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MINI-REVIEW

# Recent research on the physiological functions, applications, and biotechnological production of D-allose

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## Abstract

D-Allose is a rare monosaccharide, which rarely appears in the natural environment. D-Allose has an 80% sweetness relative to table sugar but is ultra-low calorie and non-toxic and is thus an ideal candidate to take the place of table sugar in food products. It displays unique health benefits and physiological functions in various fields, including food systems, clinical treatment, and the health care fields. However, it is difficult to produce chemically. The biotechnological production of D-allose has become a research hotspot in recent years. Therefore, an overview of recent studies on the physiological functions, applications, and biotechnological production of D-allose is presented. In this review, the physiological functions of D-allose are introduced in detail. In addition, the different types of D-allose-producing enzymes are compared for their enzymatic properties and for the biotechnological production of D-allose. To date, very little information is available on the molecular modification and food-grade expression of D-allose-producing enzymes, representing a very large research space yet to be explored.

**Keywords** D-Allose · Physiological function · Application · Biological production

## Introduction

Recently, an increasing number of people have begun suffering from health problems connected to excessive weight gain, such as obesity, hyperlipidemia, hyperglycemia, and hypertension, as a result of unwholesome dietary habits and the ingestion of high-fat and high-sucrose-containing food. Therefore, many researchers in sweetener industry have focused on low-calorie sugars that are good sucrose substitutes. Rare sugars are monosaccharides and monosaccharide derivatives that exist more sparsely in the natural world than common sugars, which were defined by the International Society of Rare Sugars (ISRS) (Izumori 2002). The majority of monosaccharides are rare sugars; only seven monosaccharides are common sugars existing abundantly in the natural environment according to the definition, such as D-glucose and D-fructose, which are well known. Although rare sugars are present in small quantities, they have great potential in the food and pharmaceutical industries because of their distinct physiological functions. The physiological effects of many rare sugars have been extensively studied, including D-form and L-form rare sugars and some sugar alcohols. For example, D-allulose, a precursor of D-allose, possesses some beneficial activities, such as anti-hyperglycemic (Hayashi et al. 2010), anti-inflammatory (Moller and Berger 2003), and anti-hyperlipidemic functions (Matsuo et al. 2001), and its physiological effects have been reviewed (Mu et al. 2012; Zhang et al. 2016).

D-Allose, an important D-form rare sugar, has 80% sweetness compared with table sugar (sucrose) but is ultra-low calorie and an ideal table sugar substitute (Mooradian et al. 2017). This rare sugar has been verified to exert plenty of beneficial physiological functions, including anti-tumor (Noguchi et al. 2016), anti-cancer (Noguchi et al. 2016), anti-inflammatory (Shinohara et al. 2016), cryoprotective (Sui et al. 2007), anti-osteoporotic (Noguchi et al. 2013), anti-hypertensive (Kimura et al. 2005), neuroprotective (Gao et al. 2013), and immunosuppressant (Hossain et al. 2000) functions. Moreover, it exhibits anti-oxidative properties by modulating the generation of reactive oxygen species (ROS) (Ishihara et al. 2011).

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D-Allose can be synthesized by chemical methods. However, these methods have many disadvantages. Recently, the enzymatic production of D-allose has drawn the attention of many researchers. So far, three main types of D-allose-producing enzymes have been studied, including L-rhamnose isomerase (EC 5.3.1.14), ribose-5-phosphate isomerase (EC 5.3.1.6), and galactose-6-phosphate isomerase (EC 5.3.1.26). In 2006, Prof. Izumori from the Rare Sugar Research Centre (Kagawa University, Japan) established the Izumori-ring strategy for the effective biological production of all hexoses (Izumori 2006). According to this strategy, D-allose could be converted from D-allulose, and D-allulose could be obtained from the epimerization of D-fructose, an inexpensive and widely available common sugar, using ketose 3-epimerase (EC 5.1.3.31). In this review, recent advances in the biochemical properties, physiological effects, applications, and biotechnological production of D-allose are reviewed from different points of view.

## Synopsis of D-allose

### Physicochemical properties

D-Allose (C-3 epimer of D-glucose) is an aldohexose and an ultra-low calorie rare monosaccharide. The molecular formula, molecular weight, and melting temperature of D-allose (CAS number 2595-97-3, 7283-09-2) are $C_6H_{12}O_6$, 180.16 g $mol^{-1}$, and 128 °C, respectively. It has a high solubility in water and is practically insoluble in alcohol. The ordinary state of high-purity D-allose is a non-toxic, odorless, white solid powder (Iga et al. 2010). In a dimethyl sulfoxide solution, D-allose exists in four ring structures, including $\alpha$-D-allose-1,4-furanose, $\beta$-D-allose-1,4-furanose, $\alpha$-D-allose-1,5-pyranose, and $\beta$-D-allose-1,5-pyranose, with proportions of 3.5:5:14:77.5, respectively (Angyal 1994; Köpper and Freimund 2003). However, in the aqueous phase, the D-allose molecule exists in the conformation of an $\alpha$ $\beta$-D-pyranose ring in $^4C_1$ (Kozakai et al. 2015).

### Existing sources

D-Allose is scarcely encountered in nature and has been found only in minute amounts in plants. It has been extracted from *Protea rubropilosa* beard (Perold et al. 1973), *Veronica filiformis* (Chari et al. 1981), *Mentzelia* (Jensen et al. 1981), potato leaves (Weckwerth et al. 2004), and the African shrub *Protea rubropilosa* (O'Neil et al. 2006). Recently, researchers isolated D-allose from Indian seagrasses used as a therapeutic (Kannan et al. 2012) and from *Acalypha hispida* leaves (Sithara et al. 2017) with ultra-low ratios (3.67% and 4.45%, respectively).

### Chemical synthesis

Chemical methods have been used for the production of D-allose. The first approach utilized for the preparation of D-allose was ribose conversion via the reduction reaction of cyanohydrin with sodium amalgam (Phelps and Bates 1934). D-Allose was prepared and isolated from the mixture obtained from 3-ketosucose after reduction with nickel (Bernaerts et al. 1963). D-Allose was obtained from D-glucose by a C-3 epimerization reaction using molybdenum as a catalyst (US Patent No. 5433793, 1995). This reaction involved complex steps including decolorization, deionization, and separation (Herber et al. 1995). However, these chemical methods show many disadvantages, such as low productivity, complex reaction steps, bad selectivity, unwanted by-products, and chemical pollution. Therefore, the enzymatic synthesis is most commonly used for the production of D-allose.

## Physiological functions

### Anti-cancer and anti-tumor effectiveness

Inhibiting cancer and tumors is the most important physiological effect of D-allose. Some researchers have focused on the potential inhibitory functions of D-allose for cancer and tumors. To date, D-allose has been found to inhibit the proliferation and metastasis of various types of carcinoma cells, such as human ovarian (SUI et al. 2005a), hepatocellular (Yamaguchi et al. 2008; Yokohira et al. 2008), pancreas (Malm et al. 2015), prostate (Jeong et al. 2011; Naha et al. 2008), head and neck (Indo et al. 2014), leukemia (Hirata et al. 2009), cervical, and skin cells (Sui et al. 2005b).

In 2008, an anti-proliferative mechanism was partially revealed using $p27^{kip1}$, a detector of the cell cycle transition, where D-allose showed an obvious induction of the expression of thioredoxin-interacting protein (TXNIP) (Yamaguchi et al. 2008). Subsequently, this result was confirmed by experiments in vitro and in vivo using head and neck cancer cell lines (Hoshikawa et al. 2010). More recently, Noguchi et al. further investigated the possible anti-cancer mechanism of D-allose. Cancer cells deregulate growth, which demands a great deal of glucose as a major energy source. Over-expression of several glucose transporters (GLUTs) facilitates the transport of glucose into cancer cells. D-Allose can up-regulate the expression of TXNIP. Inversely, the TXNIP can down-regulate the over-expression of GLUT1 in various cancer cells. Hence, D-allose suppresses the proliferation of various carcinoma cells. Furthermore, D-allose was also shown to prohibit cancer cells from absorbing glucose in this investigation (Noguchi et al. 2016).

Recent research showed that D-allose displays a more distinct anti-cancer effect in combination with other anti-

carcinogens than it does by itself. D-Allose, as a radiant sensitizer, promotes the efficacy of radiation for cancer cell apoptosis by inducing TXNIP and enhancing the level of intracellular ROS (Hoshikawa et al. 2011). The combined therapy of D-allose and docetaxel is more effective with a lower toxicity (Indo et al. 2014). The combination of D-allose and oxaliplatin manifested a synergistic anti-tumor activity (Malm et al. 2015). Together, D-allose has considerable application prospects in the clinical treatment of tumors.

### Antioxidant properties

It was reported that D-allose exhibits the anti-oxidative activity in ischemia–reperfusion (I/R) damage. In 2003, Murata et al. first reported that D-allose, but not D-glucose, has the capacity to clear ROS (Murata et al. 2003). In recent studies, rat experiments indicated that D-allose significantly attenuates brain damage and induces neuroprotection by suppressing oxidative DNA damage resulting from the assault of ROS (Nakamura et al. 2011). Synchronously, the antioxidant mechanism of D-allose was partially explained. D-Glucose induces ATP synthesis to facilitate the production of ROS in cells. However, D-allose suppresses ROS production by means of competition with D-glucose in the mitochondria (Ishihara et al. 2011).

### Anti-inflammatory effects

According to previous reports that D-allose reduces I/R injury by its antioxidant properties, Gao et al. proposed the hypothesis that D-allose may exert neuroprotection by restraining cerebral I/R damage due to its anti-inflammatory effect (Gao et al. 2011), and this hypothesis was subsequently proven to be true in rat experiments (Gao et al. 2013). Recently, some experimental results further suggested that D-allose notably decreases brain pro-inflammatory cytokines, which helps provide neuroprotection in cerebral I/R injury (Shinohara et al. 2016) and prevents nonalcoholic steatohepatitis by prohibiting progressive inflammation (Yamamoto et al. 2017). The regulatory mechanism by which D-allose protects the blood brain barrier (BBB) from I/R injury, though regulating the peroxisome proliferator-activated receptor $\gamma$ (PPAR$\gamma$) that mediates the anti-inflammatory response, was clarified (Huang et al. 2016).

### Other health benefits

In addition to these beneficial functions of D-allose, other health benefits have been investigated. In rat experiments, D-allose supplementation could suppress high blood pressure induced by high salt (Kimura et al. 2005). Researchers have discovered that D-allose has the same cryoprotective function as trehalose on cell survival during freezing (Sui et al. 2007; Yue et al. 2016). D-Allose, as an immunosuppressant, could improve allograft survival and reduce tissue injury (Hossain et al. 2000; Tanaka and Sakamoto 2011). Thus, D-allose has promising prospects for applications in food systems, clinical treatment, and the health care fields. All prominent physiological functions of D-allose are shown in Fig. 1.

## Applications

### Application in food systems

D-Allose is an ultra-low calorie monosaccharide and has 80% of the sweetness of table sugar (Mooradian et al. 2017). The acute and sub-chronic toxicity trials in rats demonstrate that D-allose is a non-toxic monosaccharide (Iga et al. 2010). These beneficial physiological properties and safety profile make it possible to apply in the food industry. D-Allose, as a table sugar substitute, is an ideal food additive and is conducive for losing weight. Furthermore, D-allose, as a reducing sugar, can participate in Maillard reactions, which may improve the color, flavor, and taste of foods. Experiments illustrated that $\alpha$-lactalbumin was glycosylated by D-allose in Maillard reactions with a faster reaction rate and with greater covalent linking compared with D-fructose or D-glucose (Sun et al. 2006). Thus, D-allose displays a promising future for application in food systems.

### Application in clinical treatment

Because D-allose exhibits many remarkable physiological functions as described above, D-allose, as a pharmaceutical agent, has an enormous potential for clinical application, such as in the clinical therapy of cancer and tumors, inflammation, stroke (Gao et al. 2013), hypertension, and obesity diseases. D-Allose has been used in surgery and organ transplantation to increase the probability of success and decrease tissue damage (US Patent No. 5620960, 1997), due to its anti-oxidative, immunosuppressant, and cryoprotective effects (Kashiwagi et al. 2016; Sui et al. 2007; Tanaka and Sakamoto 2011). D-Allose, as an antioxidant, treats various diseases resulting from oxidative stress (Ishihara et al. 2011; Nakamura et al. 2011). D-Allose ameliorates nephrotoxicity induced by cisplatin (an antineoplastic agent) due to its anti-inflammatory effects (Miyawaki et al. 2012).

### Application in health care

D-Allose has been used in health care because of its prominent physiological functions. It significantly enhances the effect of metronidazole on trichomonad parasites, which reduces the dosage of metronidazole and prevents the parasite from producing drug resistance (Harada et al. 2012). D-Allose can repress the growth of the nematode *Caenorhabditis elegans* (Sakoguchi et al. 2016). D-Allose may prevent osteoporosis


**Fig. 1** Recent researches on excellent physiological functions of D-allose, including anti-cancer, anti-oxidative, anti-inflammatory, anti-hypertensive, antianti-hypertensive, antiosteoporotic, neuroprotective, cryoprotective, and immunosuppressant effects

by inhibiting osteoclast differentiation (Noguchi et al. 2013). Moreover, it can trigger self-protection in rice by regulating the generation of ROS (Kano et al. 2013).

## Various enzymes for D-allose production

### L-Rhamnose isomerase

L-Rhamnose isomerase (L-RI, EC 5.3.1.14) reversibly catalyzes the isomerization reaction of L-rhamnose and L-rhamnulose and the additional isomerization between D-allulose and D-allose because of its extensive substrate specificity (Xu et al. 2016). To date, L-RIs catalyzing D-allose have been characterized from *Clostridium stercorarium* ATCC 35414 (Seo et al. 2017), *Caldicellulosiruptor obsidiansis* OB47 (Chen et al. 2017), *Thermobacillus composti* KWC4 (Xu et al. 2017), *Bacillus subtilis* WB600 (Bai et al. 2015), *Dictyoglomus turgidum* DSMZ 6724 (Kim et al. 2013), *Mesorhizobium loti* (Takata et al. 2011), *Caldicellulosiruptor saccharolyticus* ATCC 43494 (Lin et al. 2011), *Thermoanaerobacterium saccharolyticum* NTOU1 (Lin et al. 2010), *Thermotoga maritima* ATCC 43589 (Park et al. 2010), *Bacillus pallidus* Y25 (Poonperm et al. 2007), and *Pseudomonas stutzeri* (Leang et al. 2004).

L-RI, as the most important enzyme, is widely studied in the biological production of D-allose. L-RIs are metal-requiring enzymes and are vitally stimulated by $Mn^{2+}$ or $Co^{2+}$. The reaction temperature of L-RI is high, with a range of 60–85 °C, and the optimal pH is weakly alkaline or neutral, with a range of 7.0–9.0 (Xu et al. 2016). Conditions of temperatures too high or alkaline pH easily lead to the Maillard reaction and by-products, which are a disadvantage for the production and isolation of D-allose. In order to produce D-allose efficiently, the production condition should be optimized. Most of the L-RIs display an excellent thermal stability, which is good for the production of D-allose. The kinetic parameters of various L-RIs have been studied abundantly for D-allulose but not for D-allose. The different properties of L-RIs are compared in Table 1.

### D-Ribose-5-phosphate isomerase

D-Ribose-5-phosphate isomerase (RPI, EC 5.3.1.6) universally exists in almost all microorganisms and takes part in

the Calvin cycle and the pentose phosphate pathway (Zhang et al. 2003). It reversibly catalyzes the conversion between D-ribose 5-phosphate and D-ribulose 5-phosphate. The production of D-allose using the RPI from *Clostridium thermocellum* was the first to be reported (Park et al. 2007a). Later, other RPIs were successively identified from different strains, including *Clostridium difficile* ATCC BAA-1382D-5, *Thermotoga maritima* ATCC 43589D-5 (Yeom et al. 2010), and *Thermotoga lettingae* TMO (Feng et al. 2013). The RPI from *T. maritima* ATCC 43589D-5 exhibits a remarkable thermal stability and a high-specific activity toward D-allulose. It is a good candidate for the industrial production of D-allose. The comparison of various properties of RPIs is shown in Table 1.

### Other D-allose-producing enzymes

D-Galactose-6-phosphate isomerase (GaPI, EC 5.3.1.26), reversibly isomerizing D-galactose-6-phosphate and D-tagatose-6-phosphate, takes part in the metabolism pathway of D-tagatose. Before now, only one GPI from *Lactococcus lactis*, a D-allose-producing enzyme, had been characterized (Park et al. 2007b). The GPI from *L. lactis* shows a low optimal temperature and catalytic efficiency toward D-allose. Glucose-6-phosphate isomerase (GlPI, EC 5.3.1.9) from *Pyrococcus furiosus* has the highest optimal temperature, thermal stability, and specific activity on D-allulose, compared with reported D-allose-producing enzymes, and is a promising candidate for the production of D-allose (Yoon et al. 2009). In addition, the activities of mannose-6-phosphate isomerase (EC 5.3.1.8) toward D-allulose are too low for its use in the production of D-allose (Yeom et al. 2009). The properties of all D-allose-producing enzymes are compared in Table 1.

## Biological production of D-allose

The precursor for synthesizing D-allose, D-allulose, is an expensive rare sugar. Because of the production cost, an economical method in which D-allose is produced using a cheap sugar, such as D-glucose or D-fructose, as the starting material by serial conversion steps involving D-glucose isomerase, ketose 3-epimerase, and L-RI, has been preferred (Fig. 2). After the isomerization reaction of D-allose and D-allulose, D-allose is isolated readily from the reaction mixture by a moving-bed chromatograph separation system and ethanol crystallization (Menavuvu et al. 2006; Morimoto et al. 2006).

D-Allose is effectively produced from D-allulose using L-RI from *P. stutzeri* cross-linked with glutaraldehyde. This immobilized L-RI produces D-allose from 100 g $L^{-1}$ D-allulose with a conversion rate of 25%, but it concurrently produces 8% D-altrose as a by-product (Menavuvu et al. 2006). Meanwhile, this L-RI immobilized on chitopearl beads was used for the large-scale production of D-allose with an approximately 30% turnover rate in a continuous reaction system, and 1.65 kg D-allose crystals with 100% purity was acquired after separation and purification (Morimoto et al. 2006). The L-RI from *B. pallidus* Y25 produces D-allose from D-allulose with a yield rate of 35%, but its thermal stability is not good (Poonperm et al. 2007). Two thermostable L-RIs from *C. saccharolyticus* ATCC 43494 (Lin et al. 2011) and *T. saccharolyticum* NTOU1 (Lin et al. 2010) produce D-allose from D-allulose with a transformation proportion of 33% and 29%, respectively. The L-RI from *B. subtilis* WB600 produces D-allose from D-allulose with the highest conversion rate of 37.5%, compared with other D-allose-producing enzymes (Bai et al. 2015). Recently, two novel source L-RIs from *C. stercorarium* ATCC 35414 (Seo et al. 2017) and *T. composti* KWC4 (Xu et al. 2017) have been reported, which readily produce 199 g $L^{-1}$ and 23 g $L^{-1}$ D-allose from D-allulose with turnover ratios of 33% and 23%, respectively. To summarize, L-RI is a primary enzyme source for the efficient production of D-allose, and most of the L-RIs exhibit an enormous potential for industrial production.

The RPI from *C. thermocellum* produces 165 g $L^{-1}$ D-allose from D-allulose after a 6-h reaction with a turnover ratio of 33% (Park et al. 2007a). Subsequently, the conversion yield was increased by 7% using a site-directed mutant of the *C. thermocellum* RPI (Yeom et al. 2011). The RPI from *T. lettingae* TMO generates approximately 32% D-allose from 1.8 g $L^{-1}$ D-allulose. Moreover, 28 g $L^{-1}$ D-allose is produced from 100 g $L^{-1}$ D-allulose

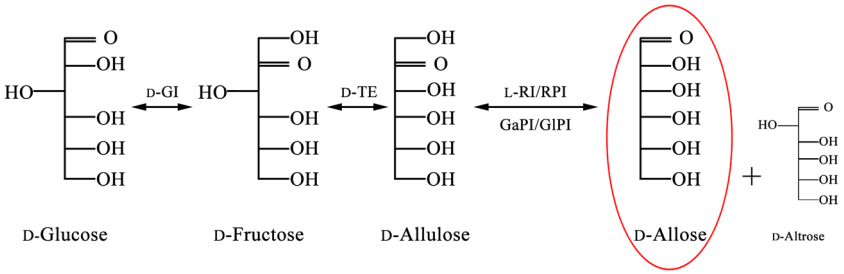


**Fig. 2** Enzymatic route for the conversion of D-glucose to D-allose using various enzymes including D-glucose isomerase (D-GI), D-tagatose 3-epimerase (D-TE), L-RI, RPI, GaPI, and GlPI

**Table 1** Comparison of enzymatic properties and kinetic parameters toward D-allose

| Enzymes | Strains | Temp. (°C) | pH | $K_m$ (mM) | $k_{cat}$ ($s^{-1}$) | $k_{cat}/K_m$ ($Mm^{-1}$ $s^{-1}$) | Specificity activities (U $mg^{-1}$) | Half-life (h) | Reference |
|---|---|---|---|---|---|---|---|---|---|
| L-RI | *C. stercorarium* ATCC 35414 | 75 | 7 | 17.2[a] | 36.3[a] | 2.11[a] | 4.5[a] | 22.8 (65 °C) | Seo et al. (2017) |
| | *C. obsidiansis* OB47 | 85 | 8 | 25.8 | 11.25 | 0.44 | 13.7 | 3.30 (85 °C) | Chen et al. (2017) |
| | *T. composti* KWC4 | 65 | 7.5 | 70.4[a] | 2.46[a] | 0.035[a] | 1.7[a] | 3.65 (65 °C) | Xu et al. (2017) |
| | *B. subtilis* WB600 | 70 | 8 | 5.98 | 0.74 | 0.12 | NR | 6.0 (65 °C) | Bai et al. (2015) |
| | *D. turgidum* DSMZ 6724 | 75 | 8 | 61.5 | 81 | 1.3 | NR | 12.7 (80 °C) | Kim et al. (2013) |
| | *M. loti* | 60 | 9 | 7.11 | 1.33 | 0.19 | 3.03 | > 1 h (50 °C) | Takata et al. (2011) |
| | *C. saccharolyticus* ATCC 43494 | 90 | 7 | 14.3 | 68.1 | 4.77 | 21 | ~ 1 h (90 °C) | Lin et al. (2011) |
| | *T. saccharolyticum* NTOU1 | 75 | 7 | 121 | 33.9 | 0.28 | 5.7 | > 2 h (75 °C) | Lin et al. (2010) |
| | *T. maritima* | 85 | 8 | NR | NR | NR | 1.1[a] | 773 h (75 °C) | Park et al. (2010) |
| | *B. pallidus* Y25 | 65 | 7 | 41.8 | 34.5 | 0.825 | 2.58 | 1 h (65 °C) | Poonperm et al. (2007) |
| | *P. stutzeri* | 60 | 9 | 42 | 2500 | 59.5 | 7.5 | NR | Leang et al. (2004) |
| RPI | *C. thermocellum* | 65 | 7.5 | 53 | 2347 | 44 | 1.9[a] | 4.7 (65 °C) | Yeom et al. (2011) |
| | *C. difficile* | 40 | 7.5 | 460 | 200 | 0.4 | 45 | 53 (55 °C) | Yeom et al. (2010) |
| | *T. maritima* | 70 | 8 | 130 | 140 | 1.1 | 12[a] | 195 (75 °C) | Yeom et al. (2010) |
| | *T. lettingae* TMO | 75 | 8 | 64[a] | 0.116[a] | $1.8 \times 10^{-3}$[a] | 0.2[a] | 3.3 (75 °C) | Feng et al. (2013) |
| GaPI | *L. lactis* | 30 | 7 | 58 | 0.0448 | $7.7 \times 10^{-4}$ | 1.8 | NR | Park et al. (2007b) |
| GlPI | *P. furiosus* | 95 | 7 | 214 | 338.2 | 0.63 | 324[a] | 7.9 (95 °C) | Yoon et al. (2009) |

[a] Acting on D-allulose

NR, not reported; L-RI, L-rhamnose isomerase; RPI, D-ribose-phosphate isomerase; GaPI, D-galactose-6-phosphate isomerase; GlPI, glucose-6-phosphate isomerase

using 2 g of dry recombinant cells harboring RPI from *T. lettingae* TMO (Feng et al. 2013). The RPIs convert D-allulose to D-allose without a detectable by-product, indicating that RPI is an ideal biocatalyst for large-scale industrial production.

The GaPI from *L. lactis* availably isomerizes D-allulose to D-allose, but a large quantity of D-allose is concurrently formed as a by-product. The GaPI from *L. lactis* produces 25 g $L^{-1}$ D-allose from 100 g $L^{-1}$ D-allulose, with 13 g $L^{-1}$ D-altrose after a 12-h reaction (Park et al. 2007b). The GlPI from *P. furiosus* can covert D-allulose to D-allose, and the conversion reaches equilibrium with the proportion of 66 (D-allulose):32 (D-allose):2 (D-altrose) after 12 h (Yoon et al. 2009). GaPI and GlPI show a non-negligible potential in the production of D-allose. The biological production of D-allose using various D-allose-producing enzymes is summarized in Table 2.

## Prospective

D-Allose shows many prominent physiological functions, in particular, anti-cancer effects, although the anti-cancer mechanism has only been partially studied. More experiments are urgently needed to explain the entire detailed mechanism of the anti-cancer effects. It is promising that the physiological functions of D-allose are expanded by investigation into the mechanism of the health benefits. Although these physiological functions of D-allose have been widely studied in rat experiments, no systematic study concerning the metabolism pathway, physiological effect, toxicity, or safety in the human body has been published yet. Therefore, it is necessary to conduct human trials to yield practical instructions, including uptake dosage, metabolism, food applications, therapeutic effects, and untoward effects.

All D-allose-producing enzymes exhibit weakly alkaline optimal pH values and metal-dependent properties, which are disadvantageous conditions for the industrial production of D-allose. To adapt for industrial production, the weak-acid-tolerant and non-metal-dependent enzymes should be scanned by molecular modification based on the reported three-dimensional structure of D-allose-producing enzymes. Furthermore, the conversion ratio and the catalytic efficiency on D-allose can be enhanced by random and site-directed mutagenesis. To solve the food safety issues that exist for the application of D-allose, the secretion and expression of D-allose-producing enzymes in a food-grade host, such as *B. subtilis*, *L. lactis*, *Saccharomyces cerevisiae*, and *Pichia pastoris*, should be imminently investigated in the future (Panghal et al. 2017).

**Table 2** Summary of the biological production of D-allose from D-allulose

| Enzymes | Strains | Biocatalyst | Substrate (g $L^{-1}$) | Product (g $L^{-1}$) | Conversion ratio (%) | Productivity (g $L^{-1}$ $h^{-1}$) | By-product ratio | Reference |
|---|---|---|---|---|---|---|---|---|
| L-RI | *C. stercorarium* ATCC 35414 | Free | 600 | 199 | 33 | 79.6 | 0 | Seo et al. (2017) |
| | *T. composti* KWC4 | Free | 100 | 23 | 23 | 1.53 | 0 | Xu et al. (2017) |
| | *B. subtilis* WB600 | Free | 10 | 3.75 | 37.5 | 0.17 | 0 | Bai et al. (2015) |
| | *C. saccharolyticus* ATCC 43494 | Free | 9 | 2.97 | 33 | 5.9 | 0 | Lin et al. (2011) |
| | *T. saccharolyticum* NTOU1 | Free | 18 | 6.12 | 34 | 1 | 0 | Lin et al. (2010) |
| | *B. pallidus* Y25 | Free | 1.8 | 0.63 | 35 | 0.013 | 0 | Poonperm et al. (2007) |
| | *P. stutzeri* | Immobilized | 100 | 25 | 25 | 2.1 | 8 | Menavuvu et al. (2006) |
| | | Immobilized | 500 | 150 | 30 | 0.37 | 1.3 | Morimoto et al. (2006) |
| RPI | *C. thermocellum* | Free | 500 | 165 | 33 | 27.5 | 0 | Park et al. (2007a) |
| | *C. thermocellum* R123E mutant | Free | NR | NR | 32 | NR | 0 | Yeom et al. (2011) |
| | *T. lettingae* TMO | Cells | 100 | 28 | 28 | 1.21 | 0 | Feng et al. (2013) |
| GaPI | *L. lactis* | Free | 100 | 25 | 25 | 2.1 | 13 | Park et al. (2007b) |
| GlPI | *P. furiosus* | Free | 0.18 | 0.058 | 32 | 0.005 | 2 | Yoon et al. (2009) |

NR, not reported; L-RI, L-rhamnose isomerase; RPI, D-ribose-phosphate isomerase; GaPI, D-galactose-6-phosphate isomerase; GlPI, glucose-6-phosphate isomerase

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** This article does not contain any studies with human participants or animals performed by any of the authors.

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