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Bioaccumulation of Yttrium in *Pseudomonas fluorescens*

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Metal toxicity has become a major ecological concern due to its negative influence on the biosphere. Industrial wastes and acid rain have led to a sharp increase of metal pollutants in the environment. The inhibitory impact of this enhanced bioavailability of metallic contaminants has been shown to affect a wide range of organisms (Haug 1984). Some microbes, however, are known to have evolved intricate strategies to circumvent the presence of high levels of metals in their ecological niches. Extracellular deposition and active extrusion of metals enable microorganisms to maintain intracellular concentration of these metallic moieties at an innocuous level (Al-Aoukaty et al.1991; Silver and Misra 1988). Intracellular sequestration and volatilization mechanisms also provide microbes reprieve from abnormal concentration of metallic elements (Stratton 1987). In this study we examined the effect of yttrium, a contaminant from nuclear reactors, on a soil bacterium. Yttrium and other radionuclides are complexed to mineral or organic matter and are eventually absorbed by plants and animals (Bocock 1981).

Bacteria belonging to the genus *Pseudomonas* are known for their economically interesting features and their versatility in nutritional requirements (Spain 1990). These attributes have made these organisms an important biotechnological tool. Our ongoing research on metal toxicity and decontamination has led us to investigate the adaptability of *Pseudomonas fluorescens* ATCC 13525 to yttrium, an effluent from nuclear reactors that may be a threat to the environment and public health. In this report we describe the bioaccumulation of the metal pollutant and the suitability of the microbe in yttrium containment is also discussed.

MATERIALS AND METHODS

All chemicals were reagent grade. Folin Ciocalteu's phenol reagent, serum albumin, D-glucose and citric acid were obtained from

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Sigma Chemical Company. The citrate assay kit was from Boehringer Mannheim. The bacterial strain *P. fluorescens* from American Type Culture Collection (Rockville, Maryland, U.S.A.) was maintained on a mineral medium solidified by the inclusion of 2% agar. The mineral phosphate deficient medium used in this study was the same as described in Al-Aoukaty et al. (1991). The metal yttrium (0.5 mM), as its nitrate salt, was complexed to citrate,the sole source of carbon prior to sterilization. The pH was adjusted to 6.8 with dilute NaOH. The media were dispensed in 200 ml amounts in 500ml Erlenmeyer flasks and inoculations were made with 1.0 ml of stationary cells grown in control phosphate-rich medium (Al-Aoukaty et al. 1991). The cultures were aerated on a gyratory water bath shaker model G76 (New Brunswick Scientific) at 26°C at 140 rev min^{-1}. Bacterial growth was measured at appropriate time intervals by monitoring the increase in total culture protein content using the method of Lowry et al. (1951). Extracellular protein and carbohydrate were measured by the Lowry et al. (1951) method and the phenol-sulfuric acid assay, respectively (Dubois et al. 1956). The pH of the broth devoid of bacterial cells was recorded with the aid of a Fisher pH meter model 610A. The mineralization of yttrium was analyzed by X-ray fluorescence spectroscopy (XRF). 50 ml samples were collected at different growth intervals. Following centrifugation at 800 rpm and 5,000 rpm, respectively, any insoluble residue, bacterial cells and supernatant were separated. These fractions were freeze dried and examined for their yttrium content in a 31mm disposable X-ray cell. The X-ray spectra and intensities were obtained using a Phillips PW1404 automatic, sequential spectrometer following standard procedures. P_{10} gas (10% methane and 90% argon) was used in the flow proportional counter. A dual Mo/Sc X-ray tube was used throughout this investigation. LIF200 was used as the analyzing crystal. The peak shifts are given in two theta degrees (2θ) and the intensities in kilocounts per second (kcps). A peak at 23.8 $2\theta^{o}$ indicative of Kα yttrium was recorded (White and Johnson 1970). Citrate utilization was monitored enzymatically with the aid of the citrate assay kit (Moellering and Gruber 1966). All experiments were performed three times in duplicate. The mean values are reported and the standard deviation ranged from 0.99 to1.75 .

RESULTS AND DISCUSSION

In the control medium without any added metal, *P. fluorescens* reached stationary phase after 36 hrs of incubation with a cellular yield of 310 µg of protein per ml of culture . The inclusion of yttrium had a stimulatory influence on growth. In this instance the microbe grew

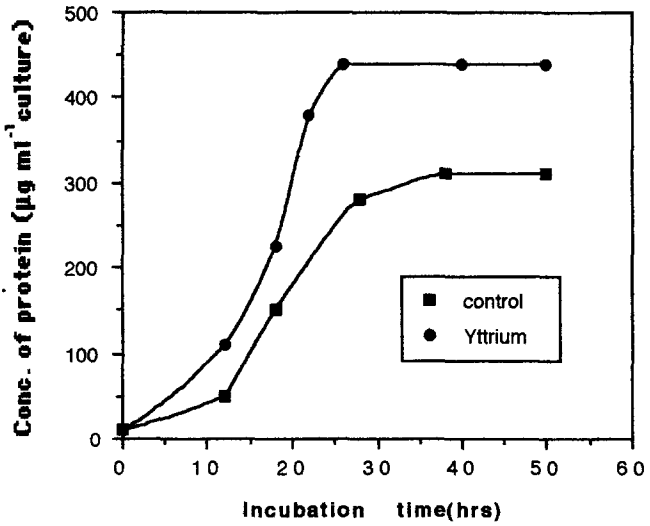

Figure 1. Effect of Yttrium on bacterial growth.

faster and a bacterial yield of 450 µg of protein per ml of culture was observed (Fig. 1). At cessation of cellular multiplication the metal-supplemented culture was characterized by a diminution in extracellular carbohydrates and proteins.

In the control medium, carbohydrate and protein content amounted to 100 µg (glucose equivalent) and 675 µg per ml of culture, respectively. Under the influence of yttrium the exocellular carbohydrate composition decreased by almost 50%, while the protein concentration was 474 µg per ml of culture. The pH of the spent fluids increased from 6.8 to 8.2-8.5. Although citrate was totally degraded in both cases, a more rapid rate of citrate utilization was recorded in the metal-enriched culture (Fig. 2). As growth progressed a gradual disappearance of yttrium from the culture medium was observed. At stationary phase of growth approximately 65% of the trivalent metal was associated with the bacterial cells. The remainder was localized in the spent fluid in soluble and insoluble forms (Fig. 3).

The foregoing data demonstrate that 0.5 mM yttrium has a beneficial influence on *P. fluorescens*. Since yttrium has a relatively similar ionic radius like calcium, an element pivotal in numerous biological reactions, it has been proposed that yttrium may substitute for calcium in diverse biochemical processes (Evans 1990). Such a substitution may indeed provide an explanation for the stimulatory influence of yttrium on this microbe. Although extracellular proteins and carbohydrates have been shown to be implicated in metal homeostasis (Higham et al. 1984;

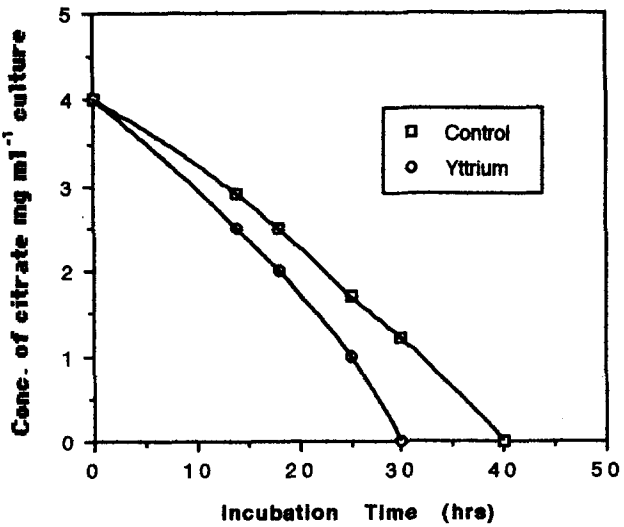

Figure 2. Citrate utilization by *P. fluorescens*

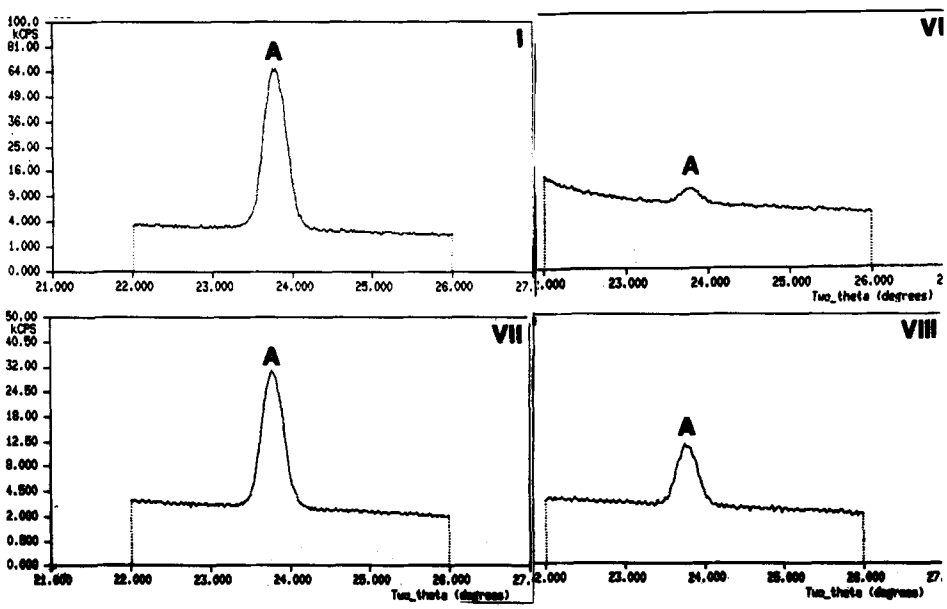

Figure 3. X-ray fluorescense analyses of yttrium metabolism in *P. fluorescens*. A=> yttrium peak: I) at incubation time, VI) from supernatant at stationary phase of growth, VII) in bacterial cells at cessation of cellular multiplication, VIII) in pellet isolated at stationary phase of growth.

Appanna 1988; Appanna and Preston 1987), these biomolecules did not appear to be directly involved in the immobilization of yttrium in this study.

P. fluorescens appears to concentrate the trivalent metal intracellularly. Metallothioneins, the cysteine-rich proteins, have been shown to regulate the intracellular sequestration of divalent metals like zinc and cadmium (Olafson 1984). Although the exact nature of the biomolecule(s) has to await further delineation, this study points to the internal sequestration of yttrium in *P. fluorescens*. Microbial models have been successfully applied in decontamination of metal-polluted environments. Meanders and impoundments abounding in metal-resistant organisms have proved effective in the removal of metals from water systems (Hughes and Poole 1989). This yttrium-accumulating microbe has the potential to eliminate soluble yttrium from the environment.

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