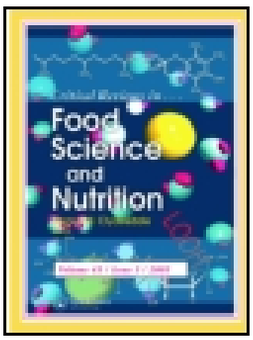

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Biocatalytic Production of D-Tagatose: A Potential Rare Sugar with Versatile Applications

Jayamuthunagai J[1]*, Gautam P[1], Srisowmeya G[1], Chakravarthy M[1]

[1]Centre for Biotechnology, Anna University, Tamil Nadu, Chennai-600025, India.

Abstract:

D-Tagatose is a naturally existing rare monosaccharide having prebiotic properties. Minimal absorption, low metabolizing energy and unique clinical properties are the characteristics of D-tagatose. D-Tagatose gained international attention by matching the purpose of alternate sweeteners that is much needed for the control of diabetes among world population. Recent efforts in understanding tagatose bioconversion has generated essential information regarding its production and application. This article reviews the evolution of D-Tagatose as an important rare sugar by appreciable improvements in production results and its significant applications resulted of its unique physical, chemical, biological and clinical properties thus considering it an appropriate product for requisite improvements in technical viability. Based on current knowledge and technology projections, the commercialization of D-Tagatose rare sugar as food additive is close to reality.

Keywords: D-Tagatose, rare sugar, galactose, hexose, sweeteners

1. Introduction:

The concept of sugar production since the establishment in seventh century is having tremendous progress in the production and application areas. Rare sugars have novel physiological functions mediating variety of recognition pathways in humans. In the classification of all hexoses and pentoses by International Society of Rare Sugars (ISRS) naturally occuring 20 hexoses and 9 pentoses were termed rare sugars and their potential for wide range of applications are already known. Various possibilities for the mass production of rare sugars from readily available raw materials such as starch, hemicellulosic waste and whey are under research (Beerens et al., 2012). The production methods of rare sugars require multi discipline approaches involving the strategies of fermentation technology, molecular biology, enzyme technology and organic chemistry (Granstorm et al., 2004). Presence of increased amount of sugars in food products raised health and body physic concerns which necessitated the research for alternatives to existing dominant sweeteners. D-Tagatose synthesized by Spherix Incorporated in early 1990's became familiar for the property of low calorie bulk sweetening. Indistinguishable taste of tagatose from sucrose showed quicker onset of research regarding the physical, chemical and biological properties of this rare sugar. D-Tagatose is 92% as sweet as sucrose and lacks cooling effect when dissolved in 10% aqueous solution (Levin and Brown, 1996). Calorific range per gm of Tagatose is 0.12kcal (Livesey and Brown, 1996). Since the first introduction of D-Tagatose almost 17 years ago (Levin, 1995), its evolution as an industrially important bio-product has prospered dramatically. After GRAS approval by U.S FDA expert panel in 2002, D-Tagatose is incorporated in confectionary, beverages, nutritional supplements and dietary products (Kim, 2004). It is also an authenticated drug additive to mask unpleasant tastes and sweetener in mouth

wash, toothpaste and cosmetics like lipsticks. Successful formulation of tagatose in a variety of industrial products has now been attracted worldwide attention resulting in commercial D-Tagatose production a nearest reality.

Tagatose is expected to create new market because of its unique properties and its capacity to compete with sugar-substituting polyalcohol markets. The commercial price of Tagatose is expected to set close to the price of competing sugar substituting sweeteners like Sorbitol (US $ 1.2/kg) and polyalcohols (US $ 3-7/kg). While other existing sweeteners are consumed in million tons every year, tagatose will be all set for the productivity competition in the near future. The bottleneck factors that will govern the productivity and price of tagatose includes raw materials, plant size, process optimization and high yielding biological solution.

2. Bio-conversion of tagatose

The chemical synthesis of D-Tagatose is an economical process but suffers disadvantage of high temperature and pressure. As a consequence of this biological production of D-Tagatose has been studied intensively in the recent years (Table 1). Possible bioconversion of hexose sugars to D-Tagatose is given in Fig 1. D- Tagatose epimerases (DTE) and L- Aldose isomerases are employed in the production of D- Tagatose. Production of tagatose using galactitol dehydrogenase with galactitol as substrate is well known. Galactitol however has low potential as a substrate for commercial production of tagatose mainly because of high cost inspite of its higher conversion rate. Galactose is also used as substrates for tagatose production by l-Arabinose Isomerase (AI).

2.1. Galactitol:

Galactitol ($C_6H_{14}O_6$) also known as dulcitol is the reduction product of galactose catalysed by aldose reductase. D-Galactitol on oxidation produces D-Tagatose. Izumori and Tsuzaki reported the 85% higher yield of tagatose from D-galactitol by the biotransformation of D-galactitol to D-Tagatose using *Mycobacterium smegmatis* grown of L-sorbose, a specific inducer of the enzyme for the fermentation of D-Tagatose (Izumori and Tsuzaki, 1988). *Arthobacter globiformis* mediated bio-conversion resulted in 14g of D-Tagatose from 1 litre of 2% dulcitol medium showing maximum yield of 85% (Izumori et al., 1984). With *Enterobacter agglomerans* 221e strain, the conversion rate of galactitol to D-Tagatose was as high as 92% when 2% galactitol was used and it was found to be 86% when 5% of galactitol was used (Muniruzzaman et al., 1994). M. Manzoni et al., (2001) reported the biotransformation of D-Galactitol to D-Tagatose by acetic acid bacteria.100-160mg tagatose /liter of galactitol was produced at 24h in non-growing conditions and 260-340mg tagatose /liter of galactitol at 48hr in case of growing cells of *Gluconobacter* strains. *Gluconobacter oxydans* DSM 2343 strain gave a notable increase in tagatose yield reaching 3160mg/l with the specific activity rate corresponding to $6.6*10^{-3}$ l/hr at 24hrs of reaction. *Klebsiella pneumonia* strain 40b showed 100% conversion of tagatose from galactitol. Consumed galactitol was 100% converted to tagatose since both galactitol and tagatose are not used by the strain (Shimonishi et al., 1995). Enzymatic synthesis of L-Tagatose with an overall yield of 78% from galactitol using galactitol dehydrogenase from *Rhodobacter sphaeroides* was studied (Huwig et al. 1997). Galactitol being a sugar alcohol is obtained from reduction reaction of aldose reductase on galactose. Galactose and glucose are obtained by the action of β-Glycosidase on lactose disaccharide. Synergistic coupling of galactose extraction

from lactose for galactitol production and later in tagatose bioconversion has got less choice for improvement in this aspect than isomerase mediated direct galactose to tagatose conversion cost constrains turns out to be a major inhibitor factor..

2.2. Galactose:

Galactose is a monosaccharide also classified as an aldose, a hexose and a reducing sugar. D-Galactose on chemical or enzymatic isomerization results in D-Tagatose. Roh et al., (2000a) reported the bioconversion of D-galactose into D-tagatose by arabinose isomerase. The crude enzyme extract obtained from *Escherichia coli* with the plasmid PTC 101 converted galactose into tagatose with a yield of 96.4%. 68% conversion of D-galactose was obtained using the recombinant enzyme L-arabinose isomerase coded by gene Ara-A (Arabinose isomerase) from the hyper-thermophile *Thermotoga neapolitana* 5068 expressed in *E.coli* system (Kim et al. 2002). The recombinant *E. coli* expressing PTC 101 (*E.coli* JM 105/PTC 101) produced L-arabinose isomerase that on covalent bonding to agarose can produce an average of 7.5gm tagatose/litre day for 7 days. Unbound L-arabinose isomerase shows productivity of 0.47-0.30mg/U-day. This immobilized enzyme system on scale up gives 99.9g tagatose/litre from galactose with 20% equilibrium in 48h. The same system on 2 other trials produced 104.1 and 103.5g-tagatose/litre of galactose thus proving galactose to be a high potential substrate for commercial production (Kim et al. 2001). The possibility of obtaining Galactose from lactose has made raw material source sustainable thus enhancing commercial AI based D-tagatose production.

3. L-Arabinose isomerase in D-Tagatose production.

An intracellular enzyme l-Arabinose isomerase (l-AI; EC 5.3.1.4) also referred to as D-galactose isomerases or D-aldose isomerases due to their ability to isomerize D-galactose to D-tagatose is widely studied (Table 2). Biological manufacture of d-tagatose from d-galactose usingl-AIs has been of great interest in the recent days. l-AIs have been identified from a number of microorganisms, such as *Thermotoga maritima, Thermus sp, Geobacillus thermodenitrificans and Geobacillus stearothermophilus* (Kim, H.J., et al., 2003; Kim and Oh, 2005; Kim, J.W., et al., 2003). l-AI at a higher temperatures and moderately low pH with stability and high activity would have the greatest potential for the production of the D-tagatose. At alkaline pH (7.5–8.5) formation of undesirable sub-products are identified (Lee et al., 2005). Though hyperthermophilic AIs are highly effective in the commercial production of tagatose certain properties limit their application namely color formation at higher temperatures leading to browning reaction (above 70°C) and requirement of metal ions (especially Co^{2+}) which are not allowed in food products (Kim, 2004; Lee et al. 2004). This can be solved using thermophilic AIs which show higher conversion yield than hyperthermophiles at commercial processing temperature using a similar enzyme to AI to limit color formation (about 60°C) (Hartley et al., 2000; Jorgensen et al., 2004; Rhimi et al., 2011; Levin et al., 1995).High conversion rate from d-galactose to d-tagatose may be difficult at lower optimum temperature (30–45^{o}C) (Kim et al., 2001). For the purpose of attaining l-AIs suitable for d-tagatose production, new organisms carrying the acidophilic and thermostable target enzyme need to be screened. With the objective of generating an enzyme with optimal characteristics for the commercial production of tagatose, mutants of *Bacillus stearothermophilus* US100 L-arabinose isomerase namely ara US100

Q268K, ara US100 N175H and ara US100 Q268K/N175H were constructed, purified and characterized. Analysis of the activity of the enzyme at different temperatures showed that the mutant N175H was optimally active at a temperature range of 50-65°C (specific activity of 153 U/mg) while for wild type and mutant Q268K it was 80°C with specific activity of 185U/mg. This revealed the key role of N175H in enzyme thermoactivity. Analysis of acidotolerance showed that Q268K was relatively more stable at pH range 6.0-6.5 while it was 7.5 for wild and N175H mutant. With the aim of generating a mutant which being both acidotolerant and optimal at low temperature, a double mutant Q268K/N175H which showed optimal activity at pH range of 6.0-7.0 and temperature range of 50-60°C at which the stability of the enzyme was independent of metal ions (Rhimi et al., 2009).

Lactic acid bacteria are well known for their acid tolerance. An araA gene from *Lactobacillus fermentum*CGMCC2921 an acidophilus bacterium encoding for l-arabinose isomerase (l-AI) was cloned and over-expressed in Escherichia coli. Activity of the purified recombinant enzyme was maximum at 65^{o}C and pH 6.5 condition is extremely suitable for industrial applications. For the thermostability and enzymatic activity divalent metal ions either Co^{2+}, or Mn^{2+} was required. The purified *L. fermentum* CGMCC2921 l-AI converted d-galactose into d-tagatose with a high conversion rate of 55% with 1mMMn^{2+} after 12 h at 65^{o}C, suggesting its excellent potential in d-tagatose production with a relatively high catalytic efficiency (K_{cat}/K_m of 9.02mM^{-1} min^{-1} for D-galactose).

Ara A gene coding for AI from thermophilic bacterium *Geobacillus thermodenitrificans* was cloned and expressed in *E. coli* with respect to its potential in tagatose production. The conversion rate of D-galactose to D-tagatose was 48% with Mn^{2+} and 46% without Mn^{2+} after 5

hours. This negligible difference indicates the potential of recombinant AI to produce D-tagatose without the addition of Mn^{2+} which is in contrast to hyperthermophlies which require Co^{2+} for the conversion of D-galactose to D-tagatose. Of all the reported thermophilic AIs *Geobacillus thermodenitrificans* AI has the highest optimum temperature for activity and gave highest yield of D-tagatose from D-galactose without metal ions, is therefore a potential enzyme for commercial tagatose production (Kim and Oh, 2005).When plasmids containing gene (*ara A*) from *Bacillus subtilis* (PTC 105) *Salmonella typhi* (PTC106) and *Escherichia coli* (PTC101) were constructed, in the recombinant culture of *Escherichia coli* with PTC 105, PTC 106 and PTC 101, galactose was converted into tagatose with a yield of 7.1,6.9 and 9.9% respectively. By the expression of L-arabinose isomerase with the plasmid PTC 101 the conversion of galactose from tagatose was 96.4% (Roh et al., 2000b). *E.coli* L-arabinose isomerase prepared from recombinant *Escherichia coli* PTC 101 had protein concentration of 1.08 mg/ml, volumetric activity of 68.0 U/ml and specific gravity of 63U/mg-protein when immobilized with agarose support. Though the initial conversion rate of galactose to tagatose by the immobilized enzyme was lower than that of the free enzyme, the production remained stable for a minimum of 7days. The productivity of the free enzyme was 0.30mg-tagatose/U-day while that of the immobilized enzyme was 0.47mg-tagatose/U day. The productivity of the free enzyme was 4 times lower than that of the immobilized enzyme as the activity of the immobilized enzyme was only 38%of the free enzyme. Immobilized L-arabinose produced as much as 104.1 tagatose g/L. the recyclability and stability of the immobilized L-arabinose isomerase shows that it has high potential for economic and commercial production of tagatose (Kim et al. 2001). L-arabinose isomerase coding gene from *Thermotoga neopolitana*, a hyperthermophile was cloned and expressed in

Escherichia coli. The recombinant enzyme by ion exchange chromatography, heat treatment and gel filtration was purified to homogenecity. The catalytic activity increased to 18 fold using L-arabinose as substrate compared with D-galactose. The recombinant enzyme converted 68% of D-galactose to D-tagatose at an optimum temperature of 80°C with divalent cations such as Co^{2+} and Mn^{2+} for its activity and thermostability (Kim et al. 2002). To increase the conversion rate of galactose to tagatose, thermostable enzyme Gali 151 from a hot spring bacteria with higher optimum temperature of 60°C compared to 30°C of *E. coli* ara A (L-arabinose isomerase) was cloned (Kim et al. 2001). Gali 151 showed enhanced reaction rate and conversion equllibrium (50% higher conversion equilibrium of tagatose) compared with *E. coli* ara A, but the reaction velocity of Gali151 with galactose was much lower than with arabinose. Since L-arabinose and D galactose have similar 3D configuration ara A can mediate both isomerisation. Using error prone PCR, it is found that the substrate specificity for arabinose is higher than for galactose and the improved galactose isomerase had 11 fold higher reaction rate for galactose to tagatose conversion comparatively (Kim et al., 2001).

Most of the L-arabinose isomerases require metal ions for their thermostability and optimum activity at a concentration of 1 to 5mM (Table 3). However these ions are unfavourable during the product downstream process which results in the increased production cost. To overcome this an effective L-arabinose isomerase with the characteristics of thermostability and independence of metal ions was required. Moez Rhimi reported the recombinant L-arabinose isomerase US100 generated by cloning, sequencing and overexpresion of AraA gene coding for L-arabinose isomerase from *Bacillus stearothermophilus* US100 in *E.coli* which showed optimum activity at pH 7.5 and at 80°C (Rhimi et al., 2009). The EDTA treatment assay proved that the enzyme

activity does not depend on any metal ions below 65°C however above 65°C addition of Co^{2+} and Mn^{2+} evidently increased the activity of the enzyme. The study on the effect of Co^{2+} and Mn^{2+}showed that the thermostability of the enzyme was totally independent of these metal ions until 65°C but above 65°C the thermostability improved obviously in the presence of 1mM Mn^{2+} and 0.2 mM Co^{2+} only. L-arabinose isomerase US100 required low metallic ions even at temperatures over 65°C leading to considerable decrease in the production cost as the metal ion elimination step is reduced or removed. The conversion rate of D-galactose to D-tagatose by L-arabinose isomerase US100 was 48% after 7 hours at 70°C. L-arabinose isomerase US100 contributes for the economical production of tagatose from galactose (Rhimi and Bejar, 2006). In another case co-expression of L-arabinose isomerase of *Bacillus stearothermophilus* US100 and mutant D-glucose isomerase from *Streptomyces SK* in *E. coli* HB101 strain was done. This recombinant strain (*E. coli* HB101/pMR20) was capable of simultaneously converting D-galactose to D-tagatose and D-glucose to D-fructose at optimized temperature of 65°C and pH 7.5. The commercial significance of this recombinant lies in the fact that is capable of producing a syrup containing a mixture of D-tagatose and D-fructose from lactoserum, an inexpensive and plentiful disaccharide using β-galactosidase activity (Rhimi et at., 2007).

4. Properties of D-Tagatose as a sweetener.

An ideal sweetener should be atleast as sweet as sugar, pure, pleasant tasting, colourless, odourless, non-carcinogenic, water soluble and compatible with a range of food ingredients. Users of these sweeteners consume an average of four type of low-calorie, reduced sugar and sugar-free products. Existing alternate sweeteners includes Acesulfame, Alitame, Neotame,

Steviocide, Dihyrochalcone, Saccharin, Cyclamate, Aspartamate etc. Despite these sweeteners, polyols (sugar alchols) continue to be important in the development of calorie free sweetening agents. All polyols have low unit calorie value (2.4kcal/g), low glycemic index and can completely or partially replace sucrose, glucose and high GI polysaccharides such as starch and maltodextrin in a wide range of food products. D-Tagatose being a ketohexose with C4 fructose epimer is considered to be a low calorie bulk sweetener. In comparison with sucrose it contains 92% of the sweetness and less than half the calories. D-Tagatose has the following beneficial properties (1) non-carcinogenic (2) off flavor potentiation (3) no cooling effect and (4) lacks laxative effect. Tagatose increases the lactic acid bacteria in the human and animal intestinal track which provides functional benefits (Levin et al. 1995). Tagatose plays an effective role in management of diabetes, controls dental carries, calorific intake control in beverages and foods.

5. Clinical properties of D-Tagatose:

Antioxidant and cryo-protective properties of D-Tagatose in cultured murine hepatocytes were compared with the equimolar concentration of glucose, mannitol or xylose. The antioxidant properties of D-tagatose was effective and concluded to result from sequestering the rodox active iron because D-Tagatose prove to be a weak iron chelator that can antagonize the iron dependent toxic consequences of intracellular oxidative stress in hepatocytes. Tagatose also afforded complete protection against redox cycling drug, Nitrofurentoin (NFT) (Paterna et al. 1998). D-Tagatose tested as a sweetner in continental breakfast had significant gastrointestinal side effects when added in excess. Rumbling in the stomach, distention, nausea, rumbling in the gut, flatulence and diarrhea scored significantly higher with excess concentration of D-Tagatose.

Otherwise the sugar was well tolerated and explained to be a low calorific alternative sweetner (Buemann et al. 1999). Genotoxicity tests on D-Tagatose were tested over murine lymphocytes by Claire L. kruger et al. (1999). D-Tagatose caused no significant increase in micronuclei, polychromatic erythrocytes, bone marrow thus proving to be non genotoxic at varying dose levels. Comparitive investigations of chemolysis induction by L-Sorbose and D-Tagatose were studied using dog erythrocytes. It is concluded that D-Tagatose unlike L-sorbose doesn't have a hemolystic effect on erythrocytes (Bar and Leeman, 1999). Ingested D-tagatose is incompletely absorbed from the small intestine, unabsorbed D-Tagatose remains in the column where it is completely fermented (Lee and Storey, 1999). Fermentation products such as alkyl chain fatty acid produce osmolytic effect in the intestine causing diarrhea (Buemann et al., 1999; Lee and Storey, 1999; Buemann et al., 2000). D-fructose has been found to increase uric acid production by accelerating the degradation of purine nucleotides probably due to hepatocellular degradation of inorganic phosphate D-Tagatose being a steroisomer of D-Fructose is found to have similar properties inspite of its putative pure absorption (Valeri et al., 1997). The iron dependent formation of super oxide anion radicals (acetylated cytochrome c reduction) induced by pro-oxidant drug remained unaffected by iron chelated fructose/tagatose, hence promising protection against oxidative cell injury (Kruger et al., 1999). Though D-Tagatose exhibits 92% of sweetening activity of sucrose it has got 25% of metabolizing energy only. On the basis of this result it is concluded that D-Tagatose could exert beneficial effect over carbohydrate toluence and fasting hyperglycemia in type 2 diabetic patients. Hence D-Tagatose could prevent diet associated weight gain in type 2 diabetic subjects suffering obesity without deleterious effects upon biomolecular metabolism.

6. Drug applications

Tagatose may be substituted for sweeteners in mouthwash, toothpaste and lipstick (Table 5). Despite the proposed use of tagatose in above products, the clinical properties of D-tagatose makes it an important ingredient in drug manufacture. Tagatose was shown not to increase the blood glucose level and the sugar was also found to be anti-hyperglycemic (Zehner et al., 1994) explaining the potential of tagatose to act as useful therapeutic adjunct in the management of type 2 diabetes mellitus. Prescribed doses of tagatose as a part of a dietary restriction program could promote active health and long living (Levin, 2002). Tagatose can also has positive impact on pregnancy and fetal development. Rats treated with tagatose produced higher percentage of live births than the controlled rats (Kruger et al., 1999). Data explaining rat feeding and safety studies summarized that the male rats receiving tagatose developed higher RBC count than the controlled rats. Tagatose fed rats of both the sexes showed regulated secretion of blood factors that would prove D-Tagatose as a beneficial drug for the treatment of hemophilia thus making tagatose a significant blood regulator. Antioxidant and cryoprotective effects of tagatose is known to protect the liver cells from lethal pro-oxidant poisons like cocaine and nitroflurentoin (NFT). Clinical experiments on normal and genetically diabetic rats was attempted to evaluate the influence of tagatose in blood glucose level (Szepesi et al., 1996). Innovative use of tagatose in organ transplants recorded positive effects when compared to transplant that lacked tagatose production. The low caloric value and taste of tagatose makes it to be employed by pharmaceutical companies as excipient use in drugs to overcome the problem of unpleasant taste which makes it difficult for consumption. Employing tagatose as a sweetening excipient in pharmaceutical products for non-human primates and canines can reduce the stress of forming

the animals to injest the unpleasant tasting substances. Tagatose having low caloric value can helping these products being used for overnight companion animals.

7. Tagatose derived products

Sorbose ($C_6H_{12}O_6$), highly water soluble monosaccharide (ketose) with a molar mass of 180.16g/mol is an important tagatose derived product of industrial and commercial importance. The commercial biosynthesis of vitamin C (ascorbic acid) often begins with sorbose. D-Sorbose can be produced from D tagatose using D-tagatose 3 epimerase (D-TE) from Pseusomonas sp. ST-24 immobilised on chitopearl beads (BCW 2503). The activity of the enzyme immobilized on beads treated with glutaraldehyde was about 80-90% that of D-TE immobilized on non-glutaraldehyde treated beads. The maximum activity of the immobilized enzyme was at 60 C and pH 7-9. At various concentration of D-Tagatose (5-30%) the reaction progressed steadily and about 70% of D-tagatose was converted to D-sorbose without significant substrate inhibition in a batch reaction. On purification 2g of D-sorbose crystals were obtained from 3g of D-tagatose. Enzymatic preparation of D-sorbose from D-tagatose is simple yet effective. Glucosyl tagatose is a potential transfer product, produced by transglycosylation of D-tagatose using *Bacilus stearothermophilus* maltogenic amylase (BSMA) with maltotriose as a donar molecule. Reaction mixture with 5% w/v of tagatose was transglycosylated to form a mixture of maltosyl and glucosyl tagatose transfer products, which on further hydrolysis with glucoamylase allowed the isolation of glucosyl tagatose as a major product. Certain properties such as glass transition temperature (-29^{o}C), considerably higher than tagatose (-45^{o}C) and greater water sorption property compared to tagatose revealed by hydroscopicity measurements showed that tagatose

has potential application as cryostabilizer (Roh et al., 2005). Muniruzzaman et al., (1994) reported the production of D-talitol, a rare polyol with organoleptic and other biochemical properties (Kim et al., 2001a; Kim et al., 2001b; Bhosale et al., 1996) from tagatose using *Aureobasidium pullulans* strain 113 B which fastened the reaction in the presence of glycerol. The conversion rate was as high as 93% at low substrate concentration (0.5%). The transformation activity was high in case of cells grown on D-glucose or D-fructose. About 0.58 g of D-talitol crystals were obtained from 1g of D-tagatose. The strain 113 B is an effective producer of D-talitol from D-tagatose.

8. Conclusion

The potential for wide use of food, non-food and drug applications is the substantial characteristic of tagatose. Despite its versatile clinical properties and inherent potential as a drug and food additive, the development of tagatose formulation to commercial viability has been impeded by various technical challenges. Forwarding D-tagatose as a marketable ingredients thus competing the existing alternative sweeteners needs upgrades such as (1)species selection balancing efficient tagatose conversion and coproduction of valuable byproducts (2) attaining higher conversion efficiency by selection strains that never uses tagatose or its raw-material in energy making pathway and production of undesirable co-products (3) providing cost efficient raw materials like galactose obtained from cheap materials in bulk quantities (4) development of mixed cultivation of microbes for automatic supply of starting materials from raw feed followed by efficient conversion (5) improvements in overexpression of genes expressing L-Arabinose Isomerase in strains compatible for intracellular bioconversion or excessive enzyme production

progressing enzymatic bioconversion. Countering the technical challenges by innovative current technological strategies and fortunate improvements in genetic engineering and biotechnological aspects in D-tagatose production can further intensify its industrial manufacturing.

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Enzyme	Advantages	Disadvantages	Significance
Isomerase	Broad specificity for substrate Substrates often unsubstituted	Formation of undesired product (mixtures)	Isomerizes D-galactose to D-Tagatose
Epimerase	Shorter route of synthesis Bridges D- and L- form of sugars	Substrates often substituted Strict substrate specificity	DTE converts D-fructose to D-psicose, D-Sorbose to D-tagatose.
Oxidoreductase	Substrates often unsubstituted Bridges D- and L- form of sugars	Need for co-factor regenration	D/L- galactitol to D-Tagatose

Table 1. Enzymes associated in D-Tagatose production

Organisms	Conversion yield (%)	Conversion conditions	References
Acidothermus cellulolytics ATCC43068	53	75°C, 12 h	Cheng et al., 2010b
Alicyclobacillus acidocaldarius	44	60°C, 6 h	Lee et al., 2005
Anoxybacillus flavithermus	60	95°C, 1 h	Li et al., 2011
Arthrobacter sp. 22c	30	50°C, 36 h	Wanaska and Kur, 2012
Bacillus stearothermophilus IAM 11001	36	65°C, 12 h	Cheng et al., 2010a
Bacillus stearothermophilus US 100	48	70°C, 7 h	Rhimi and Bejar, 2006
Escherichia coli	34	35°C, 168 h	Roh et al., 2000
Geobacillus stearothermophilus	46	60°C, 5 h	Lee et al., 2005
Geobacillus	46	65°C, 5 h	Kim and Oh,

thermodenitrificans			2003
Lactobacillus fermentum CGMCC2921	55	65°C,12 h	Xu et al., 2011
Lactobacillus plantarum NC8	30	60°C, 6 h	Chouayekh et al., 2007
Lactobacillus sakei 23K	36	40°C, 7 h	Rhimi et al., 2010
Thermoanaerbacter mathranii	25	65°C, 24 h	Jorgensen et al., 2004; Hansen et al., 2006
Thermotoga maritime	56	80°C, 6 h	Lee et al., 2004
Thermotoga neapolitana	68	80°C, 20 h	Kim et al., 2002
Thermus sp. IM6501	54	60°C, 3 days	Kim et al., 2003

*All the experiments are performed only with the enzymes obtained from the corresponding organisms.

Table 2. Enzymatic Production of D-Tagatose from D-glucose.

Organisms	Molecular mass (kDa)	Optimum Temp	Optimum pH	Co-Factors (mM)	Thermostability requirements
Acidothermus cellulolytics ATCC43068	53	75	7.5	Mn^{2+} (1.0), Co^{2+}(0.5)	NR
Alicyclobacillus acidocaldarius	44	65	6.0-6.5	Mn^{2+}(1.0), Co^{2+}(1.0), Mg^{2+}(1.0)	NR
Anoxybacillus	60	95	9.5-10.5	Ni^{2+}(1.0)	NR

flavithermus					
Arthrobacter sp. 22c	30	47-52	5.0-9.0	NR	NR
Bacillus stearothermophilus IAM 11001	36	65	7.5	Mn^{2+}(1.0), Co^{2+}(1.0)	NR
Bacillus stearothermophilus US 100	48	80	7.5-8.0	Mn^{2+}(1.0), Co^{2+}(2.0)	Strictly Mn^{2+}, Co^{2+}
Escherichia coli	34	30	8.0	Mn^{2+}(0.5)	NR
Geobacillus stearothermophilus	46	70	7.0-7.5	Mn^{2+}(1.0)	NR
Geobacillus thermodenitrificans	46	70	8.5	Mn^{2+}(5.0), Co^{2+}(3.0)	NR
Lactobacillus fermentum CGMCC2921	55	65	6.5	Mn^{2+}(1.0), Co^{2+}(2.0)	NR
Lactobacillus plantarum NC8	30	60	7.5	Mn^{2+}(1.0), Co^{2+}(0.5)	NR
Lactobacillus sakei 23K	36	30-40	5.0-7.0	Mn^{2+}(0.8), Mg^{2+}(0.8),	NR
Shewanella sp. ANA-3	-	15-35	5.5-6.5	Mn^{2+}(0.6)	-
Thermoanaerbacter mathranii	25	65	8.0	Mn^{2+}	NR
Thermotoga maritima	56	90	7.5	Mn^{2+}(5.0), Co^{2+}(1.0)	NR

Thermotoga neapolitana	68	85	7.0	Mn^{2+}(1.0), Co^{2+}(1.0)	NR
Thermus sp. IM6501	54	60	8.5	Mn^{2+}(5.0)	NR

*NR-Not required

Table 3. Properties of L- arabinose isomerase from various microorganisms (Rhimi, et al., 2011; Wanaska and Kur, 2012)

Properties	Description
Common name	Tagatose
Synonyms	D-Tagatose, α-D-Tagatose, D-lyxo-hexulose
Molecular formula	$C_6H_{12}O_6$
CAS Registry	87-81-0
Classification	Carbohydrate > Monosaccharide > Keto-hexose

Structure	3 chiral Carbons; C-4 epimerised D-fructose
Mol. Wt.	180
Physical property	Anhydrous crystalline solid
Color	White
Odor	None
Taste	Intensively sweet
Solubility in water	160g/100ml at 20^{o}C
Solubility in ethanol	0.02g/100ml at 22^{o}C
Melting point	134^{O}C
Heat of solution	-42.3 kJ/kg at 20^{o}C
Decomposition Temperature	120 ^{O}C
pH stability	2-7
Sweetness	Emulates sucrose, but faster like fructose
Relative sweetness	92% of sucrose
Intestinal absorption	25% absorbed, 75% unabsorbed
Relative energy	1/3 calories of sucrose
Calorific value	0.12kcal/g
Carcinogenicity	None
Flavor enhancer	Combined flavoring with high intensity sweetening
Bulk sweetening	Yes
Humectant	Similar to sorbitol
Caramel formation	Yes, turns brown like sucrose on high temperature

Table 4. Properties of D-Tagatose.

Products	Concentration (approx.)
Cheddar cheese	2 mg/kg

Chewing gum	60% w/w
Chocolates	15% w/w
Confectionery (hard and soft)	15% w/w
Diet drinks	1% w/w
Dietary supplements	90% w/w
Feta cheese	17 mg/kg
Frostings	15% w/w
Gjetost cheese	15 mg/kg
Ice creams	3% w/w
Laxatives	6500 mg/kg
Packed breakfast cerals	15% w/w
Parmesan cheese	10 mg/kg
Powdered cow's milk	800 mg/kg
Roquefort cheese	20 mg/kg
Sterilized cow's milk	2-3000 mg/kg
Yogurt	29 mg/kg

Table 5. Occurrence and applications of D-Tagatose in food products (Levin, 2002; Kawamura, 2004).

Fig 1. Possible biological production of D-Tagatose from D/L Hexoses.