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Cesium Stress and Adaptation in *Pseudomonas fluorescens*

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Industrialization and acid rain have led to a marked increment in the bioavailability of numerous metals. These metallic pollutants pose a serious threat to the ecosystem due to their ability to interact negatively with living organisms. Thus, considerable effort has been directed towards the development of environmentally-friendly technologies tailored to the management of metal wastes. As microbes are known to adapt to most environmental stresses, they constitute organisms of choice in the study of molecular adaptation processes. These adaptive features may be subsequently engineered for biotechnological applications.

Precipitation and accumulation mediated by bacteria may provide potential alternatives to the present methods utilized in the decontamination of metals from both nuclear and industrial wastes (Strandberg et al. 1988). Cesium, a monovalent metal with chemical similarities to potassium but with no known essential biological function, has become a major cause of environmental concern owing to its accidental release from the Chernobyl nuclear accident (Devell et al. 1986). This radionuclide that predominantly interacts with living organisms via the potassium uptake system poses a serious challenge to normal cellular activities. Thus, considerable attention has been focused on the removal and the fate of radioactive cesium in the environment (Tomioka et al. 1994).

As part of our studies on the delineation of molecular adaptation evoked by environmental stress and the subsequent development of biotechnological procedures for metal decontamination, we have examined the impact of cesium on the soil microbe Pseudomonas fluorescens. Citrate, the sole carbon source to which the test-metal was complexed, afforded a unique medium to probe the interaction between the microbe and cesium. Accumulation and/or precipitation of the monovalent metal by this microbe may provide interesting routes to the management of this nuclear waste. In this report we demonstrate the ability of this microbe to survive up to 10 mM cesium. The fate of the test-metal during growth and the suitability of P. fluorescens in cesium decontamination are also discussed.

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MATERIALS AND METHODS

P.fluorescens ATCC 13525 was obtained from the American Type Culture Collection and was maintained at 4°C by monthly subculture on a mineral citrate medium solidified by the inclusion of 2% agar. The liquid culture contained Na_2HPO_4(.06g), KH_2PO_4(.03g), NH_4Cl (0.80g), $MgSO_4.7H_2O$ (0.2g) and citric acid (4.0g) per L of deionized double distilled H_2O. Trace elements were added in the following concentrations: $Zn(NO_3)_2$. $6H_2O$ ($0.07 mgL^{-1}$), $CaCl_2$ ($0.11 mgL^{-1}$), $CuCl_2\,2H_2O$ ($0.017 mgL^{-1}$), and $Na_2MoO_4.2H_2O$ ($0.024 mgL^{-1}$) and $FeCl_3.6H_2O$ ($0.54 mgL^{-1}$. In the test cultures, the citric acid was complexed to cesium chloride (1 to 20 mM). The medium without added cesium served as control. The pH of the media was adjusted to 6.8 with dilute NaOH.

The media were dispensed in 200 mL amounts in 500 mL Erlenmeyer flasks and inoculations were made with 1 mL of stationary phase cells grown in a control mineral citrate medium (Anderson et al. 1992). The cultures were aerated on a gyratory waterbath shaker model G76 (New Brunswick Scientific) at 26°C at 140 rev. min^{-1}. At timed intervals, cells were harvested by centrifugation at 10,000 xg for 20 min at 4°C. Following disruption of the cells with 0.5M NaOH in boiling water, microbial growth was monitored by measuring soluble protein by the method of Lowry et al. (1951) and Bradford (1976). Bovine serum albumin was used as the standard. The spent fluid devoid of bacterial cells was analyzed for its protein and carbohydrate content, respectively (Bradford 1976; Dubois et al. 1956). The pH was also recorded with the aid of a Fisher pH meter model 610A.

At timed intervals cesium metabolism in P. fluorescens was followed by X-ray fluorescence (XRF) analysis on a Philips PW1404 automatic, sequential spectrometer as described in Al-Aoukaty et al. (1991). A methane/argon gas mixture was used in the flow proportional counter and a rhodium X-ray tube was employed throughout this investigation. The analyzing crystal was LIF200 and intensities and peak shifts are given in kilocounts per seconds (kcps) and in two theta degrees (28°) respectively. At various growth times bacterial cells were harvested by centrifugation at 10,000xg. These cells and the supernatant were lyophilized and analyzed for their cesium content.

Supernatant fractions isolated by centrifugation at different incubation periods, were dialyzed extensively in membranes with molecular weight cut off (MWCO) of 1 kDa, 3.5 kDa, and 24 kDa, and examined for their cesium content by the XRF technique. Citrate utilization was monitored by analyzing these supernatant fractions by the enzyme coupled assay involving citrate lyase, malate dehydrogenase and lactate dehydrogenase (Moellering and Gruber 1966).

RESULTS AND DISCUSSION

When P.fluorescens was cultured in a minimal mineral medium with varying concentrations of cesium, growth rate and cell yield observed at stationary phase were affected depending on the level of the monovalent metal. In the presence of 10 mM cesium, a 33% decrease in cell yield was recorded at stationary phase of growth and

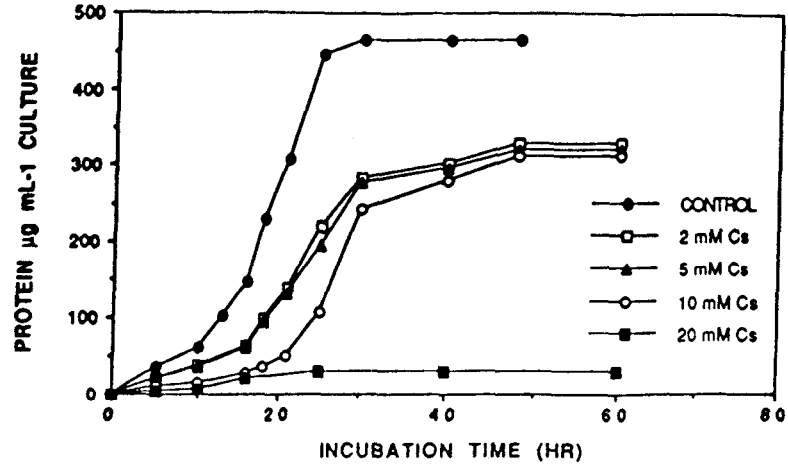

Figure 1. Growth of P. fluorescens in control and cesium-rich media. (Standard deviations ranged between 1.10 and 7.93)

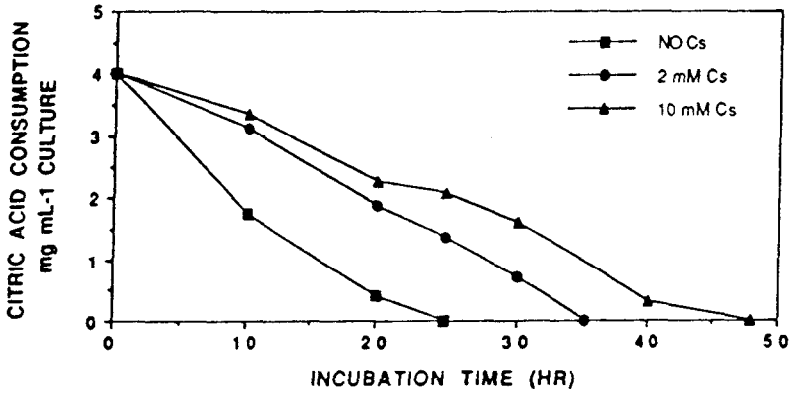

Figure 2. Citrate utilization by P. fluorescens with varying concentrations of cesium. (Standard deviations ranged between 0.018 and 0.091)

cellular multiplication was not evident until after 18 hr of incubation. Bacteria cultured in media with 2 mM cesium experienced a 24% diminution in biomass and the growth rate was markedly diminished compared with that recorded in the control cultures. Cellular growth was completely arrested in media with 20 mM cesium (Fig. 1). In control cultures, exocellular carbohydrates amounted to 70 µg glucose equivalent mL^{-1} of culture. In cesium-stressed experiments, carbohydrate content varied between 50 and 59 µg glucose-equivalent mL^{-1} of culture. The exocellular protein also did not show any marked variation and ranged between 40 and 45 µg mL^{-1} in both cultures. There was an increment of pH as growth progressed and attained a value of 8.2 to 8.9 at stationary phase of growth. Citrate was rapidly consumed and no tricarboxylic acid was recorded after 26 hr of incubation in the control culture. In cesium-rich media

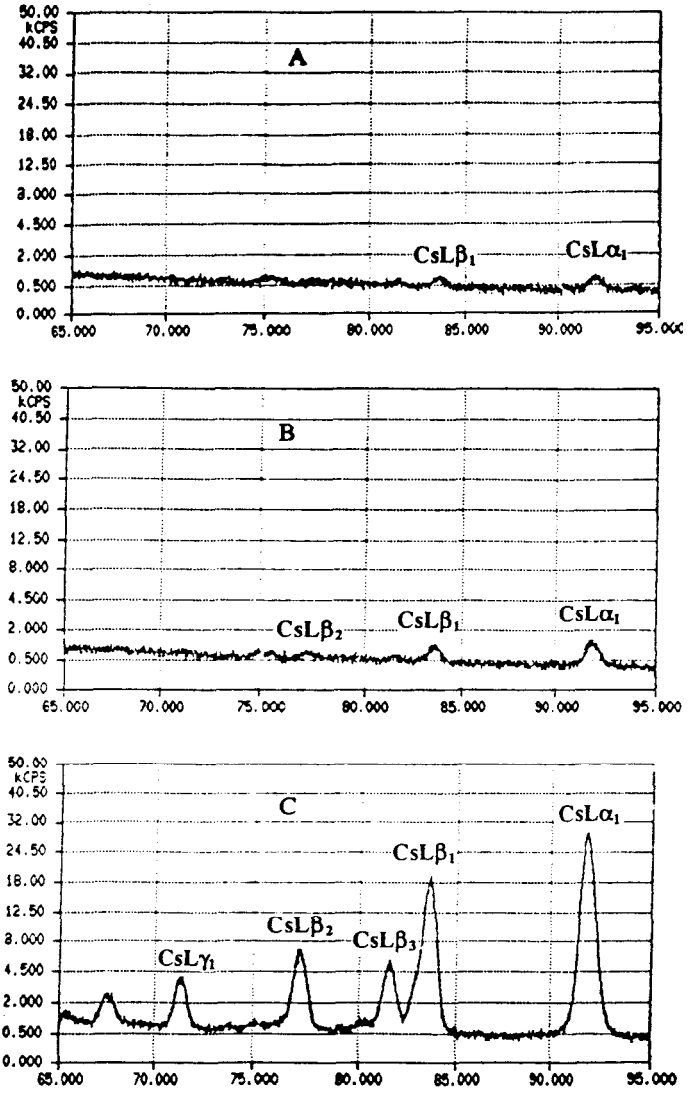

Figure 3. X-ray fluorescence analysis of cells from 10 mM Cs cultures at 20 hr (A), 48 hr (B) and supernatant from 10 mM Cs culture at 48 hr (C). Various peaks indicative of cesiurn are shown.

(2 and 10 mM), citrate was consumed within 35 and 48 hr of incubation, respectively (Fig. 2).

X-ray fluorescence studies revealed cesium was predominantly localized in the supernatant in soluble form(s). At stationary phase of growth, approximately 96% of the monovalent metal was found in the supernatant. However, there was a slight increase in cesium associated with the bacterial cells. At 20 hr of growth, 1.7% of the cesium was found with the bacterial cells while at 48 hr of incubation, the microbial

cells accounted for almost 4.0% of the monovalent metal (Fig. 3). Dialysis experiments demonstrated that cesium was associated in the supernatant component(s) with molecular mass(es) < 1.0 kDa(data not shown).

The data presented in this report show that although cesium had an inhibitory influence on both cellular yield and growth rate, P. fluorescens was able to adapt to a concentration of up to 10 mM of this monovalent metal. Cesium was predominantly localized in soluble form(s) in the spent fluid. This is in marked contrast to that observed in Rhodococcus (Tomioka et al. 1994). In this strain, relatively high amounts of cesium (690 µmol/g of dry cells) accumulated within the cells. In Synechocystis, cesium accumulation was directly proportional when extracellular cesium concentration ranged from 0.1 to 2.0 mM (Avery et al. 1991). A potassium transport system encoded by *trkD* gene was found to be responsible for the accumulation of cesium in E. coli (Bossemeyer et al. 1989).

In this study it appears cesium did not accumulate significantly in the bacterial cells but was predominantly localized in soluble form(s) exocellularly. P. fluorescens has been shown to evoke disparate detoxification strategies in order to circumvent the challenge imposed by other metallic stresses. While calcium and strontium mediate the biogenesis of crystalline carbonates (Anderson et al. 1992; Anderson and Appanna 1994), aluminum detoxification is attained by the deposition of the trivalent metal as a gelatinous lipid-rich residue (Appanna et al. 1994). Yttrium homeostasis is affected via its intracellular sequestration (Appanna and Huang 1992) and any adverse effect from millimolar amounts of iron is averted via its immobilization in a phosphatidylethanolamine residue (Appanna and Finn 1995).

Although more detailed studies are needed in order to delineate the exact mechanism(s) involved in cesium adaptation, this report clearly demonstrates that P. fluorescens can survive up to 10 mM cesium. Furthermore, as the metal is localized primarily in soluble form(s) in the spent fluid, it is evident that this experimental design is not suited for the decontamination of this radionuclide either by bioaccumulation or bioprecipitation using P. fluorescens.

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