MOLECULAR CLONING AND EXPRESSION OF A TRANSGLUTAMINASE FROM PAENIBACILLUS SP. JDR-2 IN ESCHERICHIA COLI

Barreto, M.Q.; Ayub, M.A.Z.

¹Biotechnology Laboratory, Food Technology Department, Food Science and Technology Institute, Federal University of Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil

Microbial transglutaminases are proteins that catalyse the formation of a crosslink between a free amino group and the γ-carboxamide group of protein-bound glutamine, therefore having an important role in the food industry in the production of re-structured food. Paenibacillus sp. JDR-2 is a Gram-positive microorganism, which efficiently degrades xylan. This study aims to identify transglutaminase-coding sequences in the genome of Paenibacillus sp. JDR-2 in silico and to express these sequences in Escherichia coli in order to characterize them. We looked for predicted genes encoding transglutaminase domains from Paenibacillus sp. JDR-2 that were automatically annotated and deposited at the National Center for Biotechnology Information (NCBI) database. Specific primers were designed to amplify the predicted sequence 4765, which was amplified from the genomic DNA of Paenibacillus sp. JDR-2 by PCR. This sequence was cloned into the vector pET23d+ and transformed into Escherichia coli BL21 (DE3) pLysE. The recombinant protein was induced in LB broth at 37°C and optical density 0.8 with 0.5, 1 or 1.5 mM IPTG. Total extract from E. coli was checked on a SDS-PAGE at 0, 1, 2, 3 and 24 h after induction. In silico analysis showed that nine sequences encode transglutaminase domains in the genome of Paenibacillus sp. JDR-2. The sequence 4765 is a 889-base pairs sequence predicted to encode a 33.3 KDa polypeptide containing two putative transglutaminase domains and therefore was selected for further studies. The sequence was successfully amplified and cloned into the vector pET23d+, which was confirmed by its sequencing. Expression analysis showed a band in 25 KDa under any condition tested, indicating that our product is being either degraded or processed by E. coli. Our next steps are to optimize the expression of this protein and to make functional assays in order to confirm it as a transglutaminase.

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