MICROALGAE GROWTH-PROMOTING BACTERIA: A NOVEL APPROACH IN WATER SCIENCE; A MICRO-REVIEW

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ABSTRACT

Plant growth-promoting bacteria (PGPB) of the genus *Azospirillum* are known to enhance the growth of numerous agricultural crops. We are proposing the use of these bacteria as "microalgae-growth promoting bacteria" (MGPB) to enhance the cleaning capacity of polluted water by the freshwater microalgae *Chlorella vulgaris* and *C. sorokiniana*. Deliberate inoculation of *Chlorella* sp. with a terrestrial PGPB was not reported prior to these studies, perhaps because of the different origins of the two microorganisms. *Chlorella* sp. is not known to harbor any plant growth-promoting bacteria, and *Azospirillum* sp. is rarely used for inoculation in aquatic environments. Co-immobilization of *C. vulgaris* and *A. brasilense* Cd in small alginate beads resulted in significant increases in numerous growth parameters of the microalgae. Dry and fresh weight, total number of cells, size of the microalgal clusters (colonies) within the bead, number of microalgal cells per cluster, and the levels of microalgal pigments significantly increased. Additionally, lipids and the variety of fatty acids significantly increased, and the combination of microalgae and the MGPB had superior capacity of removing ammonium and phosphorus from polluted synthetic and municipal wastewaters.

Other PGPB (like *Flavobacterium* sp., *Azospirillum* sp., and *Azotobacter* sp.) are currently studied in aquaculture; carp cultivation by enhancing phytoplankton growth, and for stabilization of mass culture of marine microalgae used as a feed for marine organisms, all with promising results.

This aspect of the effect of PGPB on water microorganisms is currently in its infancy. We propose that co-immobilization of microalgae and plant growth-promoting bacteria are an effective means of increasing microalgal populations and also their capacity for cleaning polluted water.

INTRODUCTION

In natural or artificial aquatic environments, freshwater or marine, microalgae are always associated with bacteria (Mouget et al., 1995). It is unknown whether these bacteria are associative, growth promoters, symbionts, or simply co-exist with the microalgae (like saprophytes in terrestrial environments). Apart from a few, these bacteria were seldom isolated or characterized (Suminto and Hirayama, 1997; Gonzalez-Bashan et al., 2000), and their effect on the microalgae, some used for wastewater treatment (Tam and Wong, 2000; Valderrama et al., 2002), is unknown. The working hypothesis of the studies described in this short review is that some of the aquatic bacteria are plant growth promoters. As microalgae can be considered a plant, albeit of single cells, these bacteria may promote the growth and the activity of the microalgae. As a first step, and since almost no PGPB was ever demonstrated to have an effect on microalgal growth, the use of *Azospirillum* PGPB of agricultural origins took center stage. This has been done because *Azospirillum* is the most studied PGPB, is nonspecific to plants, and can efficiently colonize roots submerged in growth nutrient solutions and consequently improve plant growth (Bashan and Holguin, 1997).

ENHANCEMENT OF MICROALGAE GROWTH BY PGPB

Co-immobilization of freshwater microalgae *Chlorella vulgaris* or *C. sorokiniana* and the PGPB *A. brasilense* Cd in small alginate beads resulted in significantly increased growth of the microalga, when growing in synthetic or municipal wastewater. Dry and fresh weight, total number of cells, size of the microalgal colonies within the beads, number of microalgal cells per colony, and the cell size of some strains significantly increased. Light microscopy revealed that both microorganisms colonized the same cavities inside the beads, though the microalgae tended to concentrate in the more aerated periphery while the bacteria colonized the entire bead (Gonzalez and Bashan, 2000; de-Bashan et al., 2002a; 2003). When *C. vulgaris* was similarly co-immobilized with its naturally-associated, N_2-fixing bacterium *Phyllobacterium myrsinacearum*, they share the same cavity in the beads as with *A. brasilense* Cd, but there was no effect on the number of cells or the biomass of the microalga (Gonzalez-Bashan et al., 2000). Transmission electron microscopy revealed that, initially, most of the small cavities within the beads were colonized by microcolonies of only one microorganism, regardless of the bacterial species cultured with the microalgae. Subsequently, the bacterial and microalgal colonies merged to form large, mixed colonies within the cavities. At this stage, the effect of bacterial association with the microalga differed, depending on the bacterium present. Though the microalga entered senescence phase in the presence of *P. myrsinacearum*, it remained in a growth phase in the presence of *A. brasilense* Cd. It appears that there are commensal interactions between the microalga and the two PGPBs, and that with time the bacterial species determined whether the outcome for the microalga is senescence or continuous multiplication (Lebsky et al., 2001).

EFFECT OF ASSOCIATION ON THE METABOLISM OF THE MICROALGAE

Co-immobilization of *C. vulgaris* with PGPB yielded significant changes in the metabolism of the microalgae and cell cytology. Pigments, lipid content, and the variety of fatty acids produced changed in comparison with microalgae immobilized in alginate without the bacterium. Co-immobilization in small alginate beads with *A. brasilense* Cd or with the N_2-fixing bacterium *P. myrsinacearum* resulted in significantly increased microalgal pigments; chlorophyll *a* and *b*, lutein, and violoxanthin (Gonzalez-Bashan et al., 2000; de-Bashan et al., 2002a). The microalgal cells accumulated large amounts of lipids in droplets within the cell and the number of fatty acids in the microalgae doubled (from 4 to 8) (de-Bashan et al., 2002a). Addition of the plant hormone indole-3-acetic acid to microalgal culture prior to immobilization of microorganisms in alginate beads partially imitated the effects of *A. brasilense* on the microalgae (Gonzalez and Bashan, 2000).

REMOVING NUTRIENTS FROM WASTEWATER

A. brasilense Cd is incapable of significant removal of nutrients from wastewater and cannot be considered a wastewater treatment bacterium, whereas *C. vulgaris* and *C.*

sorokiniana can. Co-immobilization of *C. vulgaris* and *A. brasilense* Cd in alginate beads under synthetic wastewater culture conditions significantly increased the removal of ammonium and soluble phosphorus compare over immobilization of the microalga alone (de-Bashan et al., 2002b). Co-immobilization of the two microorganisms (also with *C. sorokiniana*) was superior to removal by the microalgae alone, reaching removal of up to 100% ammonium, and 92% phosphorus within six days (varied with the source of the wastewater), compared to 75% ammonium, 84% nitrate, and 89% phosphorus by the microalgae alone (de-Bashan et al., this volume). The events occurring during this artificial association are summarized in Fig. 1.

USING PGPB ASSOCIATED WITH AQUATIC MICROORGANISMS TO SOLVE ENVIRONMENTAL PROBLEMS

PGPB may also serve as “helper” bacteria in promoting the growth of economically important aquatic microorganisms. Although some positive effects of marine bacteria on marine microorganisms (diatoms) were known for decades (Ukeles and Bishop, 1975; Riquelm et al., 1988; Suminto and Hirayama, 1996), the knowledge was not implemented. Inoculation of a marine diatom - *Chaetoceros gracilis* used as feed in pearl oyster hatchery in Japan - with the PGPB *Flavobacterium* sp. in mass culture production of the diatom, resulted in significantly higher specific growth rate than the control cultures, and the stationary growth phase in the treated cultures lasted longer until the end of the culture period (Suminto and Hirayama, 1997). Inoculation of freshwater fish aquaculture ponds in India with *Azospirillum* sp. and *Azotobacter* sp. significantly increased the phytoplankton population and consequently the yield of carp (Garg and Bhatnagar, 1999).

CONCLUDING REMARKS AND FUTURE RESEARCH PERSPECTIVES

The idea to use PGPB for enhancement of growth of economically useful aquatic microorganisms is in its infancy. The way PGPB affects the growth of these microorganisms is unknown. In wastewater treatment where information is more available, we propose co-immobilization of microalgae and PGPB as an effective means of increasing microalgal population within confined environments and increasing removal of nutrients from wastewater. However, it may be further suggested that although *Azospirillum* spp. positively affects *Chlorella* growth and nutrient removal, other PGPB should be tested for their efficiency. This man-made association is capable of improved removal of nutrients from wastewater and may serve as an important tool for novel wastewater treatments.

ACKNOWLEDGEMENTS

We thank Mr. Ira Fogel for editing the English text. This study was supported by Secretaria de Medio Ambiente y Recursos Naturales (SEMARNAT-2002-C01-0005), Mexico, and by The Bashan Foundation.

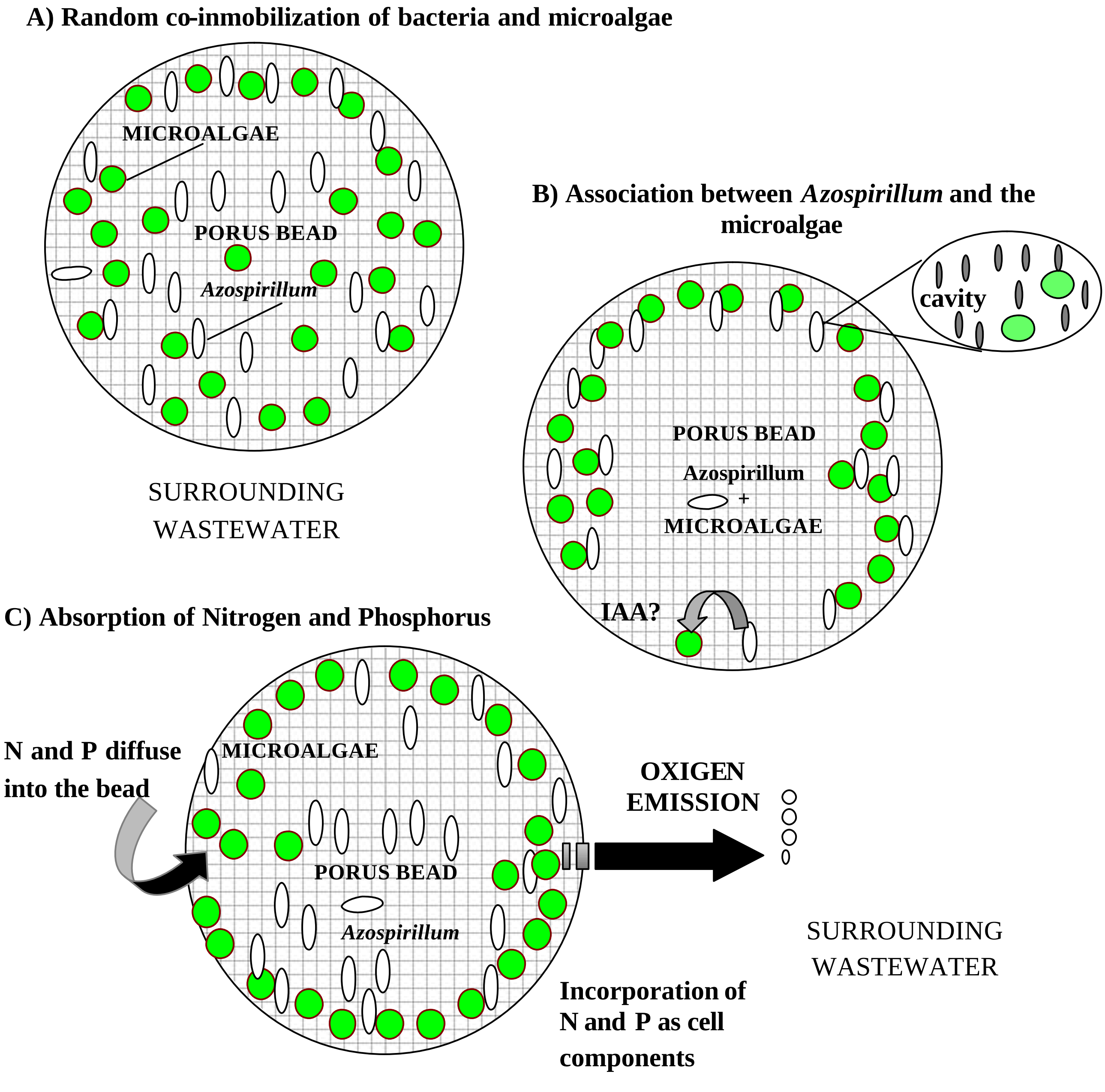

Fig 1. Model representing the events occurring during co-immobilization and co-cultivation of *A. brasilense* Cd and *Chlorella* spp. in small alginate beads. A) immediately after co-immobilization; B) effects of *A. brasilense* on multiplication on microalgae; C) removal of nutrients by the artificial biological assemblage. (Size not to scale.)

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