OnLine Journal of Biological Sciences 1 (11): 1005-1008, 2001
© Asian Network for Scientific Information 2001

The Properties of *Candida famata* R28 for D-Arabitol Production from D-glucose

Zakaria Ahmed
Department of Biochemistry and Food Science, Faculty of Agriculture,
Kagawa University, Kagawa 761-0795, Japan

Abstract: *Candida famata* R28 showed potential growth at 36 h cultivated cells (absorbance 40 at 600 nm) for D-arabitol production on substrate concentration 5~20%. Among the various carbohydrates, highest growth was observed in medium containing D-glucose. In the production of D-arabitol, reaction gave 5.0% D-arabitol from 10.0% D-glucose without producing any by-product. The product was finally identified by HPLC analysis.

Key words: *Candida famata*, D-Glucose, D-Arabitol

Introduction

Using microbial process to produce monosaccharides is more effective than that of chemical ones. Because usually, in the chemical reaction, the yield of the product is low, the by-products which are impurities often found in the product, and it needs many steps for completion of reactions. On the other hand, microbial reactions are suitable for the production of sugars, in which the yield is high and there is a few by-products in the reaction product (Izumori and Bhuiyan, 1997; Ahmed, 2001; Ahmed and Bhowmik, 2000). Extensive studies have been carried out on the production of polyols, such as glycerol, erythritol, xylitol, D-arabitol and D-mannitol, during the fermentation of soy sauce by halotolerent yeasts (Onishi and Suzuki, 1966; 1968; 1969; 1970; Jennings, 1984) and it was revealed that *Candida* sp. was one of the most potent micro organisms for D-arabitol production (Bernard *et al.*, 1982; Gold *et al.*, 1983; Kiehn *et al.*, 1979; Wong and Brauer, 1988). Saha and Bothast (1996) reported that L-arabitol could also be obtained from L-arabinose by *Candida entomaea* and *Pichia guilliermondii* (Ahmed, 2001). In laboratory, a fungal culture, named *Candida famata* R28, was isolated from soy sauce mash and characterized as a halotolarent yeast. The strain can produce D- talitol from D-psicose and possess various reduction potentials of ketoses to polyols (Sasahara *et al.*, 1998). Usually, intact cells were used to produce polyols and ketoses using polyol dehydrogenases (Izumori and Bhuiyan, 1997). To produce enough amounts of monosaccharides, we have to use natural substrates which are abundant in nature as starting materials. Thus D-glucose is very much cheap, abundant in nature (Izumori and Bhuiyan, 1997; Ahmed, 2001) and indeed, isomerization of the D-glucose and D-fructose, known as high-fructose corn syrup (HFCS), is one of the most important industrial production of a sugar sweetener. HFCS is used as an economical substitute for sucrose particularly in soft drinks. Moreover, for the optimum production of the sugar, the characterization and optimization of microbial growth is necessary. Therefore, the project was under taken to study the properties of *C. famata* R28 for D-arabitol production from D-glucose (Fig. 1).

Materials and Methods

D-Glucose, D-mannitol, polypepton, D-arabitol and yeast extract were obtained from Difco Laboratories (USA). D-Glucose oxidase was purchased from Japan and peroxidase (Horseradish) from Wako Chemical Industries Japan. 2,2'-Azino-di(3-ethylbenzthiazoline) (ABT), chloroform and other carbohydrates were purchased from Sigma Chemical Co. (USA).

Microorganism and cultivation condition: *Candida famata* R28 was cultivated in a medium containing 0.3% yeast extract, 0.5% polypepton and 10.0% D-glucose at 30°C with continuous shaking (280 rpm) as described previously (Sasahara *et al.*, 1998).

Analytical method: Accumulation of aldose in the reaction mixture was detected by the method of glucose oxidase (Herbert *et al.*, 1971) and high-performance liquid chromatography (HPLC, Nihonbunko HPLC 880 PU liquid chromatography, Shimadzu RID-6A refractive index detector and Shimadzu C-R6A chromatopac using a Hitachi HPLC column GL-611) analysis. HPLC separation was achieved at 60°C using 10mM NaOH at a flow rate of 1.0 ml/ min.

Determination of suitable growth carbon: The cells were grown aerobically with continuous shaking for 36 h at 30°C in test tubes (18 × 180 mm^2, 5.0 ml/tube) containing medium of the following composition: 0.5% polypepton, 0.5% yeast extract, 0.5% NaCl and 1.0% sugar or polyol. Test for growth was determined by measuring the absorbance at 600 nm.

Transformation at different cell concentration: *C. famata* R28 was grown aerobically with continuous shaking at 30°C in tubes containing medium described previously for 36 h. After growth, the cells were harvested by centrifugation at 9,000 rpm for 10 min. In order to determine the suitable cell concentration of *C. famata* R28 for optimum conversion of D-glucose to D-arabitol, reaction was carried out in a reaction mixture of 5.0% D-glucose, 0.05 M glycine-NaOH buffer (pH 9.0) and washed cells suspension made in the same buffer which had an absorbance 5~40 at 600 nm (total volume of 5.0 ml) in a L tube at 30°C with shaking (170 rpm). The concentration of remaining D-glucose in the reaction mixture was measured with time interval by glucose oxidase method (Herbert *et al.*, 1971) at 30°C for 15 min in absorbance of 438 nm and also by HPLC analysis. The enzyme solution for glucose oxidase method were composed of the following composition: glucose oxidase (40 U/mg), 60 mg; peroxidase (100 U/mg), 6.0 mg; 2,2-Azino-di (3-ethylbenzthiazoline) [ABT], 50.0 mg; chloroform, 2 drops and 0.1 M Na-Phosphate buffer (pH 7.0), 250.0 ml.

Production of D-arabitol: The cells of *C. famata* R28, after cultivation in above described medium for 36 h at 30°C, was harvested by centrifugation at 9,000 rpm for 10 min, washed twice with 0.05 M glycine-NaOH buffer (pH 9.0) and used for the production of D-arabitol from D-glucose. The washed cell reaction of *C. famata* R28 was carried out in a 500-ml Erlenmeyer flask at 30°C with shaking (170 rpm). The

composition of the reaction mixture was as follows: 10.0 g D-glucose, 50.0 ml of 0.1 M glycine-NaOH buffer (pH 9.0), 50.0 ml of washed cells suspension made in the same buffer which had an absorbance 80 at 600 nm (total volume of 100.0 ml). The rate of transformation of D-glucose and accumulation of D-arabitol in the reaction mixture were detected by the method of glucose oxidase (Herbert *et al.*, 1971) and HPLC analysis.

Identification of product: The product was identified by HPLC analysis and the result was compared with those of authentic D-arabitol.

Results

Effect of carbon source *C. famata* R28 was grown on various carbohydrates (1.0%) in order to determine the most suitable growth carbon source for conversion of D-glucose to D-arabitol. Among various carbohydrate tested, *C. famata* R28 showed good growth only in a medium containing 1.0% D-glucose, followed by those grown on L-sorbose, trehalose, D-mannose, D-maltose, L-arabinose and D-sorbitol (Table 1).

Effect of cultivation time: Cells of *C. famata* R28, grown for 12, 24, 36, 48, 60 and 72 h, were used to observe the effect of culture time on the conversion of D-glucose to D-arabitol. The D-arabitol production was measured by glucose oxidase method (Herbert *et al.*, 1971) by decrease in absorbance of D-glucose concentration at 438 nm and also by HPLC analysis. Cells grown for 36 h showed the highest conversion activity, followed by cells grown for 48 h (Fig. 2). However the cell yield of strain R28 at 36 and 48 h were same but significantly higher at 60 h. A culture time shorter than 36 h was found to be unsuitable for conversion. Therefore 36 h of cultivation was found suitable for D-arabitol production.

Effect of cell and substrate concentration: In the cell reaction system, suitable cell concentration of *C. famata* R28 and substrate concentration of D-glucose was optimized with respect to the potential conversion of D-glucose to D-arabitol. Cells with the absorbance of 40 at 600 nm showed most potent for the conversion of D-glucose to D-arabitol after about 80 h (Fig. 3).

Reaction was carried out in 5~20% substrate of D-glucose for the conversion of D-arabitol in the same transformation condition described previously. D-glucose conversion was prolonged with the increase of substrate concentration and can convert up to 20.0% D-glucose to D-arabitol (Fig. 4).

Effect of reaction mixture pH: Effects of pH on D-glucose degradation were studied using the following buffers (0.05 M); citrate buffer (pH 3.0-5.0), maleate buffer (pH 5.0-7.0), Na-Phosphate buffer (pH 6.0-8.0), Tris-HCl buffer (pH 7.0-9.0) and glycine-NaOH buffer (pH 9.0-11.0) in the reaction mixture. Transformation conditions was as follows: D-glucose, 5.0%; 0.05 M various buffers and cells concentration of *C. famata* R28 was adjusted at 40 in absorbance of 600 nm. Product accumulation in the reaction mixture was determined as described in methods and materials. D-arabitol production in the reaction mixture of various pHs. D-Arabitol production was the highest when the reaction mixture was carried out at an initial pH of 9.0 using the 0.05 M glycine-NaOH buffer (Fig. 5).

Confirmation of the product: The product was confirmed by analysis of the HPLC measurements. The HPLC retention time of the product was indistinguishable from that of authentic D-arabitol (Fig. 6). It should be noted that although all the D-glucose (Fig. 4) was degraded, HPLC analysis revealed that about half of D-glucose was converted into D-arabitol, the rest being consumed by the cells. Considering above results, the product formed from D-glucose was identified as D-arabitol.

Table 1: Growth and pH of *Candida famata* R 28 on various carbohydrates

Carbohydrates	Growth (Absorbance 660nm)	pH
L-Sorbose	9.40	3.82
Trehalose	9.80	4.00
D-Mannose	8.20	3.69
D-Maltose	8.20	3.69
D-Sorbitol	6.30	4.17
D-Mannitol	5.20	4.09
Glycerol	5.10	4.19
D-Fructose	5.00	3.67
Erythritol	4.70	4.10
D-Glucose	13.00	3.60
Sucrose	3.90	4.07
Galactose	0.80	3.87
D-Arabinose	0.10	6.75
L-Arabinose	7.20	5.63
D-Ribose	4.50	5.48

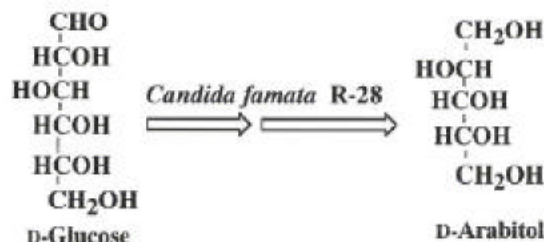

Fig. 1: Schematic diagram for the production of D-arabitol from D-glucose

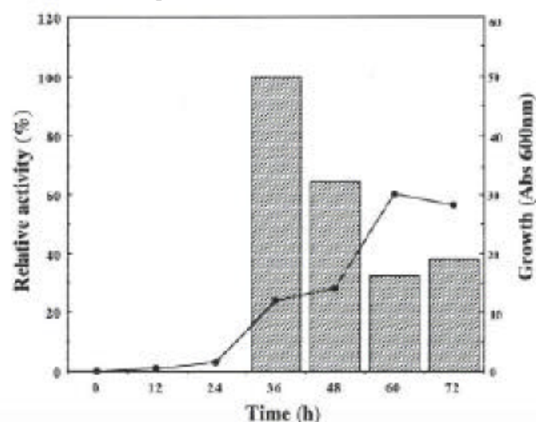

Fig. 2: Effect of culture time on the growth and D-arabitol production potential of *Candida famata* R 28. Symbols: □, Relative activity: ● Growth.

Discussion

In one report it was proposed that *Candida* spp. produced D-arabitol from D-ribulose with NAD-dependent D-arabitol dehydrogenase in which D-ribulose was derived by

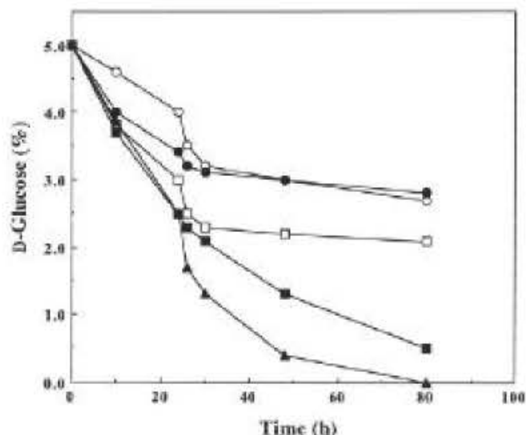

Fig. 3: Degradation of D-glucose using different cell concentration of *Candida fomata* R28, Symbols: ○, Abs = 5; ●, Abs = 10; □, Abs = 20;■; Abs = 30; ▲, Abs = 40.

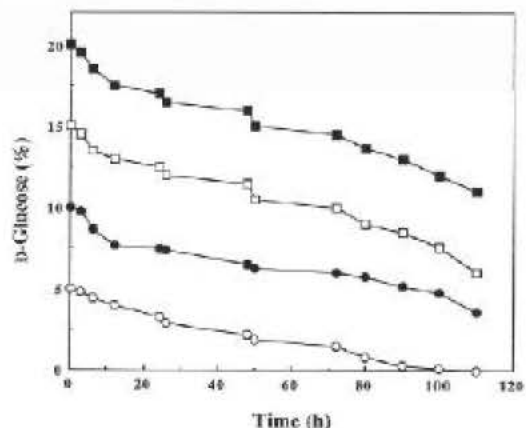

Fig. 4: Effect of substrate concentration on the degradation of D-glucose by *Candida fomata* R28. Symbols: ○, 5%; ●, 10%; □, 15%; ■, 20%.

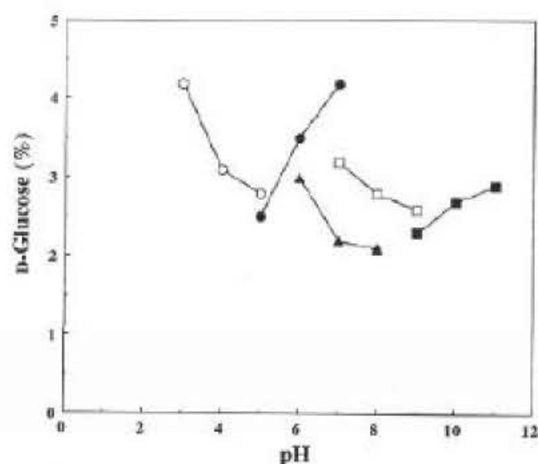

Fig. 5: Effect of reaction mixture pH on the degradation of D-glucose by *Candida famata* R28. Symbols: ○, Citrate buffer (pH 3-5); ●, Maleate buffer (pH 5-7); □, Tris-HCl buffer (pH 7-9); ■, Glycine- NaOH buffer (pH 9-11); ▲, Na-Phosphate buffer (pH 6-8).

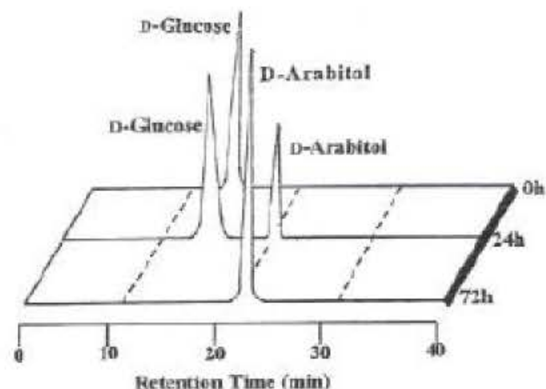

Fig. 6: HPLC of the microbial conversion of D-glucose to D-arabitol by *Candida famata* R28.

dephosphorylating D-ribulose-5-PO_4 in the pentose pathway (Wong *et al.*, 1995, Izumori and Bhuiyan, 1997). Although *Candida pelliculosa* produced D-arabitol from D-glucose but concomitantly produced by-product, D-ribose, in the reaction mixture (de-Wulf *et al.*, 1996). Onishi and Suzuki (1966) also reported that *Pichia miso*, a highly osmophilic yeast isolated from miso paste, dissimilated 30% D-glucose solution completely with the production of 50~60% glycitol identified as glycerol, erythritol and D-arabinitol. Certain osmophilic yeasts grown in the presence of high glucose concentrations produce, in addition to ethanol and CO_2 a variety of polyhydric alcohols; glycerol, erythritol, D-arabitol and mannitol (Spencer and Sallans, 1956; Blakely and Spencer, 1962). It was also reported that D-arabitol is the intermediate in the interconversion of aldose and ketose in *Candida albicans*, *C. utilis* and *Penicillium chrysogenum* (Onishi and Suzuki, 1966; Chiang and Knight, 1960). In contrast, *C. famata* R28 used in this study, produce only D-arabitol from D-glucose without any by-product formation. From Table 1 it was found that the strain R28 showed good growth in D- glucose containing media which is similar to the finding of Ramananda *et al.* (1960) experiment. This new technique is an example of interconversion between a hexose and a pentose by biochemical means.

D-Glucose is the most abundant aldose occurring in nature. In many parts of the world D-glucose is an abundant source of carbon produced enzymatically from starch, sucrose or even cellulose and also metabolized by aerobic and anaerobic organisms. Several studies have been carried out with microorganisms and their enzymes to produce various rare L- and D- sugars from inexpensive carbohydrates (Ahmed, 2001; Izumori and Bhuiyan, 1997; Chelain *et al.*, 1995). Many aldose-ketose isomerization of free sugars have also been reported previously (Izumori and Bhuiyan, 1997). Development and introduction of simple methods are extremely important for increasing the production of these sugars. Moreover, to produce enough amount of these pentoses, natural substrates have to be used which are

abundant in nature and are cheap as starting materials (Ahmed, 2001). The present studies encompass on the production of pentose from hexose by simple microbial method. An interesting phenomena observed that there was no need of product separation or purification after the reaction. In an effort to prepare 3,4-methylene-dioxyphenyl-(S)-isopropanol from 3,4-methylene-dioxyphenylacetone, Zmijewski *et al.* (1997) reported that *Candida famata* could catalyze this reaction efficiently at low substrate concentration. It has been shown that several pathogenic fungal species produce large amounts of acyclic polyols and that these polyols can be used as diagnostic markers. For example, several pathogenic *Candida* species produce large amounts of the 5-carbon polyol D-arabinitol and animals and humans with invasive candidiasis have elevated serum D-arabinitol/creatinine ratios. The D-arabinitol dehydrogenase (ArDH) of *Candida tropicalis* catalyzes the reaction D-arabinitol + NAD => D-ribulose + NADH with a high degree of substrate specificity. The pathway by which *Candida* spp. synthesizes D-arabitol is not yet known but the fact that D-ribulose-5-PO_4 from the pentose pathway is the major D-arabitol metabolic precursor limits the possibilities. A single reduction and a single dephosphorylation are sufficient to convert D-ribulose-5-PO_4 to D-arabitol; thus it is likely that D-arabitol is synthesized by one of the two pathways: (I) D-ribulose-5-PO_4 is dephosphorylated, after which D-ribulose is reduced to D-arabitol by a second arabitol dehydrogenase (ArDH) isoform, or (ii) D-ribulose-5-PO_4 could be reduced to D-arabitol-5-PO_4 by a D-ribulose-5-PO_4 redustase, after which D-arabitol-5-PO_4 is dephosphorylated to D-arabitol. In case of other osmophilic yeasts, D-arabitol is produced from D-glucose via D-xylulose-5-PO_4 with the help of NAD-dependent dehydrogenase. From the Wong *et al.* (1988) experiment, it was found that D-ribulose-5-PO_4 from the pentose pathway was the principal D-arabitol metabolic precursor. In all these case, *Candida* spp. produce by-products beside D-arabitol such as erythritol, mannitol, ethanol. On the other hand, *Candida famata* R28 produce only D-arabitol without any by-product formation. In the present report, *Candida famata* R28 was optimize for the production of D-arabitol using a simple and easy method describe in earlier (Ahmed *et al.*, 1999).

References

Armstrong, D., 1982. Rate of arabinitol production by pathogenic yeast species. J. Clin. Microbiol., 146: 353-359.

Ahmed, Z., 2001. Production of natural and rare pentoses using microorganisms and their enzymes. Electronic J. Biotech, (http://www.ejb.org/content/vol14/issue2/full/7), 4: 1-7.

Ahmed, Z. and P. K. Bhowmik, 2000. Studies on the oxidation of ribitol and D-arabitol by *Acetobacter aceti* IFO 3281. Pakistan J. Biol. Sci., 3: 1492-1495.

Ahmed, Z., H. Sasahara, S. H. Bhuiyan, T. Saiki, T. Shimonishi, G. Takada and K. Izumori, 1999. Production of D-lyxose from D-glucose by microbial and enzymatic reactions. J. Biosci. Bioeng., 88: 676-678.

Bernard, E. M., K. J. Christiansen, S. Tsang, T. E. Kiehn J. W. M. Gold, B. Wong, E. M. Bernard, T. E. Kiehn and D. Armstrong, 1983. Serum arabinitol concentrations and arabinitol/creatinine ratios in invasive candidiasis. J. Infect. Dis., 147: 504-514.

Blakely, E. R. and J. F. T. Spencer, 1962. Studies on the formation of D-arabitol by osmophilic yeasts. Can. J Biochem. Physiol., 40: 1737-1748.

Chiang, C. and S. G. Knight, 1960. Metabolism of D-xylose by molds. Nature, 18:79-81.

Chelain, E., O. Floch and S. Czernecki, 1995. New synthesis of L-ribofuranose from L-xylose. J. Carboh. Chem., 14: 1251-1256.

de-Wulf, P., W. Soetaert, D. Schwengers and E. J. Vandamme, 1996. Screening and mutational improvement of a D-ribose secreting *Candida pelliculosa* strain. J Ferment. Bioeng., 82: 1-7.

Herbert, D., P. J. Phipps and R. E. Strange, 1971. Chemical Analysis of Microbial cells. Methods in Microbiol., 5: 282-283.

Izumori, K. and S. H. Bhuiyan, 1997. Production of rare monosaccharides using microorganisms and their enzymes. Food Industry and Nutrition, 2: 16-21.

Jennings, D. H., 1984. Polyol metabolism in fungi. Adv. Microbiol. Physiol., 25: 149-193.

Kiehn, T. E., E. M. Bernard, J. W. M. Gold and D. Armstrong, 1979. Candidiasis: detection by gas-liquid chromatography of D-arabinitol, a fungal metabolite, in human serum. Sci., 206: 577-580.

Onishi, H. and T. Suzuki, 1966. The production of xylitol, L-arabinitol and ribitol by yeasts. Agric. Biol. Chem., 30: 1139-1144.

Onishi, H. and T. Suzuki, 1968. Production of D-mannitol and glycerol by yeasts. Appl. Microbiol., 16: 1847-1851.

Onishi, H. and T. Suzuki, 1969. Microbial production of xylitol from glucose. Appl. Microbiol., 18: 1031-1036.

Onishi, H. and T. Suzuki, 1970. Production of erythritol, D-arabitol, D-mannitol and a heptitol-like compound from glycerol by yeasts. J. Ferment. Technol., 48: 563-566.

Ramananda, G. R., T. Ramakrishnan and M. Sirsi, 1960. Enzymes in *Candida albicans*. I. Pathways of glucose dissimilation. J. Bacteriol., 80: 654-664.

Sasahara, H., M. Mine and K. Izumori, 1998. Production of D-talitol from D-psicose by *Candida famata* R28. J. Ferment. Bioeng., 85: 84-88.

Spencer, J. F. T. and H. R. Sallans, 1956. Production of polyhydric alcohols by osmophilic yeasts. Can. J. Microbiol., 2: 72-79.

Saha, B. C. and R. J. Bothast, 1996. Production of L-arabitol from L-arabinose by *Candida entomaea* and *Pichia guilliermondii*. J. Appl. Microbiol. Biotech., 45: 299-306.

Wong, B. and K. L. Brauer, 1988. Enantioselective measurement of fungal D-arabinitol in serum from normal adults and patients with candidiasis. J. Clin. Microbiol., 29: 1670-1674.

Wong, B., S. Leeson, S. Grindle, B. Magee, E. Brooks and P. T. Magee, 1995. D-arabitol metabolism in *Candida albicans*: Construction and analysis of mutants lacking D-arabitol dehydrogenase. J. Bacteriol., 177: 2971-2976.

Zmijewski, M. J., J. Vicenzi, B. E. Landen, W. Muth, P. Marler and B. Anderson, 1997. Enantioselective reduction of 3,4-methylene-dioxyphenylacetone using *Candida famata* and *Zygosaccharomyces rouxii*. Applied Microbiol. Biotech., 47: 162-166.