Final Report

โครงการ “การคัดเลือกหาเชื้อยีสต์และเชื้อแบคทีเรียที่ผลิต xylitol จาก Glucose”

(Screening of yeast and bacteria capable to produce xylitol from glucose)

by Prof. Watanalai Panbangred and colleagues

September 30, 2010

สัญญาเลขที่ IUG5080014

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This research project was jointly supported by Thailand Research Fund and Ueno Fine Chemicals Industry (Thailand) Ltd.

Acknowledgement

We would like to express our sincere gratitude to Thailand Research Fund and Ueno Fine Chemicals Industry (Thailand) Ltd. for their kind financial support which makes this research possible. This research funding opens the window for research activity to direct toward the industry needs which are crucial for industrial development for biotechnology in Thailand.

We are particularly grateful to Prof. Vichai Boonsaeng and Mr. Norio Kasaki for their kindness and supports. We would like to thank Ms. Patcharaporn Amornsin and Ms. Rubwad Mathurananon for their assistance on research and to Faculty of Science, Mahidol University for providing research facilities.

Abstract

Project Code : IUG5080014

Project Title : Screening of yeast and bacteria capable to produce xylitol from glucose

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Project Period : April, 2008 – September, 2010

Abstract

This research project is aimed to screen for yeast and bacteria capable to produce xylitol and/or D-arabitol from glucose. Thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) were used for preliminary screening and determination of sugar alcohol produced by selected microbes.

From TLC screening, 574 and 999 out of 4,671 and 9,664 bacterial and yeast isolates, respectively were putatively xylitol and/or D-arabitol producers. The isolates positive by TLC screening were selected for further analysis of sugar alcohol accumulated in culture broth by HPLC. Eighteen and five out of 187 bacterial isolates produced xylitol and D-arabitol at low yield at 0.006-0.12% and 0.004-0.04%, respectively. Whereas 112, 194 and 64 out of 857 yeast isolates were xylitol, D-arabitol or both sugar alcohol producers, respectively. Xylitol and D-arabitol yield of 0.071-0.344% and 0.542 - 1.464% were produced and accumulated in culture broth, among 50 and 27 selected yeast isolates, respectively. Pentitol dehydrogenase and hexose reductase activities were assayed and their activities did not correlate with sugar alcohol accumulation.

Xylitol production from glucose in 6 selected yeast strains (BK32-10-20, SB27-09-13, SS26-09-08, NR23-09-32, SS26-09-29 and NR20-09-22) were cultured in limited aeration condition at 28, 30 and 32 ^{o}C. It was found that the first 5 isolates produced xylitol at 0.2-0.87% whereas xylitol accumulation was drastically reduced or absent if they were cultured in the medium containing 5% glucose and 5% glycerol. NR20-09-22 produced only D-arabitol at 28 and 30 ^{o}C in medium containing glucose and mixture of glucose and glycerol, respectively. All 6 selected yeast strains efficiently converted 50% xylose in the medium containing 1% glucose to xylitol at the yield of 26.7-47.6% (w/v). Identification of NR20-09-22 and NR20-09-21 which are xylitol and D-arabitol producers, respectively suggested that they were *C. tropicalis*. Another yeast isolate, BK32-10-20 (xylitol producer) was identified as *Kodamaea ohmeri*. From the above study, it is suggested that optimization of culture condition and their substrates might lead to increasing xylitol and/or D-arabitol production from either glucose, xylose and their mixture.

Keywords : xylitol, D-arabitol, yeast, bacteria, glucose

บทคัดย่อ

รหัสโครงการ : IUG5080014

ชื่อโครงการ : การคัดเลือกหาเชื้อยีสต์และเชื้อแบคทีเรียที่ผลิต xylitol จาก glucose

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Project Period : เมษายน 2551 – กันยายน 2553

บทคัดย่อ

ได้ใช้วิธี Thin layer chromatography (TLC) และ High performance liquid chromatography (HPLC) ในการวิจัยที่มีวัตถุประสงค์เพื่อคัดเลือกหาเชื้อแบคทีเรียและยีสต์ที่ผลิต sugar alcohol ชนิด xylitol และ D-arabitol จากน้ำตาล glucose โดยที่ TLC ใช้เป็นวิธีการคัดเลือกเบื้องต้นขณะที่วิธี HPLC ใช้ในการหาปริมาณ sugar alcohol ที่เชื้อสร้างขึ้นและสะสมใน culture broth

จากการคัดเลือกเชื้อแบคทีเรียและยีสต์ 4,671 และ 9,664 สายพันธุ์ พบว่าจำนวน 574 และ 999 สายพันธุ์ตามลำดับให้ผลบวกโดยวิธี TLC จากนั้นได้คัดเลือกเชื้อที่ให้ผลบวกโดย TLC ไปวิเคราะห์หาปริมาณน้ำตาล D-arabitol และ xylitol โดยวิธี HPLC ผลการวิเคราะห์พบว่าเมื่อนำเชื้อแบคทีเรีย 187 สายพันธุ์ไปเลี้ยงในอาหารที่มี glucose 3% พบว่าจำนวน 18 สายพันธุ์ผลิต xylitol อย่างเดียวในระดับต่ำ (0.006-0.12%) และ 5 สายพันธุ์ผลิต D-arabitol ได้เล็กน้อย (0.004-0.047%) ขณะที่เมื่อนำสายพันธุ์ยีสต์ 857 สายพันธุ์มาเลี้ยงในอาหารที่มี glucose 10% พบว่า 112, 194 และ 64 สายพันธุ์ ผลิต xylitol, D-arabitol และน้ำตาลทั้ง 2 ชนิดตามลำดับ โดยที่ยีสต์ 50 สายพันธุ์ผลิต xylitol จาก glucose (10%) ได้ปริมาณสูงสุดในช่วง 0.071-0.344% และมี 27 สายพันธุ์ที่ผลิต D-arabitol ได้ปริมาณสูงสุดในช่วง 0.542-1.464% ได้เปรียบเทียบการวัดกิจกรรมเอนไซม์ pentitol dehydrogenase และ hexose reductase โดยการวัดค่าความเปลี่ยนแปลงของ NADH เพื่อเปรียบกับปริมาณ xylitol ที่

เชื้อผลิตโดยวิธี HPLC พบว่าค่ากิจกรรมเอนไซม์ที่วัดได้และปริมาณน้ำตาลที่เชื้อผลิตไม่มีความสัมพันธ์ต่อกัน

ในการหาสภาะที่เหมาะสมในการผลิต xylitol จาก glucose 10% ในยีสต์ 6 สายพันธุ์ ได้แก่ BK32-10-20, SB27-09-13, SS26-09-08, NR23-09-32, SS26-09-29 และ NR20-09-22 โดยเลี้ยงในสภาวะที่มี limited aeration ที่ 28, 30 และ 32 °C พบว่า 5 สายพันธุ์แรกผลิต xylitol ที่ 0.2-0.87% ขณะที่หากเลี้ยงเชื้อทั้ง 5 สายพันธุ์นี้ในอาหารที่มี glucose และ glycerol อย่างละ 5% เชื้อจะผลิต xylitol ได้น้อยลงหรือไม่ผลิตเลย ยีสต์สายพันธุ์ NR20-09-22 จะผลิต D-arabitol ที่ 28 และ 30 °C ในอาหารที่มี glucose หรือ glucose + glycerol เมื่อเลี้ยงยีสต์ทั้ง 6 สายพันธุ์ในอาหารที่มี xylose 50% + glucose 1% พบว่าเชื้อสามารถผลิต xylitol ได้ 26.7-47.6% (w/v) จากการบ่งชี้ชนิดของยีสต์ 3 สายพันธุ์พบว่า NR20-09-22 และ NR20-09-21 ซึ่งผลิต xylitol และ D-arabitol ในปริมาณสูงตามลำดับเป็นเชื้อ *C. tropicalis* ขณะที่ BK32-10-20 ซึ่งผลิต xylitol ปริมาณสูงเป็นเชื้อ *Kodamaea ohmeri* จากข้อมูลข้างต้นจะเห็นได้ว่าหากมีการหาสภาวะที่เหมาะสมในการเลี้ยงเชื้อน่าจะสามารถเพิ่มปริมาณการสร้าง xylitol หรือ D-arabitol จากน้ำตาล glucose, xylose หรือส่วนผสมของน้ำตาลทั้ง 2 ชนิดได้

Keywords : xylitol, D-arabitol, yeast, bacteria, glucose

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Introduction

Xylitol ($C_5H_{12}O_5$) is a five carbon sugar alcohol found naturally in fruits and vegetables. It has a similar degree of sweetness to sucrose but fewer calories (2.4 kcal/g compared to 4 kcal/g for sucrose), so this sugar alcohol generates lower calories compared to sucrose (1, 2). Xylitol is converted from xylose by either chemical or enzymatic processes. Xylose is a pentose sugar that conventionally prepared from lignocelluloses material by acid hydrolysis. Xylitol is extensively used in food, pharmaceutical and thin coating applications (1, 2). The most important application of xylitol is its use as an alternative sweetener in foods for diabetic patients (1). Other important uses of xylitol are : as an anticariogenic agent in tooth paste formulations as thin coating on chewing vitamin tablets, in mouth washes, in beverage and in bakery product (2, 4). Its non-cariogenic properties can inhibit the development of dental caries (1, 2). In humans, metabolism of this polyol is not insulin-mediated, so xylitol serves as a sugar substitute for diabetics. Additional auspicious qualities include its large negative heat of dissolution (greater than other sugar substitutes), resulting in a clean, refreshing sensation in the mouth, and its inability to contribute to Maillard-based food browning and caramelization, in contrast to carbony containing sugar substitutes (2). Finally, xylitol can serve as a valuable synthetic building block and was recently identified as one of the top twelve value-added materials to be produced from biomass, thereby serving as a key economic driver for biorefineries because its metabolism is not regulated by insulin nor does it involve glucose-6-phosphate dehydrogenase. Hence, this sugar alcohol has beneficial health properties.

The numerous applications of xylitol have led to its rapidly increasing consumption. Although xylitol occurs in many fruits and vegetables (4), it would be very uneconomical to extract it from such sources due to their high cost and relatively low xylitol content. On a large-scale production, xylitol is currently produced by chemical reduction of xylose derived mainly from wood hydrolysates (4). The conventional process of xylitol production includes four main steps: acid hydrolysis of plant material, purification of the hydrolysate to either a pure xylose solution or a pure crystalline xylose, hydrogenation of the xylose to xylitol, and crystallization of the xylitol (4, 5).

The critical step in this process is the purification of the xylose from the acid hydrolysate. Ion exchange chromatography is employed to remove salts and charged degradation products, and activated carbon is used to remove color. Ion exchange chromatography, however, does not remove or separate the various hemicellulosic sugars. This is a problem because acid hydrolysis releases appreciable amounts of D-galactose, D-mannose and L-arabinose in addition to D-xylose.

The exact proportions of the various sugars depend on the nature of the feedstock and the manner in which it is hydrolyzed. These contaminating sugars can complicate crystallization and purification of xylose. The yield of xylitol from the xylan fraction is about 50-60% or 8-15% of the raw material employed. The existing drawbacks of conventional xylitol production methods motivated researchers to seek alternative ways for its production. One of the most attractive procedures is xylitol production by microbes using common sugar such as glucose as substrate.

Microorganisms more readily assimilate and ferment glucose than xylose. However, although in small numbers, there are bacteria, yeasts and fungi capable of assimilating and fermenting xylose to xylitol, ethanol and other compounds. In yeast, from xylose, xylose reductase(XR) converts xylose to xylitol by a one step reaction which requires NADPH. Xylitol will be further changed to xylose by Xylitol Dehydrogenase (XDH) in the coupling reaction with NAD^+ will be reduced to NADH (Fig 1). (6).

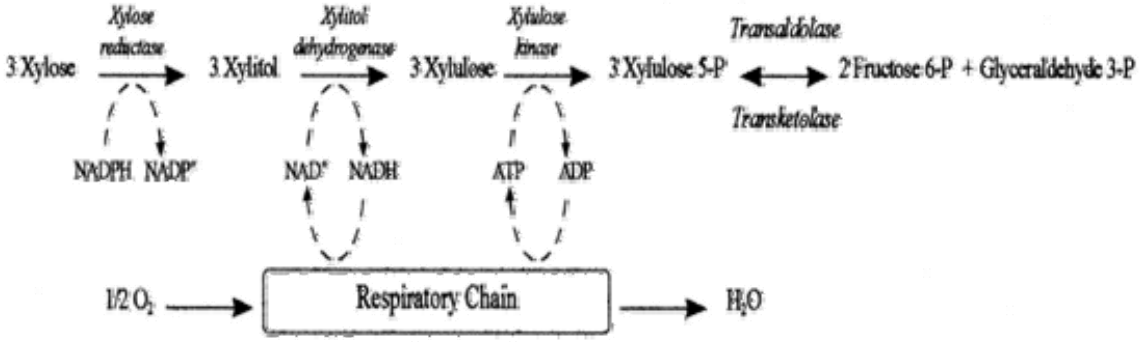


Fig. 1. Schematic diagram of the initial steps of D-xylose metabolism in *Candida guilliermondii*. (6).

A few bacteria such as *Corynebacteriurn* sp.*, Enterobacter liquefaciens,* and *Mycobacterium smegmatis* have been reported to produce xylitol (7-11). For the first two bacteria, D-xylose was mainly used as a substrate while for the last one, the substrate was D-xylulose or D-xylose isomerized by commercially immobilized D-xylose isomerase. However, due to the relatively small quantities of xylitol formed, xylitol-producing bacteria do not presently attract researchers' interest. Regarding the fungi, there is only one significant report on xylitol production by *Petromyces albertensis.* This fungus accumulated 39.8 g/l of xylitol when cultured for 10 days on 100 g/l D-xylose. Nevertheless, after initial studies regarding the effects of environmental conditions on xylitol production by this fungus, no further reports were published.

In general, among microorganisms, the yeasts are considered to be the best xylitol producers and therefore, the majority of publications deal with them. Some of the yeasts screened for xylitol

production from xylose are presented in Table 1. While considering Table 1, it should be noted that: (i) The xylitol concentrations indicated are those obtained during the screening process, that is before any optimization of the cultural conditions, (ii) different media and culture conditions have been applied and (iii) the yeasts, for which no data regarding their screening for xylitol production were available, were not included in the survey. Due to this, it is difficult here to really compare the production capacities of different yeasts. However it is obvious that the best xylitol producers belong to the genus *Candida* (5).

Table 1. Screening of yeasts for xylitol production from D-xylose.

Yeast	Xylitol g/l	Ethanol g/l
Candida boidinii NRRL Y-17213	2.9	3.9
C. *guilliermondii* FTI-20037	16.0	n.d.[a]
C. *intermedia* RJ-248	5.7	3.6
C. *mogii* ATCC 18364	31.0	n.r.[b]
C. *parapsilosis* ATCC 34078	20.0	n.r.
C. *pseudotropicalis* IZ-43 1	4.3	3.0
C. *tropicalis*	2.1	n.r.
C. *tropicalis* HXP 2	4.8	n.r.
C. *tropicalis* 1004	17.0	n.d.
C. tropicalis ATCC 7349	20.0	n.r
C. *tropicalis* ATCC 20240	5.5	n.r.
C. *utilis* ATCC 22023	1.8	n.r.
C. *utilis* C-40	3.0	n.r.
Debarvomyces hansenii C-98 M-21	0.8	n.d.
Hansenula anomala IZ-1420	6.1	n.d.
Kluyveromyces fragilis FTI-20066	4.6	3.5
K. marzianus IZ-1420	6.1	0.6
Pichia (Hansenula) anomala NRRL Y-366	2.0	n.d.
Pachysolen tannophilus NRRL Y-2460	2.2	5.2
Saccharomyces SC- 13	0.7	n.r.
Saccharomyces SC-37	2.3	n.r.
*Schizosaccharomyces pombe*16919	0.2	n.r.

[a] n.d., Not detected.

[b] n.r., Not reported

From Winkelhausen et al, 1995 (5).

Xylitol is produced commercially by chemical reduction (hydrogenation) of D-Xylose derived from hemicellulose-xylan hydrolysates of substrates such as birchwood or corn. Microbial reduction of D-xylose with yeast such as *Candida* or *Saccharomyces* species is not competitive because of their low productivity compared with the chemical process. Due to the high pollution level and requirement of extensive waste-treatment, preparation of xylose from lignocellulosic material by acid hydrohysis became diminished (12). Alternative mehod to replace either chemical reduction of D-xylose or biotransformation of D-xylose to produce xylitol with xylose reductase was reported. The method involves conversion of glucose to xylitol by enzymatic process. Firstly glucose will be converted to D-arabitol which then enzymatically oxidized to D-xylulose. Xylitol then could be formed from D-xylulose by either chemical dehydrogenation or enzymatic reduction catalysed by a NAD-dependent xylitol dehydrogenase (13). D-arabitol is a five-carbon polyhydroxy alcohol (polyols). It can be produced by many osmophillic yeast species such as *Zygosaccharomyces rouxii*, *Debaryomyces hansenii*, *Candida*, *Pichia*, *Hansenula* and others (13-16).

Two D-arabitol biosynthesis pathways have been described in fungi. In the osmophilic yeast species such as *S. cerevisiae*, *S. mellia*, and *Debaryomyces hansenii*, glucose is converted to D-ribulose-5-phosphate via the pentose phosphate pathway (14) and then D-ribulose 5-phosphate is dephosphorylated to D-ribulose and reduced to D-arabitol (14). The final step in this pathway is catalysed by NADP-dependent pentitol dehydrogenase. In contrast, another strain of *S. rouxii* and the marine fungus *Dendryphiella salina* convert glucose to D-xylulose 5-phosphate via the pentose pathway, and then this substance is dephosphorylated and reduced to D-arabitol, which is catalysed by NAD-dependent pentitol dehydrogenase (14).

It is reported that *Candida* sp. is one of the most potent microorganisms for D-arabitol production (5, 14). It has been suggested that accumulation of polyols in yeasts may be related to osmotic stress (17). In *C. albicans* (13), at the final step, D-ribulose and NADH are converted to D-arabitol and NAD by D-arabitol dehydrogenase(ArDH). In addition, most strains of *C. albicans* can also utilize D-arabitol for growth, thus D-arabitol dehydrogenase (ArDH) may also catalyze D-arabitol utilization when preferred substrates are absent (14, 17). In general, the quantity produced and pattern of polyols like D-arabitol, glycerol, erythritol, and xylitol depend strongly on the environmental conditions. This is due to the complex regulation of polyol production and to the fact that each polyol can fulfill different functions. Polyols have a role as carbohydrate reserves. Erythritol, xylitol and arabitol are produced as carbon storage compounds when the flux through the pentose phosphate pathway exceeds the need in ribulose-5-phosphate for the biomass

synthesis. At the later stage of the fermentation, all polyols are consumed as soon as the main carbon source, glucose or xylose, is depleted.

As stated above, among yeast, a wide range of yeast species belong to genus *Candida* (5, 14) are well-known for their potential industrial applications for polyol production, these include *Candida boidinii* (18), *C. guilliermindii* (6, 19, 20), *C. parapsilosis* (21), *C. peltata* (22) and *C. tropicalis* (23, 24). *Arxula adeninivorans* is a dimorphic yeast that can produce xylitol and the xylitol dehydrogenase (*AXDH*) gene was isolated and characterized (25). The *C. tropicalis* xylitol dehydrogenase gene (*XYL2*) consist of a 1,092-bp intronless ORF which codes for a 364-residue polypeptide with a predicted molecular mass of 40 kDa. This gene is present as a single copy in *C. tropicalis* genome, and the homologus genes from *A. adeninivorans* was also reported (25, 26). Several recombinant yeast were constructed and higher yield of xylitol were produced from glucose substrate. For example, recombinant *S. cerevisiae* that could produce xylitol form D-glucose in a single fermentation step was described. Expression of the xylitol dehydrogenase-encoding gene *XYL2* of *Pichia stipitis* in the transketolase-deficient *S. cerevisiae* strain resulted in a 8.5 fold enhancement of the total amount of xylitol (27).

Bioconversion of D-arabitol to D-xylulose could be occurred by oxidation catalysed by a membrane-bound D-arabitol dehydrogenase (ArDH). Many bacteria were reported to be a potent oxidizer for polyols such as *Acetobacter aceti* and *Gluconobacter oxydans.* (28, 29) *G. oxydans* posses two enzymes enabling the production of xylitol from D-arabitol. The two enzymes were membrane-bound D-arabitol dehydrogenase (ArDH) and soluble xylitol dehydrogenase (XDH). The AraDH (D-arabitol 2-dehydrogenase) from fungi and yeast oxidized D-arabitol to D-ribulose instead of D-xylulose, whereas the bacterial AraDH (D-arabitol 4-dehydrogenase) oxidized D-arabitol to D-xylulose (30). The schematic conversion of D-glucose to xylitol is shown in Fig 2.

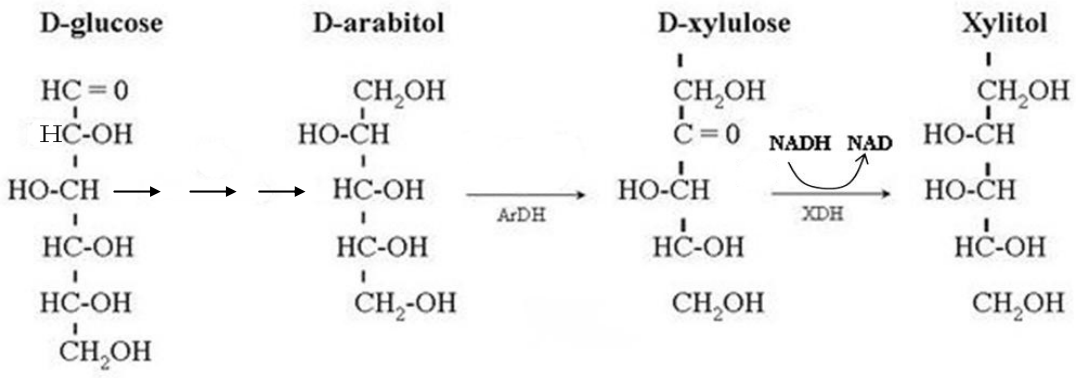


Fig. 2. Schematic representation of xylitol production from D-glucose. Various halotolerant yeasts produce D-arabitol from D-glucose though various enzymatic steps. Some bacteria such as *G. oxydans* converts D-arabitol to D-xylulose by ArDH, a membrane-bound D-arabitol dehydrogenase and use XDH, a soluble NAD-dependent xylitol dehydrogenase to convert D-xylulose to xylitol. Adapted from Sugiyama et al, (2003a) (29).

Xylitol production from D-arabitol was intensively studied in acetic acid bacteria, *Gluconobacter oxydans* (28-31). In this bacterium, D-arabitol is converted to D-xylulose by a membrane-bound D-arabitol dehydrogenase (ArDH). D-xylulose is further converted to xylitol by soluble NAD-dependent xylitol dehydrogenase (XDH). It was also reported that transaldolase/Glucose-6-phosphate isomerase bifunctional enzyme or ribulokinase could increase xylitol production from 3.8 g/l to 5.4 g/l from 10 g/l of arabitol (28). Increasing XDH activity in recombinant *G. oxydans* under controlled aeration and pH conditions, enabled the strain to produce 5.7 g/l xylitol from 22.5 g/l D-arabitol (29). The putative scheme of pentose phosphate pathway in *G. oxydans* is shown in Fig 3.

The enzymatic assay for enzymes involved in D-arabitol and xylitol production are described by Susuki et al, 2002 (31), Adachi et al, 2001 (32) and Cheng et al, 2005 (30). The detail of assay will be described in Materials and Methods. However, we found that the assay method was non-specific if the crude extract was used since the assay methods monitor the change of NADH which is the co-factor of many enzymes.

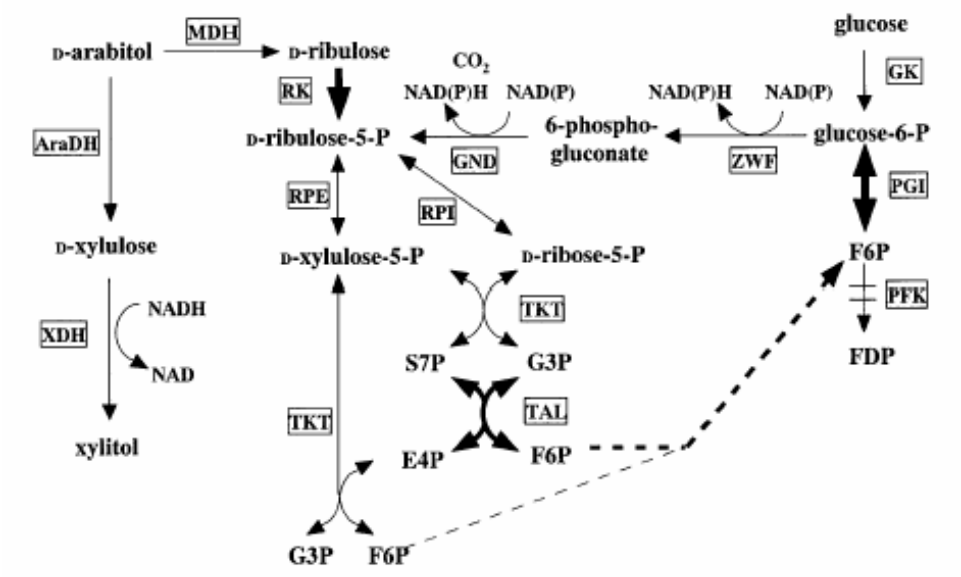


Fig. 3. Putative Scheme of the *G. oxydans* Pentose Phosphate Pathway. From Sugiyama et al,2003a (28).

The original objectives of this research project are :

1. To screen yeast capable to produce D-arabitol from glucose.
   The target for primary screening is the yeast strain that could convert D-glucose to D-arabitol with at least 20% yield (ie 8 g/l of D-arabitol from 40 g/l of D-glucose).
2. To screen for acetic acid bacteria especially *Gluconobacter* which is capable to produce xylitol from D-arabitol.
   The target for primary screening is to obtain *Gluconobacter* which is capable to produce about 3-5 g/l xylitol from 10 g/l D-arabitol.

However, after a large scale screening of several thousands strands of yeast and bacterial strains, we found that several yeast isolates could convert glucose to either D-arabitol or xylitol or to both sugar alcohols. Many strains are capable to convert D-xylose to xylitol with more than 90% yield. Whereas in bacterial isolates, very few isolates produced xylitol or D-arabitol and yield was very low.

Materials and Methods

1. Isolation of yeast and bacteria producing D-arabitol and xylitol from soil fruit and vegetable samples.

1.1 Sample collection

a. Soil samples were collected from orchards in various locations such as Nakhon Pathom, Chantaburi, Rayong, and Samutsongkhram as well as from vineyard in Chonburi, Nakhon Ratchasima and Saraburi.

b. Fruit samples were mostly collected from orchards stated above. Some fruit samples were purchased from local markets in Bangkok.

c. Vegetable samples (such as beet roots, tomatoes) were purchased from local markets in Bangkok

d. Other samples such as sugar cane, sugarcane juice, palm juice and coconut's juices were purchased from small local shops.

1.2 Isolation of yeast

Samples (1 g of soil sample or a few grams of fruit or vegetable samples) were added into 5 ml of $YEPD_{10}$ broth (10% glucose, 1% yeast extract and 2% peptone) (33) and incubated at 30 ^{o}C with shaking for 2 days. The proper diluted samples (in 0.85% NaCl) were spread on $YEPD_{10}$ and incubated at 30^{o}C for another 2 days. The isolated colonies were replicated on $YEPD_3$ agar plate (3% glucose, 1% yeast extract, 2% peptone and 2% agar) supplemented with 20 μg/ml of chloramphenicol to prevent bacterial contamination. Chloramphenicol was added to $YEPD_3$ before pouring into sterile petri-dishes. $YEPD_{10}$ was used for enrichment of osmophilic yeast and $YEPD_3$ was used for screening of xylitol product, since $YEPD_{10}$ contains 10% glucose which interferes subsequent screening of xylitol by TLC from utilized glucose. The selected colonies were grown on $YEPD_3$ at 30^{o}C for 2 days before subjection to preliminary screening for sugar alcohol production by Thin Layer Chromatography (TLC).

1.3 Isolation of bacteria

Samples (1 g of soil samples and a few grams of fruit samples) were added into 5 ml of YPG broth (0.5% yeast extract, 0.3% peptone, 3.0% glucose) (29) and incubated at 30 ^{o}C with shaking for 24 h. The proper diluted samples (in 0.85% NaCl) were spread on EYC agar plate, pH 7.0 (4.0% ethanol, 1.0% yeast extract, 1.0% Calcium carbonate and 1.5% agar) (34) and incubated at 30 ^{o}C for 24 h. The isolated colonies were replicated on GDP agar (1.0% glycerol, 0.05% D-arabitol, 0.3% yeast extract, 0.3% polypeptone and 1.5% agar) (32).

The selected bacterial colonies were grown on GDP agar plate at 30 ^{o}C for 2 days before subjection to preliminary screening for sugar alcohol production by Thin Layer Chromatography (TLC).

2. Screening of yeast and bacterial isolates capable to produce D-arabitol and xylitol or both sugars.

2.1 Preliminary screening by Thin Layer Chromatography (TLC).

After growing on $YEPD_3$ (for yeast) and GDP agar (for bacteria) at 30 ^{o}C for 2 days, agar under selected colonies (about 100-150 μl in volume) was picked and resuspended in 100 μl of distilled water, and vortexed vigourously. The solution (~ 2 μl each) was spotted two times on TLC (Silica gel 60, Merck) using toothpick. The TLC plate was placed in the glass chromatographic chamber using the solvent system of Acetonitrile : Acetic Acid : Distilled water = 63:33:5 (35)

The ascending chromatography was run twice at room temperature and TLC plates were dried completely after each run. The size of TLC plate was 10 cm x 10 cm (w x l) and time for each run is about 20 min. For sugar spot detection, dried TLC plates were swabbed thoroughly by a solvent mixture of p-anisaldehyde : sulfuric acid : ethanol = 1:1:18 (v/v) (36), then heat the swabbed TLC plate on a hot plate, till sugar spots appear. TLC plates can be heated at 120 ^{o}C for 3 min in hot air oven, however, heating on hot plate is more conveniently done in a chemical hood for evaporation of solvent.

D-arabitol, xylitol and glucose could be differentiated by either mobility distance and color of spot or both. D-arabitol gave the reddish purple spot, xylitol had the bluish spot and glucose gave the dark green spot. D-arabitol spot moved faster than xylitol and glucose. Xylitol moved a bit higher than glucose but still overlapped. The overlapped spot of xylitol and glucose gave dark brown color. However, both D-arabitol and xylitol had lower sensitivity under this detection system when compared to glucose. The authentic solutions used in the system was 1% sugar alcohol mixture of D-arabitol and xylitol, and spot by using the microtip or toothpick, soaked in the standard sugar alcohol solution and spotted two times on the TLC plate.

3. Analysis of D-arabitol and xylitol production by High Performance Liquid Chromatography (HPLC)

3.1 Yeast isolates

Positive strains from TLC method were cultured in $YEPD_{10}$ containing 10% glucose broth and incubated with shaking at 30^{o}C for 2 days. After 2 days, culture broths were centrifuged at 10,000 rpm at 4^{o}C for 10 minute. The supernatant was collected and filtered with 0.45 μm membrane. The supernatant was evaporated by Univapo 100 H for obtaining 5 fold concentrated supernatant. Xylitol and D-arabitol from concentrated supernatant were analyzed by HPLC

(Shimadzu, Japan) with a Shodex Asahipak NH_2P-50 4E column (Showa Denko, Japan) (29). The HPLC condition for separation of sugar alcohol (xylitol and D-arabitol) was performed using the column temperature at 40°C and 90% acetonitrile in water was used as a mobile phase with a flow rate at 1 ml/min. We found that at 40°C, 90% acetonitrile in water gave, this condition the best result for separation of D-arabitol and xylitol peaks.

In other experiments, YEPD broth containing different concentrations of glucose (15 or 20%) or various concentrations of xylose was used. However, HPLC analysis of sugar alcohol in the cell free supernatant was done as described above.

3.2 Bacterial isolates

Two different media were used for cultivating bacteria and analysis of sugar alcohol in culture media by HPLC (Shimadzu, Japan). For glucose utilization, YPG (0.5% yeast extract, 0.3% peptone and 3.0 % D-glucose) was used as culture media and cells were grown with shaking at 30 °C for 24 h. YPA (0.5% yeast extract, 0.3% peptone and 3.0 % D-arabitol) was used for utilization of D-arabitol in some selected isolates. Cell were cultivated at 30 °C for 24 h. Samples (5 ml) from the culture broth were centrifuged at 10,000 rpm for 3 min. The supernatant was collected, filtered through 0.45 µm membrane and evaporated at 40 °C by Univapo 100 H (UniEquip, Germany) for obtaining 5 fold concentrated supernatant in order to measure xylitol production by HPLC. Each concentrated supernatant was recentrifuged at 10,000 rpm for 3 min and filtrated with 0.45 µm membrane before injection to HPLC. A Shodex Asahipak NH2P-50 4E 4.6X250 ml column (Showa Denko, Japan) was used with the column temperature at 40 °C and 90% acetonitrile was used as mobile phase at a flow rate of 1 ml/min. Compounds were identified and quantified by comparison of their retention times with those of authentic standards (1% or 2% of either D-arabitol, xylitol in water) or its mixture.

4. Enzymatic assay

Both polyol reductase and ketose or aldose reductase which are involved in xylitol production, were assayed from membrane bound fraction (bacterial ArDh) or cytoplasm (yeast).

4.1 Enzyme from bacteria was assayed using membrane

- bound fraction. Cells were broken by bead beater.

The membrane-bound D-arabitol dehydrogenase was assayed using the following reaction mixture (final concentration) (31).

0.05 M Glycine / NaOH buffer pH 9.5 1,250 µl

1.5 M D-arabitol 100 µl

0.006 M NAD^+ 50 µl

crude enzyme 100 µl

The formation of NADH was measured at 340 nm after incubation at 25 °C for 5 min. *Gluconobacter oxydans* ATCC621 was used as standard strain.

4.2 Hexose reductase from yeast was assayed from cell extract prepared from yeast cells. Yeast cells were broken by vortexing 3x1 min with acid washed glass bead in bead beater (Biospec Product, USA). Supernatant was collected for enzyme assay.

The reaction mixture comprises of (final concentration) (37).

0.05 M Sodium Phosphate buffer, pH 6.5 750 µl

100 mM glucose 100 µl

0.002 M NADH 50 µl

crude enzyme 100 µl

The reduction of NADH was measured after incubation at 25 °C for 10 min. *C. tropicalis* DSM11953 was used as standard strain.

Note : Enzyme assay from toluene treated cells (both bacteria and yeast) was lately performed by incubating cell suspension in buffer (1 ml) with 10 µl toluene at room temperature for 1 hr before performing enzyme assay. For bacterial cells, result was good so in later experiment, toluene treated cells was used. However, for yeast cells, activity was low and not consistent, so toluene treated yeast cell was not used.

Note : The standard strains used in the enzymatic assay were shown below.

1. *G. oxydans* subsp. *suboxydans* ATCC621 was purchased from American Type Culture Collection (ATCC), USA. The strain was reported to produce highest amount of xylitol (29.2 g/l) from D-arabitol (52.4 g/l) in 27 hr from 10% D-arabitol (w/v) (31).

2. *C. tropicalis* DSM11953 or *C. tropicalis* ATCC7349 (same strain with different designation in two culture collections) was purchased from Deutsche Sammlung Von Mikroorganism and Zellkmtureen Gmbtt (DMSZ), Germany.

The strain was reported to produce xylitol (20 g/l) from D-xylose (50 g/l) (5). The strain could also produce xylitol from glucose.

5. Molecular identification

5.1 Yeast isolates

Yeast isolates which produce high amount of xylitol and D-arabitol were identified by amplifying and analysing 26S rRNA gene. The sequence was compared by BLASTN analysis (www.ncbi.nlm.nih.gov)

5.2 Bacterial isolates

Putative xyltiol-producing bacteria were identified by analysis of about 500 bp of 16S rRNA gene using UFUL primer (38). The nucleotide sequence was compared and analysed by BLASTN analysis.

Results

1. Screening of bacterial and yeast isolates capable for producing D-arabitol, xylitol or both

Several types of samples such as soil from fruit orchards, fruit samples, vegetable, fruit juice and others were collected and used for both bacterial and yeast isolation. The isolation conditions and media were described in Materials and Methods. The microbes from plates with well-isolated colonies (30-300 colonies/plates), were picked and subcultured to media containing 3% glucose. Incubation was prolonged to two days to promote glucose utilization and increased sugar alcohol accumulation in the agar under each colony before subjection to TLC analyses. Colonies that showed a reddish purple spot of D-arabitol or bluish spot of xylitol were further cultivated and sugar alcohol yield in culture supernatant was analysed by HPLC. TLC of standard sugar was shown in Fig. 3. Examples of bacterial and yeast isolates producing D-arabitol or xylitol were shown in Fig. 4 and Fig. 5.

At the beginning of the project, we screened the isolates from soil samples collected in orchard, rice field or sugar palm factories, all putative sugar alcohol producing strains were collected and HPLC assay was performed. Later on we found that strains from fruit samples gave better yield and only strains that showed clear distinquished color for xylitol and D-arabitol were collected. As shown in Table 2 and Table 3, we have screened the micro-organisms from various sources of samples, the positive strains by TLC analysis were ranged from 0 to 24.5%. High percentage of yeast isolates from soil sample gave highest positive strains by TLC, however, this high percentage was reflected by the fact that all putative positive isolates were collected at the early state of screening. Result of HPLC in subsequent screening revealed that isolates from fruit samples gave positive strains with higher yield of D-arabitol and xylitol. The other sources of samples shown in Table 2 and 3 were, Look pang, rice, sugar cane, Khao mak (fermented sticky rice), baggasses, biofertilizer, molasses, etc. For fruit samples, they were grapes, pine apple, papaya, rambutan, longan, plum, strawberries and etc but most samples were either green or red grapes collected from vineyard, or purchased from local markets.

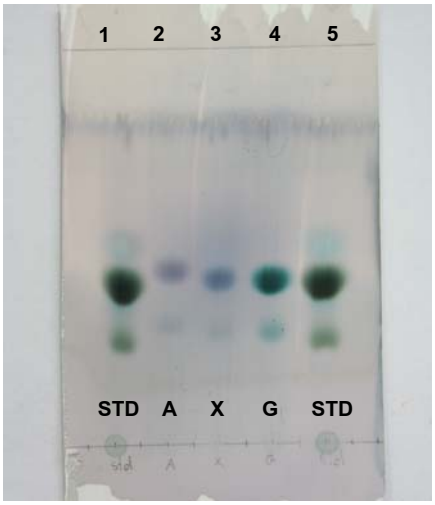


Fig. 4. TLC of standard sugars (1% w/v)
Lanes 1, 5 (STD) mixed sugar alcohol of D-arabitol (A), xylitol (x) and D-glucose (G)
Lanes 2-4 are Arabitol, xylitol and glucose, respectively.

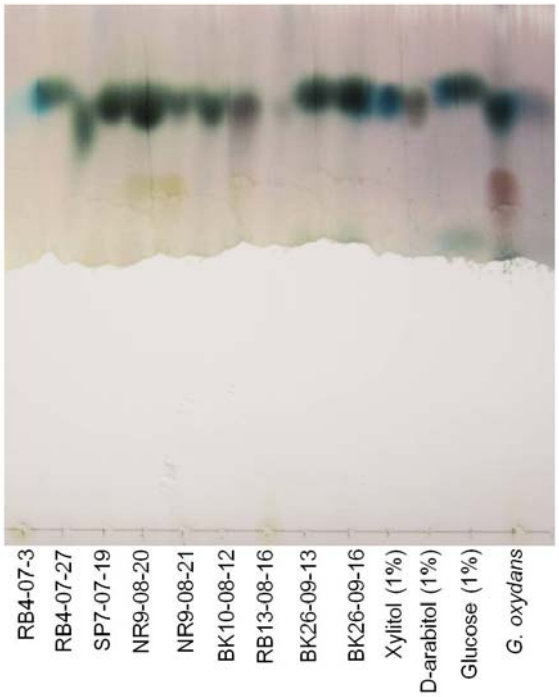


Fig. 5. TLC of sugar extracted from agar under colonies of bacterial isolates. Bacteria were grown on YPG agar plate at 30^{o}C for 2 days. Sugar and sugar alcohol in agar under each colony were extracted and analysed by TLC.

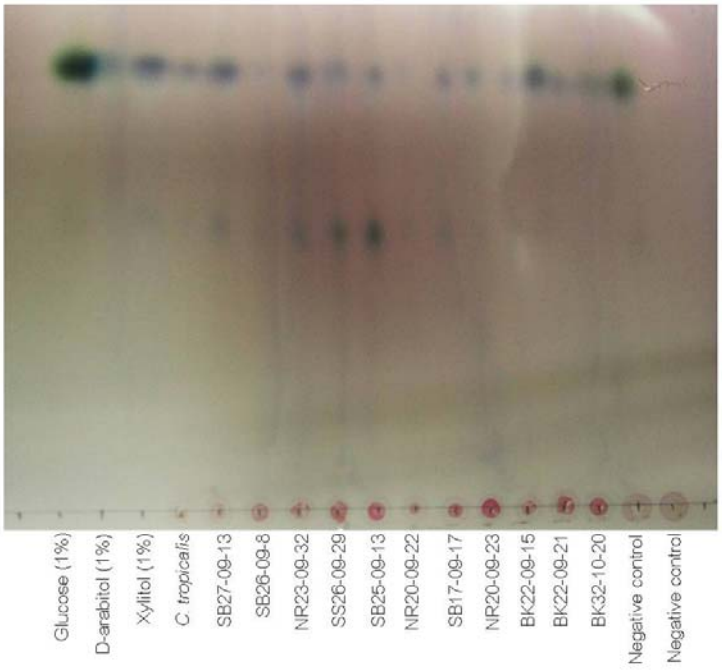


Fig. 6. TLC of sugar extracted from agar under colonies of yeast isolates. Yeasts were grown on $YEPD_3$ at 30 ^{o}C for 2 days. Only the upper part was swabbed by developing agent.

Table 2. Screening of bacteria for xylitol/D-arabitol producing strain by TLC

No.	Sources	No. of samples	No. of isolates		
			Total tested colonies	TLC positive	%
1.	Soils	75	413	40	9.7
2.	Fruits	141	3,473	467	13.4
3.	Vegetables	6	37	0	0
4.	Others	115	748	67	8.9
Total		**337**	**4,671**	**574**	**12.3**

Table 3. Screening of yeast for xylitol/D-arabitol producing strain by TLC

No.	Sources	No. of samples	No. of isolates		
			Total tested colonies	TLC positive	%
1.	Soils	114	674	165	24.5
2.	Fruits	513	8,301	725	8.7
3.	Vegetables	2	9	1	11.1
4.	Others	140	680	108	15.9
Total		**769**	**9,664**	**999**	**10.3**

2. Analysis of xylitol and D-arabitol in culture supernatant by HPLC

Most of isolates positive by TLC were cultivated and their 5 fold concentrated cell free supernatants were analysed for the presence of xylitol or D-arabitol by HPLC. Since retention time may change a bit from time to time, hence standard authentic D-arabitol and xylitol (1% w/v) solution were injected from time to time. In addition, for analysis of high xylitol or D-arabitol producing strains, concentrated supernatant alone, or mixed with either xylitol or D-arabitol where appropriate were analysed in pararel to confirm the nature of the product.

The HPLC profiles of 3 standard sugar alcohols (erythritol, xylitol and D-arabitol) at 2% (w/v) was shown in Fig 6. Under the analysed condition, erythritol was eluted first, (at 11.5 min) followed by xylitol and D-arabitol. Peaks of xylitol and D-arabitol were very close to each other at retention time of 17.5 and 19 min. The optimal condition for best separation of D-arabitol and xylitol was 90% acetonitrile in water as mobile phase, with column temperature at 40 ^{o}C and flow rate of 1 ml/min. Under this condition, glucose peak was not detected.

Acquired by	: Admin
Sample Name	: std
Sample ID	:
Vail #	: -1
Injection Volume	: 20 uL
Data File Name	: std.lcd
Method File Name	: xylitol.lcm
Batch File Name	:
Report File Name	: Default.lcr
Data Acquired	: 23/8/2553 9:31:56
Data Processed	: 23/8/2553 9:52:12

<Chromatogram>

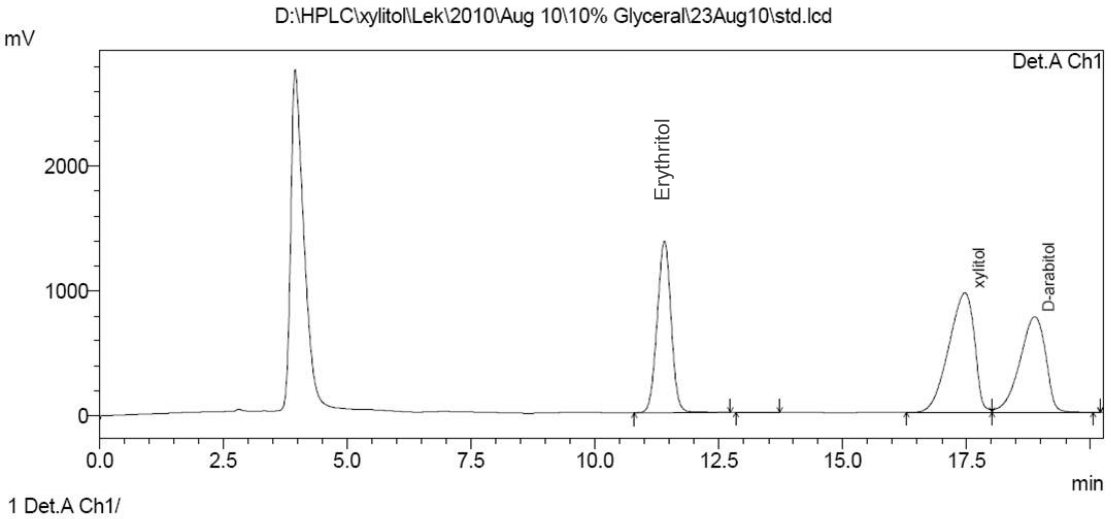
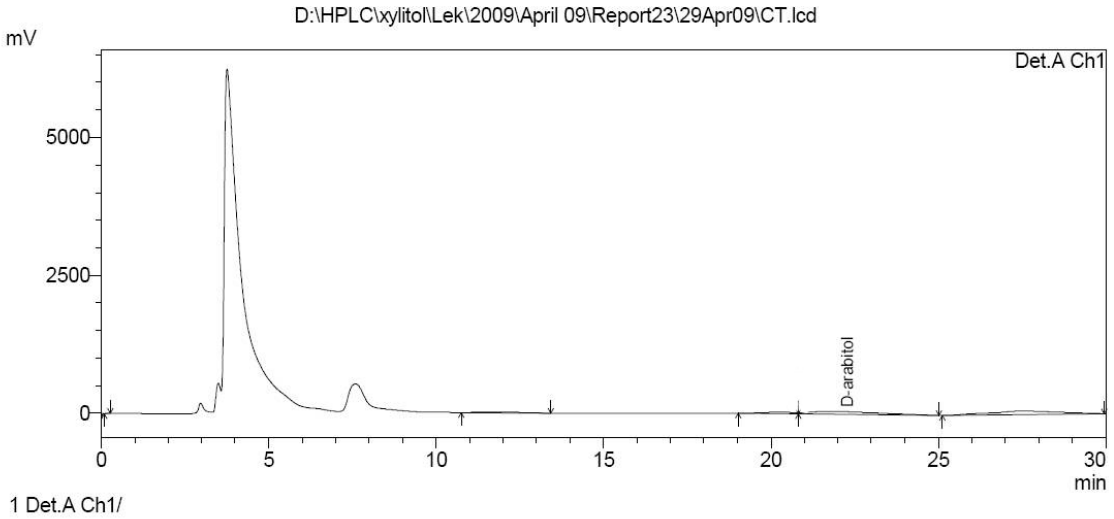


Fig. 6. HPLC profile of 2% each (w/v) of erythritol, xylitol and D-arabitol at retention times of around 11.5, 17.5 and 19 min, respectively. The first peak at around 4 min was H_2O peak.

When 5 fold concentrated supernatants from culture of bacteria or yeast isolates were analysed by HPLC, the peaks of xylitol and D-arabitol were seen at a broader profile. This might be due to the viscosity of concentrated supernatant. The presence of xylitol and D-arabitol was always confirmed by second analysis of the same sample by mixing the concentrated supernatant with equal amount of 2% xylitol and D-arabitol to obtain 1% of each standard sugar alcohol in the analysed sample. Using this analysis, though time consuming but we could identify the presence of xylitol or D-arabitol or both. The HPLC profile of the standard strain *C. tropicalis* DSM11953 (or ATCC7349) was shown in Fig. 7. The strain produced only D-arabitol from glucose at lower yield of 0.087%.

Acquired by : Admin
Sample Name : CT (*C. tropicalis* DSM11953 or ATCC7349)
Sample ID :
Vail # : 0
Injection Volume : 20 uL
Data File Name : CT.lcd
Method File Name : xylitol.lcm
Batch File Name :
Report File Name : Default.lcr
Data Acquired : 28/4/2552 13:08:53
Data Processed : 4/5/2552 15:25:38

<Chromatogram>



<Results>

Detector A

ID#	Name	Ret. Time	Area	Conc.	Units	1 Fold (0%)
1	xylitol	20.256	1950841	0.000	%	0.000
2	D-arabitol	21.861	7971530	0.435	%	0.087

Fig. 7. HPLC profile of 5 fold concentrated supernatant of *C. tropicalis* DSM11953 or ATCC7349. The yeast was grown in $YEPD_{10}$ (10% glucose) at 30 ^{o}C for 2 days.

Positive strains by TLC screening were then cultivated and 5 fold concentrated supernatant were analysed for the presence of both sugar alcohols by HPLC. Table 4 showed the number of analysed isolates, as well as number of strains producing xylitol and D-arabitol or both.

Table 4. HPLC analysis of xylitol/D-arabitol producing strains.

Microorganisms	No. of analysed strains*	No. of positive isolates for			
		Xylitol	D-arabitol	Both	None
Bacteria	187	18	5	0	164
Yeast	857	94	189	64	510
Total	1,044	112	194	64	674

*Strains which were obtained from preliminary screening by TLC.

Among the 857 positive yeast isolates positive by HPLC, 50 isolates with highest yield of xylitol was shown in Table 5. BK32-10-20, SB27-09-13 and NR33-10-37 showed highest xylitol yield at 0.334, 0.327 and 0.315% yield when they were cultivated in $YEPD_{10}$ (10%) glucose for 2 days. They all were isolated from grapes purchased in Bangkok or collected at Saraburi and Nakornrachasima vineyard, respectively. NR20-09-33, NR20-09-31, NR20-09-28, NR20-09-29 and NR20-09-21 were also isolated from grape collected at Nakornrachasima and they produced D-arabitol yield at 1.088, 1.464, 1.136, 1.221 and 1.456%, respectively. For *C. tropicalis* DSM11953 (or ATCC7349), it produced only D-arabitol at 0.087% (Table 5). The highest D-arabitol producers were shown in Table 6. Nine yeast isolates produced more than 1% D-arabitol yield from 10% glucose. Some strains also produced xylitol (Table 5 and 6).

For bacterial isolates, only 187 strains were analysed for xylitol/D-arabitol production by HPLC. Eighteen strains produced xylitol from glucose at lower yield (0.006% to 0.12%) as shown in Table 7. SP7-07-19, BK10-08-12 and BK26-09-16 produced about 0.1% of xylitol yield from 3% glucose. No strains accumulated D-arabitol (Table 7). Less number of bacteria strains were analysed since the sugar alcohol production was lower compared to those produced by yeast isolates.

Table 5. List of yeast isolates with highest xylitol yield. Cells were grown in $YEPD_{10}$ (10% glucose) at 30 ^{o}C for 2 days and analysed by HPLC.

No.	Strains	% yield	
		Xylitol	D-arabitol
1.	BK32-10-20	0.344	0.299
2.	SB27-09-13	0.327	0.030
3.	NR33-10-37	0.315	no peak
4.	SS26-09-8	0.261	no peak
5.	BK32-10-1	0.241	no peak
6.	NR23-09-32	0.232	no peak
7.	NR33-10-9	0.228	no peak
8.	SS26-09-29	0.226	no peak
9.	SB25-09-13	0.218	no peak
10.	NR20-09-22	0.202	0.915
11.	BK32-10-51	0.193	no peak
12.	NR33-10-10	0.193	no peak
13.	SB27-09-17	0.172	no peak
14.	NR33-10-11	0.171	no peak
15.	NR20-09-23	0.170	0.942
16.	BK22-09-15	0.163	no peak
17.	BK22-09-21	0.158	no peak
18.	SS26-09-7	0.156	no peak
19.	NR20-09-33	0.156	1.088
20.	NR20-09-9	0.155	0.133
21.	BK22-09-28	0.151	0.352
22.	BK32-10-52	0.133	no peak
23.	NR20-09-31	0.123	1.464
24.	NR20-09-30	0.121	0.988
25.	NR21-09-37	0.116	no peak
26.	NR22-09-26	0.111	0.461
27.	NR33-10-39	0.106	no peak
28.	NR33-10-2	0.104	no peak

Table 5. List of yeast isolates with highest xylitol yield. Cells were grown in $YEPD_{10}$ (10% glucose) at 30 ^{o}C for 2 days and analysed by HPLC (Continued).

No.	Strains	% yield	
		Xylitol	D-arabitol
29.	SS26-09-19	0.098	no peak
30.	BK20-09-3	0.093	0.512
31.	NR20-09-14	0.093	0.542
32.	NR33-10-35	0.093	no peak
33.	SB27-09-11	0.092	0.242
34.	BK19-09-12	0.089	0.655
35.	BK32-10-7	0.089	0.053
36.	BK19-09-13	0.087	0.866
37.	NR20-09-13	0.087	0.521
38.	NR33-10-34	0.087	no peak
39.	NR34-10-32	0.087	0.042
40.	NR20-09-28	0.086	1.136
41.	NR23-09-61	0.086	0.195
42.	BK20-09-2	0.085	0.508
43.	NR20-09-29	0.083	1.221
44.	NR34-10-16	0.082	no peak
45.	NR20-09-21	0.078	1.456
46.	NR21-09-38	0.072	no peak
47.	NR33-10-40	0.072	no peak
48.	NR21-09-39	0.072	no peak
49.	SB27-09-1	0.071	no peak
50.	NR33-10-15	0.071	no peak
51.	*C. tropicalis* ATCC7349 (DSM11953)	no peak	0.087

Table 6. List of yeast isolates with highest D-arabitol yield. Cells were grown in $YEPD_{10}$ (10% glucose) at 30 ^{o}C for 2 days and analysed by HPLC.

No.	Strains	% yield	
		Xylitol	D-arabitol
1.	NR20-09-31	0.123	1.464
2.	NR20-09-21	0.078	1.456
3.	BK20-09-5	0.017	1.437
4.	NR20-09-32	0.064	1.390
5.	NR20-09-20	no peak	1.341
6.	NR20-09-29	0.083	1.221
7.	BK20-09-17	0.030	1.154
8.	NR20-09-28	0.086	1.136
9.	NR20-09-33	0.156	1.088
10.	NR20-09-30	0.121	0.988
11.	BK32-10-19	no peak	0.961
12.	NR20-09-23	0.170	0.942
13.	NR20-09-22	0.202	0.915
14.	BK19-09-13	0.087	0.866
15.	BK32-10-30	no peak	0.807
16.	NR33-10-21	0.005	0.784
17.	NR20-09-12	0.012	0.781
18.	NR23-09-40	no peak	0.767
19.	NR20-09-17	no peak	0.717
20.	NR23-09-42	no peak	0.711
21.	NR20-09-15	no peak	0.695
22.	BK20-09-1	no peak	0.657
23.	BK19-09-12	0.089	0.655
24.	SS26-09-32	no peak	0.654
25.	BK32-10-23	no peak	0.631
26.	BK25-09-15	no peak	0.591
27.	NR20-09-14	0.093	0.542

Table 7. Xylitol and D-arabitol yield of bacterial isolates grown in YPG broth (3% glucose) at 30 ^{o}C for 24 h and analysed by HPLC.

No.	Strains	% yield	
		Xylitol	D-arabitol
1.	*G. oxdans* ATCC621	0.08	-
2.	SP7-07-19	0.120	0.000
3.	BK10-08-12	0.106	0.000
4.	BK26-09-16	0.100	0.000
5.	BK10-08-9	0.098	0.000
6.	RB13-08-4	0.091	0.000
7.	RB4-07-3	0.090	0.000
8.	BK26-09-13	0.090	0.000
9.	BK10-08-13	0.076	0.000
10.	RB13-08-16	0.073	0.000
11.	BK15-08-2	0.066	0.000
12.	BK15-08-7	0.061	0.000
13.	BK15-08-6	0.059	0.000
14.	SB20-08-7	0.055	0.000
15.	RB4-07-31	0.050	0.000
16.	BK15-08-3	0.049	0.000
17.	BK18-08-9	0.020	0.000
18.	RB12-08-9	0.016	0.000
19.	RB4-07-27	0.006	0.000

Examples of HPLC profile of 5 fold concentrated supernatant alone or mixed with xylitol and D-arabitol (1% w/v final concentration) were shown in Figs. 8-12. Fig. 8 was the HPLC profile of yeast strain BK32-10-20 which produced both xylitol and D-arabitol from glucose. Whereas Fig. 9 and Fig. 10 showed the HPLC profile of yeast strain NR23-09-32 and NR20-09-20 which produced only xylitol and D-arabitol from glucose, respectively.

For bacterial isolates, only few strains showed xylitol peak with very low yield. The isolates RB4-07-3 and BK26-09-16 showed small peak of xylitol at 0.087 and 0.099% (Figs 11 and 12). *G. oxydans* ATCC621 did not show any sugar alcohol peak when it was cultivated in media containing 1-2% glucose or 1-2% D-arabitol (data not shown).

Acquired by	: Admin
Sample Name	: BK32-10-20
Sample ID	:
Vail #	: -1
Injection Volume	: 20 uL
Data File Name	: BK32-10-20.lcd
Method File Name	: xylitol.lcm
Batch File Name	:
Report File Name	: Default.lcr
Data Acquired	: 3/2/2553 13:30:48
Data Processed	: 12/2/2553 12:59:42

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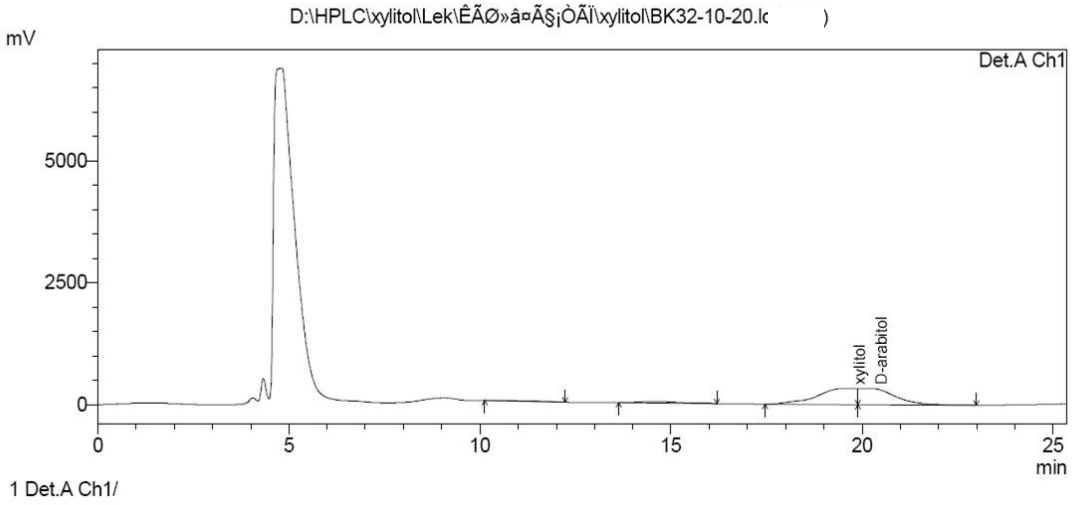

<Results>

Detector A

ID#	Name	Ret. Time	Area	Conc. 5 fold	Units	1 Fold (%)
1	Xylitol	19.580	23134367	1.719	%	0.344
2	D-arabitol	20.163	21255827	1.495	%	0.299

Fig. 8. HPLC profile of 5 fold concentrated supernatant from yeast strain BK32-10-20 grown in $YEPD_{10}$ (10% glucose) at 30 ^{o}C for 2 days. The calculated yield of xylitol and D-arabitol in supernatant was 0.344 and 0.299%, respectively.

Acquired by	: Admin
Sample Name	: NR23-09-32
Sample ID	:
Vail #	: 0
Injection Volume	: 20 uL
Data File Name	: NR23-09-32.lcd
Method File Name	: xylitol.lcm
Batch File Name	:
Report File Name	: Default.lcr
Data Acquired	: 9/4/2552 9:46:57
Data Processed	: 4/5/2552 15:10:19

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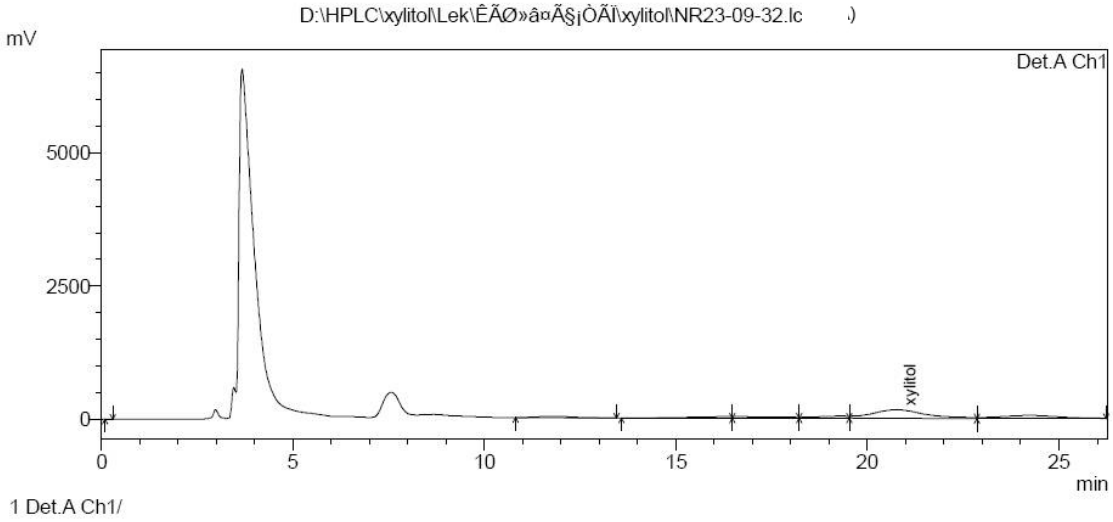

<Results>

Detector A

ID#	Name	Ret. Time	Area	Conc.	Units	1 Fold (%)
1	Xylitol	20.760	15710832	1.159	%	0.232
2	D-arabitol	0.000	0	0.000	%	0.000

Fig. 9. HPLC profile of 5 fold concentrated supernatant from yeast strain NR23-09-32 grown in $YEPD_{10}$ (10% glucose) at 30 ^{o}C for 2 days. This strain produces only xylitol. The calculated yield of xylitol in supernatant was 0.232%.

Acquired by	: Admin
Sample Name	: NR20-09-20 repeat
Sample ID	:
Vail #	: 0
Injection Volume	: 20 uL
Data File Name	: NR20-09-20 repeat.lcd
Method File Name	: xylitol.lcm
Batch File Name	:
Report File Name	: Default.lcr
Data Acquired	: 17/2/2552 17:03:39
Data Processed	: 17/2/2552 18:55:37

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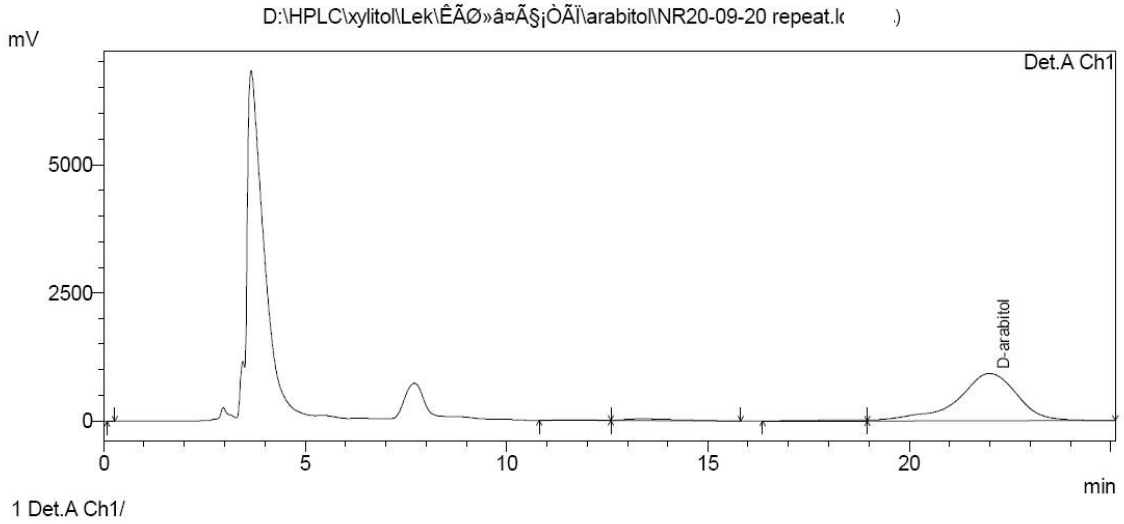


<Results>

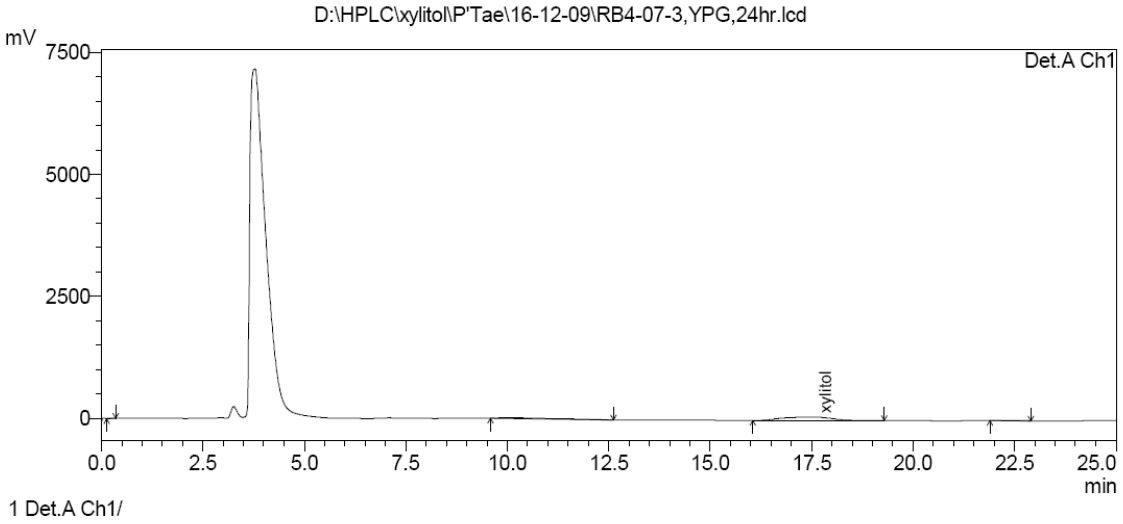
Detector A

ID#	Name	Ret. Time	Area	Conc.	Units	1 Fold (%)
1	Xylitol	0.000	0	0.000	%	0.000
2	D-arabitol	21.985	94172481	6.704	%	1.34

Fig. 10.HPLC profile of 5 fold concentrated supernatant from yeast strain NR20-09-20 grown in $YEPD_{10}$ (10% glucose) at 30 ^{o}C for 2 days. This strain produces only D-arabitol. The calculated yield of D-arabitol was 1.34%.

Acquired by	: Admin
Sample Name	: RB4-07-3,YPG,24 hr
Sample ID	:
Vail #	: -1
Injection Volume	: 20 uL
Data File Name	: RB4-07-3,YPG,24hr.lcd
Method File Name	: xylitol.lcm
Batch File Name	:
Report File Name	: Default.lcr
Data Acquired	: 11/12/2552 9:52:01
Data Processed	: 11/12/2552 10:17:02

<Chromatogram>



<Results>

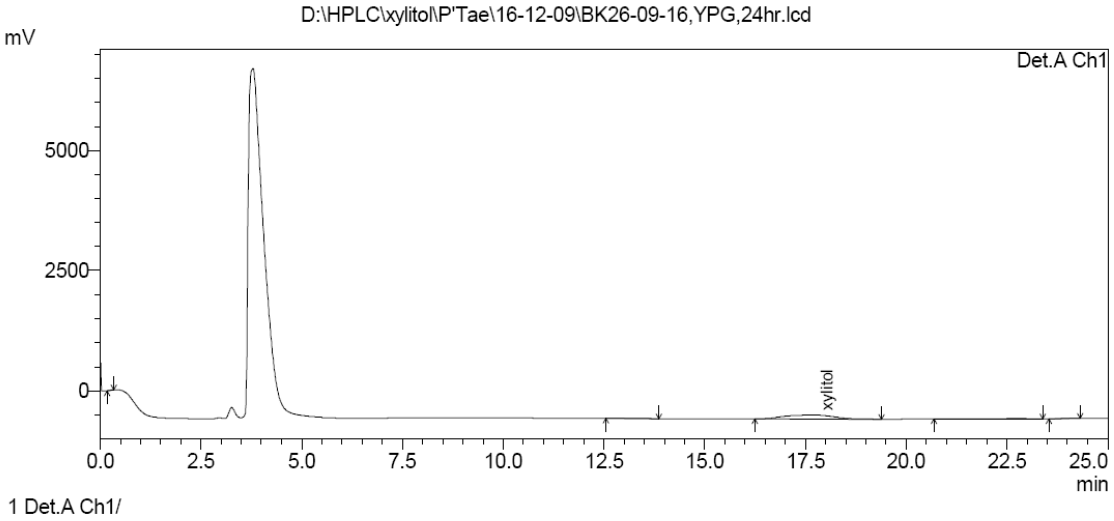
Detector A

ID#	Name	Ret. Time	Area	Conc.	Units	1 Fold (%)
1	Xylitol	17.490	6133000	0.437	%	0.087
2	D-arabitol	0.000	0	0.000	%	0.000

Fig. 11. HPLC profile of 5 fold concentrated supernatant of bacterial isolate RB4-07-3 grown in YPG at 30 ^{o}C for 24 h. The strain produces only xylitol at very low yield of 0.087%.

Acquired by	: Admin
Sample Name	: BK26-09-16,YPG,24 hr
Sample ID	:
Vail #	: -1
Injection Volume	: 20 uL
Data File Name	: BK26-09-16,YPG,24hr.lcd
Method File Name	: xylitol.lcm
Batch File Name	:
Report File Name	: Default.lcr
Data Acquired	: 11/12/2552 11:13:45
Data Processed	: 11/12/2552 11:38:46

<Chromatogram>



<Results>

Detector A

ID#	Name	Ret. Time	Area	Conc.	Units	1 Fold (%)
1	Xylitol	17.687	6893317	0.494	%	0.099
2	D-arabitol	0.000	0	0.000	%	0.000

Fig. 12. HPLC profile of 5 fold concentrated supernatant of bacterial isolate BK26-09-16 grown in YPG at 30 ^{o}C for 24 h. The strain produces only xylitol at very low yield of 0.099%.

3. Enzymatic assay

The proposed pathway for xylitol production from D-arabitol in *G. oxydans* by Suzuki et al (31) was that D-arabitol is converted to xylulose by D-arabitol dehydrogenase (ArDH) followed by reduction of D-xylulose to xylitol by xylitol dehydrogenase (XDH) which generates NAD from NADH (31). However, for assaying pentitol dehydrogenase (37), NADH will be formed from NAD when using D-arabitol or xylitol as substrate. We performed enzymatic assay of crude extract of bacterial

isolates and measured NADH formation. Comparison of pentitol dehydrogenase using D-arabitol as substrate and HPLC analysis of both sugar alcohols in culture supernatant was shown in Table 8. No correlation between enzymatic activity and yield of sugar alcohol's was observed.

Table 8. Comparison of pentitol dehydrogenase activity and % yield of sugar alcohol analysed by HPLC in bacterial isolates.

No.	Strain	Activity (U/ml) pentitol dehydrogenase	% yield by HPLC	
			Xylitol	D-arabitol
1.	RB4-07-1	4.920	no peak	no peak
2.	RB4-07-3	5.397	0.180	no peak
3.	RB4-07-9	5.800	no peak	no peak
4.	RB4-07-27	6.700	0.120	no peak
5.	RB4-07-29	9.600	no peak	no peak
6.	RB4-07-31	7.800	0.190	no peak
7.	SP6-07-6	6.700	no peak	no peak
8.	SP7-07-19	7.400	0.120	no peak
9.	SP7-07-24	13.300	no peak	no peak
10.	SP7-07-25	12.000	no peak	no peak
11.	SP7-07-27	13.200	no peak	no peak
12.	SP7-07-28	11.500	no peak	no peak
13.	NR8-08-1	14.800	no peak	no peak
14.	NR8-08-2	7.000	no peak	no peak
15.	NR9-08-2	5.500	no peak	no peak
16.	RB12-08-5	8.290	no peak	0.004
17.	RB12-08-9	11.020	0.016	no peak
18.	RB13-08-1	13.350	no peak	no peak
19.	RB13-08-2	9.830	no peak	no peak
20.	RB13-08-3	6.850	no peak	no peak
21.	RB13-08-4	6.520	0.091	no peak
22.	RB13-08-10	5.900	no peak	no peak
23.	RB13-08-11	7.640	no peak	no peak

Table 8. Comparison of pentitol dehydrogenase activity and % yield of sugar alcohol analysed by HPLC in bacterial isolates (Continued).

No.	Strain	Activity (U/ml) pentitol dehydrogenase	% yield by HPLC	
			Xylitol	D-arabitol
24.	RB13-08-12	4.270	no peak	no peak
25.	RB13-08-13	6.400	no peak	no peak
26.	RB13-08-14	8.760	no peak	no peak
27.	RB13-08-15	11.568	no peak	no peak
28.	RB13-08-16	3.320	no peak	no peak

Hexose reductase in crude extract from yeast was assayed by measuring reduction of NADH when glucose was used as substrate. No correlation between activity and product yield as analysed by HPLC (Table 9) was also found.

The use of crude extract and detection of changes of NADH which is the co-factor of many enzymes, together with reversible activity of ArDH, XDH possibly caused the uncorrelated result.

Table 9. Comparison of hexose reductase activity and D-arabitol/xylitol yield analysed by HPLC in yeast isolates.

No.	Strains	Activity (U/ml) Hexose reductase	% yield by HPLC	
			Xylitol	D-arabitol
1.	*Candida tropicalis*	24.577	No peak	0.087
2.	BK19-09-1	44.685	no peak	0.018
3.	BK19-09-2	55.856	no peak	no peak
4.	BK19-09-3	39.72	no peak	0.011
5.	BK19-09-4	48.409	no peak	0.009
6.	BK19-09-5	48.409	0.002	no peak
7.	BK19-09-6	24.329	no peak	no peak
8.	BK19-09-7	55.856	no peak	no peak
9.	BK19-09-8	71.744	no peak	no peak
10.	BK19-09-9	81.426	no peak	no peak
11.	BK19-09-10	26.315	0.002	0.124
12.	BK19-09-11	36.741	no peak	0.033
13.	BK19-09-12	114.443	0.089	0.655
14.	BK19-09-13	71.744	0.087	0.866
15.	BK20-09-1	77.702	no peak	0.657
16.	BK20-09-2	68.765	0.085	0.508
17.	BK20-09-3	65.042	0.093	0.512
18.	BK20-09-4	35.003	no peak	0.001
19.	BK20-09-5	36.493	0.017	1.437
20.	BK20-09-6	47.912	0.030	0.052
21.	BK20-09-7	26.811	no peak	no peak
22.	BK20-09-8	34.507	no peak	no peak
23.	BK20-09-9	41.458	0.004	no peak
24.	BK20-09-10	5.213	no peak	no peak
25.	BK20-09-11	21.101	no peak	0.070
26.	BK20-09-12	30.287	0.010	0.105
27.	BK20-09-13	54.863	0.022	0.112

Table 9. Comparison of hexose reductase activity and D-arabitol/xylitol yield analysed by HPLC in yeast isolates (Continued).

No.	Strains	Activity (U/ml) Hexose reductase	% yield by HPLC	
			Xylitol	D-arabitol
28.	BK20-09-14	22.343	0.009	0.094
29.	BK20-09-15	64.545	no peak	no peak
30.	BK20-09-16	0	no peak	no peak
31.	BK20-09-17	39.968	0.029	1.154
32.	NR20-09-1	44.933	0.052	no peak
33.	NR20-09-2	36.245	0.019	no peak
34.	NR20-09-3	46.175	0.031	no peak
35.	NR20-09-4	59.084	no peak	no peak
36.	NR20-09-5	32.769	no peak	no peak
37.	NR20-09-6	33.514	0.013	no peak
38.	NR20-09-7	27.804	0.027	no peak
39.	NR20-09-8	35.003	0.062	no peak
40.	NR20-09-9	44.189	0.155	0.133
41.	NR20-09-10	35.5	0.029	no peak
42.	NR20-09-11	53.622	0.015	no peak
43.	NR20-09-12	39.224	0.012	0.781
44.	NR20-09-13	42.947	0.087	0.521
45.	NR20-09-14	26.563	0.093	0.542
46.	NR20-09-15	31.28	no peak	0.695
47.	NR20-09-16	52.629	no peak	0.463
48.	NR20-09-17	49.402	no peak	0.717
49.	NR20-09-18	36.741	no peak	no peak
50.	NR20-09-19	21.101	no peak	no peak
51.	NR20-09-20	56.353	no peak	1.341
52.	NR20-09-21	46.175	0.078	1.456
53.	NR20-09-22	50.147	0.202	0.915
54.	NR20-09-23	40.713	0.17	0.942

Table 9. Comparison of hexose reductase activity and D-arabitol/xylitol yield analysed by HPLC in yeast isolates (Continued).

No.	Strains	Activity (U/ml) Hexose reductase	% yield by HPLC	
			Xylitol	D-arabitol
55.	NR20-09-24	53.126	no peak	0.229
56.	NR20-09-25	48.409	no peak	0.222
57.	NR20-09-26	62.311	no peak	0.19
58.	NR20-09-27	44.437	no peak	0.282
59.	NR20-09-28	27.804	0.086	1.136
60.	NR20-09-29	36.493	0.083	1.221
61.	NR20-09-30	42.699	0.121	0.988
62.	NR20-09-31	33.514	0.123	1.464
63.	NR20-09-32	44.437	0.064	1.39
64.	NR20-09-33	50.147	0.156	1.088

4. Optimization for xylitol production in yeast

Preliminary optimization of some selected yeast strains for their xylitol production was determined. Glucose, glycerol or its mixture as well as high concentration of xylose were used as the C-source. It was found that the 6 selected yeast strains can produced from 10% glucose at 0.2-0.9% but they could convert 50% xylose to 28-47% xylitol.

4.1 Effect of glucose, glycerol or its mixture

Six yeast strains which produced high yield of xylitol were selected. They were grown in media containing either glucose, glycerol or mixture. They were grown at either 28, 30 and 32^{o}C for 2 days in 5 ml YEPD10 broth (10% glucose, 1% yeast extract and 2% peptone). The culture condition was a limited aeration by using test tube (stand upward) and shaken at 160 rpm. Sugar alcohol in culture broth was analysed by HPLC.

BK32-10-20 produced high xylitol yield at 0.7-0.8% at all three cultivation temperatures. Other four strains (SB27-09-13, SS26-09-08, NR23-09-32 and SS26-09-29) also produced highest xylitol (0.2-0.4%) when they were cultured at these three cultivation temperatures. SB27-09-13 produced a good xylitol yield at 28^{o}C and low xylitol yield at 30 or 32 ^{o}C whereas NR20-09-22 accumulated D-arabitol (0.9%) at 28 ^{o}C (Table 10). Most strains did not accumulated D-arabitol under these conditions.

Table 10. Effect of glucose and temperature

Yeast strains	Xylitol yields (%)			D-arabitol yield (%)		
	28 °C	30 °C	32 °C	28 °C	30 °C	32 °C
1. BK32-10-20	0.869	0.868	0.767	-	-	-
2. SB27-09-13	0.438	0.027	0.061	-	-	-
3. SS26-09-08	0.328	0.296	0.435	-	-	-
4. NR23-09-32	0.276	0.349	0.254	-	-	-
5. SS26-09-29	0.295	0.204	0.337	-	-	-
6. NR20-09-22	-	0.006	-	0.986	-	-

(-) : no peak

Yeast strains were grown on 5 ml of YEPD5 supplemented with 5% glycerol broth (5% glucose, 5% glycerol, 1% yeast extract, and 2% peptone), incubated at 28, 30 and 32 °C with shaking at 160 rpm for 2 days. When 5% glycerol was supplemented in the presence of 5% glucose, xylitol yield was drastically reduced (Table 11).

Table 11. Effect of glucose (5%) plus glycerol (5%) and temperature

Yeast strains	Xylitol yields (%)			D-arabitol yield (%)		
	28 °C	30 °C	32 °C	28 °C	30 °C	32 °C
1. BK32-10-20	0.126	0.101	0.006	-	-	-
2. SB27-09-13	0.012	-	0.308	-	-	-
3. SS26-09-08	-	-	-	-	-	-
4. NR23-09-32	-	-	-	-	-	-
5. SS26-09-29	-	-	-	-	-	-
6. NR20-09-22	0.065	-	-	-	0.806	-

(-) : no peak

4.2 Effect of cultivation period

BK32-10-20 was selected and analysed its abitlity to accumulate xylitol when it was grown in YPD10 (10% glucose) for 5 days. Result in Table 12 showed that, this strain converted glucose to xylitol at highest yield (0.894%) after growing for 24 h. Prolonged incubation slightly reduced the xylitol yield.

Table 12. Effect of cultivation period on xylitol production.

Yeast strain **BK32-10-20**	Xylitol yield (%)	D-arabitol yield (%)
Day 1	0.894	-
Day 2	0.795	-
Day 3	0.745	-
Day 4	0.642	-
Day 5	0.585	-

The highest xylitol-producing yeast (BK32-10-20) was grown in 5 ml of YEPD10 broth (10% glucose, 1% yeast extract, and 2% peptone), incubated at 30 ^{o}C with shaking at 160 rpm for 5 days. At every 24 hours, the supernatant was collected for analysis of the accumulated xylitol in supernatant.

Note : HPLC condition for separation of glucose and sugar alcohol (D-arabitol and xylitol) was used to analyse the concentration of sugar alcohol in 5 fold concentrated supernatant.

Mobile phase : 90% acetronitrile in water

Column temperature : 40 ^{o}C

Flow rate : 1 ml/min

4.3 Effect of xylose and temperature

Six yeast strains (BK32-10-20, SB27-09-13, SS26-09-08, NR23-09-32, SS26-09-29 and NR20-09-22) were grown in 5 ml of YEPD 1(1% dextrose, 1% yeast extract and 2% peptone) as preculture, and then further cultivated in YEPD1 supplemented with 50% xylose with shaking until 24 hrs and subjected to HPLC analysis. Cultivation temperatures were done at 28 ^{o}C, 30 ^{o}C, and 32 ^{o}C.

Xylose at high concentration (50%) was mixed with YPD1 (1% dextrose, 1% yeast extract and 2% peptone) in main culture and xylitol yield was analysed. Culture was grown at 28, 30 and 32 ^{o}C for 2 days. The preliminary result as shown in Table 13 suggested that among the 6 selected yeast isolates, 3 strains SB27-09-13, SS26-09-29 and NR20-09-22 produced highest xylitol yield of 37.6, 47.6 and 47.3%, respectively at 28 ^{o}C. Whereas strains BK32-10-20, SS26-09-08 and NR23-09-32 produced highest xylitol yield at 45.5, 41.3 and 36.9%, respectively at 32

°C. It is clearly shown that these yeast isolates could efficiently convert xylose (50%) to xyltiol at very high yield (73.8-94.6%).

Table 13. Analysis of yeast cells for conversion of 50% xylose to xylitol.

Strain	% yield in 50% xylose at 28 °C		% yield in 50% xylose at 30 °C		% yield in 50% xylose at 32 °C	
	Xylitol	D-arabitol	Xylitol	D-arabitol	Xylitol	D-arabitol
1. BK32-10-20	37.450	no peak	36.400	no peak	45.550	no peak
2. SB27-09-13	37.650	no peak	26.750	no peak	29.600	no peak
3. SS26-09-08	35.300	no peak	28.000	no peak	41.300	no peak
4. NR23-09-32	31.550	no peak	30.700	no peak	36.900	no peak
5. SS26-09-29	47.600	no peak	41.550	no peak	31.350	no peak
6. NR20-09-22	47.300	no peak	46.150	no peak	32.450	no peak

5. Molecular identification of yeast and bacterial isolates

5.1 Molecular analysis of bacterial isolates

Both *G. oxydans* ATCC621 and BK21-09-9 were identified by analysis of its 16S rRNA gene (~500 bp). Surprisingly identification of both isolates was reported as highest identity to *Delfia* sp. as shown in Table 14. SP7-07-19 was identified as *Alcaligenes faecalis*.

Table 14. BLASTN analysis of 16S rDNA gene sequence of bacterial isolates.

Strains	Accession	Description	Identity
G. oxydans ATCC621	AM930326.1	Uncultured bacterium partial 16S rRNA gene, clone SMQ25	99%
	FJ378038.1	*Delftia* sp. JDC-3 16S ribosomal RNA gene, partial sequence	99%
BK21-09-9	EU073078.1	*Delftia* sp. TS12 16S ribosomal RNA gene, partial sequence	98%
	AM930326.1	Uncultured bacterium partial 16S rRNA gene, clone SMQ25	98%
SP7-07-19	GU181289.1	*Alcaligenes faecalis* strain WT10	100%

5.2 Molecular analysis of yeast isolates

Analysis of 26S rRNA gene (500 bp) of 2 selected isolates, NR20-09-21 and NR20-09-22 was suggested that the two isolates are closely related to *C. albicans* (Table 15) with 98-99% identity. For yeast strain BK32-10-20 which produced highest xylitol yield from glucose, it was identified as *Kodamaea ohmeri* (*Pichia ohmeri*).

Both strains of NR20-09-21 and NR20-09-22 did not produce chlamydoconidia which is the unique characteristic of *C. albicans*, hence the two strains are not *C. albicans* and they are likely identified as *C. tropicalis* from microscopic morphology, growth and biochemical characteristics (Table 16 and Table 17).

Table 15. BLASTN analysis of 26S rRNA gene sequence of NR20-09-21, NR20-09-22 and BK32-10-20

Strains	Accession	Description	Identity
NR20-09-21	GU319992.1	*Candida albicans* strain ATCC 14503 26S ribosomal RNA gene, partial sequence	99%
	AF156536.1	*Candida albicans* strain AA1622b 26S ribosomal RNA gene, partial sequence	98%
NR20-09-22	AF156536.1	*Candida albicans* strain AA1622b 26S ribosomal RNA gene, partial sequence	99%
	AY518284.1	*Candida albicans* strain SCC37 26S ribosomal RNA gene, partial sequence	99%
BK32-10-20	FM180533.1	*Kodamaea ohmeri* partial 26S rRNA gene, isolate H2S0K2	99%
	AB500890.1	*Kodamaea ohmeri* gene for 26S rRNA partial sequence, strain: CPB5	99%

Table 16. Microscopic morphology of yeasts and yeast-like fungi on cornmeal or glutineous rice tween (GRT) agar.

Organism	Pseudo-hyphae	True hyphae	Blastoconidia or yeast	Arthro-conidia	Annclio-conidia	Chlamydo-conidia	Ascospores
NR20-09-21	+	-	+	-	-	-	-
NR20-09-22	+	-	+	-	-	-	-

Conclusion: *Candida* spp. (due to characteristic of yeast and pseudohyphae production without chlamydoconidia) and they are not *Candida albicans*.

Table 17. Growth and biochemical characteristics of yeast No.21 and No.22

							Assimilation of:											Fermentation of:									
	Growth at 37°C	Pellicle in broth	Pseudohyphae or true hyphae	Chlamydoconidia	Germ tube	Capsule, India ink	Glucose	Maltose	Sucrose	Lactose	Galactose	Melibiose	Cellobiose	Inositol	Xylose	Raffinose	Trehalose	Dulcitol	Glucose	Maltose	Sucrose	Lactose	Galactose	Treahalose	Urease	KNO_3 utilization	Phenol oxidase	Ascospores
NR20-09-21	+	-	+	-	-	-	+	+	+	-	ND	ND	ND	-	ND	ND	ND	ND	F	F	F	-	-	-	-	ND	-	-
NR20-09-21	+	-	+	-	-	-	+	+	+	-	ND	ND	ND	-	ND	ND	ND	ND	F	F	F	-	-	-	-	ND	-	-

Conclusion: *Candida tropicalis* (some strains of *Candida tropicalis* do not ferment galactose and trehalose)

Sugar assimilation = use of sugar as energy source

Sugar fermentation = use of sugar with generation of gas from sugar fermentation

Discussion

A large scale screening of 9,664 and 4,671 yeast and bacterial isolates by Thin layer chromatography (TLC) and analysis of xylitol and D-arabitol by High Performance liquid chromatography (HPLC) of 5 fold concentrated supernatant of 857 and 187 strains yeast and bacterial isolates, respectively were performed from the microbial cultures grown in glucose medium. Ten and three percent glucose were used to culture yeasts and bacteria, respectively. If

more than 3% glucose was used, this high concentration of glucose inhibited growth of bacterial isolates. In this effort on screening for xylitol producers, a few points are discussed below.

1. Optimization of TLC and HPLC for simultaneous screening and detection of D-arabitol and xylitol

1.1 TLC method

Most described TLC methods were used for detection of aldose sugars. We have tested many TLC conditions and found that Silica gel 60 TLC plate with the solvent system of Acetonitrile : Acetic acid : Distilled water = 63:33:5 (v/v) and detection system of p-anisaldehyde : sulfuric acid: ethanol = 1:1:18 (v/v) gave the best result. We have modified the protocol by running TLC (ascending type) twice, swabbing detection solvent and heated on hot plate instead of using hot air oven. Under this condition, D-arabitol gave the reddish purple red while xylitol gave the bluish spot. D-arabitol moves faster than xylitol and glucose. Xylitol moves a bit faster than glucose but still overlapped. Observation of color development should be done quickly since after cooling, all spots will turn into dark green color similar to glucose spot.

Extraction of sugar from agar under colonies was effectively used for determining sugar alcohol accumulated under each microbial colonies. Using this method together with TLC, rapid screening of microbial isolates was successfully done in a large scale.

1.2 HPLC method

From literature search, several types of HPLC columns and conditions were used for analysis of sugar alcohol such as Shodex SC1211 (Showa Denko, Japan) using column temperature at 60 ^{o}C, 50% acetonitrile in 50 mg/l Ca-EDTA at a flow rate of 0.8 ml/min (31), Aminer HPX-87P column (Biorad, USA) using column temperature at 85 ^{o}C and eluted with deionized water at a flow rate of 0.6 ml/min (39). We have selected Shodex Asaki Pak NH_2P-50 4E column (Showa, Denko, Japan) since they advertises the column capacity to separate D-arabitol and xylitol. Optimal concentration of acetonitrile in water was tested using HPLC instrument and RI detection system we have (Shimadzu, Japan). We found that using the column temperature at 40 ^{o}C with a flow rate of 1 ml/min with 90% acetonitrile in water was the best condition to separate D-arabitol and xylitol peak from each other. For glucose detection, 75% acetonitrile in water was used. Under the latter condition, D-arabitol and xylitol peak was accumulated at the overlapped peak. Glucose was not detected when 90% acetonitrile in water was used as mobile phase.

2. Sources of microbes

Various types of samples such as fruit, soil, vegetable, fruit juices were collected. Fruit samples gave the best result for isolation of both bacteria and yeasts for sugar alcohol production.

Among fruit samples, grape samples yielded the microbial isolates which produced D-arabitol and xylitol or both sugar alcohols. *Gluconobacter* strains were the bacterial group reported to flourish in sugary niches such as ripen grapes, apples and dates (40). Sugar alcohol producing yeasts were isolated from soil (13), honey beehives (39, 41) and flowers (41) which are reported to convert glucose to D-arabitol.

3. Enzymatic assay for pentitol dehydrogenase and hexose reductase

Based on the assay system (37), NADH reduction and NADH formation were monitored for hexose reductase and pentitol dehydrogenase, respectively. We found that enzyme activity did not correlate with xylitol/D-arabitol yield. This might be derived from the fact that NAD and NADH are the co-factors of many enzymes, therefore the conversion between NAD and NADH are utilized by many enzymes. Hence using the crude extract with either glucose on D-arabitol as the substrates, changes of NAD and NADH are influenced by various enzymes which did not reflect the xylitol/D-arabitol yield.

4. Preliminary optimization of xylitol production from yeast

Preliminary culture conditions of selected yeast strains were tested to observe their effect on xylitol accumulation. Six selected yeast isolates were cultivated in medium containing glucose or xylose and cultivated at different temperatures (28, 30, 32 ^{o}C). It is found that limited aeration (ie cultivation in testtube with low speed shaking) gave higher xylitol/D-arabitol yield (upto ~0.9%) than using shaked flask culture. All strains coverted 50% xylose to xylitol at high yield (~90-94%). For glucose medium, the yeast strain BK32-10-20 which was identified as *Kodamaea ohmeri* produced highest xylitol yield (~0.9%) from 10% glucose. This yeast species was reported to produce D-arabitol (8.1%) from 20% glucose (41). Hence, optimization of culture condition using these yeast strains may increase the xylitol production from either glucose or xylose.

5. Future prospect

As stated above, optimization of culture condition might be performed to increase xylitol/D-arabitol yield by selected yeast isolates. Cloning of genes involved in xylitol/D-arabitol production might also enhance the sugar alcohol yield. (25, 26, 28-30). Metabolic engineering of *S. cerevisiae* allows the strain to convert D-glucose to xylitol (27). Metabolically engineered *E. coli* strains were also reported to produce xylitol from glucose-xylose mixture (42) or L-arabinose (43). Hence, stain construction by metabolic engineering should play an important role in optimization and increasing yield of xylitol / D-arabitol production.

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ภาคผนวก

Output จากโครงการวิจัยที่ได้รับทุนจาก สกว.

1. ผลงานตีพิมพ์

ขณะนี้ยังไม่มีผลงานตีพิมพ์ กำลังอยู่ในระหว่างการจัดเตรียม manuscript เรื่อง “Production of xylitol from glucose and xylose by a newly isolated *Kodamaea ohmori*”

2. การนำผลวิจัยไปใช้ประโยชน์

ยังไม่มี ขณะนี้กำลังอยู่ในช่วงที่จะต้องศึกษาหาสภาวะที่เหมาะสมในการผลิต xylitol จาก xylose ซึ่งให้ yield สูงกว่าการผลิต xylitol จาก glucose ซึ่งบริษัท Ueno Fine Chemicals company สนใจและให้ทุนนักศึกษาทำวิจัยต่อ

3. ผลงานอื่น ๆ

3.1 การผลิตนักศึกษา

- นักศึกษาระดับปริญญาโท 2 คน ได้เข้ามามีส่วนในการร่วม screen เชื้อและทำวิจัยในส่วนของการทำ molecular cloning

จบการศึกษาแล้วเมื่อวันที่ 12 เมษายน 2553

นางสาวพัชราภรณ์ อมรสิน ทำวิทยานิพนธ์เรื่อง “Cloning and expression of genes involved in xylitol production from acetic acid bacteria”

กำลังศึกษา

นางสาวรับวาสน์ มธุรันยานนท์

ทำวิทยานิพนธ์เรื่อง “Cloning gene involved in xylitol production”

3.2 การเสนอผลงานในที่ประชุม

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