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A Proteomic Approach for Discovery of Microbial Cellulolytic Enzymes

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Abstract : Environmental sustainability has taken the center stage in human life all over the world. Energy is the most essential component of our life. The conventional sources of energy are non-renewable and have a detrimental environmental impact. Therefore, there is a need to move from conventional to non-conventional renewable energy sources to satisfy the world's energy demands. The study aimed at screening for microbial cellulolytic enzymes using a proteomic approach. The objectives were to screen for microbial cellulases with high specific activity and separate the cellulolytic enzymes using a combination of zymography and two-dimensional (2-D) gel electrophoresis followed by tryptic digestion, Matrix-assisted Laser Desorption Ionisation-Time of Flight (MALDI-TOF) and bioinformatics analysis. Fungal and bacterial isolates were cultured in M9 minimal and Mandel media for a period of 168 hours at 60°C and 30°C with cellobiose and Avicel as carbon sources. Microbial cells were separated from supernatants through centrifugation, and the crude enzyme from the cultures was used for the determination of cellulase activity, zymography, SDS-PAGE, and two-dimensional gel electrophoresis. Five isolates, with lytic action on carbon sources studied, were a bacterial strain (BARK) and fungal strains (VCFF1, VCFF14, VCFF17, and VCFF18). Peak cellulase production by the selected isolates was found to be 3.8U/ml, 2.09U/ml, 3.38U/ml, 3.18U/ml, and 1.95U/ml, respectively. Two-dimensional gel protein maps resulted in the separation and quantitative expression of different proteins by the microbial isolates. MALDI-TOF analysis and database search showed that the expressed proteins in this study closely relate to different glycoside hydrolases produced by other microbial species with an acceptable confidence level of 100%.

Keywords : cellulases, energy, two-dimensional gel electrophoresis, matrix-assisted laser desorption ionisation-time of flight, MALDI-TOF MS

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