Microbial Extracellular Enzyme Activities in Natural Water

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The quantitative measurement of extracellular enzyme activities

Model substrates are used for the determination of bacterial extracellular enzyme activities. These substrates are composed of a fluorescing molecular (methylumbelliferone (MUF)) and on organic or inorganic molecule (e.g. glucose, lecuine, glucosamine, fatty acid, phosphate) (Fig.1). Both components are linked together with a specific binding (α-or β-glucoside, peptide-binding, esterbinding). The complex as a whole is non-fluorescent. After extracellular enzymetic hydrolysis the fluorescence tracer molecule (MUF) and the organic or inorganic molecule are separated and the decomposition rate (via fluorescence of MUF) is measured directly by a spectrofluorimeter. Several investigators have used MUF-substrate for the determination of extracellular enzyme activities (Hoppe, 1983; Somville, 1984). It could be demonstrate by epifluorescence microscopy that the interior of microbial cells did not exhibit fluorescence while the surrounding medium showed a strong blue fluorescence.

Water samples were supplemented with different amounts of MUF-substrates (0.01-250 $\mu M l n^{-1}$). Fluorescence measurements resulting from these experiments followed in most cases 1. order enzyme kinetics(Fing.2). Considerable differences in the substrate concentrations needed for substrate saturation were observed along with the different MUF-substrates used. Diviations from 1. order kinetics occurred sometimes at very low MUF-substrate concentrations (inhibition of enzymes by end-products?).

Attached bacteria produce nutrients for free-living bacteria

Attached bacteria may benefit from their particulate organic substrate and from the macromolecules which tend to become adsorbed on it. Specific extracellular enzyme activities (per cell) of attached bacteria seem to be much higher than those of their free-living counterparts (Kim, 1985). Nevertheless only a few percent of total bacteria (in the Baltic Sea) prefer the attached mode of life and only a few percent of organic particles are heavily colonized by bacteria. The unresolved question

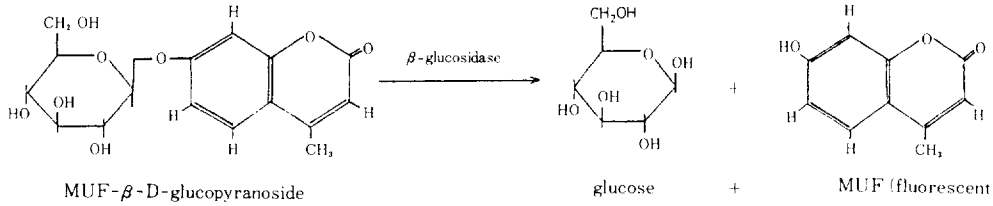

Fig. 1. The molecular structure of MUF-β-D-glucopyranoside. MUF-β-D-glucopyranoside (nonfluorescent) is hydrolyzed by β-glucosidase into equimolar concentrations of glucose and free MUF (fluorescent).

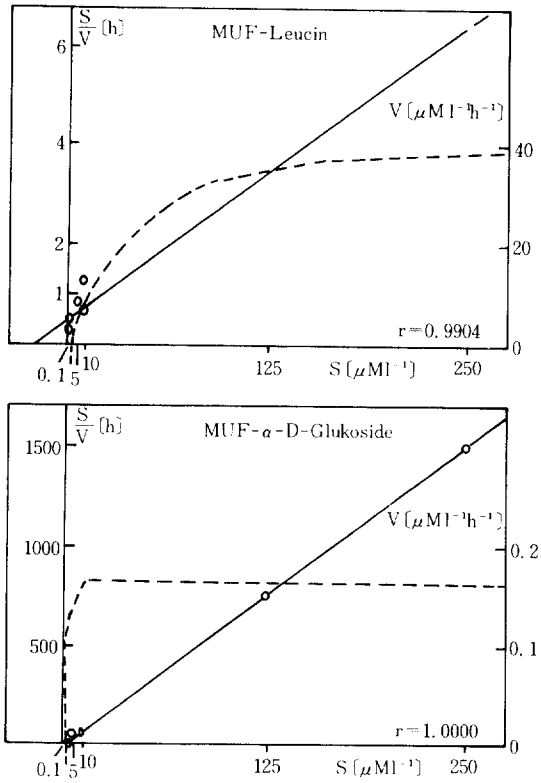

Fig. 2. Substrate saturation curves (×---×) and Lineweaver-Burk plots (○—○) of protease activity (top) and α-glucosidase activity (bottom) in a natural water sample.

here is: which factors are responsible for the observed balance between attachment and unattachement of marine bacteria? Many reasons have been discussed for explanation of this phenomenon (e.g. microzooplankton grazing on particles, production of toxic substances by microcolonies of attached bacteria, which prevent further attachement). Another possibility for explanation may arise from the fate of enzymatic decomposition products in the environment: it has been observed that (at high levels of nutrient supply) only a small portion of the monomeric decomposition products of macromolecules was directly incorporated by the enzyme producing bacteria while the major fraction entered the DOC pool of small molecules. Bacteria attached to organic particles are in a situation of nearly unlimited nutrient supply, thus their free-living counterparts may benefit from the surplus production of easily degradable molecules resulting from the extracellular enzyme activities of attached bacteria.

Bacteria which are attached to organic particles have high extracellular enzyme capacities. By the enzymatic breakdown of macromolecules they produce more easily degradable monomers than they need for their own nutrition. The surplus materials serve as nutrients for free-living bacteria. This process of nutrient transfer may lead to a better understanding of the observed distribution of attached and free-living bacteria in marine waters. Evidence for this was given by preliminary experiments, where the velocity of peptide hydrolysis versus the velocity of amino -acid uptake increased from 3 to 38 along with increasing concentrations of peptide supply.

The qualitative estimation of extracellular enzyme properties of bacterial colonies

Bacteria are responsible for the development and maintenance of anaerobic zones in the sea. The zones are characterized by a high input of sedimenting organic material. The most important factors promoting oxygen depletion and H_2S-formation are bacterial oxygen consumption and sulfate-reduction. With respect to organic substrate turnover in aerobic marine environments several changes in heterotrophic substrate uptake and extracellular enzyme activities have been observed. However, mechanisms of substrate generation and of substrate incorporation were affected by anaerobic conditions at different degrees. Bacterial uptake of monomers (glucose, amino acids) was reduced much more than the extracellular hydrolysis of polymers (provided in the from of model substrates). In this case uptake activity seems to be the growth limiting step for bacteria develoment. Consequently, as a result of less affected enzyme activities, materials of low molecular weight should accumulate in these environments. It has to be proven whether this is also the case in well adapted biotopes with perma-

nently anoxic conditions.

Conventional methods available for the detection of bacterial decomposition of macromolecules (starch, cellulose, chitin, protein, fat) are in many cases not fully satisfactory. They may be not sensitive enough or the results may be unclear (e.g. overlapping of zones of hydrolysis in the starch-test). Using MUF-substrates a quick and reliable test for the detection of enzymatic properties of bacterial colonies was developed by Kim and Hoppe (in press). In the figure 3 the procedure of the test for the identification of enzymatically active colonies on agar plates is illustrated. This test will be specially useful when it is employed together with the quantitative investigation of extracellular low enzyme activity, because it provides a possibility to quantify and the classify the bacteria which are involved in this process. The results obtained with this method are expressed in terms of numbers of bacteria colonies with special enzymatic properties (e.g. protease, α-amylase, phosphatase)

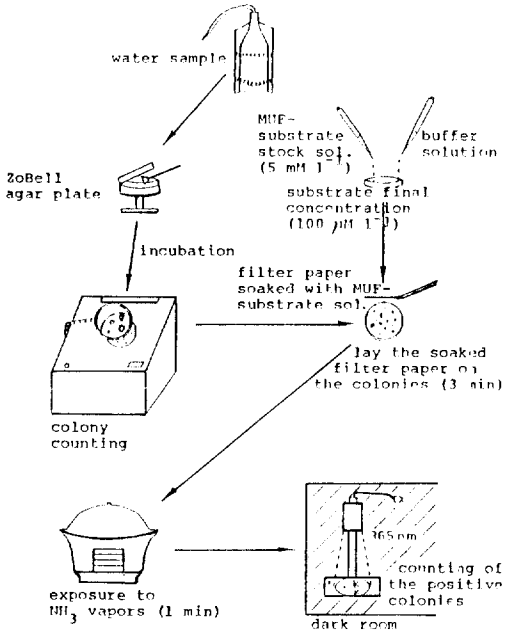

Fig. 3. Scheme of method for the identification of extracellular enzyme activities of bacteria colonies.

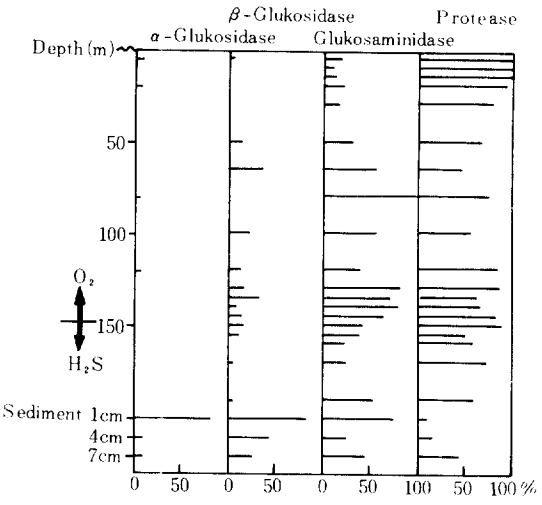

Fig. 4. The percentage of enzymatically active colonies from the total number of bacteria colonies grown under anoxic condition (Gotland deep in the Baltic sea).

or in terms of the percentage of enzymatically active colonies from the total number of colonies (ZoBell-agar).

Enzymatic properties of bacteria colonies grown under anoxic conditions were determined via fluorescent MUF-modelsubstrates. Samples for analysis were taken from water and sediment of the Gotland deep. In the figure 4 the percentage of enzymatically active colonies from the total number of bacteria colonies on the agar plates was calculated. The protion of (facultatively) anaerobic bacteria which are specialized on the degradation of carbohydrates (α-, β-glucosidase) is low in the water column even below 150m were oxygen was depleted. (Facultatively) anaerobic bacteria, capable to decompose chitin und protein modelsubstrates were abundant throughout the water column. Their contribution to the total bacteria population was expecially high in and around the boundary between oxygenated and anoxic waters. The surface layer of the sediment was dominated by bacteria with capabilities for the enzymatic decomposition of carbohydrates while the protion of proteolytic bacteria was relatively low.

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