SYNTHETIC SUCROSE ISOMERASE GENE FOR ISMALTULOSE ACCUMULATION

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Introduction. Nowadays, incorrect eating habits which highlights the excessive consumption of carbohydrates has led health problems such as increased diseases as diabetes mellitus type 2, obesity, metabolic syndrome and dyslipidemia. Sucrose is the primary sweetener or food additive used today, and perhaps contributes to metabolic disorders. It is advisable to substitute this sweetener for other with lower caloric income. However, the candidate should be soluble in food, stable at different intervals of temperature and pH, and tolerate various conditions and types of processes that are employed, should not have any adverse effect on the consumer, and particularly having a sweetness which is similar or superior to that of sucrose. Isomaltulose is a natural sweetener that has half value in calories than as sucrose, yet their physiological effects varying the sucrose (1). Due to the economic and nutritional importance of carbohydrates (2).

We are assaying isomaltulose conversion form sucrose by expression of synthetic sucrose isomerase gene expressed by *E. coli* for research purposes and then transfers this to sugarcane. The resulting development could be inserted in sugar cane genome to convert sucrose in to isomaltulose.

Methods. Sacarosa isomarase gene from *Pantoea dispersa* was recovered from NCBI**.** Restriction sites were removed and codon optimization for monocots was achieved by DNA 2.0. Synthetic gene was inserted in *E. coli*, clones were analyzed by plasmid extraction and enzyme restriction analyses. (3). Expression ability of synthetic gen was assayed and compared with standards using thin chromatography (4). To assay eukaryotic expression, synthetic SI gene was transferred to a plant vector.

Results. Clone from resulting synthetic SI gene were analyzed and those who showed correct restriction pattern were selected. IPTG induction of cloned gene was assayed and fraction of induced transfromat clones was tested. Sacarosa isomerasa accumulated in transformed clones was used convert sacarose in isomaltulose (Figure 1).
Isomalutlose containing *E. coli* induced genes transformed 100 of sucrose in isomaltulos (Figure 1, lines 4-5). To test eukaryotic expression plats transformation vector was used to insert SI gene (Figure 2). Biolistic experiments are conducted; explants bombarded were selected in hygromicin containing media.

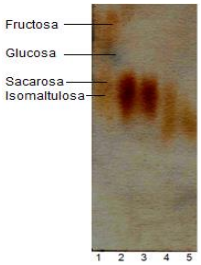

Figure 1. Thin chromatography of carbohydrates. Lane 1) Positive Controls fructose, glucose, sucrose, and isomaltulose. Lane 2) Negative control *E. coli*. Lane 3) Negative control of E. coli gene construction without SI. Lanes 4-5) Clones with SI gene construct.

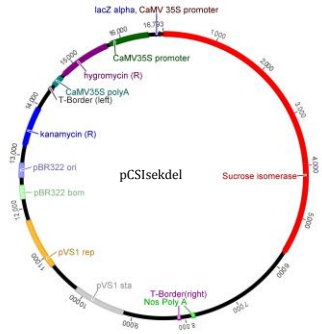

Figure 2. Plant expression vector pCSI SEKDEL with the sucrose isomerase gene.

In near future it will be possible to overexpress this synthetic SI gene in plant to increase the isomatulose content.

Conclusions. It is possible induce synthetic gene of SI in *E. coli* showed by sucrose conversion to isomaltulose, besides eukaryotic codon optimization.

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References.

1. Wu, L, Brich, R. (2005). Characterization of tha highly efficient Sucrose isomerase from *Pantoea dispersa UQ68J* and cloning of the sucrose isomerase gene. J. App. Environ Microbiol. 71:1581-1590.
2. Badui, S.(2006). *Food Chemistry.* Ed. Pearson education. Mexico. 72-75.
3. Birmboim, H,.DolLy, J. (1989). Rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucl. Acid res.7:1513-1528.
4. Schwimmer, S., Bevenue, A. (1956). Reagent for differentiation of 1,4- and 1,6-linked glucosaccharides. Science. 23:543-544.