BCIL Biotech Consortium India Limited

A novel D-allulose 3-epimerase enzyme for efficient production of D-allulose, a rare sugar of anti-diabetic and anti-obesity potential

TECHNOLOGY AVAILABLE FOR TRANSFER

UNMET NEED AND OPPORTUNITY

D-allulose is a functional sugar of nearly zero calorie, with anti-diabetic and anti-obesity potential. It is a metabolic regulator of glucose and lipid in human body. It exerts low glycaemic, blood glucose suppressive, anti-dyslipidemic, neuroprotective, and anti-oxidative effects. It enhances storage stability and texture of food products, and gives a better mouthfeel. It gives a better water-holding capacity to the gelling food material. It is a nontoxic and FDA approved molecule with Generally Recognized as Safe (GRAS) status. D-allulose is rarely found in nature, and is very expensive. The transformation of D-fructose into D-allulose by the enzymatic action of D-allulose 3-epimerase is a widely acceptable method for its biosynthesis. However, thermal stability is a major limitation for the industrial production of D-allulose. The desirable features for a biocatalyst for D-allulose production are enzymatic activity at acidic pH and high temperature, with high thermal stability, and high turnover number.

The global D-allulose market is expected to reach USD 450 million by 2030 with an annualized CAGR 8%. North America, Europe and Asia-pacific are the most common regions where D-allulose is consumed. The synthesis of D-allulose from the chemical route is possible, but chemical process require large amount of energy and it generates undesirable by-products, and the conversion yield is low. In recent years there have been efforts to establish a cost-effective, enzymatic synthesis of D-allulose from D-fructose as feedstock.

TECHNOLOGY

The present technology relates to a novel process for the production of low-calorie functional sugar D-allulose with anti-diabetic and anti-obesity potential from D-fructose employing a novel D-allulose 3-epimnerase (DaeM). The enzyme has high thermal stability with high catalytic efficiency. The enzyme has been cloned, expressed and purified using standard protocol. DaeM has been further analysed for its kinetics, stability, substrate specificity and catalytic activity for bio-conversion of D-allulose from D-fructose. By employing this enzyme either in the purified form or using recombinant *E.coli*, the bio-conversion yield of D-fructose to D-allulose is 30%. D-fructose containing feedstocks honey and grape juice when treated with this enzyme resulted into bio-conversion to D-allulose in the range of 21% to 27% respectively. Although the chemical synthesis of D-allulose is possible, high temperature, complex purification procedure, low yields and biproduct formation limits its industrial application.

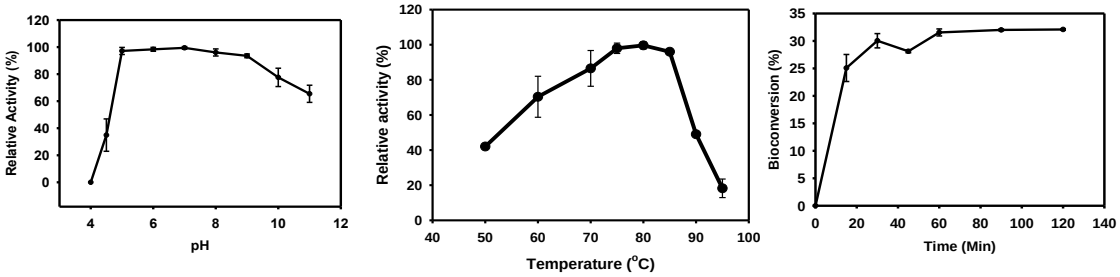
UNIQUE SELLING PROPOSITIONS

- The enzyme (DaeM) is highly potential as a biocatalyst for the cost effective and large-scale production of D-allulose.
- Functional and highly stable novel enzyme with half-life ($t_{1/2}$) 160 hrs at 60ºC compared to the currently reported maximum thermostability of an enzyme 6.8 hr at 60ºC.
- Working in the pH range of 5.0 to 10.0 which causes reduced browning of D-allulose.
- Catalyze D-allulose biosynthesis in a wide range of temperatures, 50°C to 70°C which exhibit several advantages such as minimal contamination, enhanced product solubility with higher yield.
- Whole recombinant *E.coli* catalysis with 30% bioconversion yield of D-fructose to D-allulose eliminates the need for protein extraction and purification.

To summarize D-allulose 3-epimerase (DaeM) has high stability and high efficiency of bio-conversion of D-allulose using low cost D-fructose containing feedstocks.

STAGE OF DEVELOPMENT

The technology has been validated at the laboratory scale



Temperature (˚C)	$t_{1/2}$ (min)
60 ˚C	9,900
70 ˚C	3,300
80 ˚C	49

pH and temperature activity profiles

Time point conversion of D-fructose to D-allulose

APPLICATION

The present technology is revolutionary as it involves novel extraction of D-allulose 3-pimerase enzyme from the metagenomes of the thermal aquatic habitat and showing good market commercial potential in terms of characterization and use of enzymes in the industrial downstream processes. D-allulose can be used as a therapeutic food which exerts an anti-obesity and anti-diabetic activity. D-allulose can also be used in Dairy industry (yoghurts), Food (Candies, cereals) and beverage industry.

INTELLECTUAL PROPERTY

Indian Patent Granted

LICENSING OPPORTUNITY

BCIL is looking for suitable industrial partner for commercialization of this novel recombinant enzyme technology for the production of D-allulose from D-fructose.

Reference:

1. https://www.transparencymarketresearch.com/allulose-market.html
2. Wang Y, Ravikumar Y, Zhang G, Yun J, Zhang Y, Parvez A, Qi X and Sun W (2020) Biocatalytic Synthesis of D-Allulose Using Novel D-Tagatose 3-Epimerase From Christensenella minuta. Front. Chem. 8:622325. doi: 10.3389/fchem.2020.622325

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