# Increased Erythritol Production in *Torula Sp.* By $Cu^{2+}$ and Melanin Biosynthesis Inhibitors

JungKul Lee
BioNgene Co. Ltd

Erythritol, a four-carbon polyol, is a naturally occurring substance and is widely distributed in nature. 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 (1, 2). Erythritol has sweetness with 60 to 70% of sucrose in a 10% solution. It has very high negative heat, providing a strong cooling effect when dissolved. As erythritol is as sweet as sucrose but with no bitter aftertaste, its use will improve the taste of intense sweeteners with bitter aftertaste such as aspartame (3). Erythritol can be safely used in foods as a cariogenic sweetener. This property is due to the inability of the bacteria that cause dental caries to use erythritol as a fermentation substrate. Erythritol can be produced by microbial methods using osmophilic yeasts and some bacteria (3-6).

Trace minerals, such as Cu, Mn, Fe, Co, Zn, Ni, etc. were added to the medium used to produce erythritol by *Torula sp.* The production of erythritol and the erythritol yield from glucose by *Torula* sp. were improved, in increasing order, by supplementing with 10 mg $MnSO_4$ $4H_2O$ $l^{-1}$, 2 mg $CuSO_4$ $5H_2O$ $l^{-1}$, and both 10 mg $MnSO_4$ $4H_2O$ $l^{-1}$ and 2 mg $CuSO_4$ $5H_2O$ $l^{-1}$. $Mn^{2+}$ decreased the intracellular concentration of erythritol, whereas $Cu^{2+}$ increased the activity of erythrose reductase in cells. These results suggest that $Mn^{2+}$ altered the permeability of cells, whereas $Cu^{2+}$ increased the activity of erythrose reductase in cells. $Cu^{2+}$ was the most effective in increasing the production of erythritol. Effect of $Cu^{2+}$ on the production of erythritol by *Torula* sp. was investigated in detail. The supplemental $Cu^{2+}$ markedly enhanced the erythritol production and the activity of erythrose reductase. A major by-product of the cultures with and without $Cu^{2+}$ was identified as fumarate and the fumarate production was inhibited strikingly by supplementing $Cu^{2+}$. The activity of purified erythrose reductase, a conversion enzyme from erythrose to erythritol, was markedly inhibited with increasing fumarate concentration whereas it was almost constant regardless of $Cu^{2+}$ concentration. These results suggest that the supplementation of $Cu^{2+}$ reduced fumarate production and the activity of erythrose reductase became less inhibited as the concentration of fumarate formed decreased, giving a high yield of erythritol.

Melanin is an irregular light-absorbing polymer containing indoles and other intermediate products derived from the oxidation of tyrosine (7). Melanin, a dark-brown to black pigment, is widely dispersed in the animals, plants, and microorganism such as fungi, yeast, and bacteria (8,9). This pigment is not essential for growth and development, but rather it enhance the survival and competitive abilities of species in certain environments. Melanin may be essential for protection of microorganisms for UV irradiation, radio waves, desiccation, and temperature extremes (10).

It was previously reported that many microorganisms accumulated melanin (8-10). The effect of melanin accumulation on the production of metabolite, however, has not been studied. Melanin was found during the erythritol production by *Torula* sp. as a by-product. In this study, the increase of the

erythritol production was attempted by inhibiting melanin biosynthsis. Melanin biosynthesis inhibitors, such as arbutin, kojic acid, EDTA, tricyclazole, niacin, and hydroquinone, were added to the medium used to produce erythritol by *Torula* sp. Of these inhibitors, tricyclazole was the most effective in increasing the production of erythritol. Adding tricyclazole decreased the formation of melanin and the activities of enzymes involved in melanin biosynthesis, such as tyrosinase, laccase, and peroxidase, while it remarkably increased the activity of a key enzyme in erythritol biosynthesis, erythrose reductase. The activity of erythrose reductase became less inhibited as the concentration of melanin formed decreased, giving a high yield of erythritol. This indicates that the production of metabolites such as erythritol, which accompanies the production of unnecessary melanin, can be increased by inhibiting melanin biosynthesis. This is the first report that melanin inhibits an enzyme related to the production of a sugar alcohol, lowering the production of the sugar alcohol.

## References


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