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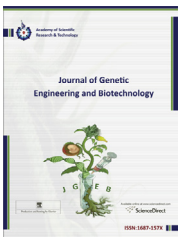

ORIGINAL ARTICLE

Isolation, characterization, optimization, immobilization and batch fermentation of bioflocculant produced by *Bacillus aryabhattai* strain PSK1

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Flocculating activity

Abstract Among others, isolate PSK1 was selected and identified by 16S rDNA sequencing as *Bacillus aryabhattai*. Growth optimization of PSK1 and physicochemical parameters affected bioflocculant production was carried out by Plackett-Burman design and resulted in increasing in the activity by 4.5%. Bioflocculant production by entrapped and adsorbed immobilized microbial cells was performed using different techniques and revealed enhancement in the activity in particular with pumice adsorption. HPLC analysis of sugars and amino acids composition, FTIR and the effect of different factors on the purified PSK1 biopolymer such as presence of cations, thermal stability, pH range and clay concentration was carried out. Scanning electron microscopy (SEM) of free, immobilized cells, PSK1 bioflocculant and formed flocs were performed. The results revealed that bioflocculant PSK1 is mainly glycoprotein consists of glucose and rhamnose with a large number of amino acids in which arginine and phenylalanine were the major. SEM analysis demonstrated that PSK1 have a clear crystalline rod shaped structure. FTIR spectrum reported the presence of hydroxyl and amino groups which are preferred in flocculation process. PSK1 was soluble in water and insoluble in all other tested organic solvents, while it was thermally stable from 40 to 80 °C. Among examined cations, $CaCl_2$ was the best coagulant. The maximum flocculation activity of the PSK1 recorded at 50 °C (92.8%), pH 2.0 (94.56%) with clay concentration range 5–9 g/l. To obtain a large amount of PSK1 bioflocculant with high flocculating activity, batch fermentation was employed. The results recorded ~6 g/l yield after 24 h of fermentation.

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1. Introduction

Bioflocculation is a process in which separation of solid liquid mixture is aided by the whole microorganisms or their byproducts so-called "bioflocculants" [17]. During the last years, there was a great interest in studying bioflocculants to replace the hazardous synthetic flocculants because of their advantages such as biodegradability and safety to human and environment [16,21]. Recently, bioflocculants are produced by various microorganisms such as bacteria, actinomycetes, fungi and algae [12,46]. Trials for isolation of microorganisms that can produce large amount of bioflocculants with high flocculating activity are important.

Optimization of the culture medium is considered as an important way to obtain high productivity [32]. It is ineffective to use the classical method of optimization that depends on changing one factor at a time but leaving the others at constant level. This method does not clarify the interaction occurs between the factors and their influence on fermentation process. Also, long time and great effort are required because the experiments that should be carried out are in large number [7,8,38]. In contrast, statistical approach allows rapid identification of factors that affect the fermentation process because of small number of the required experiments [5].

Plackett-Burman design [37] is one of the statistical approaches that can be used to screen for the most significant factors for further optimization. This design is very useful when large number of factors should be investigated.

Biopolymers production by immobilized microbial cells have many advantages compared to free cells such as that the immobilized cells can be separated easily from culture media and repeatedly used for many times also, the risk of contamination can be minimized [19,33]. Moreover, the immobilized cells can resist the surrounding toxic chemicals and retain high metabolic activities [6,44,50].

Batch fermentation has been widely applied in the industrial production of many biological products such as amino acids, vitamins and enzymes. This process provides simple operation and fewer possibilities of contamination because all of materials utilized in this process are sterilized in the vessel before starting the run [31]. However, there have been few reports on the biosynthesis of flocculants on a pilot scale. Therefore, this study dealt with the batch production of bioflocculant. Moreover, the present study was to isolate, characterize and produce microbial bioflocculant with high flocculating activity by the aid of statistical optimization method, immobilization techniques and batch fermentation.

2. Materials and methods

2.1. Sampling and screening for bioflocculant-producing microorganisms

Several samples were collected from different Egyptian agriculture soils. Screening for bioflocculant-producing microorganisms was carried out on nutrient agar medium (NA) containing (g/l): beef extract, 3; peptone, 5; agar, 15. However, the pre-culture and flocculants production media were containing (g/l): glucose, 10; yeast extract, 3.5; K_2HPO_4, 5; KH_2PO_4, 2; $MgSO_4$, 0.5; NaCl, 0.1. All flasks containing the culture media were sterilized in autoclave at 120 °C for 20 min.

To screen for bioflocculant-producing microorganisms, the soil samples were serially diluted and plated on NA agar plates, then incubated at 28 °C for 24 h. Selection of bioflocculant producers were based on the mucoid morphology of appeared and obvious microbial colonies. Subsequently, selected colonies were cultured in pre-culture broth medium at 28 °C in a rotary shaker at 150 rpm for 24 h. Then, 2% inocula of the pre-cultures ($OD_{600\ nm}$ was adjusted at 0.6) were transferred into new flasks containing 50 ml of production medium and re-incubated as described above. Kaolin suspensions (5 g/L) were then used to evaluate the flocculating activity of bacterial culture broths (the details are described in flocculation activity measurement section), and the isolates with the highest flocculating activity, were selected for further investigation.

2.2. Identification of bacteria and time course determination

Genomic DNA of the selected isolates was extracted according to the protocol of Gene Jet purification kit (Thermo K0721), which was then used as the template for 16S rDNA amplification. Primers used in the 16S rDNA amplification were 27F and 1492R [23]. The amplification process was conducted in a PCR machine using Maxima Hot Start PCR master mix (Thermo K1051). The used PCR cycles were as follow: 95 °C for 10 min; 35 cycles of 95 °C for 30 s, 65 °C for 1 min, and 72 °C for 1.5 min; and final extension at 72 °C for 10 min. The purified PCR amplicons were sequenced by ABI 3730 DNA sequencer (Applied Biosystems). Thereafter; the obtained sequences were aligned with corresponding sequences from related organisms, which were retrieved from the GenBank database using the BLAST algorithm. To construct the phylogenetic tree, ClustalX software was used to perform sequences alignment [40], and neighbor-joining phylogenetic trees were then constructed using Treeview software program.

2.2.1. Determination of flocculation activity

To determine the flocculating activity, a mixture of synthetic clay suspension (5 g Sigma clay/L distilled water) with a known volume of bacteria strain in the presence of $CaCl_2$ was stirred with rapid mixing at 230 rpm for 2 min, followed by slow mixing at 80 rpm for 3 min using Laboratory Flocculator (Flocumatic 6PLAZAS/sample, Spain) and left standing for 3 min. A sample for optical densities (OD) measurement was withdrawn using automatic pipette from a height of 3 cm below the surface of clay suspension. Relying on the upper phase OD for clay suspension that was measured at 540 nm with a spectrophotometer (7230G, Shanghai, China) the flocculation activity of the different isolated strains was screened. The flocculating efficiency was calculated according to the following equation:

$$\text{Flocculation activity } (\%) = (a - b)/a * 100$$

where a and b are the supernatant optical densities (OD) of the control (clay suspension without any bioflocculant addition) and sample respectively, at 540 nm.

Time course experiments were performed according to that described previously by Elkady et al. [14].

The measurements were measured over 5 days of incubation. The flasks containing 125 ml production media were inoculated with 2% pre-culture and incubated at 28 °C and