African Journal of Biotechnology Vol. 11(91), pp. 15798-15801, 13 November, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.2210
ISSN 1684–5315 ©2012 Academic Journals

Full Length Research Paper

Glucose (xylose) isomerase production from thermotolerant and thermophilic bacteria

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Accepted 2 October, 2012

Glucose (xylose) isomerase (GI) is one of the most important industrial enzymes. It is used widely to catalyze the reversible conversion of D-glucose to D-fructose *in vivo*. The latter is used on a wide scale in the production of the high fructose corn syrup (HFCS) from corn starch. The great need of a thermostable GI, which is still active at higher temperatures (up to 90°C), opened the door to screen more microorganisms for the production of a more efficient industrial enzyme. Whole cells of 50 thermotole-rant/thermophilic bacterial isolates were used to evaluate their potential to produce GI when they were grown in broth medium (pH 7.0) containing D-xylose as a sole carbon source. *Klebsiella* and *Pseudomonas* showed the highest enzyme activity of 0.7; *Bacillus* came second with an activity of 0.3; while *Acetobacter* and *Staphylococcus* showed moderate activity of 0.3 for both, and *Clostridium, Corynebacterium* and *Enterobacter* showed the lowest enzyme activity of 0.2 each. The results reveal the need for optimizing the conditions for enhancing the production of the enzyme from *Klebsiella* and *Pseudomonas* isolates.

Key words: Glucose isomerase, xylose isomerase, enzyme activity, *Klebsiella, Pseudomonas*.

INTRODUCTION

Glucose isomerase (GI) is a widely distributed enzyme in bacteria; it is used in industry to catalyze the reversible conversion of D-glucose to D-fructose *in vivo*, which in turn enters the process of the production of the high fructose corn syrup (HFCS) from corn starch. In many countries, consumer foods and products typically use HFCS as a sweetener. This process is facing a challenge of applying high temperature and alkaline pH (Bucke, 1983) in order to maintain a higher equilibrium concentration of fructose, faster reaction rate, and decreased viscosity of the substrate in the product stream (Tewari et al., 1985). Most of the commercially available GI was derived from mesophilic microorganisms that show optimum activity at pH range from 7.5 to 9.0 (Verhoff et al., 1985).

The importance of the GI extends also to the production of ethanol from raw materials. Hemicellulose fraction of biomass was used in the production of 1st generation bioethanol using wild strains of *Saccharomyces cerevisiae*. Isomerization of xylose to xylulose is an essential step in this process. This reaction is in turn catalyzed by GI (Silva et al., 2012). Recently, attempts to improve the enzyme activity of the enzyme have been done, and the mutants enzymes showed remarkable increase in activity over a temperature range of 40 to 90°C at pH 7.0 (Wei et al., 2009). The aim of this study was to find out suitable thermotolerant/thermophilic isolated bacteria that have the capability for the production of a thermostable, and neutral or slightly acidic GI which will be of great benefit in the industrial production of HFCS. This is helpful for food industries since it will provide the field with high glucose isomerase producer thermotolerant/thermophilic microorganisms.

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Abbreviation: GI, Glucose (xylose) isomerase.

Table 1. Alphabetical list of genera with the number of isolates of each genus and the corresponding enzyme activities range.

Organism	Number of isolate	GI activity (unit/ml of broth)
Acetobacter	5	0.0-0.6
Bacillus	10	0.0-0.8
Clostridium	2	0.0-0.4
Corynebacterium	3	0.2-0.4
Enterobacter	5	0.0-0.4
Klebsiella	7	0.0-0.8
Lactobacillus	1	0.0
Micrococcus	2	0.0
Pseudomonas	13	0.0-1.4
Staphylococcus	2	0.2-0.6

MATERIALS AND METHODS

Isolation of thermotolerant/thermophilic bacteria

Soil, water, and manure (five samples each) were collected from sites and showed remarkable high temperature (Ain Helwan and Ayon Moussa hotsprings and animal manure).

Isolation of bacteria from soil and manure

Serial dilutions of 10^{-1}, 10^{-2}, and 10^{-3} were prepared for soil and manure. Only 1 ml/each was dispensed on minimal medium containing/L: (xylose, 5.0 g; $NaNO_3$, 0.5 g; $MgSO_4.7H_2O$, 1.0 g; K_2HPO_4, 0. 5 g; KCl, 0.5 g; $FeCl_3$, 0.001 g; NaCl, 0.5 g; and bacteriological agar, 15.0 g). D-xylose was autoclaved separately and added later. The pH of the medium was adjusted to 7.0 using 0.01 M NaOH solution.

Isolation of bacteria from water

1 L of each water sample was concentrated by centrifugation at 3000 rpm for 10 min, then supernatant was discarded and the precipitate re-suspended in 10 ml. 1 ml of the concentrated suspension was dispensed on the above indicated medium. Petri-dishes were incubated at 55°C for 48 h. Growing bacterial colonies were subsequently purified onto Luria Bertani (LB) medium and preserved at 4°C. Grown colonies were then purified on the same medium and re-incubated at 65°C.

Identification of bacterial isolates

Pure cultures of all isolated colonies were identified to genus level according to the following used references, AOAC (1995), Ewing (1986) and Isenberg (1994).

Screening of individual isolates for glucose isomerase production

The medium used for screening for GI production was composed of: $(NH_4)_2SO_4$, 5 g; K_2HPO_4, 5 g; $MgSO_4.7H_2O$, 0.5 g; NaCl, 5 g; and D-xylose, 10 g. All components were dissolved in 900 ml distilled water and autoclaved for 15 min at 1.5 bar and 121°C. D-xylose was autoclaved separately in 100 ml of distilled water and added latter. pH was adjusted to 7.0 using 0.1 M NaOH.

Preparation of cells prior to enzyme assay

All isolates were grown in 5 ml of minimal medium containing D-xylose as a single carbon source, and incubated at 37°C for 24 h to induce the production of glucose isomerase. Two loopfulls of 24 h old bacterial colony of each isolate were immersed by a sterile wire loop into sterilized screw-cap test tubes. All test tubes were incubated at 37°C on shaker at 250 rpm for 24 h. Broth cultures were then assayed for GI activity at pH 7.0 according to a modified method of Bok et al. (1984) and Mezghani et al. (2005) and Borgi et al. (2004), by an indirect method.

Enzyme activity assay

An indirect method for determining glucose isomerase activity was applied. 0.5 ml of each incubated culture broth containing cells were transferred aseptically into 4.5 ml of a buffer compose of: 20% fructose, 0.1 M potassium phosphate buffer-salts solution, pH 7.0. Tubes were mixed well and incubated for 60 min at 60°C in a water-bath to allow GI enzyme which exists in the cells to convert fructose to glucose. After incubation time, mixtures were then cooled on ice-bath to stop enzyme activity. The amount of glucose which had been produced was determined by the method of glucose-oxidase (GOD-PAP) enzymatic system (Spectrum) (Mezghani et al., 2005; Borgi et al., 2004; Keston, 1956). 10 µl of each reaction mixture was incubated in 1 ml of GOD-PAP reagent for 20 min at room temperature, and then the absorption was measured spectrophotometrically at 546 nm with 100 Mg^{+2}/dl glucose mg/dl glucose as stander. One unit of glucose isomerase activity was defined as that amount of enzyme which produces 1 µmol of D-glucose per minute under the assay condition.

RESULTS

A total of 50 isolates comprising 10 genera were isolated and identified according to AOAC (1995), Ewing (1986) and Isenberg (1994). Table 1 shows the list of genera recovered, with the number of isolates of each genus arranged alphabetically in addition to the minimum and maximum enzyme activities recorded for each isolate. Some isolates showed no activity at all such as *Lactobacillus* and *Micrococcus* spp. (Figure 1). Other genera showed activities up to 1.4 (unit/ml of broth) such as: *Klebsiella* and *Pseudomonas* spp. Details of the

Table 2. Enzyme activity of individual isolates.

Organism	GI activity (unit/ml broth)
Acetobacter	
Iso I	0.6
Iso II	0.4
Iso III	0.2
Iso IV-V	0.0
Bacillus	
Iso I-II	0.6
Iso III-IV	0.4
Iso V-VI	0.2
Iso VII-X	0.0
Clostridium	
Iso I	0.4
Iso II	0.0
Corynebacterium	
Iso I	0.4
Iso II-III	0.2
Enterobacter	
Iso I	0.6
Iso II-III	0.4
Iso IV	0.2
Klebsiella	
Iso I	1.4
Iso II	0.8
Iso III-VI	0.6
Pseudomonas	
Iso I	1.4
Iso II	1.2
Iso III	0.6
Iso IV	0.4
Iso V-VII	0.2
Iso VIII-XIII	0.0
Staphylococcus	
Iso I	0.6
Iso II	0.6

*Iso, Isolate.

enzyme activity of all isolates are listed in Table 2.

DISCUSSION

On our way to offer a commercial glucose isomerase of acceptable characteristics such as high activity, pH wide range, and more resistance to higher degrees of temperature, we presented this study which can be considered the first step toward the target we planned for. In this work, we isolated culturable bacteria from different sources. It is well known that different glucose isomerases producers were typically mesophiles (Lee and Zeikus, 1991; Vieille and Zeikus, 2001). In the present study, all bacterial isolates were at thermophilic range to ensure that GI will maintain its activity unchanged during the process of high fructose corn syrup (HFCS) production. All the screened isolates were tested for their potential to grow at 65 and 75°C, although the commercially available enzyme underwent continuous improvements, and optimization (commercial GI titers have been reported to be around 35,000 U/L at p H 7.0 and 75°C) (Song et al., 1984). Our results reveal that some isolates showed comparable activity to the already used GI (1.4 unit/ml of broth to 0.035 unit/ml of broth of the commercially used enzyme). The selected isolates will be evaluated once again at slightly acidic pH to obtain higher activity of the enzyme, which will be the target of the next study.

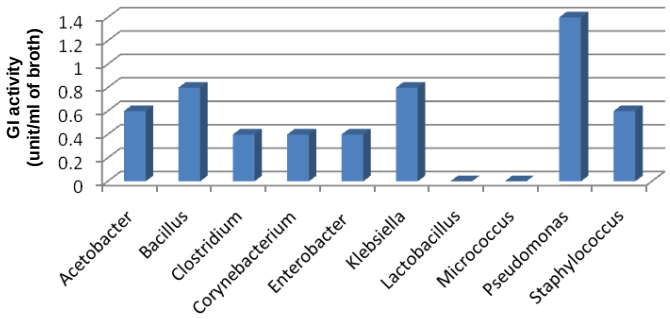

Figure 1. Both *Pseudomonas* and *Klebsiella* showed the highest GI activity, while *Lactobacillus* and *Micrococcus* did not record any activity.

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