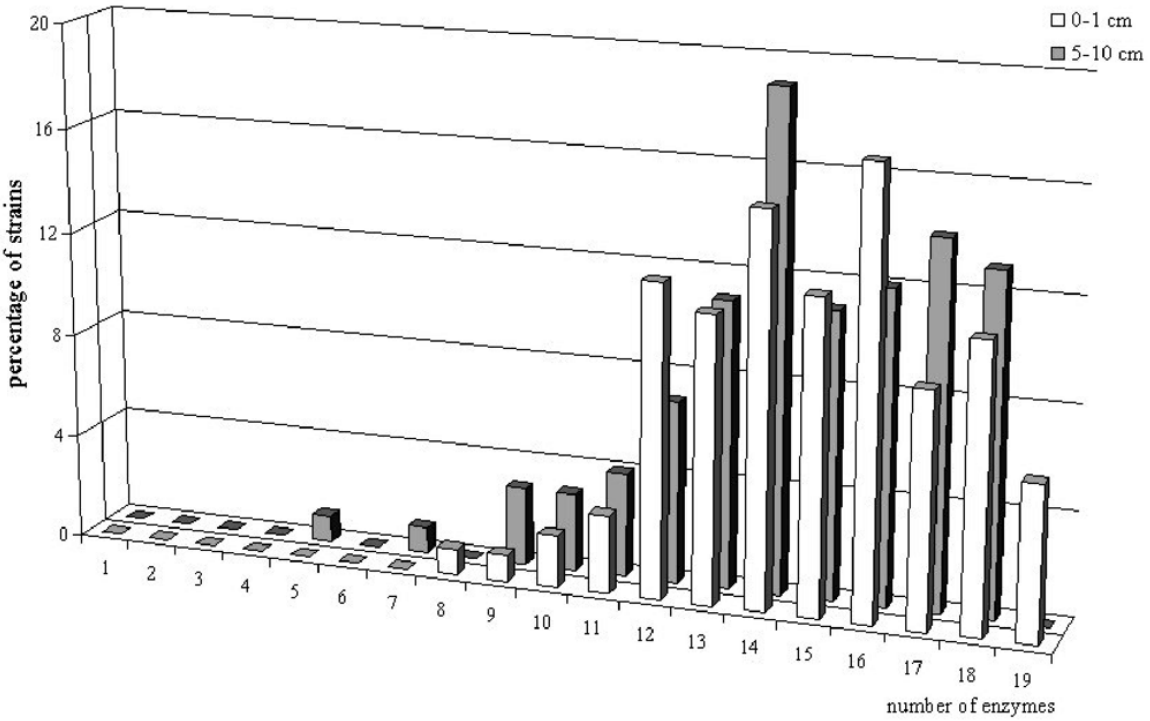


Fig.4. The number of enzyme activities produced by a strain and the proportion of strains expressing this number.

highest percentage (9-19%) of the isolated strains was capable to synthesize 14-18 enzymes; 6% of the strains from the surface (0-1 cm) sand layer produced all the 19 tested enzymes.

## Discussion

Many heterotrophic bacteria are characterized by the ability to carry out the processes of depolymerization of macromolecular compounds to mono- or oligomers [12, 25]. 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 [15, 20]. Several studies [16, 26, 27, 28] have indicated that the level of activity of particular enzymes in water basins depends on the quantity and quality of organic compounds. According to Incera et al. [29], proteins (71-84%) and lipids (25.5-27.6%) are the dominant class among labile organic compounds accumulated in marine beaches. Therefore, it can be deduced from the high protease and lipase activities that these substrates are the dominant organic compounds in Sopot beach sand.

In the Sopot beach phosphatases (alkaline phosphatases) showed a high potential level of activity. According to Hoppe and Urlich [30] and Hoppe [31], phosphatase activity is widespread in marine bacteria and is closely related with both the P and C cycles. Extracellular phosphatases can play an important role in supplying phosphorus to heterotrophic and autotrophic microorganisms. Phosphatases are synthesized most actively by those bacteria which have a very efficient system of transporting phosphorus into their cells. Phosphatase-producing capacity is shown by many species of heterotrophic bacteria mainly of the genera *Pseudomonas, Chromobacterium, Bacillus, and Flavobacterium* [32, 33]. Phosphates are the most important regulators of the synthesis and activity of bacterial phosphatases [13, 15, 18]. Phosphatases occur in two forms, as alkaline and acid; both forms are able to hydrolyse all phosphoric esters [34, 35]. Zdanowski and Donchie [36] and Mudryk [37] draw attention to the fact that, as a rule, in water bodies the activity of alkaline phosphatase is higher than that of acid phosphatase. Higher activity of alkaline phosphatase was also determined in the present study.

Results of many studies [14, 15, 28] indicate that many heterotrophic bacteria in the marine environment are also capable of synthesizing proteases. Extracellular proteases hydrolyze proteins into mono- or oligomers, mainly peptides and amino acids [38]. Those low molecular weight organic compounds are immediate precursors in the synthesis of proteins and participate in many pathways of microbial cell metabolism [39, 40]. Proteases (leucine arylaminase) were also synthesized very intensively by bacterial strains isolated from the sand of the Sopot beach. Similar results were obtained by Middelboe et al. [42] in Danish lakes, Zdanowski and Donachie [36] in arctic waters and Mudryk and Skórczewski [17] in estuarine lake Gardno. According to Jones and Lock [43], the level of leucine arylaminase activity is a good measure of the proteolytic activity of bacteria as it is a peptide bond hydrolyzing enzyme.

Boetius [19], Boschker and Cappenberg [44] and Mudryk and Skórczewski [17] draw attention to the high level of activity of esterases and lipases in aquatic environments. Those hydrolases are capable of attacking emulsified mono-, di-and triglycerides, and of splitting them with the yield of glycerol and fatty acid residues [45]. In the Sopot beach, between 96% and 98% of all bacterial strains express lipase activities. The high percentage of bacteria synthesizing this enzyme can explain the elevated the high lipase activity in fresh collected sediment samples [41]. Lipolytic enzymes are actively exported by living microorganisms or are released as free enzymes after the lysis of their cells [47]. In the studied beach, bacterial lipolytic activity against long-chain fatty acids was lower compared to lipids with short-chain fatty acids. The same results were obtained by Zdanowski and Figuerias [24] and Mudryk and Skórczewski [17] who studied lipolytic activity of heterotrophic bacteria isolated from marine and estuarine basins.

According to Boetius [19], production and activity of bacterial hydrolytic enzymes depends on the availability, distribution and concentration of organic substrates. Therefore, activity of enzymes in horizontal profiles can be expected to mirror the distribution of organic matter in water basin sediments. Results of the present study do not indicate clear differences in the level of potential activity of bacterial enzymes among different parts of the studied beach. According to Martinez et al. [25] this might indicate no horizontal shifts in both the availability and degradability of organic compounds in the Sopot beach.

Many authors [15, 26, 48] draw attention to considerable variability in the activity of extracellular enzymes in the vertical profiles of sediments in the sea. In most cases, activity of hydrolytic enzymes decreased with depth [11, 19]. Results of the present study do not indicate clear differences in the level of potential activity between enzymes in the surface and subsurface sand layers. This absence of vertical gradients can be explained by destruction of the sediment profile by people (about 3000 people can pass there daily) leading to an increased enzymatic activity in the deeper sediment layers.

The authors realize that the semi-quantitative API ZYM technique used in this study allows measurement of only the potential enzymatic activity of bacterial strains which is never identical to bacterial activity in natural environments. In laboratory conditions it is not possible to generate and monitor short-term changes commonly occurring in situ. The results obtained in the present study and their ecological interpretation can, however, be an important source of new information on the potential activity of bacterial enzymes in a marine sandy beach and therefore in the process of transformation of organic matter in marine ecosystems.

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