Production of erythritol by a newly isolated strain of *Pseudozyma tsukubaensis* (BN75E)

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Erythritol, a four-carbon polyol, is a naturally occurring substance and is widely distributed in nature[1]. Like most other polyols, it is a metabolite or storage compound for seaweed, mushrooms, and fruits. It occurs frequently in fermented food including wines and beers, and processed vegetables such as soy sauce and oriental miso bean paste[2-4]. Erythritol has sweetness with 60 to 70% of sucrose in a 10% solution and has very high negative heat, providing a strong cooling effect when dissolved[1]. Erythritol has been safely used in foods as a non-cariogenic sweetener in many countries. This property is due to the inability of the bacteria that cause dental caries to use erythritol as a fermentation substrate[5]. Erythritol can be produced by microbial methods using osmophilic yeasts and some bacteria[1,6-11].

A novel erythritol-producing yeast, which is capable of growth at high osmolarity, was isolated from fermentation sludge in Korea. Characteristics of the strain include asexual reproduction by multilateral budding, the absence of extracellular starch-like compounds, and a negative Diazonium blue B colour reaction. Phylogenetic analysis based on the 26S rDNA sequence and physiological analysis indicated that the strain belongs to the species *Pseudozyma tsukubaensis*, and has been named *Pseudozyma tsukubaensis* BN75E. Under aerobic conditions, it grows well in the pH range of 4.5-6.5 and produces erythritol, ethanol, and small amount of organic acids. When *Pseudozyma tsukubaensis* BN75E was cultured aerobically in medium containing 400 g l^{-1} glucose as carbon source, 223 g l^{-1} of erythritol was produced. *Pseudozyma tsukubaensis* showed the highest erythritol yield ever reported by an erythritol-producing microorganism.

Supplemental Cu^{2+} enhanced the production of erythritol and the activity of erythrose reductase, a key enzyme that converts erythrose to erythritol, while it significantly decreased the production of a major by-product that accumulates during erythritol fermentation, which was identified as fumarate by instrumental

analyses such as NMR and APCI-MS. Erythrose reductase from *Pseudozyma tsukubaensis* was purified to homogeneity by chromatographic methods, including ion-exchange, and affinity chromatography. *In vitro,* purified erythrose reductase was significantly inhibited uncompetitively by increasing fumarate concentration. In contrast, the enzyme activity remained almost constant regardless of Cu^{2+} concentration. This suggests that supplemental Cu^{2+} reduced the production of fumarate, a strong inhibitor of erythrose reductase, which led to less inhibition of erythrose reductase and a high yield of erythritol. This is the first report that suggests catabolite repression by a TCA cycle intermediate in *Pseudozyma tsukubaensis*.

Melanin, a dark-brown to black pigment, is widely dispersed in the animals, plants, and microorganism such as fungi, yeast, and bacteria[12,13]. This pigment is not essential for growth and development, but rather it enhance the survival and competitive abilities of species in certain environments[14]. The effect of melanin accumulation on the production of metabolite, however, has not been studied. Melanin was found during the erythritol production by *Pseudozyma tsukubaensis* as a by-product. In this study, the increase of the erythritol production was attempted by inhibiting melanin biosynthesis. Melanin biosynthesis inhibitors, were added to the medium used to produce erythritol by *Pseudozyma tsukubaensis* BN75E. Tricyclazole was the most effective inhibitor in increasing the production of erythritol and in decreasing the production of melanin. Supplementation with tricyclazole enhanced the production of erythritol and the activity of erythrose reductase, while it significantly inhibited the production of DHN melanin and the activities of enzymes involved in the biosynthesis of DHN melanin such as trihydroxynaphtalene reductase. *In vitro,* purified erythrose reductase was significantly inhibited noncompetitively by increasing DHN melanin concentration. This is the first report that DHN melanin inhibits an enzyme in the production of a sugar alcohol, lowering the production of the sugar alcohol.

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