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Comparative study on the biosorption of aluminum by free and immobilized cells of *Bacillus safensis* KTSMBNL 26 isolated from explosive contaminated soil

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ABSTRACT

The study describes the sorption of aluminum by *Bacillus safensis* isolated from an explosive contaminate site. In a batch system, the optimum pH, temperature and contact time on biosorption of Al and cell growth were found to be 6.0, 35 °C and 24 h, respectively with initial metal concentration of 100 mg/l Al. In immobilization studies, sodium alginate was used as supporting material for cell entrapment under optimized conditions. The variation in the protein banding pattern, functional groups and nature of Al treated and untreated bacterial cells were studied using SDS-PAGE, FTIR and XRD. While comparing the results of batch and immobilized sorption studies, the metal removal efficiency of free cells (92 mg/l) was slightly higher than the immobilized cells (84 mg/l). But the maintenance of high cell density, low processing cost, repeated and continuous uses are all recommends the immobilization technique for large scale water treatment.

1. Introduction

Industrial development has an important role in the economic growth of the country but increased incidence of pollutants such as heavy metals causes deteriorating effect in the ecosystem. Heavy metals are the main toxic and persistent pollutants encountered in our environment. The most crucial characteristic that distinguished heavy metals from other pollutants is their non-biodegradability and their adverse condition to accumulate in living material [1,2]. Major producers of heavy metal wastes are tanneries, mines, electroplating units, manufacturing of paints, metal pipes, batteries and ammunition [3].

Aluminum is the most abundant metal in the earth's crust which occupies 8.8% of its weight and it is most commonly used in water supplies, medicines [4], explosives, construction of siding, aircrafts and motor vehicles and in food industry as cans, packaging materials, kitchen utensils and vessels [5]. Consequently, the environmental exposure of aluminum to human and other animals is obviously possible. This metal ion causes Parkinson's disease, Alzheimer's disease [6], encephalopathy/dialysis dementia, osteomalacia [7,8] and also several clinical diseases particularly in patients with chronic renal failure [9]. The Environmental Protection Agency (EPA) sets the secondary permissible standard for aluminum in drinking water as 0.05–0.2 mg/l [10]. Therefore it is necessary to remove aluminum from its source, before it enters into the environment.

Physico-chemical methods such as chemical precipitation, chemical oxidation or reduction, filtration, ion exchange, electrochemical treatment, reverse osmosis, membrane technology and evaporation recovery have been widely used to remove heavy metal ions. These technologies usually produce wastes with high concentrations of metals which are a significant source of environmental pollution. Furthermore, the above methods may be ineffective or expensive, especially when the heavy metal concentrations are less than 100 mg/l [11]. For these reasons, interest has arisen recently in biological method as an innovative technology for heavy metal removal. Various microbial detoxifying mechanisms such as bioaccumulation, biotransformation, biomineralization or biosorption can be applied either *ex situ* or *in situ* to design economical bioremediation processes [12].

Biosorption consists of accumulation, predominantly by metabolism independent interactions, such as adsorption or ion exchange processes. It also involves physiochemical interactions between the metal and functional groups such as ketones, aldehydes, carboxyls *etc.*, present on the microorganism's cell surface [13]. Commercial application of microbial biomass as a

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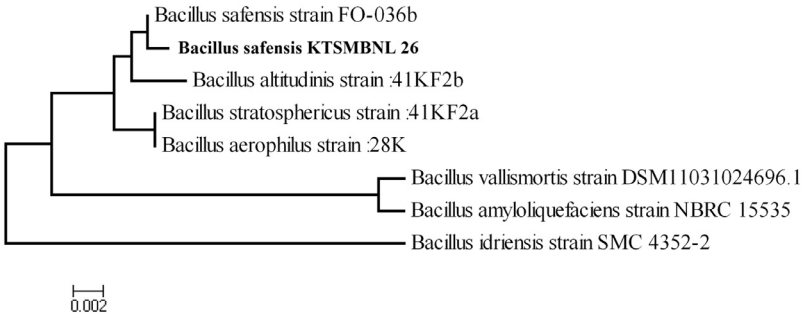

Fig. 1. Phylogenetic tree of *Bacillus safensis*.

Table 1
Comparison for *Bacillus safensis* with other aluminum tolerant microbes.

S. no	Organism mame	Isolation site	Tolerance level	Growth media	Growth time	Reference
1.	*Lactobacillus rhamnosus*	Human gastrointestinal tract	11.52 mg/l	MRS media	48 h	[23]
2.	*Alkalophilic bacteria*	Paper and pulp mill effluent	3106 µm	King's B agar	24 h	[24]
3.	*Streptomyces rimosus*	Lab culture	30 mg/l	Nutrient media	24 h	[25]
4.	*Pseudomonas aeruginosa*	–	30 ng/l	Nutrient media	24 h	[26]

biosorbents can suffer from problems associated with physical characteristics of the biosorbing materials such as small particle size with density, poor mechanical strength and rigidity. As a possible solution to this problem, immobilization technique such as encapsulation, cellular aggregation or surface fixations [14–16] are used to overcome these drawbacks.

Alginate is so far the most commonly used polymer for immobilization and microencapsulation technique. Alginate is a seaweed extract which consists of alternating unit of α-L-guluronic acid and β-D-mannuronic acid residues. A cross-linking between the α-L-guluronic acid of alginate with divalent cations such as calcium ions make alginate as a strong supporting matrix [17]. Calcium alginate beads are generally used as a supporting material in immobilization studies because of the good biocompatibility, low cost, easy availability and simplicity of preparation [18].

The aim of our study is to select the microorganism that is able to biosorb aluminum (100 mg/l) in aqueous system for the application of wastewater bioremediation processes. Our work is structured in seven stages: (1) screening and identification of indigenous Al resistant microorganisms from explosive (firework industry) contaminated site; (2) Selection of strains with high concentration of metal retention; (3) Aluminum biosorption using the batch studies by live biomass for bioremediation process; (4) Study on Al biosorption using metal resistant immobilized cells; (5) Antibiotic susceptibility of Al resistant strain; (6) Protein variation between the aluminum exposed and unexposed bacteria; (7) FTIR and XRD analysis of Al loaded and Al unloaded bacterial biomass.

2. Material and methods

2.1. Sample collection and analysis

Soil samples were collected from a firework industry in Sivakasi, Tamil Nadu, India. The collected sample was transferred into a sterile plastic container and maintained at 4 °C till use. Soil samples were digested with HNO_3 and 30% H_2O_2 to extract the aluminum from the soil and it was analyzed using Atomic Absorption Spectrophotometer (AAS), Thermo Scientific Model iCE-3000.

2.2. Isolation and screening of aluminum resistant bacteria

The metal resistant bacteria were isolated from soil using serial dilution method. The isolated pure colonies were streaked on LB agar plate containing peptone – 0.5 g, yeast extract powder – 0.3 g, NaCl – 0.5 g and agar agar – 2 g in 100 ml of double distilled water with different concentrations (50, 100, 150 and 200 mg/l) of aluminum (AlK $(SO_4)_2.12H_2O$) and the plates were incubated at 35 °C for 24 h. The most resistant bacterial isolate that had the ability to grow on highest concentration of aluminum metal was selected for further investigation. All the microbiological media and chemicals used in this study were sterilized in an autoclave at 120 °C for 20 min.

2.3. 16S rRNA gene sequencing

The metal resistant bacteria were grown on LB broth at 35 °C for 24 h. DNA was extracted from bacterial colonies using EZ-10 Spin column Genomic DNA kit (Bio Basic Inc., Canada). Taxonomic identity of the isolate was ascertained by 16S rRNA sequence analysis. PCR was performed from live cells cultured in LB medium and the 16S rRNA were amplified with bacterial universal primers 8f (AGAGTTTGATCCTGGCTCAG) and 1492r (TACGGCTACCTTGTTACGACTT). Amplification was performed for 28 PCR cycles with denaturation at 94 °C for 1 min, annealing at 52.3 °C for 1 min and extension at 72 °C for 1 min. PCR product was sequenced by ABI Solid 3730xl – bigdye terminator version 3.1 (Xcelris labs, India) and the 16S rRNA sequence was compared against the GenBank database using the NCBI Blast program. The sequence alignment was done with Clustal X software program [19]. The tree was constructed using the neighbor – joining program and the phylogenetic analysis was carried out by MEGA version 4.0.

2.4. Biosorption of aluminum by free cells

Biosorption experiments were conducted to determine the optimum pH, temperature and contact time. The effect of pH on aluminum biosorption was investigated in the range of 2.0–10.0. The pH of the each solution was adjusted to the required value with NaOH and HCl prior to the addition of metal resistant bacteria. For temperature effect, cultures were incubated at 25–45 °C for 24 h. The time required for reaching the equilibrium state was evaluated by performing the experiment at different time intervals (2–36 h). After incubation, the cells grown in the LB medium were centrifuged (10,000 rpm) for 10 min at 4 °C [20]. The residual aluminum ions in the media were determined using AAS and the biomass of metal resistant bacteria were measured using UV–visible spectrophotometer (UV 1700, Shimadzu) at 540 nm.

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