

Review Article

Arabitol production by microbial fermentation - biosynthesis and future applications

Himabindu Kumdam, Shweta Narayana Murthy and Sathyanarayana N Gummadi*

Applied Industrial Microbiology Laboratory, Department of Biotechnology, Indian Institute of Technology Madras, Chennai 600 036, India

*Corresponding Author: Sathyanarayana N Gummadi; Applied Industrial Microbiology Laboratory, Department of Biotechnology, Indian Institute of Technology Madras, Chennai 600 036, India

Abstract

Arabitol is a 5 carbon sugar alcohol which exists in both D and L forms. Arabitol was produced by chemical synthesis which was both tedious and expensive and hence microbial production gained significant interest. Fermentation of natural substrates such as lignocellulose, hemicelluloses etc and by using osmotolerant organisms such as yeast have resulted in higher yield and quality than chemical synthesis. Osmophilic yeasts often accumulate polyols when exposed to many environmental stress and arabitol is one such polyol that is produced which prevents the yeast from degradation. Several species of yeasts such as *Zygosaccharomyces*, *Pichia*, *Debaryomyces* are used and have produced up to 84 g/L of arabitol with yields up to 56% and the productivity up to 30g/L. Metabolic engineering for the production of arabitol is extensively studied which will eventually lead to the construction of a novel arabitol producing organism.

Keywords: Arabitol, Fermentation, Osmophilic yeast, Osmolyte

Introduction

Arabitol also called as arabinitol or lyxitol is a pentitol, being five carbon sugar alcohol exists in both D and L forms. It is sweet, colorless, crystalline, soluble in water, melts at 103 °C (Tourneau, 1966; Talja and Roos, 2001). Naturally arabitol is present in mushrooms and lichens (Lindberg, 1952). Alongside xylitol, arabitol is popularly used as a natural sweetener. In the recent times the demand for alternative sugars has been on rise owing to their beneficial properties (Gare, 2003; Moskowitz, 1971; Yamaguchi, 1970). Following are the properties that alternative sugars have to possess; low caloric content of 0.2 kcal/g, diabetic reducer, control of dental caries, sweetness similar to sucrose, colourless, odourless,

should be metabolized normally (Kirk and Othmer, 1981; Fabre et al., 2002).

D-arabitol is mostly produced by the catalytic reduction of D-arabinose or lyxose or chemical reduction of lactones of arabinonic and lyxonic acids. This process uses expensive catalyst and high temperatures of 100 °C. D-arabitol has been produced by chemical reduction of D-arabinose or D-lyxose with sodium amalgam. Catalytic hydrogenation of L-arabinose gives L-arabitol which is carried out in slurry reactors, and both the process require elevated hydrogen pressure (40-60 bar), use of Ru/C catalyst and temperature (90 – 120 °C), thus making the chemical production very expensive and time consuming (Ruff, 1899). Many natural substrates such as lignocellulosic

hydrolysates and sugar cane bagasse are rich in hexoses and pentoses. These hydrolysates can be effectively used for production of value added products like arabitol and other polyols (Ruff, 1899). Glucose is the commonly used substrate for conversion to arabitol (Kumdam et al., 2013). Arabitol is used as an alternative sweetener which has other beneficial medicinal properties.

Applications of D and L-arabitol

D-arabitol is used for synthesis of compounds of significance which are enantiopure and are used in immunosuppressive glycolipids, herbicides and antipathogenic disease medicines (Levin et al., 1995; Levin, 2002). Oral flora cannot metabolize this rare sugar alcohol, and hence protects from cavities and is popularly referred to as anticariogenic and possess a cooling effect. Arabitol also plays an important role in energy controlled diets since it is absorbed slowly by the human digestive tract and has a low caloric content (Levin, 2002). L-arabitol has similar physiological effect as xylitol and it can reduce the deposition of fat in digestive tract and adipose tissue in body. Arabitol has lower caloric content than xylitol. Arabitol is a commonly used ingredient in chewing gums owing to this use (Horwitz and Kaplan, 1964; Levin et al., 1995; Ho Park et al., 2001; Levin, 2002; Jiang et al., 2011)

Arabitol as a source of electricity power

Electricity can be directly generated from polyols. Arabitol, xylitol and sorbitol are found to be the three pentitols from which power can be generated and is of high interest, owing to the fact that polyols are byproducts of ethanol fermentation. These polyols are derivatives of lignocellulosic carbohydrates. Arabitol generated a maximum power density of 2070 ± 10 mW/m² at a current density of 0.68mA/cm². An effective use of this polyol could constitute an important step

of utilization of biomass for economical production of renewable energy. Direct electricity production from arabitol was demonstrated for using single chamber, air- cathode and mediator less MFC. Arabitol was utilized by bacteria with COD removal efficiency in the range of 91% (Catal et al., 2008).

Micro-organisms that produce arabitol

Many osmophilic yeast species such as *Zygosaccharomyces*, *Saccharomyces*, *Debaryomyces*, *Metschnikowia*, *Candida*, *Pichia*, *Hansenula* (Blakley and Spencer, 1962; Ingram and Wood, 1965; Hanssens et al., 1972; Bernard et al., 1981; Nobre and Costa, 1985; Van Eck et al., 1989, 1993; Groleau et al., 1995; Bisping et al., 1996; Nozaki et al., 2003) produce D-arabitol when grown on D-glucose. Table 1 summarizes the fermentation parameters of arabitol production by several yeast species. Certain bacteria such as *Aerobacter aerogenes* have the ability to utilize D-arabitol as sole source of carbon. Earlier studies in the laboratory have shown that *Debaryomyces nepalensis* NCYC 3413, osmotolerant yeast, is capable of producing arabitol when grown on glucose. Culture conditions which lead to the intracellular accumulation of arabitol was studied. It was also found that arabitol was produced in the stationary phase and beyond the stationary phase, the organism consumed arabitol for survival. In the present study, an attempt was made to develop a suitable medium for fermentative production of D-arabitol as a part of which several macro and micronutrients were tested for their ability to improve the product yield (Kumdam et al., 2012; Kumdam et al., 2013). Glucose and arabinose were used by the organism as suitable substrates.

Table 1. Summary of arabitol production by various yeast species under batch and fed-batch fermentation.

Organism	Substrate (g/l)	Arabitol (g/l)	Arabitol yield (g/g)	Fermentation conditions	Reference
<i>Metschnikowia reukaufii</i>	Glucose (200)	81.4	0.41	pH 5.0, 34 °C, 600 rpm, 225ml/min aeration rate; batch fermentation in 1L jar fermenter	Nozaki et al., 2003
<i>Zygosaccharomyces rouxii</i> NRRL 27264	Glucose (175)	83.4	0.48	pH 5.0, 30 °C, 350 rpm, 5% inoculum	Saha et al., 2007
<i>Kodamae ohmeri</i> NH-9	Glucose (200)	81.2	0.41	pH 7.0, 37 °C, 220 rpm batch fermentation in shake flask	Zhu et al., 2010
<i>Hansenula polymorpha</i>	Glucose(<30)	18.8	0.14	pH 4.8, 45 °C, 1000 rpm, 1 vvm, fed batch fermentation in 2 L fermenter	Escalante et al., 1990
<i>Pichia guilliermondii</i>	L-arabinose (50)	33.4	0.54	pH 4.0, 34 °C, 200 rpm; batch fermentation	Fonesca et al., 2007
<i>Candida entomaeae</i>	L-arabinose (50)	34.7	0.77	pH 5.0, 34 °C, 200 rpm; batch fermentation in shake flask	Saha and Bothast 1996
<i>Kluyveromyces lactis</i>	Lactose (188)	14	0.25	30 °C, 450 rpm, 0.5 vvm; batch production in 2.5 L bioreactor	Toyoda et al., 2011
<i>Endomycopsis chodatii</i>	Glucose (100)	35-40% of the sugar utilized	0.34	pH 4.8, 30 °C, 200mm/L/h; batch fermentation in shake flasks	Hajny et al., 1964
<i>Debaryomyces nepalensis</i> NCYC 3413	Glucose (150)	14	0.1	pH 6.0, 30 °C, 180 rpm; batch fermentation in shake flasks	Kumdam et al., 2012
	Glucose (100)	11.5	0.15		
	Arabinose (100)	24.6	0.32		

Fermentation factors that affect production of arabitol in various organisms

Environmental factors such as temperature, aeration, phosphate, nitrogen, sugar concentration and osmotic stress have been found to affect the rate and yield of arabitol by yeasts. In case of *C. entomaeae* NRRL Y -7785 and *P. guilliermondii* NRRL Y- 2075 temperature and pH were responsible for production of arabitol. Maximum yield of arabitol was produced by them at temperatures between 30°C - 35°C. The two organisms produced around 0.70 g/g from L-arabinose (50 g/l) at pH 4 and 5 respectively. Both the strains preferentially utilized glucose to L-

arabinose. Their biomass yield was at its maximum between 22 °C - 28 °C (Saha and Bothast, 1996). The optimal temperature for *Endomycopsis chodatii* was also 35 °C (Hajny et al., 1964). In *Zygosaccharomyces rouxii*, arabitol production was optimal at 30 °C and the optimum pH was 5.0. The inoculum percentage influenced the D-arabitol production where the increase in the inoculum increased the product formation. The percent of inoculums used ranged between 1.0 – 10% and 10% was proved the best. Similarly the use of high agitation of about 450 rpm also favored the production of arabitol. *Z. rouxii* also preferentially used D-glucose, and there

was no growth observed in L-arabinose. The degree of aeration is integral in arabinol production, at levels of aeration between 0.6vvm – 1vvm. Under these conditions, the glucose consumption was higher and arabinol was also produced higher. At different agitation rates, different products get accumulated. In case of *Z. rouxii* ATC12572, requires about 500 rpm for arabinol to be accumulated (Groleau et al., 1995; Saha et al., 2007).

In *M. reukaufii* AJ14787, when D-arabitol fermentation was done in medium with excess nitrogen sources or with pH controlled by ammonia gas decreased arabinol yield and increased biomass. D-arabitol productivity was improved by a balance feed of the right balance of glucose and nitrogen source such as ammonium sulphate. The alteration of these components i.e. the nitrogen or by producing extra source of amino acids did not favor the cell growth as well as formation of arabinol. *M. reukaufii* also utilized fructose and produced arabinol, probably by the pentose phosphate pathway, the efficiency of production was

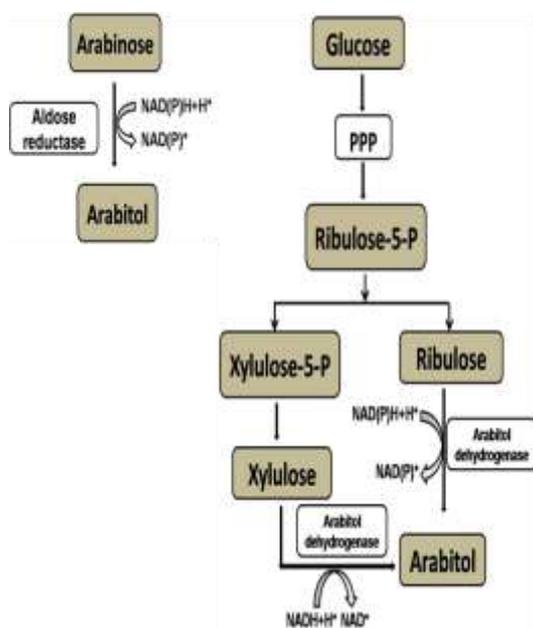


Figure 1: Pathways for arabinol synthesis from glucose and arabinose

not the same (Nozaki et al., 2003). The effect of nutrients on arabinol production has been only to a limited extent. However in case of this organism, the yeast extract concentration exerted tremendous influence on fermentation. Concentration of yeast extract also corresponded to the amount of arabinol produced by this yeast. At 0.5% yeast extract concentration, it was considered optimal since high yield of arabinol was obtained in a reasonable fermentation time. Aeration needed was intermediate, the yield of arabinol rises and subsequently drops as well. Excessive nitrogen concentration in the media resulted in decreased arabinol production and a high biomass. The right balance of nitrogen in medium gave adequate arabinol and appropriate cell growth. Phosphorous also proved essential, without this component the organism did not produce much of a biomass and the conversion was low as well.

An osmophilic yeast called *Kodamaea ohmeri* produces D-arabitol as the main metabolic product from glucose requires about 37 °C for growth and production of arabinol. *P. guillermondii* PYCC3012 produced more arabinol from L-arabinose to *C. arabinofermentans* PYCC5603. At 5 g/l sugar concentration, *P. guillermondii* accumulated arabinol whereas only biomass was formed for *C. arabinofermentans* (Fonseca et al., 2007). With the increase in the substrate concentration (80%) and oxygen limiting conditions, it led to arabinol production.

Biosynthesis of D-arabitol

The biochemistry of D-arabitol synthesis has been elucidated to some extent in several organisms. Fig.1 shows the possible pathways for arabinol synthesis from glucose and arabinose. Most of these studies were based on comparison of labelling patterns of D- arabinol when grown on glucose radio labelled at different positions. D-arabitol pathway in *D. hansenii* was determined by Adler et al

using high resolution ^{13}C NMR. Pentose phosphate pathway was found to play a major role in the formation of arabinol, ribulose-5-phosphate being the precursor, as indicated by the labelling pattern (Inga Tunblad-Johansson, 1990).

Arabinol biosynthesis has not been studied extensively. Many aspects regarding the enzymes involved in the pathway and its regulation are still obscure and a thorough research in this respect is warranted. It was earlier proposed that arabinol dehydrogenase (ArDH) catalyses the reduction of D-ribulose to D-arabinol in *C. albicans*. But later it was found that the enzyme mediates the first step in arabinol catabolism. This observation was evidenced by the inability of mutants lacking ArDH to grow on minimal medium containing D-arabinol. On the other hand, these mutants were able to synthesize D-arabinol when grown on glucose medium, which proved that ArDH was not involved in the synthesis of D-arabinol dehydrogenase. A probable pathway suggested by Wong et al (1995) involves the reduction of ribulose-5-phosphate to arabinol-5-phosphate by a reductase and subsequent dephosphorylation of arabinol-5-phosphate. Arabinol dehydrogenase has been used as diagnostic marker. D-arabinol concentration has been found to be higher in humans and animals with invasive candidiasis than colonized and uninfected controls. Recombinant arabinol dehydrogenase has been over expressed and purified. The reaction is based on oxidation of D-arabinol to D-ribulose by rArDH, with the simultaneous reduction of NAD to NADH. The initial rate of NADH is proportional to amount of D-arabinol in the serum. Thus, D-arabinol is a quantitative diagnostic marker for invasive *Candida* infections (Roboz et al., 1980; Deacon, 1986; Soyama and Ono, 1987). *Candida* species were generally potent organisms for production of D-arabinol.

Zakaria Ahmed has worked on *Candida famata* R28 which produced D-arabinol from D-glucose without producing any byproduct. This organism had the ability to give about 5% D-arabinol from 10 % D-glucose. *Candida pellicose* produced D-arabinol from D-glucose but D-ribose is produced as the by-product (De Wulf et al., 1996). D-arabinol was also formed as an intermediate product in the interconversion of aldose and ketose in *C.albicans*, *C.utilis*, and *P.chrysogenum* (Saha et al., 2007). Wong et al (1993) using ^{14}C labelled glucose, concluded that *C. albicans* synthesizes D-arabinol by dephosphorylation and reduction of ribulose-5-phosphate (Wong et al., 1993). A similar pathway that proceeds through the reduction of ribulose was suggested by Weimberg for the synthesis of arabinol in *S. mellis* (Weimberg and Orton, 1965).

In case of osmophilic yeasts, D-arabinol is produced from D-glucose via xylulose 5 P_4 with the help of NAD-dependent dehydrogenase (Fig. 1). Blakely et al reported that synthesis of arabinol in *S.rouxii* P3a occurs using a different pentose phosphate pathway intermediate, xylulose-5-phosphate, as precursor. The proposed pathway was validated by the labelling pattern of arabinol and also by the presence of large amounts of arabinol in cells grown on xylulose 5 ^{14}C (Blakley and Spencer, 1962). The synthesis of D-arabinol in *Dendryphiella salina* also takes the route of xylulose 5 phosphate. Glucose is initially converted to xylulose 5 P via PPP and the latter is dephosphorylated and reduced to arabinol (Lowe and Jennings, 1975).

Onishi and Suzuki in 1960s described a 3 step process for production of arabinol from glucose by an Osmophilic yeast strain, *D.hansenii* followed by the oxidation of D-arabinol into D-xylulose with *Acetobacter suboxydans* and subsequently reduced to xylitol by *C.guilliermondii*. The microbial conversion of D-arabinol to xylitol is an alternative

process that is independent from D-xylose involving oxidation of D-arabitol to D-xylulose by D-arabitol dehydrogenase followed by reduction of D-xylulose to xylitol by xylitol dehydrogenase. However productivity was low because the reactions were carried out separately. Strains of acetic acid bacteria belonging to the genus *Gluconobacter* had a membrane bound D-arabitol dehydrogenase (AraDH) and a soluble xylitol dehydrogenase that had the potential to directly convert to xylitol from D-arabitol.

Metabolism of L-arabitol

L-arabinose catabolism shows two kinds of redox reactions showing different cofactor specificity. The reduction is catalysed by NADPH – linked aldose reductase which is unspecific, and then subsequently it gets converted to L-xylulose by L-arabitol dehydrogenase using NAD as cofactor and subsequently it gets reduced to xylitol. Cofactor imbalance during L-arabinose pathway is understood by the accumulation of arabitol. Similarly fungi such as *Aspergillus* and *Penicillium* use NADH as cofactor. The catabolic pathways in them, reduce L-arabitol and is subsequently converted to xylitol in two consecutive redox steps catalyzed by L-arabitol dehydrogenase and L-xylulose reductase (Saha and Bothast, 1996).

In yeasts, *C.arabinofermentens* and *P.guillermondii* L- arabinose is metabolized via redox catabolic pathway. The resulting D-xylulose proceeds through pentose phosphate pathway and is further converted to D-ribulose, yielding D-arabitol. These yeasts have been characterized in terms of physiology, including the influence of sugar and oxygen in L-arabinose transport and early steps of L-arabinose catabolism. Arabitol production by the respiratory chain from the impairment of NAD⁺ was seen in *C.arabinofermentens*, but in *P.guillermondii* L-arabinose was utilized and arabitol was accumulated by resting

cells. *P.guillermondii* is known to have synthesized high amount of L-arabitol from L-arabinose (Saha and Bothast, 1996; Fonseca et al., 2007).

Regulation of arabitol synthesis

Kayingo and Wong studied the effect of environmental stresses on the production of arabitol and glycerol in *C. albicans*. The synthesis of D-arabitol in *C. albicans* was shown to be partly regulated by MAP kinase Hog1p of the HOG pathway. It was observed that *C. albicans* produces glycerol in response to osmotic and citric acid stress, and D-arabitol in response to temperature and oxidative stress. The HOG1 gene product which is involved in the regulation of glycerol synthesis was also found to be responsible for the regulation of arabitol formation under oxidative stress but not temperature stress. This implies that arabitol synthesis in *C. albicans* is regulated by both HOG1-dependent and HOG1 independent pathways (Kayingo and Wong, 2005).

Arabitol as compatible solute

Osmophilic yeasts accumulate polyols under osmotic stress. The mechanism being when external osmolarity increases it causes hyperosmotic stress on yeast cells, which in turn results in outflow of water. Under this circumstance, the yeast adapts to the surrounding environment by accumulation of osmolytes such as glycerol, arabitol, xylitol and mannitol in the cytoplasm of the cell preventing the yeast from dehydration. Many osmophilic and osmotolerant yeasts synthesize and accumulate soluble, low molecular weight compounds in response to stress induced by high salt and sugar concentration. These compounds are known as compatible solutes, because they are compatible with cell metabolism. Polyols or sugar alcohols constitute an important class of compatible solutes along with other substances like proline and trehalose. Glycerol is the major polyol synthesized

by many types of yeast. Arabitol, albeit a secondary osmolyte, also plays an important role in osmoregulation in several yeast species and fungi.

Nobre et al studied the effect of hyperosmotic stress on the halophilic yeast *D. hansenii* in relation to polyol accumulation. The yeast, *Debaryomyces hansenii*, synthesized and accumulated glycerol and arabitol when grown in the presence of glucose, KCl & NaCl. The cells produced only arabitol when grown on 1% glucose. Growth on 1% glucose and NaCl/KCl resulted in the intracellular formation of both glycerol and arabitol. Glycerol was predominant during logarithmic phase whereas arabitol accumulation was the highest in stationary phase. A similar pattern was observed when grown in medium containing 25% glucose or PEG (Nobre and Costa, 1985).

Arabitol production by *Zygosaccharomyces* sps in response to osmotic stress and factors affecting arabitol production was investigated in detail by Peterson et al. Out of the 11 species studied, *Z. nussbaumeri*, *Z. richteri*, and *Z. rugosus* gave good yields of arabitol. *Z. barkeri* was found to be the best arabitol producer with 55 per cent conversion of glucose to arabitol. Later Brown and Simpson reported the accumulation of arabitol along with glycerol in *Z. Rouxii* under conditions of low water activity (Brown and Simpson, 1972). The accumulation of arabitol by *Z. rouxii* was found to be influenced by the solute concentration of the medium. The intracellular composition of *Z. rouxii* was compared with that of *S. cerevisiae* which is non osmotolerant and showed no accumulation of arabitol. Based on these findings, Brown hypothesized that arabitol functions as a compatible solute in osmotolerant strains (Brown and Simpson, 1972).

Moran and Witter (1979) found that when glucose concentration in the medium was increased, the amount of arabitol produced in *Z. rouxii* was also increased. The enhanced specific activity of D-arabitol dehydrogenase with increased glucose concentration justified the above observation. Sucrose was found to have no effect on enzyme activity and hence arabitol production (Moran and Witter, 1979). Van Zyl and Prior observed that glycerol and arabitol were accumulated in *Z. rouxii* during growth in continuous culture at lowered water potential. Arabitol was only found during the initial phase of adaptation and hence was considered an auxiliary compatible solute (Zyl et al., 1990). *Candida sake*, the biocontrol yeast, was also shown to accumulate glycerol and arabitol as main compatible solutes in response to lowered water activity. Intracellular concentration of these polyols was found to be dependent on the solute used to modify the water activity. When the water activity of the molasses media was lowered with glucose, arabitol was the major polyol accumulated (Abadias et al., 2001).

Polyols were also known to be secreted under hypo osmotic conditions in order to maintain the osmotic balance, thus preventing water influx and consequent cell swelling. Kayingo et al investigated the effect of hypo osmotic shock on *S. cerevisiae*, *Zygosaccharomyces rouxii* and *Pichia sorbitophila* and observed that *Z. rouxii* released arabitol and glycerol while *P. sorbitophila* released erythritol in addition to arabitol and glycerol (Kayingo and Wong, 2005). Arabitol is favored in culture medium containing nitrate as sole nitrogen source. The yeast *D. hansenii* accumulates arabitol during the stationary phase of growth in glucose medium and glycerol during exponential growth in response to large concentrations of external sodium chloride. The intracellular concentration of arabitol does not appear to be regulated by the solute concentration

of medium. *D. hansenii* produced better arabitol from glycerol as the substrate with the yield 55 % and a productivity of 0.2 g/L-h. The initial glycerol concentration favored the production of arabitol from 14 to 40% (Koganti, 2012).

Fungi

Accumulation of polyols like arabitol and glycerol is one of the several mechanisms employed by fungi to overcome the adverse effects of low water activity. Arabitol is common intracellular constituent of higher fungi, and accumulate in sexual or asexual spores or in stationary phase cells when play a role as carbon and energy reserves mobilized during germination or regrowth. Arabitol commonly occurs during mycelia growth and its intracellular concentration is regulated by the solute concentration of glucose for growth in the medium. During fungal infection, there is a rise of D-arabitol which is explained with a role of polyol in carbohydrate storage. Carbohydrate storage function is also supported by large quantities of arabitol found in spores, which disappeared on germination. In some fungus such as *P. graminis* the arabitol was found in germinated and ungerminated endospores. Thereby concluding that arabitol has a role in stress tolerance (Link et al., 2005). The fungus *Geotrichum candidum*, was also reported to accumulate arabitol during vegetative growth in media containing 10 % (w/v) to 30 (w/v) glucose, or 25 % (w/v) fructose or L-sorbose and the intracellular concentration of arabitol increased with increasing solute concentration in the medium (Costa and Niederpruem, 1982). *A. oryzae* accumulated high concentrations of glycerol, erythritol and arabitol in solid state fermentation, where the water activity is very low (0.96–0.97) (Ruijter, 2004). Holligan and Lewis reported that arabitol was the major soluble carbohydrate accumulated in *Aspergillus*

clavatus (Holligan and Jennings, 1972). Arabitol and mannitol are the major polyols produced in the marine fungus *Dendryphiella salina* and their accumulation was shown to be influenced by the carbon and nitrogen sources in the growth medium (Holligan and Jennings, 1972).

Recovery of arabitol

The recovery of arabitol from the fermentation media has been complicated since there is high concentration of dissolved solids, low arabitol concentrations in the media which have hindered the recovery process. The broth was removed of the biomass, and the supernatant was treated with activated charcoal initially to remove the colorants present. Arabitol was subsequently removed using phase equilibrium systems. Abdul lohman has performed by selectively extracting glycerol using acetone. The supernatant was dehydrated, to remove acetone and arabitol was extracted using hot butanol in 1:0.9 proportions at 90 °C. Recovery has been only 65 % of the total arabitol present in the system but the arabitol crystals were around 95 % pure (Loman and Ju, 2013). Jiang et al also decolourized the supernatant by using activated carbon and passed it through an ion exchange resin to facilitate removal of metal ions which reached a conductivity of 20 µs/cm. The solution was concentrated and cooled from 70 °C to 4 °C. The precipitated white powdery crystals were suspended in the solution and were washed with ethanol and dried subsequently which resulted in about 90 % purity of arabitol. A combination of ion exchange resins and ethanol distillation removed around 90 % ionic impurities and also removed unwanted byproducts of fermentation. It was also found that arabitol loss occurred largely during the extraction procedure while removing glycerol, thereby making it very essential

to minimize the glycerol production during the recovery process (Jiang et al., 2011).

Purification of arabitol from xylitol mother liquor

Xylitol mother liquor is a product of xylose mother liquor after chemical hydrogenation of xylose from corn cobs. L-arabitol constitutes of about 28-33 % in this liquor. L-arabitol was prepared from L-arabinose commercially by hydrogenation and high pressure, but it incurs high production cost. Xylitol mother liquor constitutes about 33 % of L-arabitol, but since the mother liquor constitutes other polyols, purification of the same was not efficient when used simulated moving bed chromatography. Recently it was found that *Bacillus megaterium* BM314, an aerobic bacterium, cannot use L-arabitol but can use other polyols in the mother liquor. The organism first converts xylitol and sorbitol to D-xylulose and D-fructose by dehydrogenases with NAD^+ as the co-factor and then metabolized via HMP shunt and glycolytic pathways for survival and energy. The supply of NAD^+ regenerated from NADH produced, is sufficient for complete oxidation of xylitol and sorbitol from the mixture. The bacterium *B.megaterium* BM314 requires high aeration for its rapid cell growth and metabolism. L-arabitol from this mixture is subsequently purified by decolourization of the fermentation media and ion exchange, L-arabitol was crystallized with a purity of 98.5 % (Jiang et al., 2011).

Conclusion

There have been many organisms that have been characterized and screened for production of arabitol. Enzyme studies pertaining to arabitol dehydrogenase have to characterized, which could serve as potential field as it could assist in maximizing the production of arabitol. Glucose and arabinose is the preferred substrate for yeasts. Arabitol

accumulation, owing to stress has been another important feature of study. Subjecting the organisms to various stress conditions lead to the desired production of arabitol and other polyols. The commercial chemical process for arabitol has been studied in the early 1960's. Microbial production of arabitol has been studied as an alternative chemical process. Redox balances of *Candida* and other yeast strains have been studied. Also the affinity for the yeast strains in the uptake of glucose and arabinose for production of arabitol has been studied. Microbial reduction of many raw materials such as hydrolysates has been an important feature of study for commercial production of many polyols. Arabitol may also find its use in creation of many drugs, owing to its properties of being easily metabolized in the body.

Acknowledgement

This work was supported by research grant from Department of Biotechnology, Government of India

References

- Abadias, M., Benabarre, A., Teixidó, N., Usall, J., Viñas, I., 2001. Effect of freeze drying and protectants on viability of the biocontrol yeast *Candida sake*. *Int. J. Food Microbiol.* 65, 173–182.
- Bernard, E.M., Christiansen, K.J., Tsang, S.F., Kiehn, E., Armstrong, D., 1981. Rate of arabinitol production by pathogenic yeast species. *J. Clinic. Microbiol.* 14(2), 189-94.
- Bisping, B., Baumann, U., Simmering, R., 1996. Effects of immobilization on polyol production by *Pichia farinosa*, in: R.H. Wijffels C. Bucke and J. Tramper BT - Progress in Biotechnology, R.M.B. (Ed.), Elsevier, pp. 395–401.
- Blakley, E.R., Spencer, J.F.T., 1962. Studies on the formation of D-arabitol by osmophilic yeasts. *Can. J. Biochem. Phys.* 40, 1737–1748.
- Brown, A.D., Simpson, J.R., 1972. Water relations of sugar-tolerant yeasts: the role

- of intracellular polyols. *J. Gen. Microbiol.* 72, 589–91.
- Catal, T., Xu, S., Li, K., Bermek, H., Liu, H., 2008. Electricity generation from polyalcohols in single-chamber microbial fuel cells. *Biosens. Bioelectron.* 24, 855–860.
- Costa, M.S., Niederpruem, D.J., 1982. Arabitol accumulation in *Geotrichum candidum*. *Arch. Microbiol.* 131, 283–286.
- De Wulf, P., Sotaert, W., Schwengers, D., Vandamme, E.J., 1996. Screening and Mutational Improvement of a D-Ribose Secreting *Candida pelliculosa* Strain. *J. Ferment. Bioeng.* 82, 1–7.
- Deacon, G., 1986. Estimations of serum arabinol for diagnosing invasive candidosis. *J. Clin. Pathol.* 39, 842–50.
- Escalante, J., Caminal, G., Figueredo, M., de Mas, C., 1990. Production of arabitol from glucose by *Hansenula polymorpha*. *J. Ferment. Bioeng.* 70(4) 228-231.
- Fabre, L., Gallezot, P., Perrard, A., 2002. Catalytic hydrogenation of arabinonic acid and lactones to arabitol. *J. Catal.* 208 (1), 247-254.
- Fonseca, C., Spencer-Martins, I., Hahn-Hägerdal, B., 2007. L-Arabinose metabolism in *Candida arabinofermentans* PYCC 5603T and *Pichia guilliermondii* PYCC 3012: influence of sugar and oxygen on product formation. *Appl. Microbiol. Biotechnol.* 75, 303–310.
- Groleau, D., Chevalier, P., Yuen, T.L.S.T., 1995. Production of polyols and ethanol by the osmophilic yeast *Zygosaccharomyces rouxii*. *Biotechnol. Lett.* 17, 315–320.
- Hajny, G.J., Products, F., Service, F., 1964. D-Arabitol Production by *Endomycopsis chodati*. *Appl. Microbiol.* 12, 87–92.
- Hanssens, L., Van Regenmortel, a, Verachtert, H., 1972. pH-dependent polyol production in *Moniliella tomentosa*. *Appl. Microbiol.* 24, 831–3.
- Park, H.N., Yoshida, S., Takakashi, A., Kawabata, Y., Jin Sun, H., Kusakabe, I., 2001. A new method for the preparation of crystalline L-arabinose from arabinoxylan by enzymatic hydrolysis and selective fermentation with yeast. *Biotechnol. Lett.* 23, 411–416.
- Holligan, P.M., Jennings, D.H., 1972. Carbohydrate metabolism in the fungus *Dendryphiella salina*. II. The influence of different carbon and nitrogen sources on the accumulation of mannitol and arabitol. *New Phytol.* 71, 583–594.
- Horwitz, S.B., Kaplan, N.O., 1964. Hexitol dehydrogenases of *Bacillus subtilis*. *J. Biol. Chem.* 239, 830–838.
- Ingram, J.M., Wood, W.A., 1965. Enzymatic basis for D-arabitol production by *Saccharomyces rouxii*. *J. Bacteriol.* 89, 1186–1194.
- Jiang, M., Wang, B., Yang, L., Lin, S., Cheng, H., 2011. Microbiological purification of L-arabitol from xylitol mother liquor. *J. Microbiol. Biotechnol.* 21, 43–49.
- Jovall, P. A., Tunblad-Johansson, I. and Adler, L., 1990. ¹³C NMR analysis of production and accumulation of osmoregulatory metabolites in the salt-tolerant yeast *Debaryomyces hansenii*. *Arch. Microbiol.* 154, 209–214.
- Kayingo, G., Wong, B., 2005. The MAP kinase Hog1p differentially regulates stress-induced production and accumulation of glycerol and D-arabitol in *Candida albicans*. *Microbiol.* 151, 2987–99.
- Kirk, R.E., Othmer, D.F., 1981. Kirk-Othmer encyclopedia of chemical technology: Index Vol. 9 to 12. J. Wiley & Sons.
- Koganti S (2012) Conversion of biodiesel byproduct glycerol to arabitol and sophorolipids through microbial fermentation. 207.
- Kumdam, H., Murthy, S.N., Gummadi, S.N., 2013. Production of ethanol and arabitol by *Debaryomyces nepalensis*: influence of process parameters. *AMB Express* 3, 1–12.
- Kumdam, H., Narayana Murthy, S., Gummadi, S.N., 2012. A statistical approach to optimize xylitol production by *Debaryomyces nepalensis* NCYC 3413 in

- vitro*. Food Nutri. Sci. 03, 1027–1036.
- Levin, G., Zehner, L., Saunders, J., Beadle, J., 1995. Sugar substitutes: their energy values, bulk characteristics, and potential health benefits. Am. J. Clin. Nutr. 62, 1161S–1168.
- Levin, G. V., 2002. Tagatose, the new GRAS sweetener and health product. J. Med. Food. 1, 23–36.
- Lindberg, B., Wachtmeister, C. A., and Wickberg, B., 1952. Studies on the chemistry of lichens. 1052-1055
- Link, T., Lohaus, G., Heiser, I., Mendgen, K., Hahn, M., Voegelé, R.T., 2005. Characterization of a novel NADP(+)-dependent D-arabitol dehydrogenase from the plant pathogen *Uromyces fabae*. Biochem. J., 389, 289–95.
- Loman, A. Al, Ju, L.-K., 2013. Purification of arabitol from fermentation broth of *Debaryomyces hansenii* using glycerol as substrate. J. Chem. Tech. Biotech. 88, 1514–1522.
- Lowe, D., Jennings, D., 1975. Carbohydrate metabolism in the Fungus V. The pattern of label in arabitol and polysaccharide carbon sources. New Phytol. 74, 67–69.
- Moran, J.W., Witter, L.D., 1979. Effect of sugars on D-arabitol production and glucose metabolism in *Saccharomyces rouxii*. J. Bacteriol. 138, 823–31.
- Moskowitz, H.R., 1971. The Sweetness and Pleasantness of Sugars. Am. J. Psych. 84, 387–405.
- Nobre, M.F., Costa, M.S. da, 1985. Factors favouring the accumulation of arabinitol in the yeast *Debaryomyces hansenii*. Can. J. Microbiol. 31, 467–471.
- Nozaki, H., Suzuki, S., Tsuyoshi, N., Yokozeki, K., 2003. Production of D-arabitol by *Metschnikowia reukaufii* AJ14787. Biosci. Biotechnol. Biochem. 67, 1923–1929.
- Onishi, H., N. Saito AIK (1961) Various factors affecting on polyalcohol production by *Pichia miso*. Agri. Biol. Chem. 25:124–130.
- Onishi, H. and Suzuki, T. (1966). The production of xylitol, L-arabinitol and ribitol by yeasts. Agri. Biol. Chem. 30:1139-1144.
- Roboz, J., Suzuki, R., Holland, J.F., 1980. Quantification of arabinitol in serum by selected ion monitoring as a diagnostic technique in invasive candidiasis. J.Clin. Microbiol. 12, 594–601.
- Ruff, O., 1899. D- und R-Arabinose. Ber. Dtsch. Chem. Ges. 32, 550–560.
- Ruijter, G.J.G., 2004. Polyol accumulation by *Aspergillus oryzae* at low water activity in solid-state fermentation. Microbiol. 150, 1095–1101.
- Saha, B., Bothast, R., 1996. Production, purification, and characterization of a highly glucose-tolerant novel beta-glucosidase from *Candida peltata*. Appl. Environ. Microbiol. 62, 3165–3170.
- Saha, B., Sakakibara, Y., Cotta, M.A., 2007. Production of D-arabitol by a newly isolated *Zygosaccharomyces rouxii*. J. Ind. Microbiol. Biotechnol. 34, 519–523.
- Soyama, K., Ono, E., 1987. Improved procedure for determining serum D-arabinitol by resazurin-coupled method. Clin. Chim. Acta. 168, 259–260.
- Talja, R.A., Roos, Y.H., 2001. Phase and state transition effects on dielectric, mechanical, and thermal properties of polyols. Thermochim. Acta. 380, 109–121.
- Toyoda, T., Ohtaguchi, K., 2011. Effect of temperature on D-arabitol production from lactose by *Kluyveromyces lactis*. J. Ind. Microbiol. Biotechnol. 38(9):1179-85.
- Tourneau, D. Le, 1966. Trehalose and Acyclic Polyols in Sclerotia of *Sclerotinia sclerotiorum*. Mycologia 58, 934–942
- Van Eck, J.H., Prior, B.A., Brandt, E. V., 1989. Accumulation of polyhydroxy alcohols by *Hansenula anomala* in response to water stress. Microbiol. 135, 3505–3513.
- Van Eck, J.H., Prior, B.A., Brandt, E. V., 1993. The water relations of growth and polyhydroxy alcohol production by ascomycetous yeasts. J. Gen. Microbiol. 139, 1047–1054.
- Weimberg, R., Orton, W.L., 1965.

Synthesis and breakdown of the polyphosphate fraction and acid phosphomonoesterase of *Saccharomyces Mellis* and their locations in the cell. J. Bacteriol. 89, 740–7.

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

Wong, B., Murray, J.S., Castellanos, M., Croen, K.D., 1993. D-arabitol metabolism in *Candida albicans*: studies of the

biosynthetic pathway and the gene that encodes NAD-dependent D-arabitol dehydrogenase. J. Bacteriol. 175, 6314–20.

Zhu, H-Y., Xu, H., Dai, X-Y., Zhang, Y., Ying, H-J., Ouyang, P-K., 2010. Production of d-arabitol by a newly isolated *Kodamaea ohmeri*. Bioproc. Biosyst. Eng. 33(5), 565-571.

Zyl, P., Kilian, S., Prior, B., 1990. The role of an active transport mechanism in glycerol accumulation during osmoregulation by *Zygosaccharomyces rouxii*. Appl. Microbiol. Biotechnol. 34, 231–235.