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Purification of Bacillus Lipopeptides for Diverse Applications

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Abstract : Bacillus lipopeptides are biosurfactants with wide ranging applications in the medical, food, agricultural, environmental and cosmetic industries. They are produced as a mix of three families, surfactin, iturin and fengycin, each comprising a large number of homologues of varying functionalities. Consequently, the method and degree of purification of the lipopeptide cocktail becomes particularly important if the functionality of the lipopeptide end-product is to be maximized for the specific application. However, downstream processing of Bacillus lipopeptides is particularly challenging due to the subtle variations observed in the different lipopeptide homologues and isoforms. To date, the most frequently used lipopeptide purification operations have been acid precipitation, solvent extraction, membrane ultrafiltration, adsorption and size exclusion. RP-HPLC (reverse phase high pressure liquid chromatography) also has potential for fractionation of the lipopeptide homologues. In the studies presented here, membrane ultrafiltration and RP-HPLC were evaluated for lipopeptide purification to different degrees of purities for maximum functionality. Batch membrane ultrafiltration using 50 kDa polyether sulphone (PES) membranes resulted in lipopeptide recovery of about 68% for surfactin and 82 % for fengycin. The recovery was further improved to 95% by using size-conditioned lipopeptide micelles. The conditioning of lipopeptides with Ca2+ ions resulted in uniformly sized micelles with average size of 96.4 nm and a polydispersity index of 0.18. The size conditioning also facilitated removal of impurities (molecular weight ranging between 2335-3500 Da) through operation of the system under dia-filtration mode, in a way similar to salt removal from protein by dialysis. The resultant purified lipopeptide was devoid of macromolecular impurities and could ideally suit applications in the cosmetic and food industries. Enhanced purification using RP-HPLC was carried out in an analytical C18 column, with the aim to fractionate lipopeptides into their constituent homologues. The column was eluted with mobile phase comprising acetonitrile and water over an acetonitrile gradient, 35% - 80%, over 70 minutes. The gradient elution program resulted in as many as 41 fractions of individual lipopeptide homologues. The efficacy test of these fractions against fungal phytopathogens showed that first 21 fractions, identified to be homologues of iturins and fengycins, displayed maximum antifungal activities, suitable for biocontrol in the agricultural industry. Thus, in the current study, the downstream processing of lipopeptides leading to tailor-made products for selective applications was demonstrated using two major downstream unit operations.

Keywords : bacillus lipopeptides, membrane ultrafiltration, purification, RP-HPLC
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