MUTAGENESIS AND PROTOPLAST FUSION OF *Arthrobacter* sp. FOR IMPROVED GLUCOSE ISOMERASE PRODUCTION

By

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B.Sc. Agric. Sci. (Food Science), Fac. Agric., Cairo Univ., 2002
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APPROVAL SHEET

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Title of Thesis: Mutagenesis and Protoplast Fusion of *Arthrobacter* sp. for Improved Glucose Isomerase Production.
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ABSTRACT

In attempts to construct superior glucose isomerase-producing strains, four bacterial strains (*i.e. Arthrobacter* sp. B-3728, *Actinoplanes missouriensis* B-3342, *Streptomyces phaeochromogenes* B-1131 and B-1517) were screened for their glucose isomerase (GI) synthesis. Both *Arthrobacter* sp. and *A. missouriensis* were proved as the highest producers (16.8 and 15.6 U.ml^{-1}, respectively). Ultra Violet (UV) and Ethyl Methane Sulfonate (EMS) were used for mutagenesis. Induced mutants having antimicrobial resistance markers generated from *Arthrobacter* sp. and *A. missouriensis* (wild types) were screened for their GI production and compared with wild types. About 78 mutants from each treatment and each strain were examined. The mutant EMS 60-28 D generated from *Actinoplanes missouriensis* exhibited the highest activity (33.6 U.ml^{-1}) amongst the isolated mutants from this strain with 1.99-folds. While, the mutant EMS 60-25 D generated from *Arthrobacter* sp. exhibited the highest GI production in this study (49.7 U.ml^{-1}) with 3.2 folds improvement than its wild type.

Protoplast fusion technology was successfully applied using hyper-producing GI mutants generated from *Arthrobacter* sp. According to their antimicrobial responses, 4 mutants were selected to perform 6 crosses. Eight fusants were obtained from each cross and their GI activities were determined. The fusant (C 3-2) exhibited the highest GI synthesis (2.75 folds the wild type).

For optimal GI synthesis by the mutant EMS 60-25 D and fusant C 3-2, batch fermentation system was optimized. Optimization of production fermentation resulted in an additional 10% improvement in enzyme synthesis by mutant EMS 60-25 D. On the other side, GI of fusant C 3-2 was increased after optimization from 42.4 to 60.1 U.ml^{-1} with 3.85 times the activity of the wild type. Enhanced glucose conversion ratio (48 and 48.8%), respectively was also noted by the studied strains compared to 35.3% for the wild type.

Glucose isomerase of fusant C 3-2 was extracted, then purified by ammonium sulfate fractionation followed by gel filtration on Sepharose 4-B. The total yield was 17.8%. SDS-PAGE of the purified GI showed one band with a molecular weight of 47 kDa. Optimum temperature; pH; substrate and Mg^{+2} concentration of the purified enzyme were 75°C, 8, 500 mM, and 0.05 M, respectively. K_m value as calculated from Lineweaver-Burk plot was 285 mM. The enzyme was stable for 1h at 80-90°C and pH 5.

The highest GI producing fusant C 3-2 was successfully immobilized within K-carrageenan gel, hardened with glutaraldehyde, for the continuous production of HFCS. The immobilized preparation exhibited a maximal glucose conversion of 36% after 12h of isomerization at 60°C and the half life of such beads was 408h of continuous operation.

Key words: Glucose isomerase, mutation, protoplast fusion, high fructose corn syrup, purification, immobilization.

DEDICATION

I dedicate this work to my father's sole, mother, sisters and brothers whose love and encouragement helped me through many long nights; to my little family: my beloved husband Hany and my son Basem for all the support; prayers and patience they offered to complete my thesis and in all my life.

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INTRODUCTION

D-glucose/xylose isomerase (D-xylose ketol isomerase, EC 5.3.1.5) referred to glucose isomerase (GI), is one of the three highest tonnage value enzymes after amylases and proteases. It catalyzes the reversible isomerization of D-glucose and D-xylose to D-fructose and D-xylulose, respectively (Bhosale *et al.*, 1996). By far the most successful application of GI in the industrial scale is the production of high fructose corn syrup (HFCS). The annual world consumption of HFCS was estimated to be 12 million tons with a world market price of 230 €/kg (Angardi, 2011). HFCS could be used as a replacer of sucrose in beverages, baking, canning and confectionary industries.

Enzymatic isomerization of glucose to fructose received considerable attention due to the high sweetening index of fructose. HFCS is manufactured from totally non-sweet substance, namely starch. This conversion requires the sequential use of three processes *i.e.*: 1) the starch liquefaction using a bacterial α-amylase, 2) the saccharification of the liquefied starch using glucoamylase to give a solution where 94-96% of the carbohydrate present is in the form of D-glucose, and 3) the isomerization of the produced glucose solution into fructose using D-xylose/glucose isomerase. HFCS is preferred by some food industries since it does not pose the problem of crystallization as sucrose, and as well as its stability in temperature fluctuation and wide pH range (Brakett, 2008). D-fructose also plays an important role as a diabetic sweetener because it is only reabsorbed by the stomach and does not influence glucose level in the blood (Bhosale *et al.*, 1996).

The use of this sweetener will continue to grow because of the sugar price increase due to the acute shortage of water resources required for the cultivation of sugar cane and the enormous human population growth (Bhosale *et al*., 1996). In Egypt, sugar production is still insufficient to cover consumption as the sugar is used directly by human, and also in many food industries. A vast gab between sugar production (sucrose) and consumption is reported annually as illustrated in Fig. 1. This gab decreased from 852000 ton in 2011 to 700000 in 2014 (CAPMAS, 2014 and El-Shatla and Refaat, 2015). To decrease this gab, different sucrose replacers are produced *i.e.* glucose syrup and HFCS (annually production is 45000 and 100000 ton, respectively) that are produced only by National Company for Maize Products (NCMP, 2015). Besides importing from many countries such as Brazil and China (FAO, 2015).

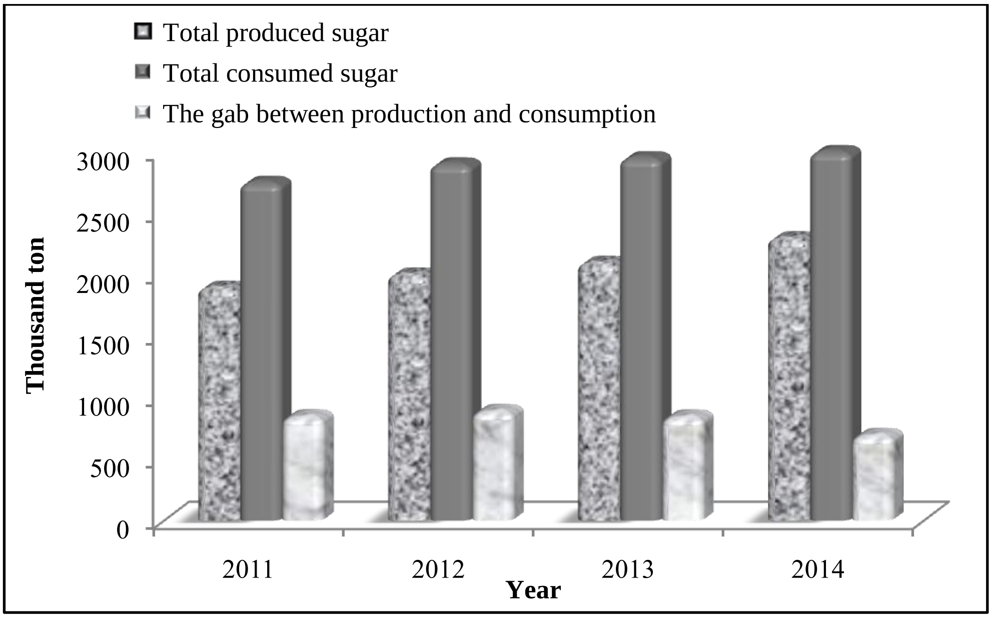

Fig.1. Total production, consumption and gab value of sugar in Egypt through 2011-2014 (El-Shatla and Refaat, 2015).