2006 Ocean Sciences Meeting, Honolulu, Hawaii, 20-24 February.

Phosphatase Activity of Heterotrophic Bacteria at Single Cell Level. First Assays in Mediterranean Sea and on Marine Bacterial Cultures.

Van Wambeke, F., Nedoma, J., Duhamed, S., Strojsova A., Moutin, T., Garcia N., Goutx, M.

Laboratoire de Microbiologie Geochimie et Ecologie Marines, CNRS - UMR 6117
Campus de Luminy, case 901, 13 288 Marseille cedex 9, France

Hydrobiological Institute, Academy of Sciences of the Czech Republic, Na Sadkach 7, CZ-370 05 Ceske Budejovice, Czech Republic

Laboratoire d'Oceanographie et de Biogeochimie, CNRS - UMR 6535
Campus de Luminy, case 901, 13 288 Marseille cedex 9, France

Email: wambeke@com.univ-mrs.fr

Microbial (algal, bacterial) extracellular alkaline phosphatases (AP) are inducible enzymes localized on cell surfaces, where they catalyse hydrolysis of ambient organic phosphorus, yielding inorganic phosphate capable of transport into the cells. Presence of AP serves as an indicator of phosphorus deficiency. ELF97 phosphate (ELFP; Molecular Probes, USA) is an artificial substrate capable of tagging AP at single cell level by forming green fluorescent precipitates at the membrane sites of AP activity. While observation of ELF-labelling on micro-sized algal cells is easy, it is complicated for bacteria because of their small size and necessity to counterstain them with another fluorochrome (DAPI). Bacterial cultures of marine strains were followed in conditions of P or C limitation. AP activity (total activity determined by spectrofluorometry with MUF-P and cell-specific detection of ELF spots by epifluorescence microscopy) was also followed {\it in situ}in oligotrophic, P-limited Mediterranean Sea (French PROOF PECHE program). The degree of AP expression is highly variable according to strains. Cell-specific activities in P limited conditions ranged from 0.01 to 2 femto mole MUF-P hydrolyzed per cell per hour. While ELF labelling was significant in P-limited cultures (ELF-targeted cells to total DAPI counts in P-limited cultures reached 26-100 \% according strains), the ratio of ELF-targeted spots to bacterial abundance never exceeded 0.01 \% {\it in situ}. However the response was consistent with global fluxes of phosphatase activity. Detection at the heterotrophic bacterial cell level is promising to explain co-limitation processes (C versus P) but needs further improvement for the ELF methodology.