Abstract

The marine strain of *Bacillus circulans* produced a maximum of 1.64±0.1 gL^{-1} of lipopeptide biosurfactants with a μ_{max} of 0.20 h^{-1}, when grown on a glucose mineral salts medium (GMSM) and caused reduction in surface tension of the medium up to 27 mN m^{-1}. The lipopeptide nature of the products was confirmed by primulin and ninhydrin assays by High Performance Thin Layer Chromatography (HPTLC). Further characterization of the biosurfactants was done by Fourier Transform Infrared spectroscopy (FTIR) and matrix assisted laser desorption ionization time of flight (MALDI-ToF) mass spectral analysis. The biosurfactant concentration was determined by HPTLC and gravimetric methods. An efficient method for separation and purification of the biosurfactant isoforms was developed in High Performance Liquid Chromatography (HPLC) by optimizing solvent gradient and flow rate. The optimized method drastically reduced the time of purification to 20 min from an initial run time of 60 min. A combinatorial screening strategy was adopted to develop a Modified Marine Medium (MMM) for the improved biosurfactants production by our marine isolate. The MMM induced more growth as evident from higher μ_{max} (0.42 h^{-1}) and enhanced biosurfactants production to 2.58±0.05 gL^{-1}, as compared to GMSM**.** Biosurfactants production was further augmented by medium optimization using response surface methodology (RSM) and artificial neural network modelling coupled with genetic algorithm (ANN-GA), separately, in shake flask studies. An enhancement by approximately 70% (4.35±0.6 gL^{-1}) was achieved by ANN-GA optimization procedure, as compared to about 17% by RSM. Biosurfactants concentration of 4.61±0.07 g L^{-1} was achieved using the same medium as optimized by ANN-GA technique in 3.7 L fermenter in batch mode. The production level was further enhanced to 6.21±0.25 g L^{-1} by fed batch operation. Subsequently, the critical process variables were optimized in a batch fermenter using ANN-GA. A 52% improvement in biosurfactants concentration (6.98±0.14 g L^{-1}) as opposed to unoptimized conditions was achieved by this optimization strategy. The extracellular biosurfactant products were concentrated and purified in a single step by ultrafiltration (UF) using two membranes of different molecular weight cut-off. The Omega 10 kDa membrane recovered more (89%) product than YM30 kDa (73%) membrane in a stirred cell UF system. The product purity, as adjudged by the extent of decrease in critical micelle concentration (CMC), was greater (80%) for the product purified by UF than that by TLC (55%). However, the purity of the isoforms as achieved by HPLC was about 85%. The marine lipopeptides showed potential antimicrobial and cytotoxic activities against different pathogenic bacteria and cancer cell lines respectively. Thus, the thesis showcases systematic development and optimization of a bioprocess for the characterization, enhanced production and recovery of the marine lipopeptide biosurfactants in shake flask and fermenter studies, employing a judiciously designed marine medium.

Keywords: *Marine biosurfactant; HPTLC analysis; Bioprocess development; Bioreactor strategies; Process optimization, Response surface methodology; ANN–GA; HPLC purification; Recovery by ultrafiltration; Product characterization; FTIR; MALDI–ToF*