SPHINGOMONAS SP. MCT13, ITS ECOLOGICAL ROLE AND ENZYMATIC ACTIVITIES WITH POTENTIAL BIOTECHNOLOGICAL APPLICATION

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The genus *Sphingomonas*, originally proposed by Yabuuchi and coworkers, has been later subdivided into the genera: *Sphingomonas "sensu stricto"*, *Sphingobium*, *Novosphingobium* and *Sphingopyxis*. Sphingomonads are widespread in aquatic and terrestrial environments. Some strains are able to degrade a variety of refractory contaminants, including pollutants produced by industrial processes, polycyclic aromatic hydrocarbons and dibenzofurans, and other toxic chemicals such as insecticides, herbicides and heavy metals.

In the first year of my PhD project I performed several tests in order to better characterize the *Sphingomonas* sp. MCT13.

The draft genome of MCT13 was obtained and the 16S rRNA gene sequence analysis placed the isolate within the *Sphingomonas* (*sensu stricto*) cluster and showed the closest relative to be *Sphingomonas koreensis* (98.52%). MCT13 has a peculiarity than other characterized *Sphingomonas* species: the ability to degrade agar by producing agarase.

MCT13 is a rod-shaped, Gram-negative, oxidase- and catalase positive, aerobic, non-spore-forming and non-motile strain. Its growth occurred between 20 and 35 °C, with optimum at 28-30 °C, and in the pH range 6.5-9 with optimum at 7.5. NaCl was tolerated at concentrations in the range 0-1.5%, with the optimal growth occurring with no salt at all.

The morphological and physiological features of the MCT13 isolate were studied and compared with the characterized *S. koreensis* type strain JSS26. Several culture media were evaluated to obtain the best MCT13 growth; the strain was able to grow on TSA and MHA within 48h, but it took 3-4 days on ZoBell w/o NaCl, and did not grow on MacConkey agar; the growth of the isolate did not occur in home-made chemically defined medium M9, with glucose as the carbon source, and was quite scanty also in Brunner (DSMZ 457) medium: among the different combinations of aminoacids and/or vitamins solution tested to improve the growth, the best results were achieved by adding 0.02% of L-methionine to the formula.

MCT13 agarase activity was observed on LBA after 48-72h of incubation, as clearing of agar and pitting of the colonies and is more apparent on ZoBell or agarized Brunner medium. The clearing was also observed by flooding the plates with Lugol's iodine solution.

Analysis of respiratory quinones and polar lipids were carried out by the Identification Service and Dr. Brian Tindall, DSMZ, Braunschweig, Germany, so as the fatty acids determination.

In the second year of my PhD project I will complete MCT13 biochemical characterization.

Moreover, I will clone agarases genes in order to better understand the MCT13 potential interest for biotechnological applications.

In fact, over the last decades, agar degrading enzymes have found medical and nutraceutical relevance through the production of biologically active oligosaccharides useful to human health, as anticoagulant, anti-inflammatory, antioxidant or immunostimulating activity.