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Differential response to the filamentous induction among various strains of *Sphaerotilus natans*

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Background and aims:

The good efficiency of the activated sludge process is based on the gravity separation of purified water and bacterial biomass in the clarifier. The proliferation of filamentous bacteria preferentially occurring at low F/M ratio can be promoted by inadequate shortage of oxygen, by C / N imbalances or by the nature of the influent. Filamentous bulking caused by an overabundance of filamentous micro-organisms, severely disrupts the ability of sludge to settle and degrade the effluent quality. The most commonly employed curative treatments use oxidizing compounds such as chlorine. They are not selective. A targeted strategy could be considered when molecular mechanisms of the filamentous growth of the major strains involved will be better known.

Among the filamentous bacteria described in activated sludge, *Sphaerotilus natans* is especially favoured by shortage of oxygen, nitrogen or phosphorus, as well as by high concentrations of readily metabolizable soluble substrates (Eikelboom, 2000). This filament is dominant in 12% of bulking cases in the United States (Jenkins *et al*., 2004). It occurs mainly in waste water treatment plants (WWTP) treating industrial influents as from agro-food industry. This rod shape bacteria can grow as single cells when the media is rich. The filamentous form needs to synthesize a polysaccharide sheath to grow (Suzuki *et al*. 2002). This bacterium was chosen as test micro-organism because its filamentous form, can be induced reversibly from a culture of dispersed cells.

The emergence of a bulking could depend on the mode of filament elongation from a single cell pre-existing population. This study was first conducted to determine whether the *S. natans* filamentous development proceeds by linkage of pre-existing single cells or by cell divisions within proto filaments. The different easily degradable carbon substrates in the common culture media used to cultivate *S. natans*, do not show the same ability to induce filamentous growth. This ability has been characterized by experience plan in the purpose of defining an effective inducer media.

It was finally determined whether different strains of *S. natans* from collection, exhibited an equivalent capacity to grow as filaments. This last point will allow to select the most adequate strains on which filamentous growth mechanisms could be subsequently studied.

Methods and results

In order to determine the *S. natans* filament elongation mode, a culture of dispersed cells was transferred in filamentous inducing media (Takeda *et al*. 1998), to which was added an inhibitor of cell division: nalidixic acid 20 mg. L^{-1}. The cells were stained with cFDA / SE. After 48 hours of incubation at 30°C under agitation, the intensity of fluorescence per cell was identical to the original state, indicating that the cells have not entered into division. No filament structure was observed. The cells were locally clustered.

To determine their involvement in the morphological differentiation of *S. natans*, the main sources of organic carbon present in the culture media, an experiment plan involving 7 factors, based on Hadamard matrix was used in broth and solid agar plates (Larpent-Gourgaud and Sanglier, 1992). The 7 factors combined in this experiment correspond to the

following: peptone, casitone, tryptone, glucose, glycerol, yeast extract and mineral salts. They were examined at two different concentrations on 3 different strains of *S. natans*: ATCC 13338T, 13929 and 15291. They were isolated respectively from river water, activated sludge and paper mill effluents. The 3 strains studied show differences in behaviour.

Nearly all tested agar media tend to promote dispersed growth form. In liquid broth, at low concentration, all carbon sources tested induce a filamentous growth. However, at high concentration, growth as single cells is favoured by the association of peptone and tryptone for strain 15291. This strain present the highest degree of differentiation. 13338T strain grows mainly as single cells and strain 13929 as filaments.

To clarify these behaviour differences, 5 different strains of *S. natans* have been studied by varying access to oxygen or carbon substrate concentration of nutrient medium. These strains were: 13338T, 13929, 15291, 29329 and 29330. The last two were isolated from pond. It appears that low concentrations of oxygen or substrate promote growth of filamentous form. However all strains show variable degrees of differentiation. Strains 29329 and 29330 grow mainly as single cells form very few filaments at low concentration of oxygen or substrate. Conversely, the strain 13929 grows mainly as filaments and high concentrations of substrate or oxygen are necessary for the emergence of single cells.

Conclusions:

The pre-existence of a large amount of single cells of bacterial species able to form filaments in activated sludge at the onset of inducing factors, represents a potential risk to the WWTP. This biomass is currently very difficult to detect. It is shown here that *S. natans* dispersed single cells, could cause a filamentous proliferation by cell divisions and not by simple cell linkage. In pure culture, this morphological duality expressed preferentially with peptones and tryptone as carbon substrates. The ATCC 15291 strain presents broader dynamics of morphological differentiation, closely followed by the strain 13338T. The strains 29329 and 29330, growing mainly as single cells and strain 13929 growing mainly as filaments, are less suited for a study on filamentous growing mechanisms. For instance, a comparative study of proteomes requires cultures of the same strain in both morphological states. These studies should be complemented by using strains of filamentous bacteria frequently encountered in bulking. Unfortunately, the latter are generally much more difficult to grow.

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