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Introduction of Mutation on *Aspergillus niger* by Chemical and Physical Methods for Improved Bioleaching of Bauxite

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The wild strains of *Aspergillus niger* isolated from soils are found to be capable of removing siliceous and iron impurities from bauxite ore in laboratory conditions. Mutation was induced in the wild strain to achieve better leaching. Chemical mutation by ethylene imine produced mutant strain *A. niger* AB101 which could effect a leaching of 22.7 and 25% of silica and iron, respectively, from bauxite ore. In order to improve the leaching further, mutation with X-ray was tried which could enhance the leaching appreciably to 49.8 and 56.5% for silica and iron, respectively from bauxite ore.

Key Words: Mutation, *Aspergillus niger*, Bioleaching, Bauxite.

INTRODUCTION

The role of microorganisms on the removal of silica from different ores has been recognized[1–3]. A number of organisms are used for different types of leaching processes[4, 5]. Use of fungi in the leaching of different ores has been reported by many workers[6–8].

We also have used fungus as an effective agent for removal of silica and iron from bauxite ore, but the wild type strain is limited in its leaching ability. So, the possibility of exploiting the mutant strain in leaching should be resorted to. As the spontaneous rate of mutation is very low, the idea of treatment of microorganisms with various mutagens and screening of the surviving progeny has now been recognized as a means to obtain strains of desirable potency. In the present communication, the development of a mutant strain of A. *niger* for the leaching of silica and iron from bauxite ore is reported. A strain of *A. niger* was treated with ethylene imine and X-ray with the primary objective of improving the leaching efficiency of *A. niger.*

EXPERIMENTAL

Various samples of bauxite ore of Araku valley site of Orissa and Madhya Pradesh both in India, were collected and crushed in a ball mill to 200 mesh size for silica and iron solubilization experiments.

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Microorganisms: A pure culture of *A. niger* isolated from sandy and loamy soils was used in this investigation. This *A. niger* is capable of releasing silica and iron from bauxite ores (9.8 and 10.1%) where the silica and iron are present as the major impurity (15%) in the form of silica (SiO_2) and Fe_2O_3.

Mutagenic Treatment: The present culture used in the experiment was *A. niger* strain as selected out of sixty isolates of the fungus from various sources. Spore suspension was prepared in sterile normal saline water from the spores scraped from 10 days old Czepek Dox (CD) agar slants. The preparation was thoroughly shaken and filtered twice through sterile cotton to remove spore clumps. The concentration of the spore suspension was finally made up to 12×10^6 per mL. The mutant strains were maintained on malt extract and yeast extract agar slants at 4°C. The Czapek-Dox agar medium was used for the mutational studies. The medium used for the fermentation of bauxite ores for leaching of silica and iron consisted of $NaNO_3$ (0.2%), KH_2PO_4 (0.15%), KCl (0.05%), $MgSO_4 \cdot 7H_2O$ (0.05% and yeast extract (0.001%), pH 4.0, henceforth abbreviated as FM. It was sterilized at 120°C for 15 min. Glucose (4%) was sterilized separately and added to the medium aseptically.

When the cultures were needed for mutational studies, they were transferred to slants of malt-extract and yeast extract agar and incubated at 30°C for 6 d for sufficient sporulation. Spore crops were harvested by washing the slants with sterile normal saline water and filtering the resulting spore suspension through several layers of sterile absorbent cotton. The spore density was adjusted to 12×10^6/mL.

(a) Treatment with ethylene imine: Spore suspension of 1 mL was added to 9 mL of ethylene imine solution of different concentrations resulting in the dilution of ethylene imine to 1 : 3000, 1 : 5000 and 1 : 7000. At an interval of 1 h, 1 mL of the sample was diluted to 10 mL with sterile normal saline. The diluted spores were then plated in CD agar medium and kept at 30°C for 6 d to develop distinct colonies. After sporulation, colonies were transferred to malt- extract and yeast extract agar slants and stored at 4°C.

(b) Treatment with X-rays: 2 mL of the spore suspension containing 12×10^6 spores per mL taken in a pertridish (5 cm diameter) was exposed to X-rays (35 kV and 10 mA) at a distance of 10 cm for different periods of time. The treated *A. niger* spores were plated on the CD agar medium and plates were incubated at 30°C for 6 d. Selected colonies were transferred to malt extract and yeast extract slants and stored at 4°C.

The spores selected from different stages of treatment with mutagens (ethylene imine and X-rays) were initially tested for leaching of silica and iron from bauxite ores by submerged culture fermentation process.

Determination of silica and iron concentrations

At the end of fermentation 50 mL of fermentation broth was taken out of the flasks aseptically and centrifuged at 10,000 rpm for 10 min in Sorvall Re-5 super speed centrifuge at room temperature to remove biomass and unsolubilized ore matters. The clear solution was digested with 1 : 1 H_2SO_4 and after digestion the solution was diluted with demineralised water and filtered for separating SiO_2. The filtrate was made up to the mark in a 250 mL volumetric flask. The quantity of SiO_2

was determined by firing the residue in a muffle furnace at a temperature of 900°C for 1 h and then by its subsequent hydrofluorization with HF and H_2SO_4. The amount of Fe_2O_3 was estimated by titrating a definite volume of the above filtrate with standard $Hg_2(NO_3)_2$ solution. The amount of Al_2O_3 content in the sample was determined by titrating the filtrate with standard EDTA solution using xylenol orange indicator

RESULTS AND DISCUSSION

Effect of ethylene imine concentration: A comparative study was made on the survivality of *A. niger* with exposure to ethylene imine. Ethylene imine diluted to 1 : 3000 showed greater killing effect with a steep slope in the survival curve. The 1 : 5000 dilution with a 5 h exposure gave a survival rate of 0.3% (Fig. 1). The distribution of the productive variant of this treatment is shown in Fig. 2. The

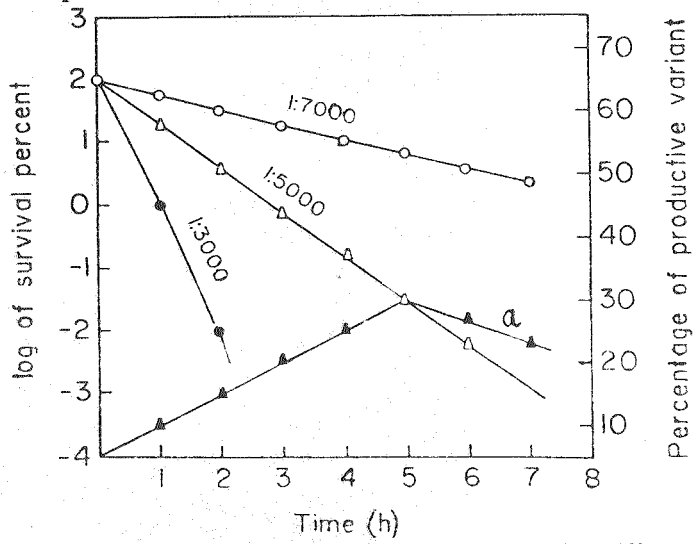

Fig. 1. Survival curve of A. niger AB obtained by treatment with different dilutions of ethyleneimine and the development of productive variants at different intervals of time (curve *a* represents development of productive variants for the treatment with 1 : 5000 dilutions)

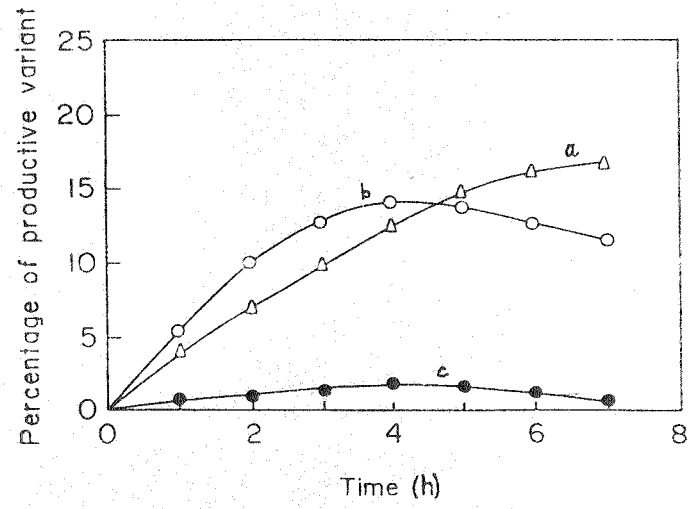

Fig. 2. Distribution of productive variants obtained by ethylene imine treatment: (a) –ve variants, (b) +ve variants and (c) (0) variants

development of (+) variants shows a maximum and then declines, while negative variants show an increasing pattern even up to 7 h. In all 1,200 isolates after treatment with ethylene imine were tested for leaching of silica and iron from bauxite ores. *A. niger* AB101 gave higher leaching capacity of silica and iron (22.7 and 25.2%, respectively) while the parent strain *of A. niger* AB leached only 9.8% silica and 10.1% iron from bauxite ore.

Effect of X-ray treatment: The spore suspension of A. *niger* AB was treated with X-rays (35 kV and 10 MA) for different periods of time. The scoring of survivors and productive variant is depicted in Fig. 3. Complete inactivation of spores took place in 120 min. Productive variants were detectable only upon treatment for 20 min and their number increases with increasing period of exposure. A maximum of 30% productive variants were obtained on exposure to X-rays for 6 min. After different treatments with X-rays, 850 isolates were selected for leaching of silica and iron from bauxite ores; it was observed that the mutant *A. niger* x_1 was capable of leaching (49.8% of silica and 56.4% of iron) from bauxite ore.

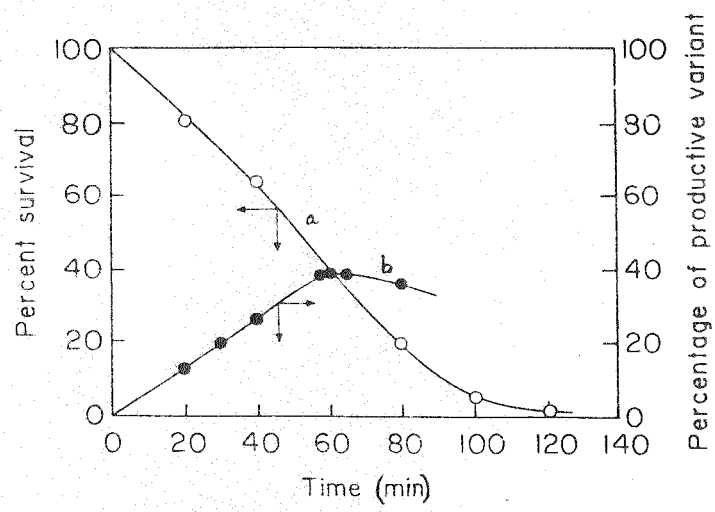

Fig. 3. The scoring of survivors and productive variants obtained by treatment with X-ray

A total of 501 productive mutants were obtained and studied in the present work. It was observed that ethylene imine treatment produced 35% productive variants including 15% (+) variants.

The effect of ethylene imine, an alkylting agent, is to modify the DNA nucleolide permanently by chemical modification and this alkylating process is accompanied by an enzymatic breakage of the alkylated DNA *in vitro*[10]. Therefore, in the course of self-duplication of DNA, this type of change will induce some permanent change in the DNA structure and as a result the stability of the mutant is appreciable. These are supported by the stability test of the variants, whereas X-rays act by producing free radicals which attack the DNA, breaking the chain.

Conclusion

Chemical mutation of the wild strain of *A. niger* can yield better leaching of bauxite ore. Mutation of the same strain by X-ray yields still better results enhancing the leaching to 49.8 and 56.5% of silica and iron, respectively in our laboratory conditions.

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