ENZYMATIC CHARACTERISATION AND DETECTION OF PROBIOTIC ORGANISMS, THE BIFIDOBACTERIA

Hasanah Mohd. Ghazali, Mohd. Yazid Abd. Manap, Raha Abd. Rahim, Abd. Manaf Ali and Khalid Ghazi

Faculty of Food Science and Biotechnology
Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

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Introduction

Bifidobacteria are probiotic microorganisms claimed to have beneficial effects on human and animals when they are present in the bowels; their activities help eliminate or reduce harmful bacteria. Natural sources are infant faeces, suckling animals, chicken faeces, the human vagina, dental cavity and the hindgut of honeybees. Currently, the bacteria are incorporated into foods such as yoghurt and bifido milks. Identifying the origin and strains of the organisms is important because of regulatory issues since both human food and animal feed are involved, and in marketing. The presence of the organisms in food can be determined by detecting the activity of the enzyme, fructose-6-phosphate phospho- ketolase (F6PPK), which is unique only to bifidobacteria. It catalyses the cleavage of F6P to form erythrose-4-phosphate and acetyl-1-phosphate, which are then converted in a multistep reaction into acetic acid. The objective of this project was to purify F6PPK from *Bifidocaterium* spp., determine the amino acid sequence and construct DNA probes, or produce polyclonal antibodies, so as to develop an enzyme- and DNA-based detection systems for bifidobacteria.

Materials and Methods

Fructose-6-phosphate phosphoketolase (F6PPK) was extracted, characterised and purified from *Bifidobacterium asteroides* (ATCC 25909). The activity of the enzyme was determined by the amount of acetyl-1-phosphate formed when fructose-6-phosphate was used as the substrate. Release of the enzyme from the bacterial cells was done using bead milling, after which the enzyme undergo various purification steps such as ammonium sulphate precipitation, ultrafiltration, gel filtration using FPLC and gel electrophoresis. The presence of EDTA during isolation, purification and storage of the enzyme was necessary to prevent losses due to proteolytic activity. The N- and C- terminals of the purified enzyme have been sequenced. Oligonucleotide probes are being prepared to help isolate the F6PPK gene from various bifidobacterial species. Partially purified F6PPK are also being used to produce polyclonal antibodies, which will then be used in the development of an ELISA-based identification system.

Results and Discussion

Microscopic examination of the bacterium, which was isolated from the intestine of a honeybee (*Apis mellifera* L.) showed that it is a gram-positive rod. Older cultures contained cells that were either Y or V shaped. A scanning electron photomicrograph shows that the Y and V branching is caused by the adhesion of at least two cells. Results obtained also show that has an optimum pH and temperature of 6.0 and 37°C, respectively. The enzyme was stable at temperatures below 55°C when exposed for 10 minutes at various incubation temperatures. The enzyme was also stable at pH 5.0-7.0. Storage of a crude preparation in phosphate buffer at -20°C for 7 days led to a loss of 50% of the initial activity. The presence of 0.1mM EDTA in the preparation reduced the loss of activity significantly, and was due to inhibition of metalloproteases. The enzyme was purified to homogeneity using a series of techniques including ammonium sulphate precipitation and gradient filtration by FPLC fitted with a Mono-Q anion exchanger. Unlike previously reported, the enzyme was found to be relatively tolerant to the salt. The estimated molecular weight of the enzyme is between 110 000 – 1120 000 D using gel filtration chromatography on Sephadex G-200. Native polyacrylamide gel electrophoresis (PAGE) examination of the active fraction from FPLC revealed a single protein band. However, when the protein was subjected to sodium dodecylsulphate PAGE, two bands were observed. The molecular weights of the two polypeptide chains were estimated to be 58 000 and 54 000 D, respectively. The purified enzyme has been partially sequenced, where the 10 amino acids at the N-terminal is ENLVIIGELD.

Conclusions

Fructose-6-phosphate phosphoketolase is an intracellular enzyme, and hence its extraction required the lysis of the organism. A series of techniques were needed to purify the enzyme to homogeneity before partial sequencing of the enzyme can be made. As the protein is composed to two unsymmetrical polypeptide chains, only one was used to obtain the partial sequence, which will be used to design PCR primers, needed to isolate the F6PPK gene from several *Bifidobactrium* spp.

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