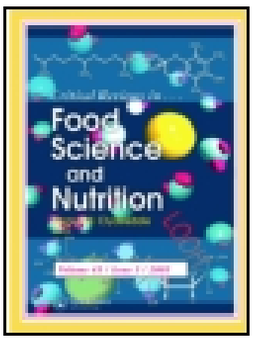

Critical Reviews in Food Science and Nutrition

ISSN: 1040-8398 (Print) 1549-7852 (Online) Journal homepage: http://www.tandfonline.com/loi/bfsn20

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To cite this article: Muhammad Bilal, Hafiz M. N. Iqbal , Hongbo Hu, Wei Wang & Xuehong Zhang (2017): Metabolic engineering pathways for rare sugars biosynthesis, physiological functionalities, and applications – A review, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2017.1341385

To link to this article: http://dx.doi.org/10.1080/10408398.2017.1341385

Accepted author version posted online: 29 Jun 2017.

Metabolic engineering pathways for rare sugars

Metabolic engineering pathways for rare sugars biosynthesis, physiological functionalities, and applications -- A review

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Abstract

Biomolecules like rare sugars and their derivatives are referred to as monosaccharides particularly uncommon in nature. Remarkably, many of them have various known physiological functions and biotechnological applications in cosmetics, nutrition and pharmaceutical industries. Also, they can be exploited as starting materials for synthesizing fascinating natural bioproducts with significant biological activities. Regrettably, most of the rare sugars are quite expensive, and their synthetic chemical routes are both limited and economically unfeasible due to expensive raw materials. On the other hand, their production by enzymatic means often suffers from low space-time yields and high catalyst costs due to hasty enzyme denaturation/degradation. In this context, biosynthesis of rare sugars with industrial importance is receiving renowned scientific attention, across the globe. Moreover, the utilization of renewable resources as energy sources via microbial fermentation or microbial metabolic engineering has appeared a new tool. This article presents a comprehensive review of physiological functions and biotechnological applications of rare ketohexoses and aldohexoses, including D-psicose, D-tagatose, L-tagatose, D-sorbose, L-fructose, D-allose, L-glucose, D-gulose, L-talose, L-galactose, and L-fucose. Novel *in-vivo* recombination pathways based on aldolase and

phosphatase for the biosynthesis of rare sugars, particularly D-psicose and D-sorbose using robust microbial strains are also deliberated.

Keywords

Rare sugars; biotechnological applications; biosynthesis; metabolic engineering; DHAP-dependent aldolases

ACCEPTED MANUSCRIPT

Introduction

The International Society of Rare Sugars (ISRS) has defined the namesake ‘rare sugars, and their derivatives are monosaccharides that hardly exist in nature (Ahmed, 2011; Beerens et al., 2012; Shuang-Yan, 2012). Despite their quite low natural abundance, rare sugars display various known biological functions and hold enormous potential for exploitations in synthetic, cosmetics, pharmaceutical, food and flavor industries (Granström et al., 2007; Kano et al., 2010; Mäki-Arvela, et al., 2011; Moghaddam and Van den Ende, 2012; Li et al., 2013; Wen et al., 2016). Some unnatural rare sugars, for instance, D-psicose have shown potential to inhibit hepatic lipogenic enzymatic activity, and resultantly reduce abdominal fat accumulation (Chung et al., 2012). D-tagatose is an imperative drug for alleviating type-2 diabetes, and also has been certified by the U.S. Food and Drug Administration (FDA) as a valuable food additive (Espinosa and Fogelfeld, 2010). D-allose has diverse physiological functionalities, as an anti-carcinogenic, anti-inflammatory, and anti-oxidative agent (Ueki et al., 2008; Lim and Oh, 2011; Yamada et al., 2012). Compared with their D-enantiomer counterparts, several L-form rare sugars have been extensively employed for developing antiviral and anticancer drugs in therapeutic fields (Mathé and Gosselin, 2006; Perali et al., 2011). L-sorbose has been utilized to synthesize glycosidase inhibitor (1- Deoxygalactonojirimycin) and L-ascorbate (Itoh and Izumori, 1996). L-fructose is identified as a nonnutritive sweetener, glycosidases inhibitor, and an insecticide for house flies and ants (Muniruzzaman et al., 1996). Similarly, in the food industrial sectors, rare sugars have been recognized as low-calorie supplements and nonnutritive sweetener (Matsuo et al., 2002; Oshima et al., 2006). Nevertheless, the insufficient natural availability of these compounds may hamper their industrial perspective (Börgel et al., 2012).

Given limited availability from natural sources, a variety of chemo-enzymatic methods has been developed to produce unnatural sugars from abundant carbohydrates (Wei et al., 2015). Together with low sugars yield, the chemical routes also tend to involve multiple protection/deprotection and tedious purification stages (Emmadi and Kulkarni, 2014). Alternatively, enzymatic biosynthesis under gentle working environment and high efficiency have distinctive advantages regarding both regio- and stereoselectivity (Beerens et al., 2012). Prof. Ken Izumori (Kagawa University Rare Sugar Research Centre) developed a novel and efficient “Izumoring” strategy

for biosynthesis of all hexoses from cheap and abundantly available feedstocks (Izumori, 2006). Encouraging by the Izumoring strategy, many D/L-sugars, such as D-psicose and L-fructose, have now been synthesized using various enzyme-assisted biotransformation processes (Men et al., 2012). Nevertheless, the conversion rates are not convincing (less than 40%) in most of the investigated cases. Moreover, due to the inevitability of expensive cofactor NAD(P)H, these oxidation reactions are preferred to carry out inside microbial platform to supply reductive power by the cellular metabolism (Shuang-Yan, 2012).

Aldolases are the unique class of biocatalysts that have been widely investigated for catalyzing stereoselective C-C bond formation (Brovetto et al., 2011). Dihydroxyacetone phosphate (DHAP)-dependent aldolases evidenced particular specificity towards DHAP carrying out the condensation reactions between DHAP and a broad range of aldehydes to produce poly-hydroxylated rare sugars (Brovetto et al., 2011). Figure 1 illustrates a detailed process of rare sugar synthesis from a bioreactor and metabolic engineering perspective to purification and analyses. Owing to instability and elevated price of DHAP, the glycolytic pathway has been engineered by inactivating triose phosphate isomerase (TPI, EC 5.3.1.1) to augment intracellular accumulation of DHAP. Green production from sustainable resources is the method of choice since microbial conversions exploit cheaper catalysts evading purification and cofactors recycling. Efficient uses of these microorganisms are, however, limited due to the uncertain background, non-safety or restricted capability to form an invaluable array of industrially-pertinent compounds. As a result, tailoring of a food-grade organism involving in the synthesis of rare sugars might display a more convincing approach (Yang et al., 2015). Several aldolases and phosphorylases-assisted recombination pathways are assembled in *Escherichia coli* or *Corynebacterium glutamicum* to efficiently produce unnatural sugars and their derivatives (Wei et al., 2015; Yang et al., 2015). Glycerol, a major by-product of the biodiesel production process, as an attractive carbon sources displays higher potential for the microbial biosynthesis of value-added bioproducts. It not only transformed to L-glyceraldehyde and 3-hydroxypropionaldehyde but can also be converted to DHAP through the glycerol utilization pathway (Yang et al., 2016). In this review study, the recent advances regarding physiological functionalities as well as biotechnological potentialities of rare sugars are summarized. A particular emphasis was given to

the ketohexoses and aldohexoses (Figure 2). The state-of-the-art biosynthetic strategies for rare sugars by microbial fermentation as well as recent advances are also reviewed.

Rare sugars -- Ketohexoses

Physiological and biotechnological functions of D-psicose

D-Psicose (D-allulose, or D-ribo-2-hexulose; $C_6H_{12}O_6$) is a low-calorie monosaccharide sugar that hardly exists in natural products. It is a carbon-3 epimer of D-fructose and has proven to exhibit the anti-glycemic effect. Matsuo and Izumori (2009) conducted animal-based experiments to explicate glucose suppression mechanism caused by the inhibition of α-glycosidase. D-psicose conferred similar behavior as D-fructose in glucose uptake from the liver. It is demonstrated that D-psicose can markedly repress the elevated blood glucose level in healthy adults (Tetsuo et al. 2008; Hayashi et al. 2010). Supplemental D-psicose in the food ingredients has shown improved insulin sensitivity and glucose tolerance while decreasing the glycemic response (Hossain et al. 2011). Moreover, D-psicose can conquer hepatic lipogenic enzymatic activity and reduces intra-abdominal fat accumulation (Matsuo and Izumori 2009). It displayed superior reactive oxygen species (ROS) scavenging capacity than any other rare sugars (Murata et al. 2003).

D-psicose has important perspective in clinical/medical applications such as anti-obesity, anti-hypertension, anti-hyperlipidemia and anti-atherosclerotic agent (Kawakami et al., 2014). Investigators have explored the anti-obesity activity of D-psicose by diminishing weight of adipose tissue in humans and animals (Chung et al., 2012). The anti-obesity properties not merely ascribed to decreasing food intake but also associated with reducing fat mass and adipose tissue weight. Additionally, a variety of investigations have already ascertained the anti-hyperlipidemic effects of D-psicose; nevertheless, the exact mechanism underlying anti-hyperlipidemic is still unclear necessitating further clinical trials. It is however hypothesized that D-psicose affects lipogenic and lipolytic enzyme activities (Ochiai et al., 2014).

Due to its unique physicochemical properties (i.e., sweetness, high solubility, extremely low calories, and low glycemic response), D-psicose has promising market potential as an ideal sucrose substitute in the food industry (Oshima et al. 2006; Fukada et al. 2010). Various functionalities of D-psicose are diagrammed in Figure 3. Amalgamation of D-psicose in food ingredients could upgrade the gelling behavior and improve food quality through Millard

reactions with food proteins. Amongst all the D-ketoses (D-tagatose, D-sorbose, D-allulose, and D-fructose), D-allulose presented inimitable enhancement in food properties of egg white protein through the Maillard reactions, such as excellent gel strength, emulsifying and foaming properties, and antioxidant activities (O'Charoen et al., 2015). A syrup containing D-psicose can be commercially procured from Matsutani Chemical Industry Co. Ltd., (Hyogo, Japan) as a source of functional food (Zhang et al., 2016).

Of most importantly, D-allulose has been certified as Generally Recognized as Safe (GRAS) status by FDA, USA and is approved as a dietary supplement and food ingredient (Zhang et al., 2016). According to the toxicity ranking chart, it occupies the "relatively harmless" category, which is the lowest toxicity grade (Mu et al., 2012). D-psicose has been documented as the foremost anthelmintic sugar exerting parasite growth suppressing effects (Harada et al., 2012).

Physiological and biotechnological functions of D-Tagatose and L-tagatose

D-Tagatose is a naturally occurring rare hexoketose with low metabolizing energy, minimal absorption, and unique medicinal properties. Following GRAS approval by U.S FDA, D-tagatose has been incorporated in a range of food ingredients, beverages, confectionary and dietary products (Kim, 2004). D-tagatose is a ketohexose of C-4 fructose epimer with low-calorie bulk sweetener. D-tagatose is 92% as sweeter as sucrose but with less than half the calories (25% of metabolizing energy only). Non-carcinogenicity, no cooling effect, off flavor potentiation, and lack of laxative effect are some of the valuable properties D-tagatose. Tagatose plays a key role in regulating postprandial blood glucose level, restricting dental plague and high energy intake in foods and beverages. Jayamuthunagai et al., (2016) documented that D-tagatose can exert favorable effects on carbohydrate tolerance and hyperglycemia, particularly in type-2 diabetic patients. Noteworthy antioxidant and cryoprotectant properties of D-tagatose consequence from sequestering the active redox iron since D-tagatose is a weak iron chelator and can antagonize the iron-dependent toxic effects of intracellular oxidative stress in liver cells. These known properties are useful in protecting hepatocytes from lethal oxidant poisons such as cocaine and nitrofurantoin. Since D-tagatose did not cause any obvious increase in micronuclei and polychromatic erythrocytes; it proved to be non-toxic at different dose levels. Further, D-tagatose

does not exert any hemolytic effect on RBCs (Bär and Leeman, 1999). Various functionalities of D-tagatose are diagrammed in Figure 4.

Clinically, D-tagatose is a vital ingredient in drug manufacturing (Levin, 2002). It can also have positive impacts on pregnancy and fetal growth. Tagatose-treated rats showed regulated secretion of blood factors that verified D-tagatose as a suitable drug for hemophilia management. State-of-the-art uses of tagatose in organ transplantation exhibited positive effects in comparison to tagatose-deficient transplants. Based on low caloric value and sweat taste, pharmaceutical companies assimilate tagatose in drugs to circumvent unpleasant taste which makes it unappealing for consumers. Low caloric values in tagatose can support tagatose-based products to be used for overnight companion animals (Jayamuthunagai et al., 2016). L-tagatose like many other rare sugars may have potential as a functional sweetener and therapeutic purposes. Unlike technologically highly appreciated rare sugar, i.e., D-tagatose, L-tagatose has not yet been extensively investigated or commercially utilized inarguably due to inadequate biosynthetic routes, high process cost and expensive raw materials (Rao et al., 2008; Li et al., 2013).

Physiological and biotechnological functions of D-sorbose and L-fructose

D-sorbose ($C_6H_{12}O_6$) is a highly water-soluble, tagatose-derived ketose monosaccharide with remarkable industrial and commercial significance. It can be exploited as a non-nutritive sweetener, pest-controlling agent, and an essential building block for developing an invaluable array of technologically useful products (Li et al., 2013). L-fructose is an unnatural monosaccharide that possesses many useful applications. It is a well-known non-nutritive sweetener and can be employed as an inhibitor of various glycosidases as well as an insecticide for ants and houseflies (Muniruzzaman et al., 1996; Franke et al., 2003). Also, L-fructose is useful as starting raw feedstock for the production of bioactive compounds. Deoxy sugars constitute one of the pivotal classes of carbohydrates that are found in liposaccharide glycoproteins and glycolipids on bacterial cell surfaces. In addition to the function as central metabolism, it is also involved in signal transduction, immunological recognition, and host-pathogen communications. 2-deoxy- L-ribose has long been employed as therapeutic agents to suppress tumor growth. Similarly, 1-deoxy-L-fructose and its analogs have proven to act as metabolic inhibitor and antimetabolites (Gullapalli et al., 2007). Bearing in mind the extensive

applications, various biosynthetic routes for nucleotide sugars would be contemplated as potential targets for novel antimicrobial agents. Various functionalities of D-sorbose and L-fructose are diagrammed in Figures 5 and 6, respectively.

Rare sugars -- Aldohexoses

Physiological and biotechnological functions of D-allose

D-allose is an aldohexose made up of *β*-D-allo-1, 5- pyranose (77.5%), *α*-D-allo-1, 5-pyranose (14%), *β*-D-allo-1, 4-furanose (5%), and *α*-D-allo-1, 4-furanose (3.5%). Despite seldom existence in nature, it has fascinated a profound research interest in recent years owing to numerous pharmacological activities, including anti-cancer, anti-tumor, anti-inflammatory, anti-oxidant, anti-hypertensive, cryoprotectant, and immunosuppressive agents, as shown in Figure 7 (Lim and Oh, 2011). D-allose is a low-caloric rare sugar with no known toxicity effects (Iga et al. 2010). It is readily miscible in water and insoluble in alcohol and physically appeared as a white odorless crystalline solid. Remarkably, D-allose impedes carcinogenesis by suppressing the propagation of cancer cells, predominantly hepatocellular, cervical, ovarian, head and neck, skin and prostate cancer etc. (Sui et al. 2005; Naha et al. 2008; Yamaguchi et al., 2008; Mitani et al. 2009; Hirata et al. 2009). In combination with radiation, D-allose has been demonstrated as a convincing agent for cancer therapy. Applying as an anti-oxidant agent, it prevents the ROS-mediated oxidative damage, reduces free radical's generation, and consequently retards deterioration (Sun et al. 2006; Nakamura et al. 2011). D-allose prevents ischemia-reperfusion injury, reduces segmented neutrophil production, and lowers platelet count by acting as an anti-inflammatory agent (Murata et al. 2003; Hossain et al. 2003; 2004; Gao et al., 2011). Looking forward the potential cryoprotective role of D-allose on biological cells and tissues, it could be postulated as a valuable agent in surgery and transplantation (Sui et al. 2007; Ueki et al. 2008).

Physiological and biotechnological functions of L-glucose

Like D-sugars, L-sugars often possess enormous agricultural, biological and therapeutic activities, since L-sugars are the essential component of many antibiotics, glycopeptides, oligosaccharides, terpene glycosides, steroid glycosides and clinically valuable nucleosides (D'Alonzo et al., 2009; Zulueta et al., 2013; Xia et al., 2014). As other rare sugars, L-glucose also does not exist naturally in living organisms, even though its immense industrial applicability, such as a non-

calorie sweetener, and suppressant for various glucosidases and bacterial growth (Figure 8). It can also be explored as an intriguing raw material for the development of vaccines against *Shigella sonne* (Livesey and Brown, 1995; Bautista et al., 2000). L-gulcopyranosides are the major components of the antitumor drug, Bleomycin A2 and the nucleoside antibiotic, Adenomycin (Segerman et al., 2013; Yu et al., 2013).

Physiological and biotechnological functions of D-gulose and L-talose

D-gulose and L-talose are the aldohexose monosaccharides. Given their natural scarcity, both sugars and their derivatives are very expensive, but exhibit potential applications in many industrial sectors. Crystalline form of D-gulose can be utilized as a drug-formulating agent and food additives; whereas L-talofuranosyl adenine, a derivative of L-talose, was investigated to be a slow-reacting substrate for calf intestinal adenosine deaminase (CIAD) enzyme and therefore might be exploited to suppress the *in-vitro* growth of leukaemia L1210 cells (Lerner et al., 1994). Various functionalities of D-gulose and L-talose are diagrammed in Figures 9.

Physiological and biotechnological functions of L-galactose and L-fucose

L-galactose (C-4 epimer of glucose) is a rare sugar that is less sweet than glucose and fructose. It has been reported as a suitable precursor for L-ascorbic acid biosynthesis (Wheeler et al., 1998). Some recent studies documented that galactose might have a role in managing focal segmental glomerulosclerosis (a kidney disorder resulting in proteinuria and kidney failure) (Figure 10). It is also a constituent of antigens present on blood cells determining blood type within the ABO blood group system (McCarthy et al., 2010). L-Fucose is a naturally occurring rare sugar that belongs to the family of deoxy sugar. This sugar is found in biomass, particularly in the plant, but insufficient quantities. It can be found on the mammalian cell surface and several carbohydrate antigens as a vital core moiety (Guo and Wang, 2009; Yin and Huang, 2012). In mammals, deoxy sugars such as fucose-containing glycans manifest noteworthy functions in blood transfusion reactions, selectin-mediated leukocyte-endothelial adhesion, host-microbe communications, and numerous other signaling events. Any alterations in the expression of fucosylated oligosaccharides led to severe pathological dilemmas, including atherosclerosis and cancer. Various functionalities of L-fucose are diagrammed in Figure 11.

DHAP-dependent aldolases

New carbon-carbon bond formation holds remarkable significance in chemical reactions, particularly in organic syntheses. Notably, aldol condensation reactions designate a powerful avenue to accomplish this transformation. Nevertheless, constituting aldol condensation with pronounced stereoselectivity is the most imperative and problematic concern in this process. In recent years, two approaches including biocatalysis and small molecule catalysis have been envisioned in organic methodology for catalyzing aldol reactions. Amongst both methods, enzymatic approach (especially aldolases) is receiving increasing researcher's attentions for multifunctional preparative synthetic applications, due to their chemoselectivity, regiospecificity, and stereoselectivity as well as functioning under mild working conditions.

Aldolases are a fascinating class of lyases catalyzing the reversible enantioselective addition of a ketone donor onto an aldehyde acceptor, a fundamental reaction in organic chemistry. Until now, more than 30 different types of aldolases have been identified (Machajewski and Wong, 2000). Though aldolases display strict specificity for their donor molecules in aldol reactions, they frequently tolerate a broad range of aldehyde acceptors. Based on their donor specificity, the aldolases are categorized into different classes as summarized in Table 1. Among several aldolase families, DHAP-dependent aldolases are particularly well-developed and most extensively studied enzymes with massive synthetic potential. They catalyze the reversible aldol reaction of DHAP to a wide-variety of aldehyde acceptors (Machajewski and Wong, 2000; Clapés et al., 2010). Outstandingly, four types of DHAP-dependent aldolases have been reported and successfully employed for the synthesis of several carbohydrates and their derivatives (Machajewski and Wong, 2000; Samland and Sprenger, 2006). For instance, DHAP-dependent aldolases have been used for synthesizing various glycosidase inhibitors (iminocyclitols and polyhydroxylated pyrrolidines). Furthermore, rare sugars, thio- sugars, long-chain sugars and cyclitols which are not easily prepared by traditional methods could be obtained by DHAP-dependent aldolases (Schümperli et al., 2007). Despite great promise, however, their applicability in an industrial arena hinges on the sustainable availability of DHAP. Though, the DHAP-dependent aldolases are now commercially available but very expensive. Numerous chemical and enzymatic routes have been attempted to synthesize DHAP; however, the product

yield and purity are unsatisfactory. Chemical routes obligated the usage of toxic and expensive chemicals, while most of the enzymatic strategies have not advanced beyond small scale. Therefore, the aptitude to produce DHAP from low-cost sources could eventually expand the usefulness of aldolase reactions rendering it an attractive challenge (Schümperli et al., 2007).

Biosynthesis of rare sugars with DHAP-dependent aldolases

As mentioned above, the rare sugars are very expensive, as they cannot be extracted/ isolated from natural sources in desirable quantities. Evidently, chemical approaches are applied to synthesize the rare sugars of interest commercially. However, chemical-based routes are mainly plagued with some serious disadvantages as long coupling reaction time, and poor to moderated overall yields after several economically unfeasible and labor-intensive steps of the catalytic reaction. On the other hand, enzymatic strategies which could address the afore-mentioned drawbacks suffer from inadequate availability of the major pathway enzymes or restricted substrate acceptability of the enzymes. In this respect, biotechnological synthesis of rare sugars using metabolically engineered strains provides a new platform that is often superior to chemical methods once suitable enzymes acting on rare sugars are found. This state-of-the-art approach reveals the promising potential for the large-scale biosynthesis of unnatural/rare sugars in which dihydroxyacetone phosphate (*DHAP* or glycerone phosphate) is produced in-vivo and then coupled with aldehydes on site within engineered bacterial machinery (*i.e. E. coli* or other). More interestingly, identical metabolic pathway engineering approach has been documented previously as a proof-of-concept yielding a high titer of 1 g/L of taxadiene (the first committed Taxol intermediate) in a metabolically engineered *E. coli* strain (Ajikumar et al., 2010).

Engineering recombination pathways

Although rare sugars such as D-psicose and D-sorbose could be synthesized from DHAP and D-glyceraldehyde via the one-pot enzymatic reaction (Li et al., 2011). However, very expensive and unstable nature of DHAP restricted its application for commercial biosynthesis of theses sugars. Numerous enzyme-assisted approaches have been established to synthesize DHAP from glycerol or dihydroxyacetone (DHA); nonetheless, the resulting yield was not satisfactory and has not advanced beyond the small-scale (Schümperli et al., 2007). The key idea behind this metabolic approach was *in vivo* synthesis of one of the substrate, DHAP through *E. coli*

metabolic pathways from a cheap carbon source, i.e., glucose or glycerol. Inevitably, a large surplus of glycerol is generated as a by-product of biodiesel production. Thus it has become a very attractive and promising carbon source for the synthesis of value-added industrial products including rare sugars (Pagliaro et al., 2007; Trinh and Srienc, 2009; da Silva et al., 2009). In glycolysis pathway, glucose is converted into fructose-1, 6-bisphosphate (FBP) via three enzymatically-catalyzed steps. The resulting FBP is cleaved reversibly by fructose-1, 6-bisphosphate aldolase (FBA, EC 4.1.2.13) to two triose phosphates, DHAP and D-glyceraldehyde-3-phosphate (Romano and Conway, 1996). Alternatively, DHAP is obtained through the process of glycerol dissimilation. In wild-type *E. coli*, glycerol is first internalized into the cells by glycerol facilitator and then phosphorylated by glycerol kinase, generating *sn* glycerol 3-phosphate (G3P). Subsequently, G3P is oxidized to DHAP catalyzed by glycerol 3-phosphate dehydrogenase under aerobic conditions (Truniger and Boos, 1994; Krämer and Steckhan, 1997) (Figure 12) which is processed through glycolytic pathway. Another three-carbon substrate (glyceraldehyde) is incorporated in the medium continuously since glyceraldehyde could easily diffuse into the cells via glycerol diffusion facilitator (GlpF). The resulting DHAP is coupled with D-glyceraldehyde *in situ* by DHAP-dependent aldolase to furnish sugar-1-phosphate which is further dephosphorylated by phosphatase *in vivo* to yield the target sugar product.

Similarly, the recombination pathways based on fructose 1, 6-bisphosphate *aldolase* (*Fru A;* EC 4.1.2.13) or tagatose-1, 6-bisphosphate *aldolase* (*Tag A,* EC 4.1.2.40) and YqaB phosphatase were developed by Yang et al., (2015) in *E. coli* BL2 (DE3) and a *C. glutamicum* strain to synthesize rare sugars utilizing glucose and L-glyceraldehyde as feedstock's. Both the recombinant *E. coli* and *C. glutamicum* strains presented the expected ability to synthesize L-psicose/L-sorbose; nevertheless, it was worth noting that the bio-synthetic proficiency of recombinant *E. coli* was lower in contrast with the corresponding *C. glutamicum* strain. After evaluating the different host cells and combinations of FruA or TagA with YqaB as well as optimizing gene expression, recombinant *C. glutamicum* strain was able to produce 2.53 g/l total ketoses, with a corresponding yield of 0.50 g/g L-glyceraldehyde. Interestingly, the inactivation of Zn-dependent alcohol dehydrogenase encoding gene *cgl0331* in *C. glutamicum* significantly

enhanced the yield of target compounds. Finally, fed-batch fermentation strategy of the engineered strain led to 3.5 g/L L-sorbose and 2.3 g/L L-psicose, with a final yield of 0.61 g/g L-glyceraldehyde suggesting the effectiveness of a strategy to synthesize rare L-sugars.

In another study, Yang and coworkers, (2015) constructed a recombination pathway in *C. glutamicum*. Multiple aldehydes (formaldehyde, glycolaldehyde, L-glyceraldehyde, and D-erythrose) were coupled with DHAP to produce rare ketoses (D-sorbose and D-psicose) by rhamnulose-1-phosphate aldolase (RhaD) and fructose-1-phosphatase (YqaB) respectively. The *C. glutamicum* strains harboring the recombination pathway accumulated total rare ketoses of 6.24 g/L with a yield of 0.34 mol/mol D-glyceraldehyde. Additionally, knocking out *tpi* gene, encoding triose phosphate isomerase led to nearly 20-folds elevated DHAP concentration than wild-type. Following optimization of expression levels from rhaD and yqaB genes and fermentative conditions, the metabolically engineered strain displayed excellent potential for rare ketoses synthesis, and as a consequence, the yield increased to 0.59mol/mol D-glyceraldehyde with corresponding productivity to 2.35 g/L h from 0.58 g/L h. Under the optimized conditions in a fed-batch culture mode, the resulted modified strain accumulated massive production of 19.5 g/L of D-sorbose and 13.4 g/L of D-psicose.

Of most recent, a novel rare ketoses synthesis pathway from glycerol using DHAP-dependent aldolases with no external aldehydes was developed by Yang and workers, (2016). The pathway was divided into three modules, namely endogenous DHAP synthesis, heterologous aldehydes synthesis, and aldol reaction. A new glycerol assimilation pathway was constructed to synthesize DHAP. The enzymes responsible for converting glycerol to 3-hydroxypropionaldehyde and L-glyceraldehyde were chosen, and their analogous aldehyde synthesis pathways were designed *in-vivo*. Some alcohol dehydrogenases (ADHs) from horse liver and *Saccharomyces cerevisiae* and glycerol dehydrogenases (GDHs) from *Gluconobacter oxidans* and *Neurospora crassa* were screened and tested their catalytic efficiency to glycerol (Viswanath-Reddy et al., 1978; Quaglia et al., 2012). Notably, ADH derived from horse liver (HLADH) displayed the improved catalytic activity towards glycerol than other ADHs and GDHs (Yang et al., 2016). Based on higher oxidization of glycerol to 3-hydroxypropionaldehyde, the glycerol dehydratase (DhaB) from *K. pneumonia* was also preferred and selected for this study (Jung et al., 2014). Four different aldol

reaction pathways depending on various aldolases (RhaD, FucA, FruA, TagA) and phosphorylase were assembled. Figure 13 illustrates a schematic representation of aldolases (RhaD, FucA, FruA, TagA). Three pathways were subsequently gathered and the resulting engineered strains synthesized 5-deoxypsicose, 5-deoxysorbose, and 5-deoxyfructose from glucose and glycerol and produce L-fructose, L-tagatose, L-sorbose, and L-psicose with glycerol as the sole carbon source. In total, 18% improved the 5-deoxysorbose and 5-deoxypsicose yields of strain to 0.92 mol product per mol glycerol following the elimination of *tpi* gene. Under optimized fermentation conditions in a fed-batch culture, the recombinant strains accumulated 38.1 g/L 5-deoxypsicose with a corresponding yield of 0.91 mol product/mol of glycerol and able to synthesize 20.8, 10.3, 1.2, and 0.95 g/L of L-fructose, L-tagatose, L-sorbose, and L-psicose, respectively. Three aldol pathways using aldolases (RhaD, FruA, or TagA) and YqaB have already been constructed to synthesize many C4−C7 rare ketoses in *C. glutamicum* (Izumori, 2006; Börgel et al., 2012; Men et al., 2014).

Concluding remarks and future perspectives

Biosynthesis, as well as the functional studies of rare sugars, has become an emerging research theme because of their versatile applications in different industrial sectors. However, the crucial factor limiting their exploitation is insufficient availabilities, resulting from limited synthetic routes. Chemical methods entail multiple steps and are expensive with low yields. The biotechnological production methods of rare sugars seem superior to the chemical methods known. Bio-catalysis often offers advantages over chemical synthesis, since enzyme-assisted reactions are highly enantio- or region-selective. Besides, enzyme catalyzed reactions are usually carried out under moderate and environmentally-safe conditions. A major problem in producing rare sugars biotechnologically is finding specific enzymes that can act as catalysts. Use of advanced multidisciplinary approaches such as metabolic engineering, synthetic biology, enzyme biotechnology, and bioengineering could potentially address the inadequacies above by improving the catalytic properties (activity, thermostability, or substrate- binding affinity) of existing enzymes or discovering new and robust microbial candidates with a novel biocatalytic system for industrial-scale applications. The microbial strategy could be applied to the commercial-scale biosynthesis of many other rare ketoses or deoxy-sugars with several

configurations by modifying the type of DHAP-dependent aldolase. In the future, there is a dire need to explore as of yet unrevealed novel characteristics from these rare sugars which would surely have profound effects on the daily life and health of people.

Conflict of interest

We do not have any conflicting, competing and financial interests in any capacity.

Acknowledgements

The authors are grateful to the Shanghai Jiao Tong University, Shanghai 200240, China, and Tecnologico de Monterrey, Mexico for providing literature facilities.

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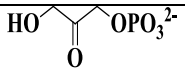
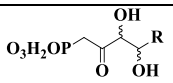
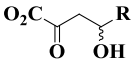
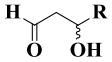
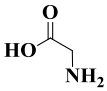
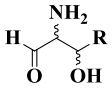
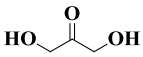
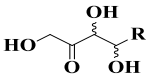
Table 1 Classification of Aldolases based on their donor specificity (Reproduced with permission from Cai, 2011).

	Donor	Acceptor	Product	Example
DHAP				FucA RhaD
Pyruvate				NeuAc aldolase
Acetaldehyde				DERA
Glycine				Threonine aldolase
DHA				FSA

DHAP-dihydroxyacetone phosphate, Fuc-Fuculose 1-phosphate aldolase, Rhad-Rhamnulose-1-phosphate aldolase, NeuAc aldolase-N-acetylneuraminic acid aldolase, DERA-2-deoxyribose-5-phosphate aldolase, DHA-dihydroxyacetone, FSA-D-fructose-6-phosphate aldolase.

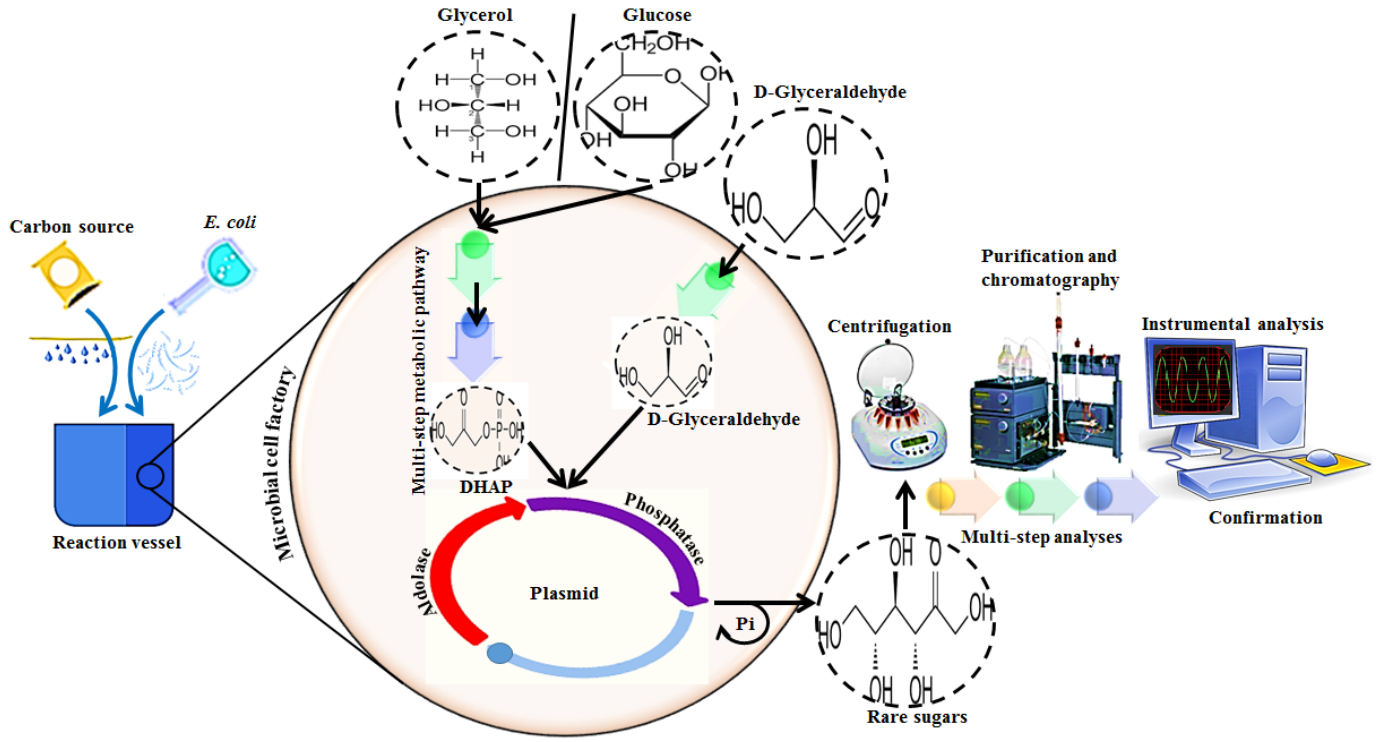


Figure 1 A detailed process of rare sugar synthesis from a bioreactor and metabolic engineering perspective to purification and analyses.

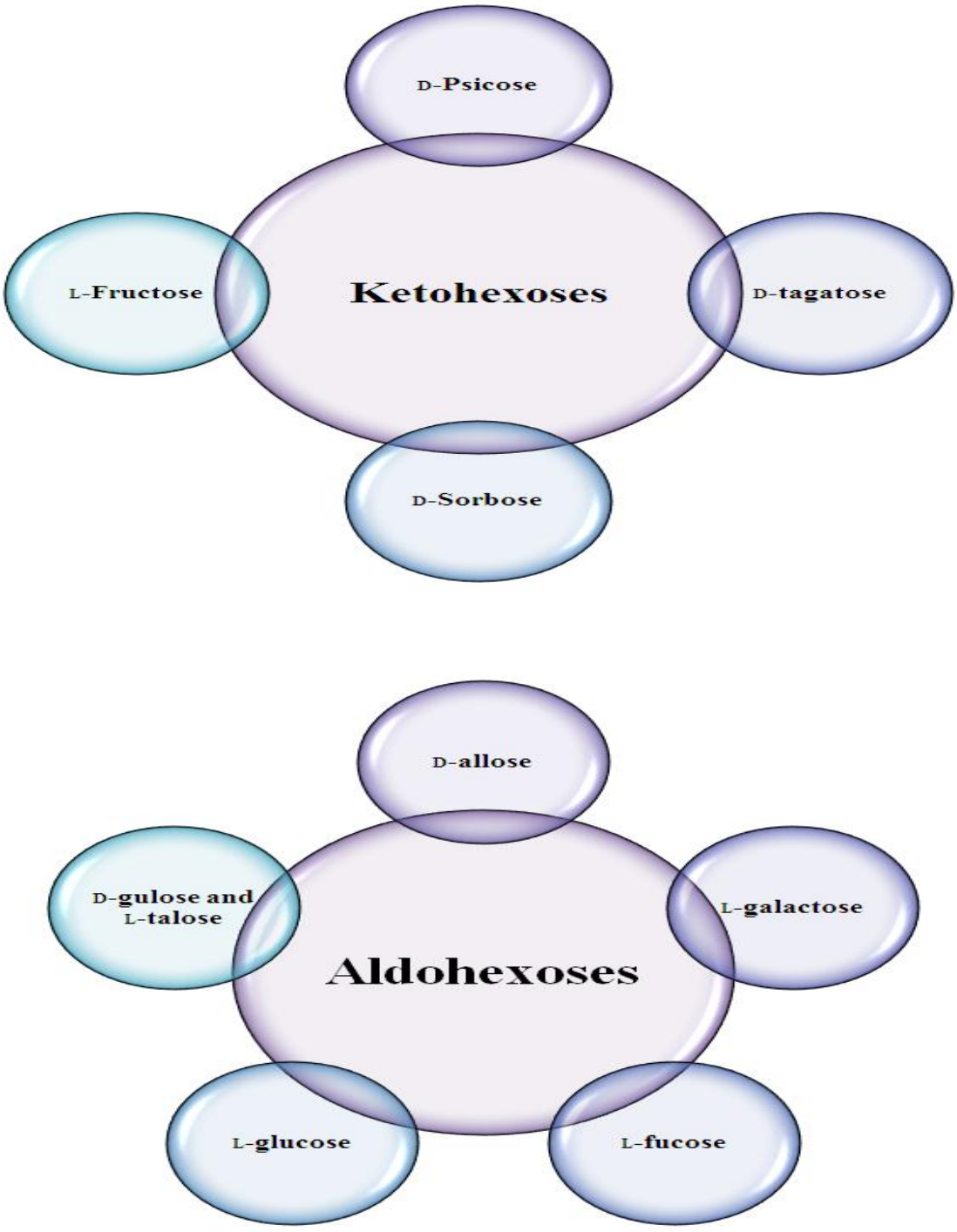


Figure 2 Two major classes of rare sugars i.e. ketohexoses and aldohexoses and their sub-members.

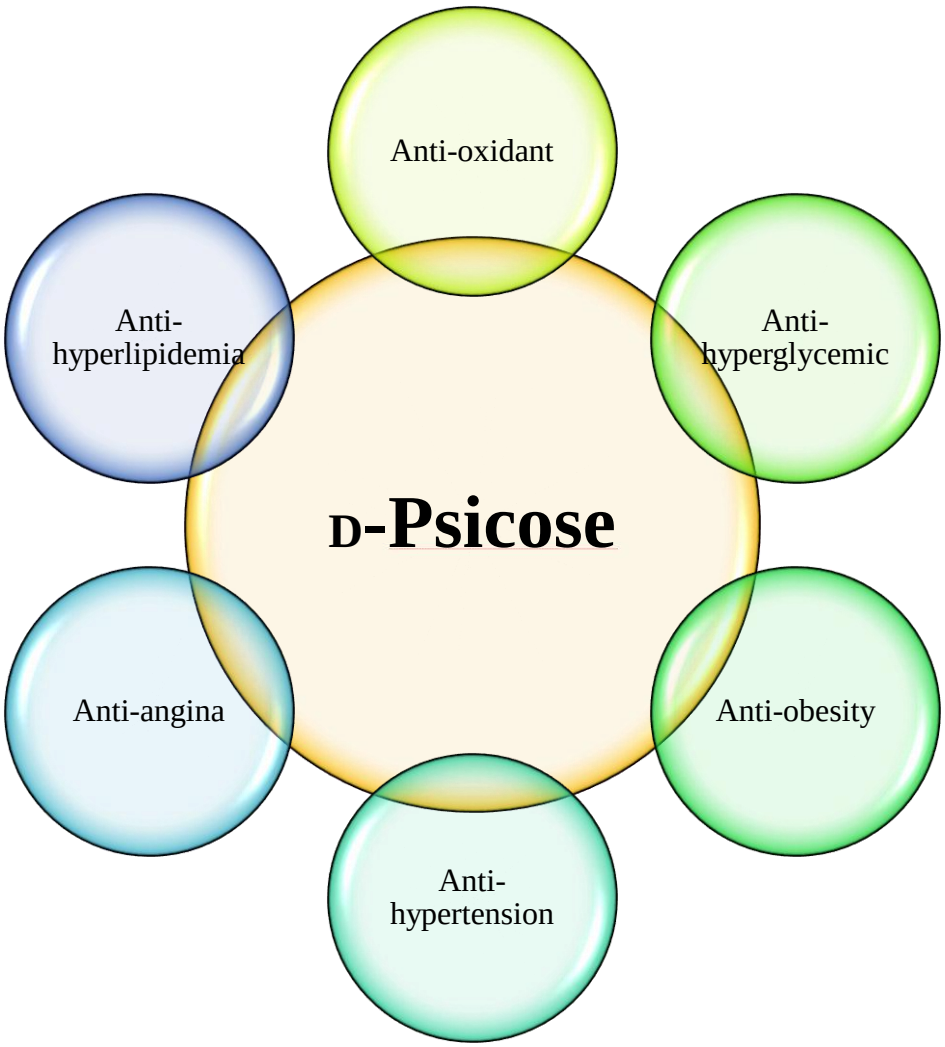


Figure 3 Various functionalities of D-psicose.

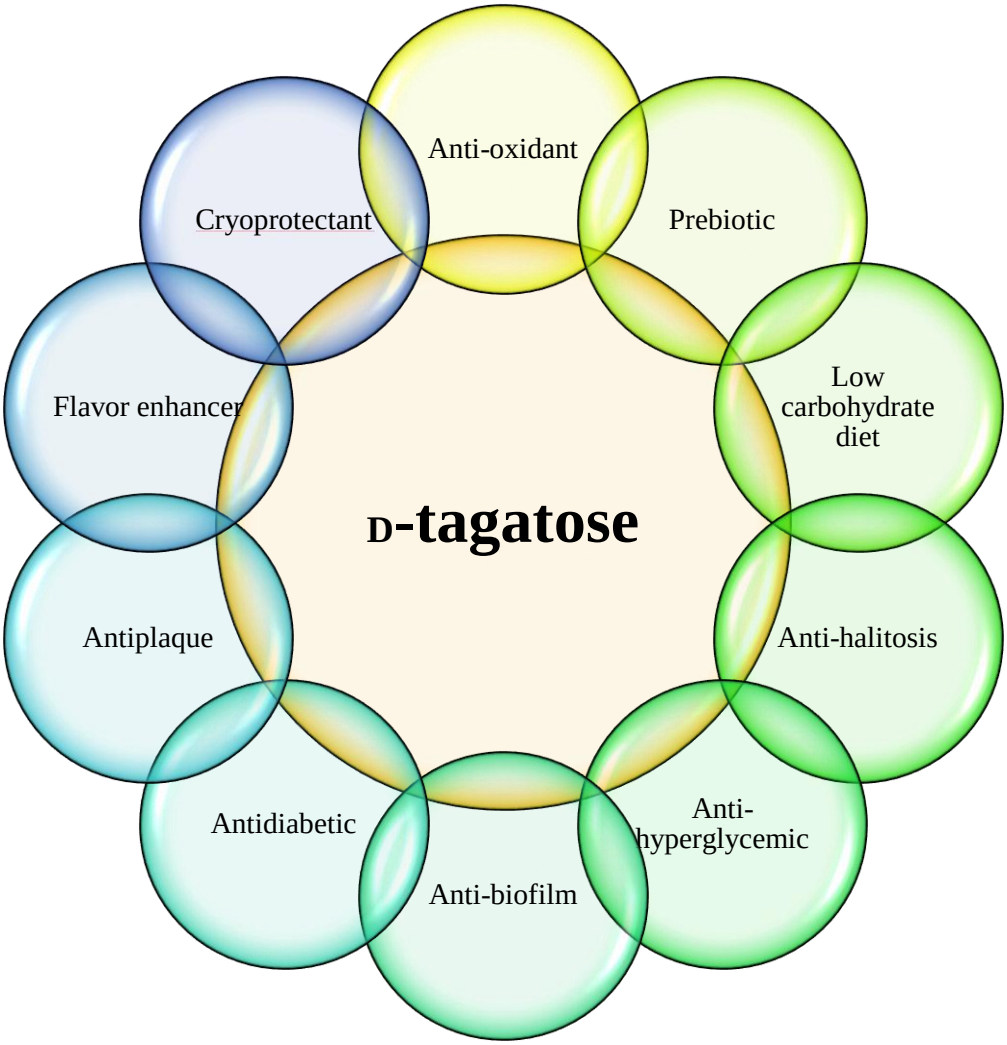


Figure 4 Various functionalities of D-tagatose.

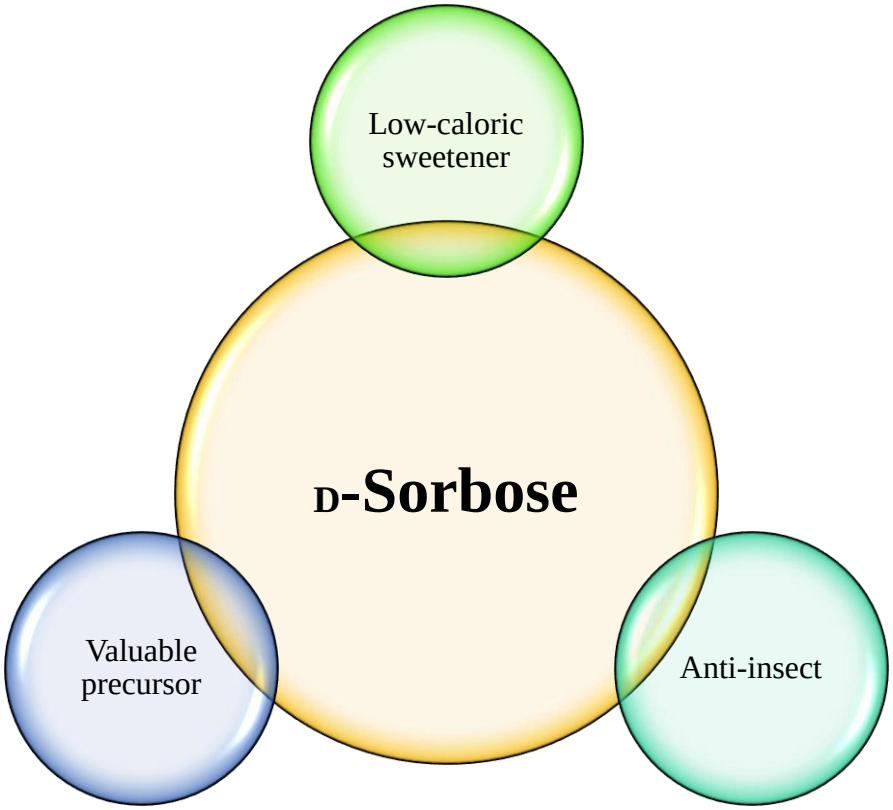


Figure 5 Various functionalities of D-sorbose.

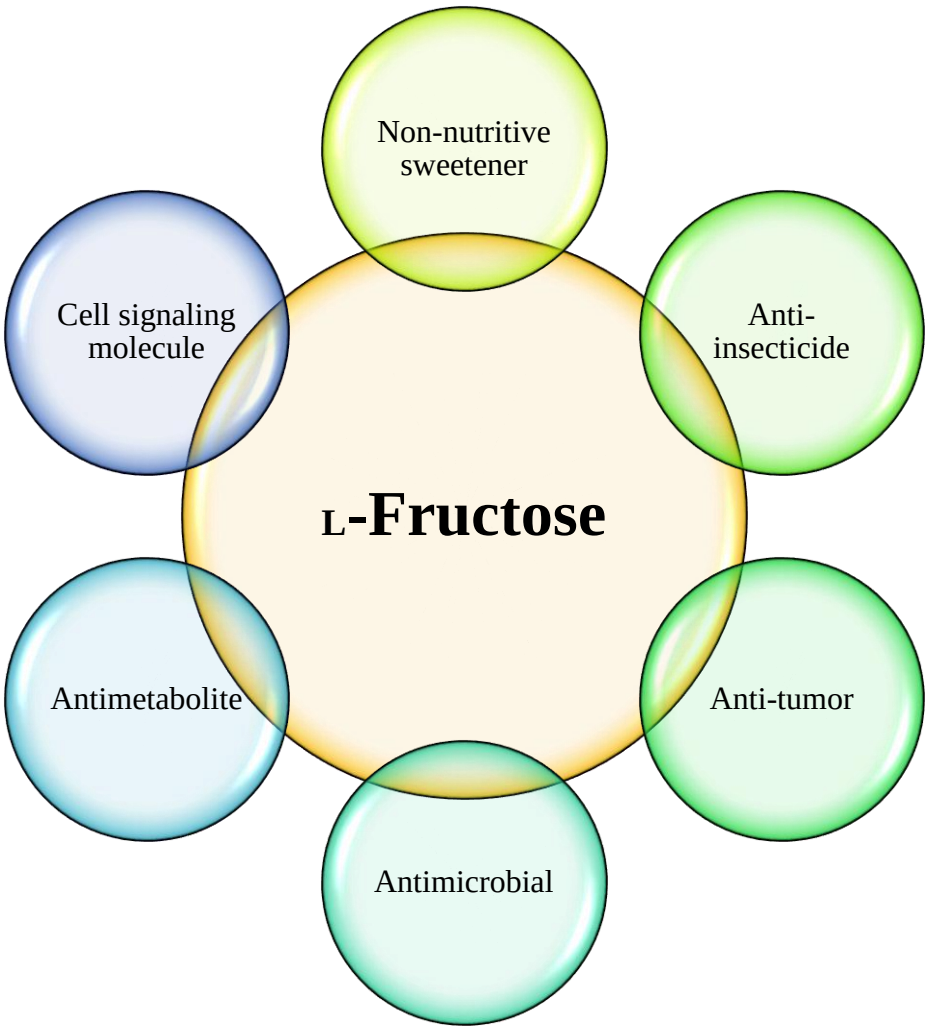


Figure 6 Various functionalities of L-fructose.

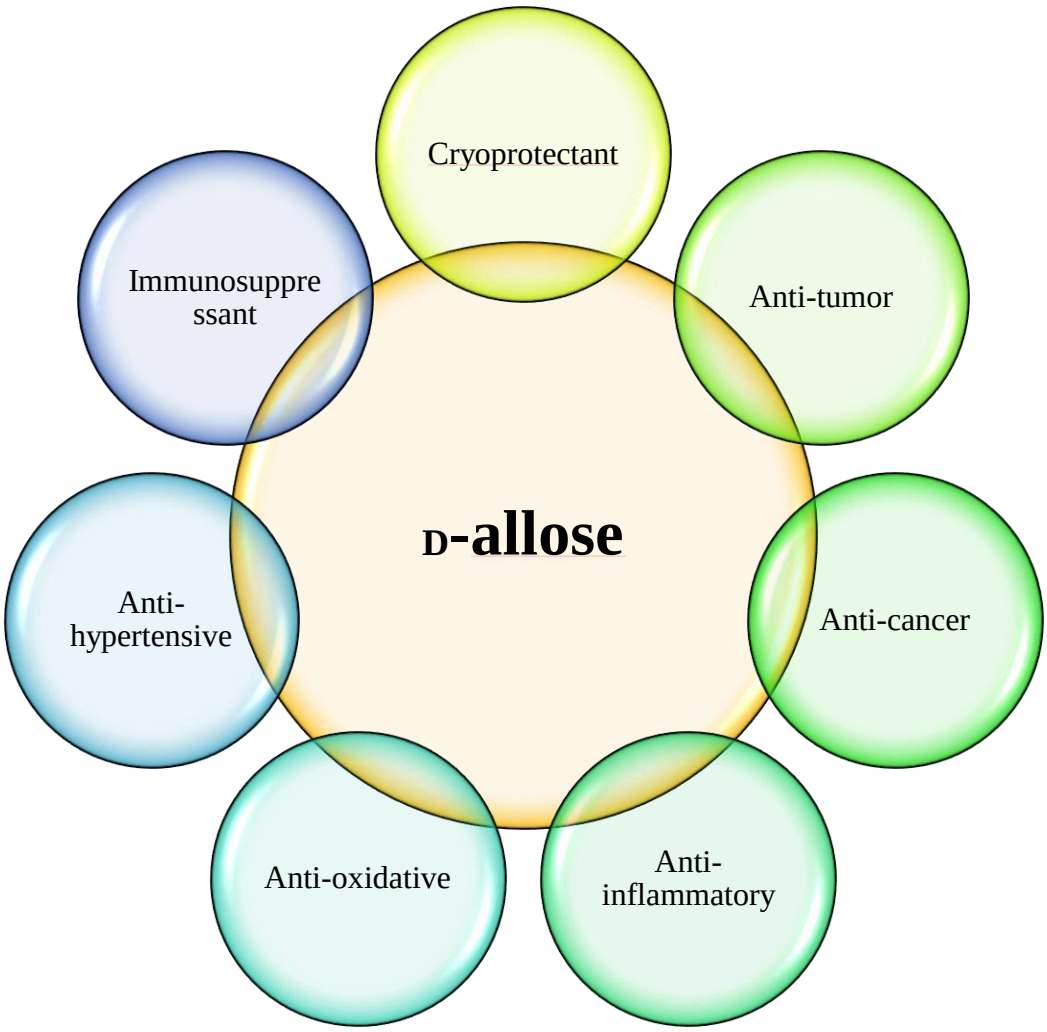


Figure 7 Various functionalities of D-allose.

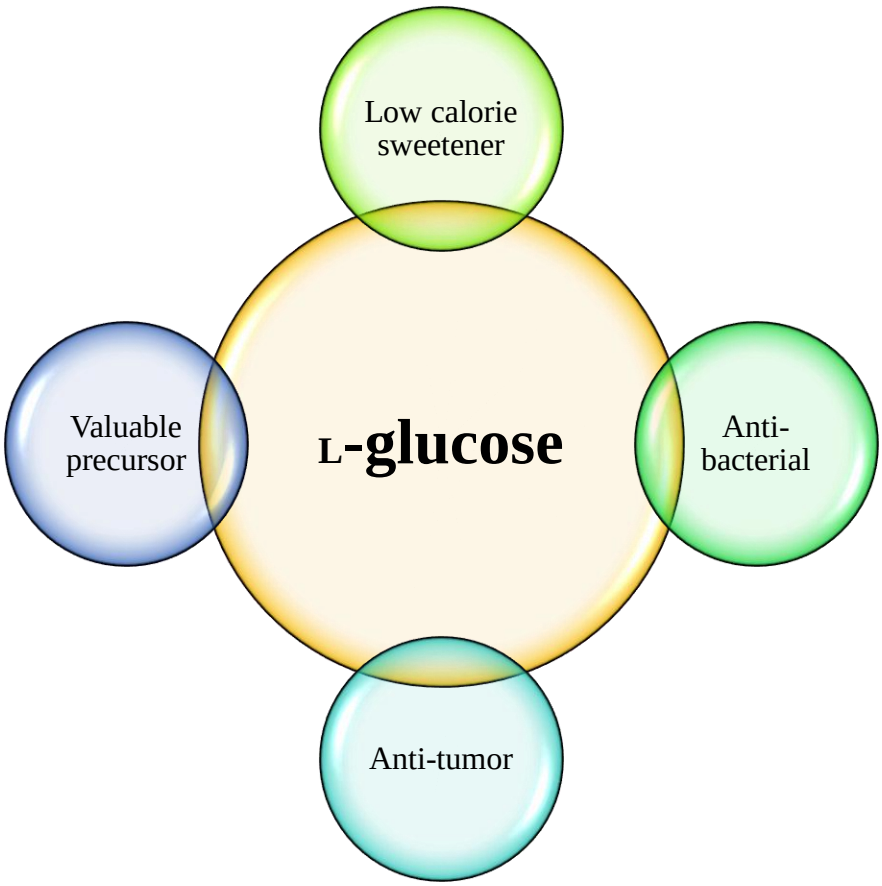


Figure 8 Various functionalities of L-gulose.

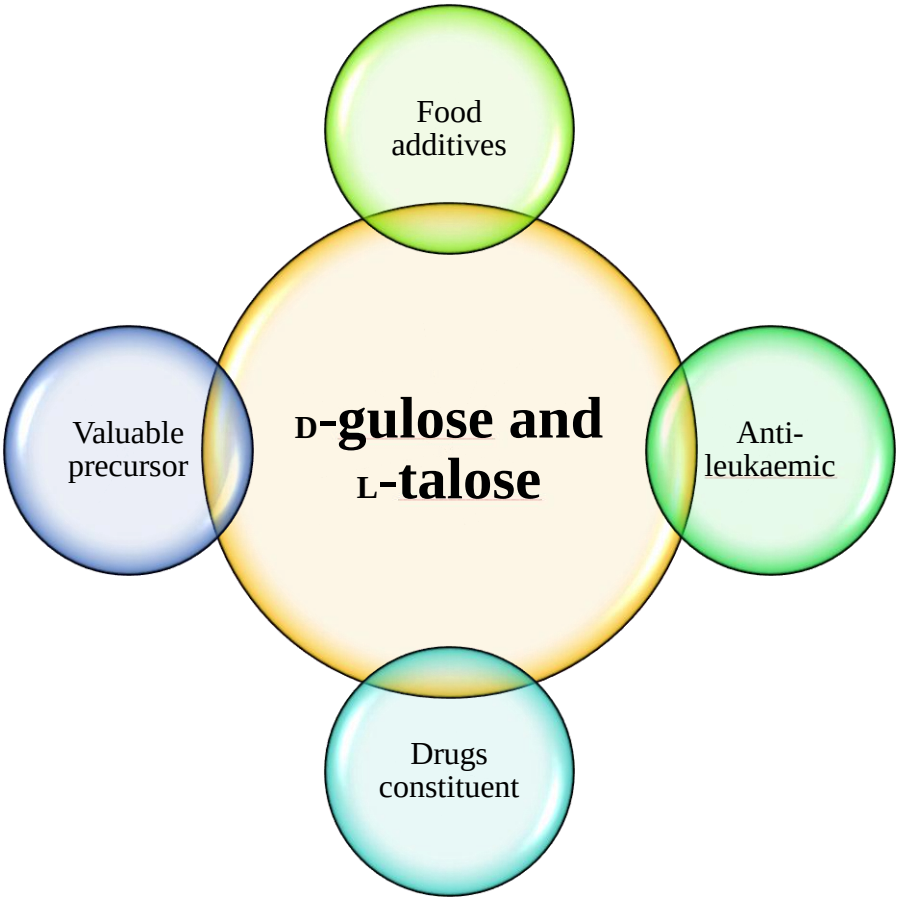


Figure 9 Functional characteristics of D-gulose and L-talose.

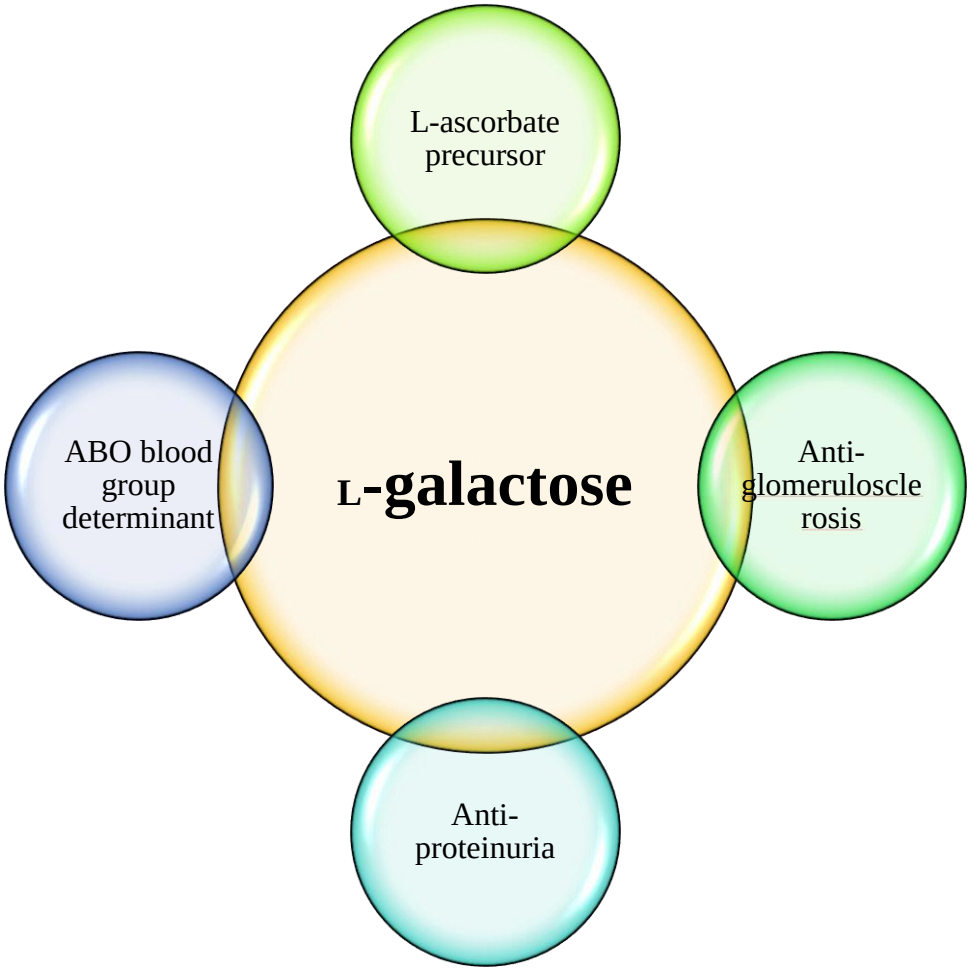


Figure 10 Functional characteristics of L-galactose.

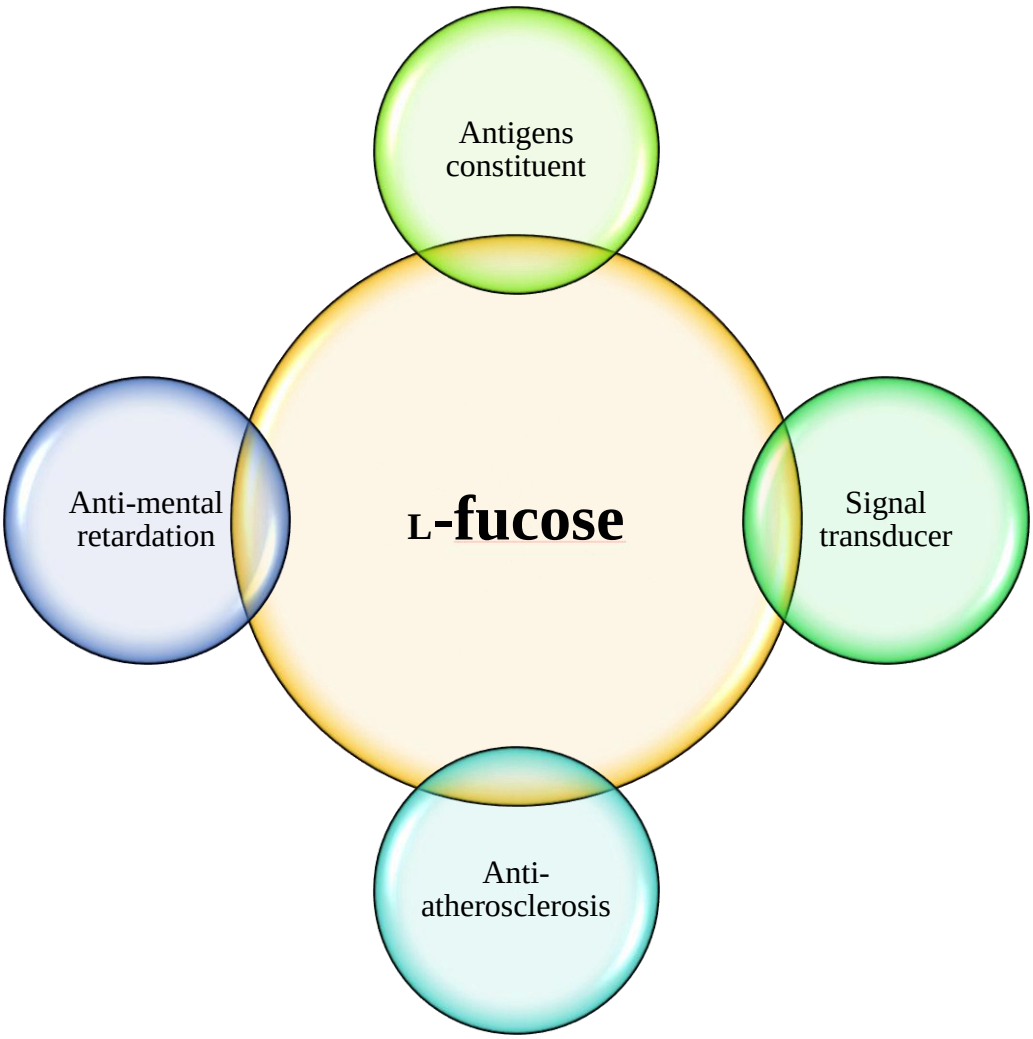


Figure 11 Various functionalities of L-fucose belongs to the family of deoxy sugar.

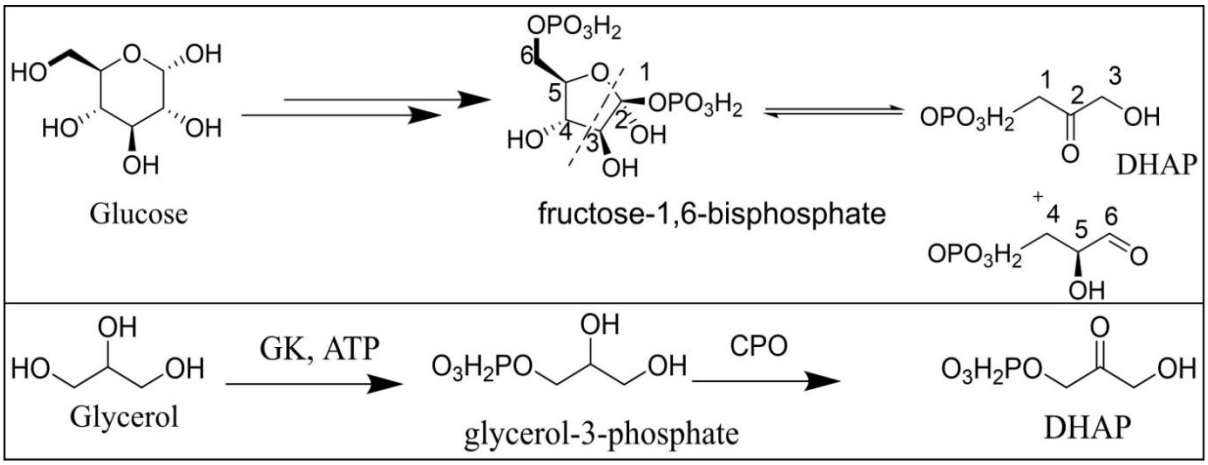


Figure 12 Production of DHAP from cheap carbon source (Reproduced with permission from Cai, 2011).

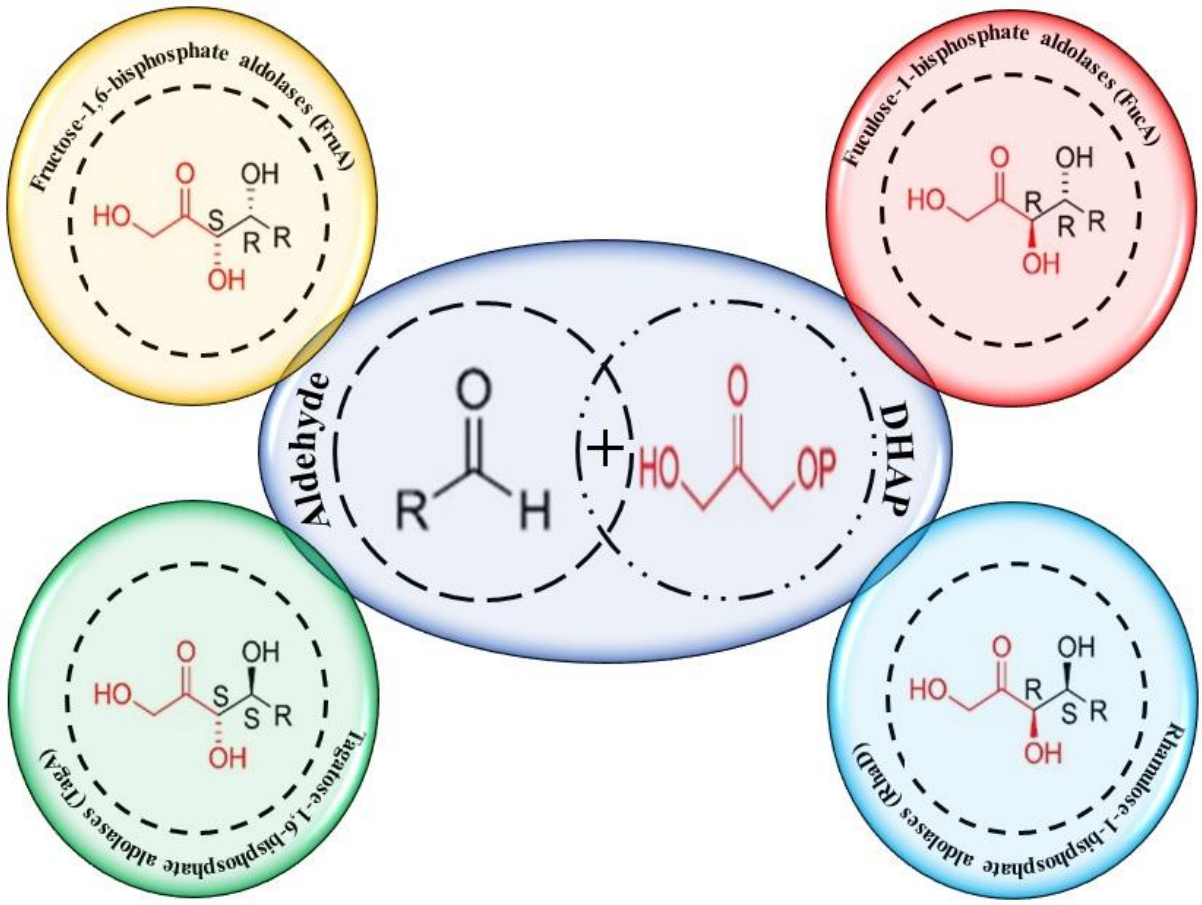


Figure 13 A schematic representation of aldolases (RhaD, FucA, FruA, TagA).