**Note**

**Microbial Degradation of Lipid by Acinetobacter sp. Strain SOD-1**

Daisuke Sugimori,† Masatoshi Nakamura, and Yuma MiHara

Department of Chemistry and Biology Engineering, Fukui National College of Technology, Geshi, Sabae, Fukui 916-8507, Japan

Received January 7, 2002; Accepted March 13, 2002

Acinetobacter sp. strain SOD-1, capable of rapidly degrading salad oil, was isolated from soil. Strain SOD-1 showed good growth and degraded 68.7 ± 2.7 and 83.0% of an initial 3000 ppm salad oil suspension in 24 h at 20°C and pH 7.0 and at 35°C and pH 8.0, respectively. The degradation rate depended on pH, temperature, phosphate concentration, and initial cell density.

Key words: Acinetobacter sp.; lipid degradation; lipid-degrading bacterium; microbial degradation; wastewater treatment

Much oleaginous material such as fats and greases of animal and vegetable origin is contained in wastewater from food industries, restaurants, and kitchens. These materials (lipids) cause many problems in systems for handling wastewater and pollution of sewage and water resources and the environment. Especially, lipids accumulating in the grease trap result in disposal and sanitary problems. Therefore, the development of a microorganism with high degradation activity for lipids is essential for the solution of these problems. So far, there are many reports on microbial degradation of lipids.1–6 However, we propose that it is essential to construct many microbial libraries for effective degradation of all kinds of lipids in the wastewater treatment system under various conditions such as pH, temperature, and nutrients. We report here a characterization of Acinetobacter sp. strain SOD-1, capable of rapidly degrading salad oil for practical use.

The salad oil was a commercial vegetable oil, consisting of rapeseed and soybean oil, from Nisshin Oil Mills, Ltd. (Tokyo, Japan). Lard was obtained from Snow Brand Milk Products Co., Ltd. (Tokyo, Japan). A basal medium (pH 7.0) contained 2 g of (NH₄)₂HPO₄, 2 g of K₂HPO₄, 1 g of NaH₂PO₄, 0.2 g of MgSO₄·7H₂O, and 0.1 g of yeast extract (Difco) per liter of distilled water. Soil samples were added to a test tube containing 5 ml of the medium and 10% salad oil and incubated for several days at 28°C with reciprocal shaking.

One hundred fifteen microorganisms were isolated from 54 soil samples. Strain SOD-1, which grew well and had a high degradation rate for salad oil, was chosen from the isolates for further characterization. Strain SOD-1 was taxonomically identified by NCIMB Japan (Shizuoka, Japan). The characteristics of strain SOD-1 are summarized in Table 1. Strain SOD-1 was a Gram-negative aerobic cocobacillus and was identified as an Acinetobacter sp.

The lipase activity of 24-h culture supernatant was measured with a Lipase Kit S (Dainippon Pharmaceutical Co., Ltd., Osaka, Japan). One unit of enzyme activity (IU) was defined as the amount of enzyme that liberated one μmol of free thiol groups per min. Lipase activity of the culture supernatant was 1.34 IU/l, indicating that strain SOD-1 produces an extracellular lipase.

A loopful of one of the plate cultures was inoculated into a test tube containing 5 ml of the medium and 30 μl of salad oil. These cultures were incubated at 28°C for 24 h with shaking. Colony forming units (CFU) were counted on nutrient agar plates. The cell density of the cultures averaged 4 × 10⁶ CFU/ml. Typically, 1 ml of a culture was transferred to a 500-ml baffle-walled shaking flask with 100 ml of the medium containing 0.3 g of salad oil. The cultures were incubated at 20°C for 24 h on a rotary shaker.

<table>
<thead>
<tr>
<th>Table 1. Characteristics of Strain SOD-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram stain</td>
</tr>
<tr>
<td>Motility</td>
</tr>
<tr>
<td>Spores</td>
</tr>
<tr>
<td>Requirement of free oxygen</td>
</tr>
<tr>
<td>Oxidase activity</td>
</tr>
<tr>
<td>Catalase activity</td>
</tr>
<tr>
<td>Anaerobic fermentation of glucose</td>
</tr>
<tr>
<td>Colony color</td>
</tr>
<tr>
<td>Growth at:</td>
</tr>
<tr>
<td>44°C</td>
</tr>
<tr>
<td>41°C</td>
</tr>
<tr>
<td>37°C</td>
</tr>
</tbody>
</table>

† To whom correspondence should be addressed. Fax: +81-778-62-3415; E-mail: sugimori@fukui-nct.ac.jp
**Abbreviations:** CFU, colony forming unit; PFB medium, K₂HPO₄ and NaH₂PO₄-free basal medium
Fig. 1. Effects of Various Conditions on Salad Oil Degradation by Strain SOD-1.

The cultures were incubated in the basal medium containing 3000 ppm salad oil for 24 h with shaking under the following conditions: (A) pH 7.0, (B) 20°C, (C, D, and E) 20°C and pH 7.0; (D), in the presence of 2 g/l (11.5 mM) K$_2$HPO$_4$ and 1 g/l (8.33 mM) NaH$_2$PO$_4$, (E), K$_2$HPO$_4$ and NaH$_2$PO$_4$ concentration was changed in a molar ratio of 1:1.38 (NaH$_2$PO$_4$/K$_2$HPO$_4$) at 15.1 mM (NH$_4$)$_2$HPO$_4$. Bar represents standard deviation of the mean ($n = 5$).
Acinetobacter sp. Capable of Rapidly Degrading Lipid

(140 rpm). The cultures were then autoclaved. The residual lipids in the cultures were measured as n-hexane extracts by the method JIS K0101-1991. For pH 4–7 and 8–10, the initial pH of the medium was adjusted with 1 N HCl or 10% Na₂CO₃, respectively.

Salad oil degradation by strain SOD-1 depended on the salad oil content. Strain SOD-1 had a high degradation rate at the content of less than or equal to 3000 ppm of salad oil. Strain SOD-1 degraded 85.0% of an initial 2000 ppm salad oil suspension in 24 h at 28°C and pH 7.0. A report has shown that Acinetobacter sp. strain SK0402A degraded 83.5% of an initial 2000 ppm salad oil suspension in 24 h at 22°C. Strain SOD-1 degraded 49.1% of an initial 5000 ppm salad oil suspension in 24 h at 28°C, and Okuda et al. reported that Bacillus sp. strain 351 degraded about 90% of an initial 5000 ppm used a salad oil suspension in 24 h at 30°C. We thus believe that the salad oil degradation ability of strain SOD-1 will be comparable or superior to that of these other microorganisms.

As shown in Fig. 1A, strain SOD-1 efficiently degraded salad oil over a wide temperature range (20–40°C). The optimum temperature was found to be around 35°C. Interestingly, strain SOD-1 had high degradation activity even at 20°C. Strain SOD-1 degraded 68.7 ± 2.7% of an initial 3000 ppm salad oil suspension in 24 h at 20°C and pH 7.0. In contrast to this, the degradation rate decreased significantly below 20°C.

Since the temperature of wastewater seems to be mostly low (about 10–30°C), further investigations were done at 20°C. The optimum pH was around 8.0 (Fig. 1B). Figure 1B shows that strain SOD-1 will be applicable for the wastewater treatment between pH 7 to 9. The degradation rate was very sensitive to initial cell density (Fig. 1C). The experiment showed that the effective degradation requires an initial cell density of above 1 × 10⁶ CFU/ml. The degradation rate was strongly affected by phosphate concentration (Fig. 1D and E). As shown in Fig. 1D, the optimum (NH₄)₂HPO₄ concentration was around 10.6 mM. At concentrations below 6.06 mM, the degradation rate decreased remarkably, and the medium pH declined (data not shown). In addition, strain SOD-1 degraded 37.1% of an initial 3000 ppm salad oil suspension in 24 h at 20°C and pH 7.0 in the medium containing 2 g/l (NH₄)₂SO₄ instead of (NH₄)₂HPO₄.

The medium pH fell markedly in K₂HPO₄- and Na₂HPO₄-free basal medium (PFB medium), in which a concentration of (NH₄)₂HPO₄ is 15.1 mM (Fig. 2). The pH drop must result from production of fatty acid with lipid degradation by strain SOD-1 and from decline of buffering capacity of the PFB medium. Strain SOD-1 had significant growth at 20°C and pH 7.0 on the basal medium. The doubling time was 1.04 h. The degradation rate was 68.7 ± 2.7% in 24 h.

On the other hand, the growth on the PFB medium was slow and poor with a doubling time of 1.62 h. The degradation rate was 27.0% in 24 h (Fig. 1E). These results proved that the salad oil degradation by strain SOD-1 is strongly related to the medium pH and the cell growth. The buffering capacity seemed to be sensitive to the cell growth and lipid degradation by strain SOD-1. From these results, we concluded that pH control will be required for effective salad oil degradation by strain SOD-1. Since the hydraulic retention time of wastewater is in general short, the good growth of strain SOD-1 is likely effective for the wastewater treatment.

Under the optimum conditions (35°C and pH 8.0), strain SOD-1 degraded 83.0% of an initial 3000 ppm salad oil suspension in 24 h. At pH 7.0, strain SOD-1 degraded 20.7 and 42.0 ± 4.24% of an initial 3000 ppm lard suspension in 24 h at 20 and 28°C, respectively. This result suggests that the high melting point of lard (28–48°C) and its fatty acid composition is likely responsible for insusceptibility to enzymatic and microbial attack in the lipid degradation by strain SOD-1.

Further work is in progress to construct microbial libraries for effective treatment of lipid-contaminated wastewater.
Acknowledgments

We are indebted to Professor F. Hasumi for many helpful discussions. The authors acknowledge helpful discussions with Mr. N. Tsubouchi, Dr. T. Hozumi, and Mr. T. Tsubouchi of GATE Co. Ltd. This work was supported, in part, by the Hokuto Foundation for Bioscience.

References