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Short communication

A novel method for the extraction of prodigiosin from bacterial fermenter integrated with sequential batch extraction reactor using magnetic iron oxide

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ABSTRACT

A novel method for the extraction of prodigiosin from bacterial fermentation was achieved using an integrated sequential batch extraction reactor (SBER) containing functionalized iron oxides ($[Fe_3O_4]_F$). The $[Fe_3O_4]_F$ was prepared using diethanolamine to impart quaternary amine group onto Fe_3O_4. The quaternary amine group imparted to Fe_3O_4 enhanced the prodigiosin adsorption efficiency. Two SBER were integrated with the continuous fermenter for the continuous extraction of prodigiosin. The present investigation established a method to conserve solvent by about 95% during the prodigiosin extraction step. The proposed extraction method can be regarded as solvent free direct extraction of bacterial bioactive compounds from bacterial fermented medium.

1. Introduction

Prodigiosin is a red pigmented alkaloid, produced by several bacterial strains including *Serratia marcescens* [1]. These compounds have been extensively used in pharmacological applications [2,3]. However, the high cost towards consumption of solvents and energy in the extraction step limit their application. In general, polar and non-polar solvents are being used for the extraction of prodigiosin from fermented medium [4–9]. Solvent extraction is considered to be disadvantageous because the toxic organic solvents such as chloroform, diethyl ether, petroleum ether etc., used in the solvent extraction process severely affect the fertility of the soil and carcinogenic hazardous to the biota [10]. Certain polymers such as chitin and resin materials have been suggested for the extraction of prodigiosin from the fermented medium [11–13] along with addition of alum, solvents and surfactants in the pretreatment step [14–16]. The discharge of pretreated fermented solution causes environmental damage and also pretreatment cost become very high. Hence, it is important to develop an environmentally safe and cost effective methodology for the extraction of prodigiosin from fermentation medium. Iron oxides have been considered for the extraction of prodigiosin from fermented medium for their high sorption capacity due to reactive surfaces and regeneration property. It is essential to introduce functional groups onto the surface of Fe_3O_4 to facilitate their bioapplication. $[Fe_3O_4]_F$ have been employed for biological applications, exploiting their intrinsic magnetic property and favorable biocompatibility [17,18]. The presence of functional groups onto the surface of the iron oxide enhance the selective interaction with prodigiosin. The present investigation was focused on the preparation of $[Fe_3O_4]_F$ using diethanolamine as a stabilizing agent and its application to the extraction of prodigiosin from the fermented medium without secondary pollution emission.

2. Materials and methods

2.1. Materials

Ferric chloride, ammonia solution (25% w/v), and diethanolamine were of analytical grade resourced from Merck chemicals, India. Microbiological chemicals were purchased from

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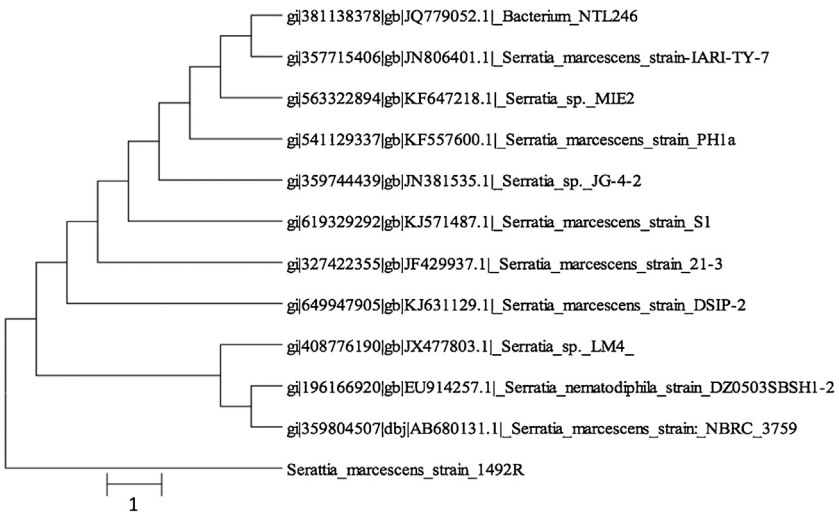

Fig. 1. Characterization of isolated red bacteria using phylogenetic tree by Neighbor joining method.

HiMedia chemicals, India. Milli-Q water was used in all the experiments.

2.2. Microorganism and biosynthesis of prodigiosin

The bacterial strain employed in this study was isolated from the proteinacious solid waste, known as tannery fleshing discharged from leather industry and the isolated strain was identified using 16s rRNA. The rRNA gene was amplified using 27F- AGAGTTTGATCMTGGCTCAG and 1492R-TACGGYTACCTTGTTACGACTT primers, and then performed 35 amplification cycles at 94 °C for 45 s, 55 °C for 60 s, and 72 °C for 60 s. The PCR product was sequenced using the 518F-CCAGCAGCCGCGGTAATACG and 800R-TACCAGGGTATCTAATCC primers [19]. The 16S rRNA sequence was analyzed for the similarity and homology with the existing sequences available in National Center for Biotechnology Information (NCBI) data base using BLAST search. The phylogenetic tree was constructed based on Neighbor joining method using ClustalW software (Fig. 1). A loop full of the isolate *S. marcescens* was inoculated in nutrient broth medium and incubated at 37 °C for 24 h and named as mother culture. The mother culture (1% v/v) was transferred to sterilized fermentation medium for the biosynthesis of prodigiosin. The fermentation medium consisted of delimed tannery fleshing (1% w/v) in M9 mineral (1%) medium of volume 100 mL [20]. The fermentation medium was incubated for 96 h at 30 °C. The prodigiosin biosynthesized fermented solution (PBFS) was directly used for the extraction of prodigiosin.

2.3. Preparation of functionalized iron oxide

$[Fe_3O_4]_F$ was prepared by chemical precipitation method with little modification as described by Lu et al. [21]. In details, ferrous sulphate (1% w/v) solution of volume 100 mL in a 500 mL glass beaker was agitated at 600 rpm to obtain homogeneous ferrous sulphate solution. Diethanolamine of volume 10 mL was added to ferric chloride solution under agitation at 1000 rpm for 15 min. Ammonia solution (30% w/v) of volume 100 mL was added gently and the resultant solution was heated at 80 °C for 30 min under stirring at 80 rpm. The change in colour of the solution from brown to black indicated the formation of functionalized iron oxide $[Fe_3O_4]_F$. Similarly, Fe_3O_4 was prepared by following the above steps without the addition of diethanolamine and named as reference iron oxide, Fe_3O_4. The Fe_3O_4 and $[Fe_3O_4]_F$ were separated from the suspension by centrifuging at 8000 rpm for 20 min. The separated Fe_3O_4 and $[Fe_3O_4]_F$ were thoroughly washed with distilled water until the wash water showed negative answer to NH_4^+-N. The separated products were dried and stored at room temperature until to carry out in further experiments. The functionalized iron oxide was characterized by XRD.

2.4. Extraction of prodigiosin from continuous fermenter

The solvent free extraction of prodigiosin was achieved by integrating two SBER (SBER-1 and SBER-2) with bacterial fermenter. The prodigiosin was biosynthesized in bacterial fermenter and incubated for 96 h. After biosynthesis of prodigiosin in fermenter, the sterilized tannery fleshing was slowly added to the fermenter through inlet and simultaneously outlet of the fermenter was connected to SBER-1 (Fig. 2). The flow rate of the solution was maintained by peristaltic pump (Watson Marlow, Switzerland) at hydraulic retention time (volume of the reactor/flow rate) of 24 h. The inlet of SBER-1 was closed after it received one liter of PBFS and the outlet of fermenter was connected to SBER-2. Meanwhile, the SBER-1 containing PBFS was subjected to extraction step under batch mode. The optimized dosage of $[Fe_3O_4]_F$ was added to PBFS in SBER and agitated at 150 rpm using mechanical stirrer for 30 min. The same experiments was repeated using Fe_3O_4 as control. After discoloration (prodigiosin extraction by magnetic oxides) of the PBFS solution, the iron oxides were separated from the fermenter by the application of external electromagnetic field. The prodigiosin adsorbed onto Fe_3O_4 or $[Fe_3O_4]_F$ was allowed to settle at the bottom of the reactor and the supernatant was discarded. The applied magnetic field was removed and the settled iron oxide was dispersed in double distilled water under agitation at 150 rpm for 10 min. The same procedure was repeated for three times to get the required purity of prodigiosin. The prodigiosin was extracted from iron oxides using acetone of volume 50 mL. After the extraction of prodigiosin, the recovered iron oxides were used in the subsequent cycles for prodigiosin extraction. The acetone was dried from the extracted prodigiosin by evaporation under vacuum condition and it was re-dissolved in methanol for further characterization. 1 mL (10 mg/mL) of prodigiosin solution was used for analysis of electron spray ionization mass spectrometry (ESI–MS) by Thermo fisher instrument (LCQ Advantage Max).