Synthesis of Citronellyl Acetate via a Transacetylation to Citronellol from Acetyl Coenzyme A Produced from Glucose and Acetate in Growing Yeasts

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A novel coupling system, which is an acetylation system of primary alcohols with acetyl coenzyme A [acetyl-CoA] formed via the metabolism of glucose and acetate, was developed with *Hansenula* and *Pichia*. The supplementation of sodium acetate to glucose as the source of acetyl-CoA was effective to enhance the reaction rate and yield of acetylation of citronellol.

The coupling of transacetylation from acetyl-CoA to primary alcohols by the aid of alcohol acetyltransferase [AATFase] and metabolism of glucose to acetyl-CoA is a unique procedure for the production of various acetic esters without any acetyl donor (we referred to this system as coupling system).[1–3] In this system, a high concentration of glucose in a hydrophilic carrier of an interface bioreactor, which is a nonaqueous bioreactor using a microorganism growing on an interface between a hydrophilic carrier and a hydrophobic organic solvent,[4] contributes as the source of acetyl-CoA and also depresses the unfavorable oxidation of the substrates, primary alcohols. However, the excess amount of glucose in the carrier generally leads to decrease of growth rate of cells and the coupling activity. For example, the activity of *Hansenula saturnus* IFO 0809 decreases at over 5% (w/v) of glucose content.[1] Moreover, the production rate of acetyl-CoA via the metabolism of glucose is limited because of multiple steps of its pathway. In this study, it is clarified that the supplementation of sodium acetate to glucose in the carrier is effective to increase the reaction rate and the yield of the coupling system (Figure 1).

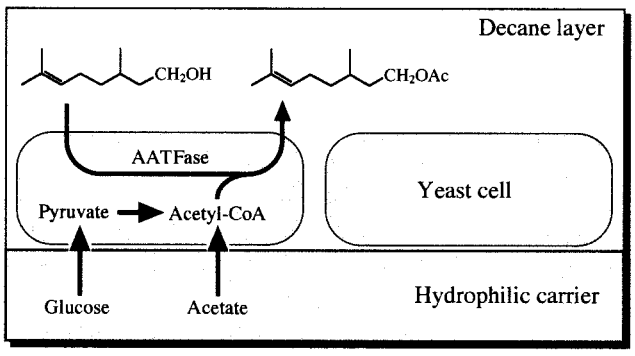

Figure 1. Principle of the coupling system supplemented with sodium acetate. Citronellyl acetate is useful as a perfume.

Three type culture yeasts, *H. saturnus* IFO 0809, *Pichia quercuum* IFO 0949, and *P. heedii* IFO 10019 having the coupling activity,[2] were used. The basal medium (pH 6.0) consisted of 5.0 g of peptone, 3.0 g of malt extract, 3.0 g of yeast extract, 1.0 g of $MgSO_4 \cdot 7H_2O$, 40.0 g of glucose, 0–50.0 g of sodium acetate, and 1.0 L of distilled water. Agar powder (15.0 g) was added to 1.0 L of the medium for the preparation of agar plates. Three hundred μL of a cell suspension (1 loopful/mL medium) of each strain was spread on the agar plate prepared in a glass petri dish (surface area, 38.5 cm^2; volume, 25 mL), and the plate was incubated at 30 °C for 1 day. Then, 8 mL of a 1% (w/v) solution of citronellol in decane was added, and incubation was performed at 30 °C by allowing the dish to stand. The concentrations of citronellyl acetate and citronellol in the decane layer were directly determined by gas chromatography: the column (ϕ, 2.6 mm; length, 3 m) contained Thermon-3000 /Chromosorb W (Chromato Packing Center, Kyoto); the column temperature was raised from 100 to 240 °C at a rate of 7 °C/min; the carrier gas was N_2 (flow rate, 60 mL/min).

In spite of strong biotoxicity of citronellol,[5] three strains tested could produce citronellyl acetate from 1% (w/v) of citronellol due to toxicity alleviation effect of the incubation system (growth on a solid–liquid interface).[6] As shown in Figure 2, adequate supplementation of sodium acetate was effective to enhance the production rate of citronellyl acetate for all strains. Especially, as for *H. saturnus*, supplementation of 3% (w/v) of sodium acetate led to the most drastic increase. It is easily supposed that the added acetate is readily converted to acetyl-CoA and works as the acetyl donor in the transacetylation step. On the other hand, oxidation of citronellol was not observed in any strains because of repression of the production of citronellol-oxidizing enzymes by the presence of high content of glucose (4%, w/v).[1–3,7] Thus, glucose must be used as a repressor of the oxidation of primary alcohols in the coupling system with sodium acetate.

Although the use of sodium acetate is effective as shown above, the excess addition of sodium acetate inhibited the coupling activity, i.e., the activities of *P. quercuum* and *P. heedii* were reduced at over 4 and 3% (w/v) of sodium acetate, respectively. It is well known that acetate exhibits strong biotoxicity on many microorganisms. Indeed, while only 20 mM potassium acetate strongly inhibits the uptake of L-serine into *Bacillus subtilis* cells, growth of the cells is completely supressed by 100 mM of the salt, acetate.[8] Furthermore, the uptake of phosphate into *Saccharomyces cerevisiae* cells is completely inhibited by the presence of 80 mM acetate because of destruction of a cell membrane.[9] In conclusion, the addition of sodium acetate is

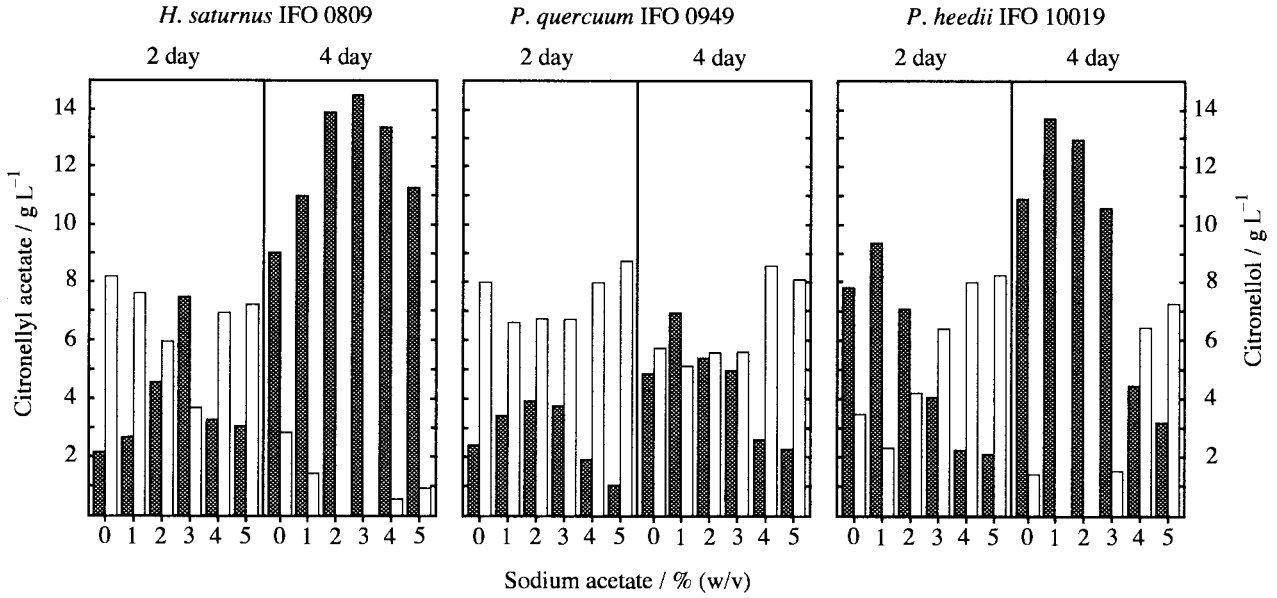

Figure 2. Effects of sodium acetate content in the carrier on the coupling activity. ▩, Citronellyl acetate concentration; □, citronellol concentration. Each strain was inoculated on the nutrient agar plate and precultivated for 1 day. Then, 8 mL of a 1% (w/v) solution of citronellol in decane was added, and incubation was carried out at 30 °C by allowing the plate to stand.

effective for the elevation of the coupling rate and yield, although its toxicity was observed at high concentration.

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