CLONING AND EXPRESSION OF GENES INVOLVED IN XYLITOL PRODUCTION FROM ACETIC ACID BACTERIA

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ABSTRACT

Xylitol is a low-calorie sweetener widely used in chewing gum and confectionaries. Xylitol is found naturally in fruits and vegetables. Moreover, it is also produced from either glucose or xylose by bacteria and yeasts. In this study, acetic acid bacteria were isolated from fruits, fruit juices and flowers, and were screened for sugar alcohol (D-arabitol and xylitol) production from glucose by thin layer chromatography (TLC). From among 558 strains, 84 putative sugar alcohol producers were obtained. The sugar alcohol product was determined by high performance liquid chromatography (HPLC), from 71 selected isolates, but no D-arabitol or xylitol peaks were detected. Genes involved in production of both sugar alcohols were screened. Specific primers for Arabitol dehydrogenase (ArDH) and Xylitol dehydrogenase (XDH) genes were designed and successfully amplified to a 319 bp of ArDH and 1.2 kb XDH gene from 4 out of 18 and 13 out of 66 selected isolates, respectively. The DNA sequence homology of the amplified products of selected strain BK21-09-9 to the ArDH and XDH genes of G. oxydans strains GCMCC1.110 and ATCC 621 was at 75% and 92%, respectively. The presence of both genes indirectly suggested that the strain might produce xylitol. Cloning of genes harboring ArDH and XDH was performed. The plasmid harboring ArDH and XDH were designated as pArDH3 and pXDH2, respectively. The 4.6 kb inserted fragment in pArDH3 presented 5 ORFs with the highest similarity to hypothetical protein GDI_3711, ferric iron siderophore receptor, TonB-dependent siderophore receptor, D-arabitol dehydrogenase and an incomplete ORF of manitol/sorbitol ABC transporter permease protein. The ArDH encoded 257 amino acid residues with a molecular mass of 29 kDa. No promoter sequence was found on the upstream sequence of ArDH gene. The pXDH2 harbored a complete XDH gene with a single open reading frame of 786 bp, which encoded 262 amino acid residues of xylitol dehydrogenase with a molecular mass of 27.8 kDa. XDH was produced at highest yield in an E.coli transformant cultivated at 37 °C for 16 h. XDH activity from E.coli DH5α harboring pXDH2 grown at various temperatures were analyzed. XDH activity in E.coli (pXDH2) was 88 fold higher than E.coli pBlueISK(+) when cells were cultivated at 37 °C. No xylitol production was detected in BK21-09-9 incubated with cell extract from E.coli transformants harboring pArDH3 and pXDH2 in the presence of 1 M D-glucose.

KEY WORDS: XYLITOL/D-ARABITOL/XYLITOL DEHYDROGENASE/D-ARABITOL DEHYDROGENASE