

Iosporus JCM5269, *Xanthophilomyces dendrorhous* IFO10129, *Xanthobacter flavus* JCM1204, *Paecilomyces lilacinus* ATCC10114, *Acremonium chrysogenum* ATCC11550, *Hyphomonas hirschiana* ATCC33886, *Rhizobium meliloti* ATCC9930, and the like.

As the reduced coenzyme Q₁₀-producing microorganisms, not only the wild species of the above-mentioned microorganisms but also microorganisms in which the transcription and translation activities of the genes relevant to the biosynthesis of reduced coenzyme Q₁₀ in the above-mentioned microorganisms, or the enzyme activity of the expressed protein are modified or improved can be used preferably, for example.

As the means for modifying or improving the transcription and translation activities of the genes or the enzyme activity of the expressed protein, there may be mentioned gene recombination (including gene improvement, amplification and destruction by itself, external gene introduction, and gene improvement and proliferation of thus-introduced external genes) and mutagenesis by mutagens. In particular, the mutagenesis by mutagens is preferred.

The more preferable microorganisms usable for the present invention are microorganisms containing reduced coenzyme Q₁₀ at a ratio of not less than 70 mole %, preferably not less than 75 mole %, more preferably not less than 80 mole %, still more preferably not less than 85 mole %, and particularly preferably not less than 90 mole %, among the entire coenzymes Q₁₀ in the case where the above-mentioned modified or improved microorganisms, preferably microorganisms mutated by mutagens, are evaluated by the above-mentioned proliferation method and the measurement method. In the fermentation production on the industrial scale, it is preferable to use microorganisms having a productivity of reduced coenzyme Q₁₀ per unit culture medium of not less than 1 µg/mL, preferably not less than 2 µg/mL, more preferably not less than 3 µg/mL, still more preferably not less than 5 µg/mL, particularly preferably not less than 10 µg/mL, much more preferably not less than 15 µg/mL, and most preferably not less than 20 µg/mL.

The mutagenesis may be carried out by a single mutagenesis; however, mutagenesis is preferably carried out not less than 2 times. That is because it was found that the productivity of reduced coenzyme Q₁₀ can be improved in the respective mutagenesis steps. It is needless to say that the candidates of the microbial cells to be mutated are, generally, those having a productivity of reduced coenzyme Q₁₀ as high as possible in the case where the evaluation is carried out by the above-mentioned proliferation method and measurement method.

The mutagenesis can be carried out by using optional and proper mutagens. The term "mutagen" encompasses, in a board definition, not only chemical agents having mutagenesis effects, for example, but also treatments such as UV radiation having mutagenesis effects. As examples of proper mutagens, there may be mentioned ethyl methanesulfonate, UV radiation, N-methyl-N'-nitro-N-nitrosoguanidine, nucleotide base analogues such as bromouracil, and acridines; however, they are not limited to these examples.

According to a conventional mutagenesis technique, successively to the mutagenesis, a proper selection of microbial cells having high productivity of reduced coenzyme Q₁₀ is carried out. For that, the culture obtained from a single colony should be evaluated, for example, by the above-mentioned proliferation method and measurement method. Since a reduced coenzyme Q₁₀ crystal forms a white solid layer or a colorless liquid phase, a productivity of reduced

coenzyme Q₁₀ can be suitably evaluated by the above-mentioned measurement method at the time of selection of the colony.

In the processes of the present invention, high productivity of reduced coenzyme Q₁₀ in the fermentation production on the industrial scale can be achieved partially by using the microbial cells containing reduced coenzyme Q₁₀ at a ratio of not less than 70 mole % among the entire coenzymes Q₁₀ and, partially, by using the suitable conditions of culture (fermentation) for increasing a productivity of reduced coenzyme Q₁₀ per unit culture medium as described below. It is particularly preferable to combinedly use suitable microbial cells described above and the suitable conditions of culture (fermentation) as described below.

The culture is carried out, in general, in a culture medium containing major nutrients and micronutrients suited for microorganism proliferation. As the above-mentioned nutrients, there may be mentioned, for example, carbon sources (e.g. hydrocarbons such as glucose, sucrose, maltose, starch, corn syrup and molasses; alcohols such as methanol and ethanol), nitrogen sources (e.g. corn steep liquor, ammonium sulfate, ammonium phosphate, ammonium hydroxide, urea and peptone), phosphorus sources (e.g. ammonium phosphate and phosphoric acid) and micronutrients (e.g. minerals such as magnesium, potassium, zinc, copper, iron, manganese, molybdenum, sulfuric acid and hydrochloric acid; vitamins such as biotin, desthiobiotin and vitamin B₁; amino acids such as alanine and histidine; and natural raw materials containing vitamins such as yeast extract and malt extract); however, these are not limitative ones, and commonly used ones may be used. Incidentally, in natural components of a culture medium, such as yeast extract, phosphorus sources such as phosphates are contained. The above-mentioned nutrients can be appropriately used in combination.

The culture is generally carried out at a temperature range of 15 to 45° C., preferably 20 to 37° C. If it is below 15° C., the proliferation speed of microorganisms tends to be too slow to allow the industrial production and at high temperatures exceeding 45° C., the viability of microorganisms tends to be easily hindered.

In general, the culture is carried out at a pH range of 4 to 9, preferably 5 to 8. If the pH is not more than 3 or not less than 10, proliferation of microorganisms tends to be easily inhibited.

In the fermentation production on the industrial scale, although it depends on the microorganism species, the concentration of the carbon sources (including the produced alcohols) during the culture is preferably controlled to a concentration that no adverse effects are substantially caused on the productivity of reduced coenzyme Q₁₀. Accordingly, it is preferable to control the culture so as to have the concentration of the carbon sources that no adverse effects are substantially caused on the productivity of reduced coenzyme Q₁₀, that is, generally to not more than 20 g/L, preferably not more than 5 g/L, and more preferably not more than 2 g/L in the broth.

To control the concentration of the carbon sources, a fed batch culture method is preferably used. The carbon source concentration in the broth can be controlled by adjusting the supply of nutrient sources (especially carbon sources) based on the culture control indexes such as pH, the dissolved oxygen concentration (DO) or the remaining saccharide concentration. Although it depends on the microorganism species, the supply of the nutrient sources may be started from the initial stage of the culture or during the culture. The supply of the nutrient sources may be continuous or inter-