Asian Journal of Chemistry Vol. 19, No. 7 (2007), 5513-5517

Accumulation of Heavy Metals on *Bacillus cereus* and *Pseudomonas aeruginosa* Strains Isolates from the Van Regional Soils in Turkey

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This investigation was conducted in order to determine the overall intracellular accumulation of Co^{2+}, Cd^{2+} and Pb^{2+} on *Bacillus cereus* and *Pseudomonas aeruginosa* which isolated from the soil samples collected from Van region. According to the results of the investigation, intracellular accumulation was observed to increase in wild type strains of *B. cereus* T 124 and *P. aeruginosa* N 48 through the applications of Co^{2+}, Cd^{2+} and Pb^{2+} (0.25, 0.5, 1, 2 and 4 mM). However, both the type of bacteria and the effect of various heavy metals caused different accumulation in two bacteria. In the three of the heavy metals put into application, *B. cereus* T 124 was found to have much more accumulation than *P. aeruginosa* N 48. The intracellular accumulation of Cd and Co metal was observed to be greater in *B. cereus* T 124 strain whereas the intracellular accumulation Pb^{2+} metal was seen to be much greater in *P. aeruginosa* strain. The highest amount of accumulation was observed for Pb^{2+}.

Key Words: Heavy metals, *Bacillus cereus*, *Pseudomonas aeruginosa*.

INTRODUCTION

Heavy metals infiltrated into the agricultural lands after various activities are accumulated in the micro fauna or colloidal elements of soils. Such accumulation negatively affects the soil microorganisms, which play an important role in plant nutrition. On the other hand, it poses some dangers to animal feeding on the plants grown in such soils. Today, some organisms, which gained resistance against toxic chemicals, scattered in natural environment at various rates and thus have become mutant, could also be found. Various bacteria and fungi have the ability to accumulate a wide variety of metals to a great extent[1-3]. The increase of heavy metals in natural environment leads to intracellular accumulation in bacteria. Therefore, bacteria developed different mechanisms to relatively keep heavy metal ions at an intracellular level[4]. Such activities were reported by the

interaction between microbial cells and metals adsorbs ion from surface of the cell. The formation of metal complexes with extra cellular, microbial metabolites or metabolically through intracellular accumulation[5]. The elimination of the heavy metals from the environments in which the industrial wastes are abundantly found, paved the way for cost-effective and sustainable measures to be taken in the environment[6]. The discharge of heavy metals into the environment due to agricultural, industrial and military operations and the effect of this pollution on the ecosystem and human healthy are growing concerns. Recent research in the area of heavy metal removal from waste-waters and sediments has focused on the development of materials with increased affinity, capacity and selectivity for target metals[7,8].

In this investigation, it is aimed to determining the effects of Co^{2+}, Cd^{2+} and Pb^{2+} metals at increasing rates being applied to the *Bacillus cereus* T 124 and *Pseudomonas aeruginosa* N 48 strains, isolated from the soil.

EXPERIMENTAL

The organisms used in the study were *Pseudomonas aeruginosa* N 48 and *Bacillus cereus* T 124 wild-type strains derivative from the Van regional soils, the properties of which are pH 8.10, saltless and sandy clay loam texture. Media containing 4 mM concentrations of Cd^{2+}, Co^{2+} and Pb^{2+} were used for resistant test strains isolated from the soil. *P. aeruginosa* and *B. cereus* strains at the medium including Cd^{2+}, Co^{2+} and Pb^{2+} metals was observed developing a dirty white colour in the shape of colony.

In order to reproduce and identify the bacteria cultures LB (Luria Bertani) Agar, Media-1 and GSP Agar were used[9,10]. For the preparation of LB Agar, 10 g of tripton, 5 g of yeast extract, 10 g of NaCl and 15 g of agar were solved in 1000 mL distilled water.

In order to preparing Media-1 Agar, 10 g water-soluble starch, 5 g polipeptone, 5 g yeast extract, 1 g K_2HPO_4, 0.2 g $MgSO_4 \cdot 7H_2O$ and 20 g agar were solved in 1000 mL distilled water.

To prepare the GSP Agar, 10 g of water-soluble starch, 10 g of sodium glutamate, 2 g of KH_2PO_4, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.36 g of phenol red and 15 g agar were solved in 1000 mL distilled water. The pH values of all these three media were adjusted to 12 through NaOH and sterilized in the autoclave. LB (Luria Bertani) Agar medium 1 and GSP Agar nutrition place were used to proliferate bacteria cultures.

Cd^{2+}, Co^{2+} or Pb^{2+} accumulation assay: The stock solutions of Cd^{2+}, Co^{2+} and Pb^{2+} were prepared in 100 mM concentrations and were sterilized in 0.45 µm pore size filters. After the growth of bacterial strains, to measure Cd^{2+}, Co^{2+} and Pb^{2+} uptake, cells were incubated in at 37ºC shaking water bath for 10 min. To bacterial culture was added Cd^{2+} ($CdCl_2$), Co^{2+}

($CoCl_2$) and Pb^{2+} ($Pb(NO_3)_2$) at the concentration of 0.25, 0.5, 1, 2 and 4 mM/100 mL. The mixture was incubated in a 37ºC shaking water bath for 1 h. Cells were harvested and washed three times at 4ºC with GSP broth by centrifugation. The cell pellets were lyophilized and the dried cells were digested overnight in 70 % nitric acid at 45ºC. The digestion mixture was diluted with water to a final nitric acid concentration of 10 to 15 %[13]. The total Cd^{2+}, Co^{2+} and Pb^{2+} content of the cells were measured with a Unicam 929 model atomic absorption spectrophotometer.

RESULTS AND DISCUSSION

The intracellular heavy metal uptake according to the concentrations and bacteria types were found to be different (Figs. 1 and 2). In both types of bacteria the intracellular heavy metal uptake where observed to be increased with the increased rates of heavy metal concentrations. However a proportionate decreased was seen in the amounts of intracellular uptakes of Cd^{2+}, Co^{2+} and Pb^{2+}, as the concentration increased. At the beginning, it was adsorbed that *B. cereus* T 124 strain accumulated Cd^{2+} metal in 0.25 mM much last than in *P. aeruginosa* N 48 strain, the intracellular metal uptake in the other concentrations were seen to be heavier and *B. cereus* T 124 had as similar amount of intracellular metal uptake along with *P. aeruginosa* N 48 strain. Gram-positive *B. cereus* T 124 accumulated Cd^{2+} in much greater amounts than *P. aeruginosa* N 48 strain depending on the increased concentrations of Cd^{2+}. Such as similar, at the studies, it was asserted that the Cd uptake in tolerance of Gram-positive bacteria was much batter than Gram-negative bacteria[11,12].

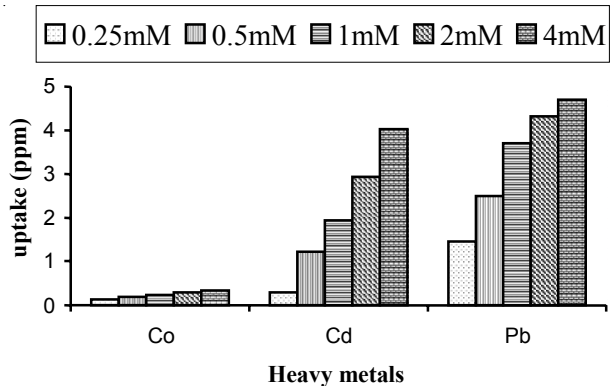

Fig. 1. Heavy metals uptake by *B. cereus*

The intracellular uptake in *B. cereus* T 124 and *P. aeruginosa* N 48 was found to be increased as Co^{2+} matter concentration was elevated Co^{2+} uptake in *B. cereus* T 124 strain was determining to be higher compared with *P. aeruginosa* N 48.

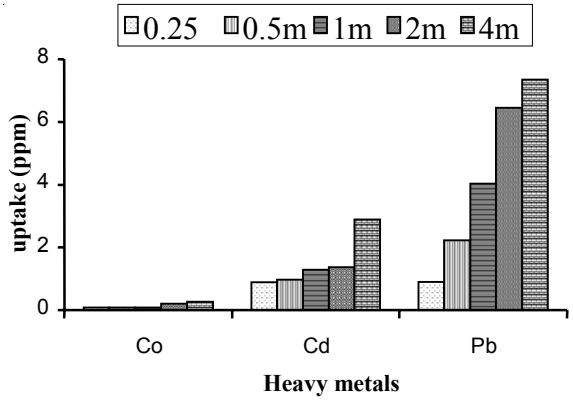

Fig. 2. Heavy metals uptake by *P. aeruginosa*

After the applications of the increased rates of Cd^{2+} metal, whereas a little amount of increase was observed in the intracellular accumulation in *P. aeruginosa* N 48 strain of the 2 mM concentrations, the increase became two-fold when the application of the Cd^{2+} metal was conducted in 4 mM of concentration. Hao *et al.*[13] reported the ability of mutant and wild type cells of *L. plantarum* to accumulate Cd in a concentration of 10 µM was studied. In the final course of the investigation, the mutant cells were found to accumulate 5 % of Cd while the wild type cells accumulate 20% of Cd[13].

A difference was observed in the intracellular uptake of Pb^{2+} by test bacteria whereas the intracellular uptake of Pb^{2+} by *B. cereus* T 124 strain at 0.25 and 0.5 mM concentrations was found to be much higher in comparison with *P. aeruginosa* N 48 strain. This parameter was found to be much last in the other concentrations.

The investigation conducted for determining the accumulating ability of alcalo-tolerant *B. cereus* T 124 and *P. aeruginosa* N 48 strains over metals, the lowest concentration was used as 0.25 mM and the highest concentration as 4 mM. Therefore, the accumulation rates of heavy metal compounds for both of the different bacteria strains changed between 1 and 2 %. This result could be associated with different microorganisms and exceeding metal concentrations used in the trial.

In present study, it is aimed to determine the abilities of alcalo-tolerant *P. aeruginosa* N 48 and *B. cereus* T 124 strains to accumulate metals. The lowest concentration was maintained as 0.25 mM and the highest concentration was 4 mM. Therefore, the accumulation rate of the metal compounds for both of the various bacteria strains changed between 1 and 2 %. This might be due to different organisms and the excess in the metal concentrations used in the trial.

In conclusion the total intra-cellular accumulation amounts of Cd^{2+}, Co^{2+} and Pb^{2+} metals prepared in 0.25 and 4 mM concentrations were observed to have gradually increased in test bacteria depending on the increase of concentration. However, when such increase is analyzed in percentage, a relatively proportional decrease was observed as the concentration was elevated whereas a small amount of accumulation was observed in *B. cereus* T 124 strain through the increased rates of Co^{2+} metal applications. A greater amount of accumulation in linear direction was observed in the applications of Cd^{2+} and Pb^{2+} metals compared with the applications of Co^{2+} metal. In the application of 2 mM Pb^{2+} metal, intra-cellular metal uptake has reached to saturation. In the applications of the increased rates of Co^{2+} metal in *P. aeruginosa* N 48 in 0.25, 0.5 and 1 mM concentrations no varying accumulations were determined relatively and greater amount of accumulation was observed in 2 and 4 mM concentration of Co^{2+} compared with the former one.

In the final stage of experimental trials, it is concluded that serious ecological and health problems will occur if no precaution are taken in order to eliminate those industrial wastes from the natural environmental and local environment.

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(*Received*: 22 November 2006; *Accepted*: 19 June 2007) AJC-5732