ABSTRACT

Due to the importance of *Brevibacterium linens* in the industrial production of enzymes, amino acids and vitamins, the vast majority of research carried out has focused on expression of extra cellular proteins and amino acid biosynthesis. Most research to date on *B. linens* has demonstrated that the physiological and metabolic activities of the bacterium are significantly strain dependent. The heterogeneity among different stains of *B. linens* has also been confirmed by studies of DNA-DNA homology and partial 16S rDNA sequence analysis. A large number of questions remain to be answered in many areas of the physiology, metabolism, genetics, and taxonomy of *Brevibacterium linens* DSM 20158. As far as we are aware, there has not yet been a report on the global analysis of cellular proteins associated with *B. linens* DSM 20158. It may be because of the unsequenced genome of this strain. However in the present study due to the establishment of the *Brevibacterium linens* BL2 shotgun genome sequence, we have focused on three aspects of this bacterium in order to understand the physiology and biochemistry of the organism as a whole (i) by optimizing production, purification and characterization of industrially important extra cellular enzymes such as alpha-amylase, protease, lipase and an important amino acid L-Lysine which this strain produces naturally, (ii) by isolation, purification and characterization of respiratory chain complexes (iii) by charting the cellular and extra cellular proteome analysis of *Brevibacterium linens* DSM 20158 which lacks a sequenced genome by mass spectrometry-driven sequence similarity searches. One factor optimization is time consuming, laborious and does not give information about interactions between various fermentation variables. Therefore, we used a statistical approach to optimize factors that influence the production of extra cellular enzymes and amino acids. We used solid state fermentation due to its preference over submerged fermentation. Various cultivation parameters were optimized using a statistical approach to improve the alpha amylase, protease, lipase and L-lysine yield by *Brevibacterium linens* DSM 20158. The Plackett-Burman design was used to screen the fermentation variables followed by the optimization of significant parameters by response surface CCD in each case. Using the optimal factors, alpha amylase and protease yield was found to be twofold higher than that obtained in the unoptimized reference medium whereas lipase and L-lysine production was also found to be improved by using statistical

approach. The closeness of optimized values to experimental values proved the validity of the statistical model.

B.linens was found to have a branched electron transport chain (Respiratory chain), in which electrons can enter the respiratory chain either at NADH (complex I) or at complex II level. In the present study, we were able to isolate and purify the complex-II (succinate dehydrogenase), complex III (menaquinole cytochrome *c* reductase cytochrome *c* subunit, complex IV (cytochrome *c* oxidase) and complex V (ATP synthase) of the plasma membrane of *Brevibacterium linens* strain DSM 20158. Oxidized, reduced and pyridine ferrohemochrome spectra of membrane-bound complex-II, III and IV of this bacterium showed the presence of cytochrome *b*, cytochrome *c* and cytochrome aa3 respectively which were further confirmed by the heme staining. The complex II isolated from *Brevibacterium linens* strain DSM 20158 seems to contain three subunits of 64.8-, 30- and 12- kDa. The enzymatic activity of succinate dehydrogenase showed that it is highly active in this microorganism. The complex III, also known as Menaquinol-cytochrome *c* reductase cytochrome *c* subunit, was identified with a single band of about 26 kDa. The complex IV (cytochrome *c* oxidase) was seen to be composed of two subunits at 62.8-, 32- kDa and was further confirmed by its enzymatic activity. The Complex V (F_1F_o-ATP synthase), essential for ATP generation by oxidative phosphorylation, is isolated, purified and appears to be biphasic in nature during its kinetic studies.

Brevibacterium linens DSM 20158 is an industrially important actinobacterium, but the lack of a genome sequence limits the applicability of conventional protein identification methods to the proteome of this bacterium. Although a shotgun genome sequence for the BL2 strain of this microbe, it does not cover the entire scope of its proteome. This study has established the first comprehensive proteomic reference map of *B. linens* DSM 20158. The present study is carried out first by identification of proteins by homology database MASCOT followed by the advanced approach of *de novo* genome sequence assembly and MS BLAST to drive the expanding *B. linens* scope of proteomics. This study will help to enhance the usability of this strain of *B. linens* in different areas of research in future rather in food industries only.