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Mutation effect of MeV protons on bioflocculant bacteria *Bacillus cereus*

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Abstract

A 3.2 MeV proton beam was used to irradiate bioflocculant bacteria (*Bacillus cereus*) to achieve mutation. The ion fluence ranged from 10^{11} to 10^{14}/cm^2. Most of the bacteria were killed when the ion fluence reached 10^{12} ions/cm^2. The survival ratio drops in an exponential way on further increasing the ion fluence. The flocculating activity of 7 samples out of 51 showed a positive change, and a perfect mutant C7-23 with a stable high capacity of bioflocculant production was found. RAPD measurements showed that a new lane appears in this sample. The flocculating activity of the C7-23 bacteria increased by factors of 22%, 54% and 217% under pH values of 4, 7 or 10, respectively.

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Keywords: Irradiation; Bioflocculant; Survival ratio; Heavy ions; RAPD

1. Introduction

Flocculating agents are widely used in wastewater treatment and other industrial processes because of their high efficiency and low cost. However, most synthetic polymer flocculants have been proved to cause health and environmental problems [1–4]. Bioflocculants as an alternative choice have gained more attention recently since they are environmentally friendly, biodegradable and nontoxic [5,6] . Therefore, bioflocculants have the potential to be applied in a wide range of industrial processes, such as wastewater treatment [7,8], food and fermentation processes [9], removal of heavy metals, dye [10,11] and so on.

Up to now, none of those bioflocculants have been practically applied due to their low flocculating activity [12]. Screening new microorganisms with high flocculating activity is the key point [13]. Conventional methods of screening flocculant-producing strains have been used [1,2,5–16]. Mutational methods including ultraviolet radiation, chemical mutagenesis nitrosoguanidine and ethylme thane sulphonate were assessed [17–19], and a 30% increase of flocculating activity at most indicates these methods are unadaptable for industrial use.

Heavy ions have been used to induce mutation. They have a wider mutation spectrum and are very efficient due to their high LET [20]. There have been many reports that the use of keV heavy ions can produce designed physical changes [21,22], and also some findings on the effects of ion irradiation on crop seeds, cells and microbes in recent years [23]. However, low energy heavy ions irradiation experiments must be performed in vacuum conditions which will cause the death of most bacteria, and the treatment works only at the surface region. For example, the range of 30 keV N ions is only several hundred nm. Compared with keV heavy ions, MeV H^+ irradiation in atmosphere has a high LET and can penetrate the whole bacteria, which means their effect should not be limited to the surface.

Randomly amplified polymorphic DNA (RAPD) analysis is a promising, simple and rapid tool to detect genomic

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DNA damages and mutations [24]. Changes occur in RAPD profiles including variation in band intensity as well as gain or loss of bands [25]. The mutated genomic DNA was analyzed by RAPD in this study.

2. Materials and methods

2.1. Sample preparation

Original flocculant-producing strains of C7 was isolated by conventional methods, and identified as *Bacillus cereus* according to the 16S rDNA sequence in TaKaRa Biotechnology (Dalian) Co., Ltd. The GeneBank accession number is DQ207562.1, the similarity is 100%.

It was cultivated on LB agar medium and inoculated into 150 mL fermentation medium to harvest the bioflocculant. The culture temperature was kept at 30 °C for 66 h with a shaking frequency of 150 rpm. The fermentation medium contained (per litre): glucose 10 g, urea 0.5 g, yeast extract 0.6 g, NaCl 0.6 g, K_2HPO_4 6 g, KH_2PO_4 3 g, water 1000 ml and pH value was adjusted to 7.

Before the irradiation experiment the original strains were cultured in a LB fluid medium at 30 °C with a shaking frequency of 150 rpm for 10 h. Samples were placed in a centrifuge at 4000 rpm for 5 min, then washed three times with sterilized distilled water. The concentrations of bacterial suspensions were modulated to 10^7/mL. A 0.3 mL bacterial suspension was put on a 34 mm diameter Petri dish, then dried at room temperature.

2.2. Proton irradiation

Irradiation experiment were performed using a 2×6 MV tandem accelerator and the fluence of 3.2 MeV H^+ ions ranged from 5×10^{11} to 1×10^{14} ions/cm^2. The ions were extracted from the vacuum chamber through a window made of 5 μm thick titanium foil film to irradiate the sample in atmosphere and at room temperature. The distance between the sample and the exit window was about 2 mm.

2.3. Mutation selection

After irradiation the samples were washed with sterilized distilled water and diluted into serial dilutions according to the ratio of 1:10. The mutated strains were selected and purified after a 1–2 day cultivation in a LB medium at 30 °C. The flocculating activity of the bacteria was measured with Kurane's method [18].

Eight milliliter of bioflocculants and 10 mL of $CaCl_2$ solution were mixed with 800 mL of kaolin solution in a 1000 mL beaker, and the pH value was adjusted with HCl or NaOH to specific values. Sampling was performed in the middle of the beaker and the decrease of turbidity (OD_{550}) measured. The test beaker was stirred for 1 min at 200 r/min and then 2 min at 80 r/min, left to stand for another 5 min. The flocculating activity can be calculated as following:

$$\text{flocculating activity } (\%) = \frac{A - B}{A} \times 100,$$

where A and B are the optical densities of the control and measured sample at 550 nm.

The selected bacteria were passed for four generations to test their genetic stability, The flocculating activities were measured at every generation, and the pH value of kaolin suspension was adjusted to 4, 7 or 10, respectively.

2.4. RAPD analysis

The method of genomic DNA extraction was used according to [26], and the RAPD was measured as in [25]. Amplifications were carried out in a 25 μL system of reaction mixture containing 2.5 μL of 10 × assay buffer (with $MgCl_2$), 2.5 μL PCR dye, 4 nM dNTPs, 10 pM primer, 2.5 unit Taq DNA polymerase and 1 μL of genomic DNA. 8 random primers were tested in this experiment. DNA amplification was performed in a ABI 2720 Thermal Cycler PCR for 40 cycles, for 1 min at 94 °C, 1 min at 36 °C, 1 min at 72 °C, followed by a final extension cycle of 10 min at 72 °C. The RAPD products were resolved on agarose gel (1.4%).

3. Results and discussion

3.1. Survival rates

Bacteria survival ratios with different ion fluences have been measured (Fig. 1). Compared with keV ion irradiation where ion fluence can be as high as 10^{15} ions/cm^2 [27], the use of 2.3 MeV H^+ ions is more efficient in killing the bacteria. Even at a fluence of 1×10^{12}/cm^2 , more than 90% were killed. With increasing ion fluence the survival ratio drops exponentially. This exponential relationship between fluence and survival ratio is very common in radiation

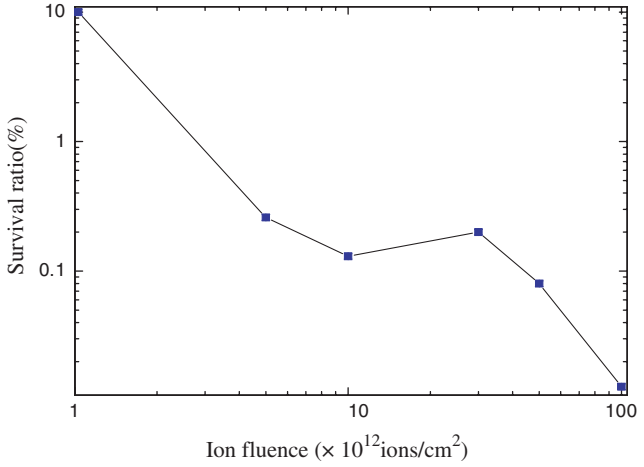

Fig. 1. Bacteria survival ratio as a function of ion fluence.