

Aerobic condition accelerates carotenoid production in *Enterococcus gilvus*

Lactic acid bacteria (LAB) is industrial important bacteria because LAB are used in fermented foods and probiotics. The clarification of the stress tolerance mechanism of LAB is beneficial for probiotic and molecular breeding to equip LAB with stress tolerance. The increased stress tolerance of LAB can lead to efficient productivity of metabolites and high survivability in the gut environment. The objective of this study is the clarification of stress response mechanism including carotenoid in LAB considered as novel stress tolerance mechanism in LAB. In general, carotenoid plays role in photo-biosynthesis and antioxidant in plants and microorganisms. A part of LAB belonging to *Enterococcus* and *Lactobacillus* have yellow pigment, and the yellow pigment is carotenoid named as diaponeurosporene which can enhance oxidative stress tolerance as well as bile acid and lysozyme. However, the gene expression response of carotenoid and its precursor biosynthesis pathways, such as the isoprene biosynthesis pathway via mevalonate, to oxidative stress is not understood in detail.

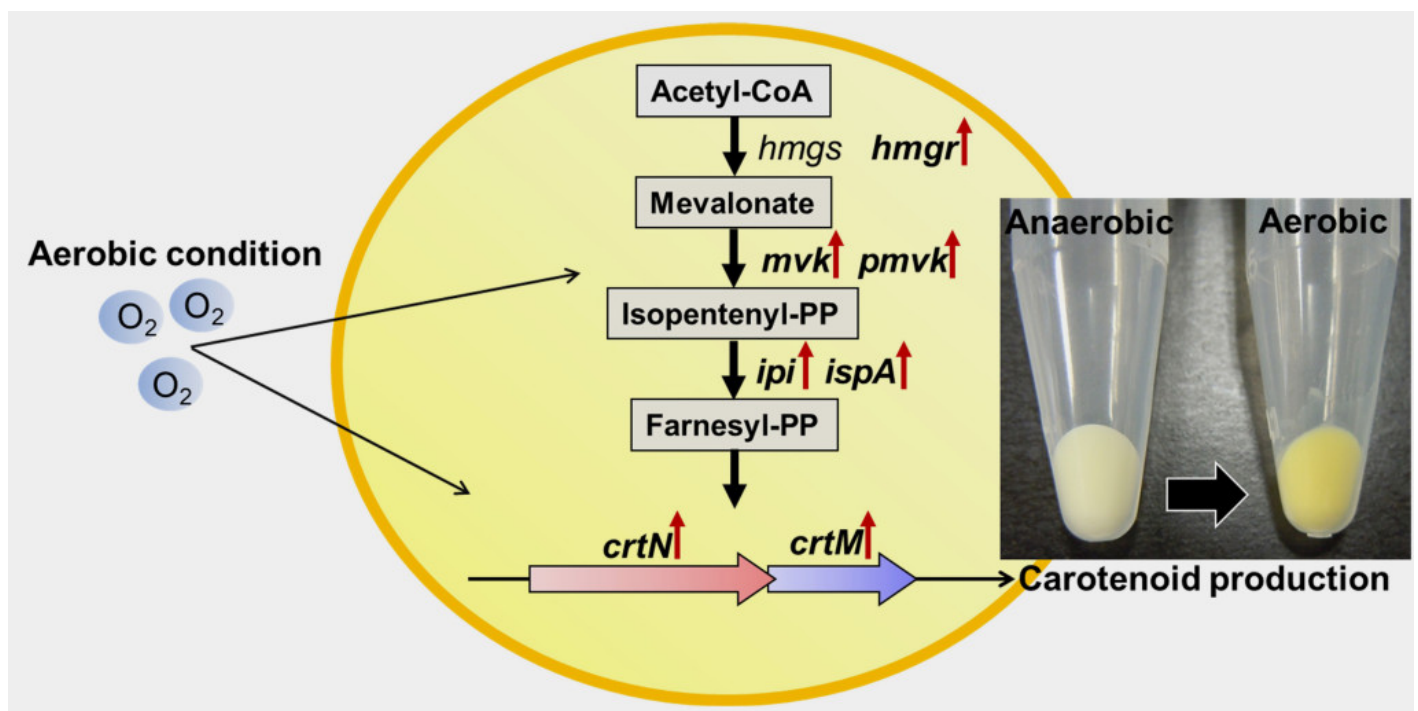


Fig. 1. The dynamics of isoprenoid biosynthesis pathway and cell pigment under aerobic condition in *Enterococcus gilvus*. Gene names in bold are upregulated under aerobic condition (red arrows). The yellow pigment level of cell pellets in aerobic conditions (right) was higher in anaerobic (left).

To clarify stress response gene expression associated with high carotenoid production, we used

Enterococcus gilvus isolated from cow's raw milk which is able to produce high amount of carotenoid. As stress treatment, *E. gilvus* cells, which were incubated anaerobically, were shaken (aerobic condition). On the other hand, anaerobic incubation of *E. gilvus* is continued as non-stress treatment (anaerobic condition). To investigate stress response genes associated with carotenoid production, the gene expression levels of the mevalonate biosynthesis pathway (*hmgs* and *hmgr*), the isoprene biosynthesis pathway (*mvk*, *pmvk*, *mpd*, *ipi* and *ispA*) and the carotenoid biosynthesis pathway (*crtM* and *crtN*) were examined using real-time PCR. Compared with anaerobic condition, all genes excluding *hmgs* were upregulated in aerobic condition (Fig. 1.). We could show that upregulation of isoprenoid biosynthesis genes occurred under aerobic condition and led to enhancement of carotenoid production. To enhance stress-tolerant LAB, mutagenesis in the isoprenoid biosynthesis pathway is considered to be a useful. Furthermore, LAB with high levels of carotenoid production may contribute to supply fermented products with carotenoid considered as antioxidant.

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