Factors Influencing the Growth of Debaryomyces hansenii and Saccharomyces servazzii Isolated from Salted “Daikon” (Japanese Radish)

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Influences of carbon dioxide (0–91%), oxygen (2.0–20.8%), sodium chloride (0–12%), pH (4.5–6.5) and ethanol (0–2%) on the growth of Debaryomyces hansenii and Saccharomyces servazzii were studied. Carbon dioxide had no effect on the growth of S. servazzii, while an inhibition of the growth of D. hansenii was observed with an increase in the concentration of carbon dioxide (5–40%) and its growth was completely inhibited when the concentration of carbon dioxide was increased over 50%. Although sodium chloride had a little effect on the growth of D. hansenii, it was much effective on that of S. servazzii which was inhibited completely at 12% sodium chloride.

The effects of these factors on the growth of D. hansenii and S. servazzii, which are grown in the cover brine during the storage of salted “Daikon”.

1. Materials and Methods

Bacterial strains

The bacterial strains used in this study were D. hansenii (JCM 5203) and S. servazzii (JCM 5200)

Cultivation media

A basic medium was composed of 3 g of Bacto Yeast Extract, 3 g of Malt Extract, 5 g of Bacto Peptone, 10 g of Bacto Dextrose and 1000 ml of distilled water. The medium pH was adjusted to 6.5. The media containing 6, 8, 10 and 12% of sodium chloride were prepared by adding it to the basic medium and sterilized. The media containing 0, 1 and 2% of ethanol were prepared by adding it to the sterilized basic medium. The media with various pHs were prepared by adding 2 N H2SO4 or 2 N NaOH to the basic medium and sterilized.

Experimental procedure

Ten ml of the sterilized medium which was prepared for each test were pipetted into a 20 ml sterilized test tube with 14 mm diameter. The yeasts, which were precultured in YM agar at 28°C for 2 days, were transferred into the test tube to attain the cell number of about 1 × 10^5 cells/ml. For fermentation in the air as controls, 20 ml–test tubes with cotton plugs containing the medium and yeast were incubated in an incubator at 28°C.

Cultivation under controlled atmosphere was performed as follows (Fig. 1); 20 ml–test tubes with cotton plugs containing the medium and yeast were...
placed in an anaerobic box and the air was removed from the box, then a mixed gas whose composition was adjusted by the injection of nitrogen, carbon dioxide and oxygen gases was introduced into the box (Fig. 1), in which the pressure was controlled to 1 atm.

After the anaerobic boxes were placed in an incubator, incubation was started at 28°C.

**Calculation of cellular growth**

Growth of cells was monitored by measurement of optical density at 660 nm with a Spectro-photometer (Shimazu Spectronic 20).

**Measurement of concentration of oxygen and carbon dioxide**

The concentration of oxygen was measured by oxygen meters (Oxygenmeter-SR-500, Iijimaseimitsu and Oxygen meter, Mitaka instrument). That of carbon dioxide was measured by a carbon dioxide meter (RG-100, Tore-engineering).

2. Result and discussion

Carbon dioxide had no effect on the growth of *S. servazzii*, while an inhibition of the growth of *D. hansenii* was observed with an increase in the concentration of carbon dioxide (5 ~ 40%) (Fig. 2) and its growth was inhibited completely when the concentration of carbon dioxide was increased over 50%.

The growth patterns of *D. hansenii* were similar during the incubation in the media containing 0 ~ 6% sodium chloride, whereas at the higher levels of sodium chloride (8 ~ 12%), the growth of *D. hansenii* was inhibited with an increase in the concentration of sodium chloride. The growth of *S. servazzii* was inhibited with increasing the concentration of sodium chloride (0 ~ 12%).

At the concentration of 12% sodium chloride, the growth of *S. servazzii* was inhibited completely (Fig. 3). The influences of the concentration of oxygen (2.0 ~ 20.9%) and ethanol (0 ~ 2%) on the growth of both *S. servazzii* and *D. hansenii* were not so high as those of carbon dioxide on that of *D. hansenii* and as those of sodium chloride on that of *S. servazzii*. The influences of pH (4.5 ~ 6.5) on the growth of both *S. servazzii* and *D. hansenii* were small. The inhibitory effect of carbon dioxide on the growth of *D. hansenii* was smaller at the concentration of 6% sodium chloride than at the concentration of 0% sodium chloride. No reason can be given for this phenomenon but we were interested in it5,6).

The lag phase and logarithmic phase of *S. servazzii* were short compared with those of *D. hansenii*. It was considered that the growth inhibition of *D. hansenii* in the nitrogen gas packing system for the storage of salted
"Daikon" was attributable to inhibitory effect by carbon dioxide in addition to the lack of oxygen, and the concentrations of sodium chloride and ethanol.

References

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