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Trehalose Synthase and Genetic Engineering to the Producing Bacteria

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Many microorganisms produce the trehalose synthase. The trehalose synthase and the producer bacteria are overviewed in order to research them in the next step. Trehalose is composed of two glucose molecules and it is a disaccharide, bound the two units by alpha-1ÿ1 linkage. The trehalose has many properties and it is known to be one of the sources of energy in bacteria espically extremophiles. Microorganisms containing trehalose can live in cold or dry environment, so it is used widely in industrial. Trehalose is also used for various applications in the field of pharmacy. The producing of trehalose rely on the chemical synthesis and biosynthesis, the first method is worse than biosynthesis because of the too low productivitying. Then the industries usually use the biosynthesis to produce trehalose. Biosynthesis produces trehalose using enzyme. Now trehalose synthase is used widely to produce trehalose because of its high productivitying and simple process. The main source of the trehalose synthase is bacteria, but the trehalose also can be found in fungi, insects, invertebrates, and plants.

Keywords: Trehalose synthase, bacteria, trehalose.

Trehalose is a disaccharide with an alpha-1, 1 linkage and is distributed in plants, insects, yeast, and bacteria without reducing. In organisms producing the trehalose, it serves as a storage for energy and a protectant from freezing, heating[1], the desiccation, hyperosmosis[2] and other stresses[3]. Trehalose also can stabilize the cell structures[4] and avoid the salt damage[5]. It is used widely in food and pharmaceuticals as a sweetener and a stabilizer.

Trehalose synthase (TreS) which converts maltose to trehalose is considered to be a better biocatalyst in biosynthesis for producing trehalose because of its high productivity and simple process. At present, many trehalose synthesizing enzymes systems have been discovered in many microorganisms. There are three main pathways among them: Phosphate based enzyme complex systems[6]; Trehalose synthase synthesizing trehalose from maltose and a two-step enzyme system with maltooligosyl trehalose trehalohydrolase (TreZ) and maltooligosyl trehalose synthase (TreY).

Although trehalose is found in many species, the main producer for producing trehalose synthase is bacteria. As the recent studies showed, *Rhodococcus opacus* ACCC 41021[7], *Thermomonospora curvata* DSM 43183[8], *Corynebacterium glutamicum* ATCC13032[9] and *Meiothemus* sp. SK3-2 GU129930.1[10] contain the TreS. They are the producers for trehalose synthase. Many studies are researching to put the gene into other microorganisms and constructure the engineering bacteria.

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Charateristics of trehalose synthase

Trehlose synthase can catalyze to produce trehalose using maltose as substrate. Trehlose synthase, with simple technological process, is a widely used catalyzer for microbial conversion in producing trehalose. As studied by Ran Zhang, the catalytic reaction of trehalose synthase is intramolecular rearrangement process, not intermolecular[11]. The trehalose synthase reacts with 5-fluoroglycosyl fluorides results in the trapping of a covalent glycosyl enzyme intermediate consistent with trehalose synthase being a member of the retaining glycoside hydrolase family. Both of the general independence of k_{cat}(catalytic rate constant) according to the leaving group ability and the absence of a secondary deuterium kinetic isotope effect point to a rate-determining conformational change, maybe they are the closing and opening of the enzyme active site.

From the contrast of trehalose synthase amino acid sequence, it has four conserved regions in α-amylase, so trehalose synthase is one member in the α-amylase family. Mechanism of trehalose synthase action is similar to α-amylase. At first, maltose molecular is integrated with catalytic site of the enzyme; Glu in the active centre put a proton to oxygen atom in 1, 4- glucosidic bond, meanwhile Asp attacks carbon atom to form transition state.

In recent years, scientists found the trehalose synthase from *Pyrococcus horikoshii* had reversible catalysis in trehalose and nucleoside 52 -diphosphate-glucose (NDP-Glc) synthesis with enzyme recycling[12]. They wanted to regenerate NDP-Glc from NDP with trehalose as a glucose resource. In addition, the directed site mutagenesis can promote the thermo stability of trehalose synthase in *Picrophilus torridus*[13]. The mutant type strain showed about 39% higher activity and productivity of trehalose than that of the wild type in same conditions. The high thermo stability of the enzyme have the important property for the industrial producing. The proline site replacement technology in the study is good for changing the trehalose synthase properties for applications. A trehalose synthase was found from *Corynebacterium nitrilophilus* NRC and puried by ammonium sulphate precipitation [14]. The trehalose synthase specific activity was increased 200-fold from 0.14 U/mg protein to 28.3U/mg protein. This purified enzyme was stable and can prolong itself thermal stability, but it can be inhibited strongly by metal ion.

Because of the vulnerability of enzymes, many articles studied the enzyme immobilization. Purify and immobilize the poly (His)-tagged trehalose synthase using the highly porous crosslinked polystyrene divinylbezene-based metal chelator [15]. The result Co(II)-loaded adsorbent has the relative highest specificity for the adsorption of the trehalose synthase.

In relation to metabolism of carbohydrate, some trehalose synthesis-related genes including TreS, TreC and TreY were highly expressed during the metabolism of carbaryl[16]. The strain they used is *Burkholderia* sp. C3, it can produce many proteins in metabolisms when degrading N-methylcarbamates. This study focused on contrast proteins and metabolisms in C3 utilizing carbary with those using nutrient broth. The study showed that the trehalose synthase has a contribution in degrading N-methylcarbamates.

Trehalose synthase from *Pyrococcus horikoshii* could be applied to a new sugar nucleotide cycling process for the synthesis for the functional α-galactose epitopes and the α-galactose epitopes with alactulose acceptor showed very strong inhibitory activity of anti-adhesion[17]. So it may be can overcome antibiotic resistance.

UdP-glucose 4-epimerase in *Pyrococcus horikoshii* could be coupled with trehalose synthase from *P. horikoshii* to regenerate UDP-galactose from UDP[18]. UDP was able to be converted to UDP- glucose with trehalose by trehalose synthase. Then we can get one-pot two-enzyme system with UDP-glucose 4-epimerase, trehalose synthase and trehalose for the regeneration of UDP-Gal to achieve a sugar nucleotide cycle.

The industries need a stable and reusability of enzyme system to produce trehalose. Maltooligosyl-trehalose synthase (MTSase), Amylosucrase (AS), and maltooligosyl-trehalose trehalohydrolase (MTHase) were used in combined cross-linked enzyme aggregates to complete one-step bioconversion of maltose to trehalose[19]. Co-aggregated serum albumin with enzymes as a protein feeder to improve trehalose production. The combi-enzymes used in practice showed re-

stability of five cycles without losing activity.

P. aeruginosa can replicate in the intercellular spaces in a leaf because of the trehalose biosynthesis in it. The study of Slavica Djonovic explained how *P. aeruginosa* repurposed a conserved "house-keeping" pathway of trehalose biosynthesis as a potent virulence factor that permits it to replicate in environment of a leaf[20]. The result of their study shows trehalose produced by PA14 is required for virulence in Arabidopsis and the data suggest that required for plant but not for pathogenesis of metazoan.

Gene engineering of the strain for producing the trehalose synthase

Physiological role of trhalose is a key to improve the stress resistant of bacteria by metabolic and genetic engineering. The main aim of the gene engineering of the strain is to obtain the high productivitys for trehalose synthase. Some genes of trehalose synthase producer strains screened from soil were obtained using degenerate PCR. Amplify the TreS gene using thermal asymmetric interlaced PCR from *Enterobacter hormaechei*[21]. *Escherichia coli* is the recipient bacterium for the trehalose synthase gene from *Rhodococcus opacus*[22]. Use bacteria genome DNA extracting kit to obtain the genomic DNA of *R.opacus* and amplification the target gene. Then the recombinant *E.coli* containing the TreS gene and can produce the recombinant TreS.

Moreover, overproduce trehalose synthase from a thermo acidophilic archaea *Picrophilus torridus* (PTTS) in *Escherichia coli*[23]. They found that whenever the T7 promoter-driven PTTS gene (P_{T7}-PTTS) was employed in *E. coli* on a multicopy plasmid, the overproduction of PTTS would be hampered. The study overcomes these difficulties, *E. coli* strain is improved with P_{T7}-PTTS inserting into chromosome and genomic argU tRNA and ilex trRNA (truncated RNA) genes strengthened expressing. The constructed producer strain can produce a high-level and stable production of Tres. Besides the study, some articles show the *E. coli* is the ideal bacteria for constructing the recombinant bacteria. A earlier study putted a thermo stable trehalose synthase gene from *Meiothermus ruber* CBS-01 into *E. coli*, cloned and over expressed[24]. Kinetic analysis showed that the re-trehalose synthase had a twofold higher catalytic activity for maltose than for trehalose, then perorating maltose as preferred substrate.

Besides obtaining the high productivitying for trehalose synthase by genetic engineering, improve the stress resistance of species is also the aim because of the characteristics of trehalose. To further improve trehalose production, an osmotic sensitive mutant of *Propionibacterium freudenreichii* subspp. with high trehalose productivity was isolated[25]. In mutant, trehalose productivitys were 3 and 4 times higher with respect to substrate and biomass consumed as compared to parent strains when using the crude glycerol as a carbon source.

The plant growth- promoting bacterium *Pseudomonas* sp. UW4 also has the ability to produce trehalose production. The strain can promote the growth of plant in different environmental stresses, such as cold, heavy metals, drought and flooding. Use pyrosequencing to obtain the genome sequence of UW4 and use directed PCR to find that the contigs gaps were much closed. Identified thirty one putative insertion sequences and predicted nineteen genomic islands. Moreover, genes in UW4 that contribute to the environment fitness of the strain were found with genes responsible for heavy metal resistance[26]. Phylogenetic analysis showed that UW4 belongs to the *fluorescens* group, *jessenii* subgroup. When studying the *Streptomyces roseosporus* with the transcriptional analysising to the decanoic acid stress effect. They found it can promote the productivitys of trehalose production[27]. The genes coding expressed for the putative maltose transporter, and productivitys of TreS was elevated.

Now there are some studies aims to research the trehalose from the trends in bacterial metabolism of trehalose. The trends and nodes of metabolic pathway in trehalose accumulation was overviewed[28]. In addition, Annette A. Angus found the *Burkholderia tuberum* can produce trehalose and researched the DNA sequences in the recently of four *Burkholderia* species with including the strain with ability to synthesize trehalose[29].

DISCUSSION

Many microorganisms have abilities to synthesize the trehalose synthase. Trehalose is

composed of two glucose molecules and it is a disaccharide, bound the two units by alpha-1-1 linkage. The trehalose has many properties and it is one of the sources of energy in bacteria especial extremophiles. Microorganisms containing trehalose can live in cold or dry environment, so it is used widely in industrial.

Trehalose is also used for various applications in the field of pharmacy and food. The producing of trehalose rely on the chemical synthesis and biosynthesis, the first method is worse than biosynthesis because of the too low productivitying. Then the industries usually use the biosynthesis to produce trehalose in practice. Biosynthesis produces trehalose using enzyme. Now trehalose synthase is used widely to produce trehalose because of its high productivitys and simple process. The main source of the trehalose synthase is bacteria, but the trehalose also can be found in fungi, insects, invertebrates, and plants. The recent studies of trehalose biosynthesis under stress environment are incomplete and needs further research.

Now many studies focus on the strains isolated for producing trehalose and has few studies about penetrating into mechanisms of trehalose synthase action. In industrial practice for trehalose can not use pure maltose as substrate because of taking into account the cost problem. The industries usually add glucose or other oligosaccharides into the starch hydrolysate or high maltose syrup as substrate. But the glucose has inhibitory effect on the trehalose synthase activity and reducing the production rate of the trehalose. So that is unfavourable for producing trehalose. The molecular weight of trehalose trehalose from extremophiles is more large than usual and need to dissolve the bias of codon to improve the expression quantity in *E. coli*. It is difficult to improve the charateristics of the trehalose synthase via reconstructing the space of molecular structure because there's few data relevant to the sequence, structure and the characterics of biochemical about trehalose synthase recently. At present, the studies about the influence of the oligosaccharide to catalytic activity of trehalose synthase are few too. Aim at the problem, scientists usually use Tail-PCR[30]to screen the novel genes and combine with the mutagenesis to improve the enzyme characteristics.

There're difficulties to separate and purify the trehalose, to a certain extent, because of the similar physical natures of the trehalose and maltose. According to the difference between maltose and glocuse in activities of absorption to activity carbon and solube in alcohol solution, translating the other oligose and untransformed maltose into glucose using saccharifying enzyme before the separation and purification of trehalose[31]. The study recently takes advantage of the activated carbon column chromatography or gradient elute[32] into practicing.

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