Degradation of Water-Soluble Fraction of Jellyfish by a Marine Bacterium, *Brevibacterium* sp. JCM 6894

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We used *Brevibacterium* sp. JCM 6894, which was isolated from seawater, to degrade the water-soluble fraction of jellyfish. After 27 h of incubation, the protein content in the supernatant was reduced from 1.97 to 1.31 mg/ml with an increase in cell yield of 0.40 mg cell protein/ml. The missing protein content in the fraction was 0.26 mg/ml. By the cell growth, the content of NH₃ was increased from 0.38 to 4.57 µmol/ml. The value of chemical oxygen demand, which is a marker of remaining organic compounds in the fraction, was reduced from 890 to 431 mg O₂/liter.

Key words: microbial degradation, Jellyfish, marine *Brevibacterium* sp. JCM 6894

Generally, organic wastes containing water are burned with fossil fuel in a furnace. During this process, greenhouse gases, mainly CO₂, which trap the heat radiating from the earth's surface, are released into the atmosphere. To preserve the natural environment and to conserve natural resources, it is important to use environmental microorganisms to degrade organic wastes^{2,4,5}.

Actually, some marine life forms such as blue mussels and jellyfish are degraded efficiently by microorganisms. In summer at atomic power plants for generating electricity, shells grow fast on the inside of the pipes used for pumping in cooling seawater, and a lot of jellyfish become trapped on the wire-mesh filter in the pipes. To prevent operational problems, these organisms are removed at intervals. An excess of organic waste results in spoilage on the ground. Consequently, deodorization is also a factor to be taken into consideration⁸.

Using a marine microorganism to degrade organic wastes taken from seawater seems to be an ecologically friendly approach. Therefore, in this study, we examined the extent to which a marine *Brevibacterium* sp. JCM 6894^{6,7} is able to degrade the water-soluble fraction of jellyfish. To assess

this ability, we used *Escherichia coli* ATCC 9637 as a reference strain because the type strain grew in seawater¹, and *E. coli* itself survived in the marine environment³.

Jellyfish were collected from a coastal area near Himeji, Hyogo prefecture, Japan, in early July 1999. The tissues of the jellyfish were immediately destroyed with a juice mixer (MJ-700, Matsuden), centrifuged (3,000×g, 5 min) and filtrated (pore size, 5.0 and 0.45 µm) with a membrane filter (47 mmφ, Advantec). The mixture of water-soluble and insoluble fractions obtained just after the destruction of jellyfish had a dry weight of 32.2 mg per ml. By filtration with a pore size of 5 µm, the dry weight was reduced to 32.0 mg per ml, where the turbidity was 0.07 at 650 nm (DU 640, Beckman Instruments, CA, USA). To minimize contamination by microorganisms as well as to obtain a pure water-soluble fraction, we re-filtrated the solution with a pore size of 0.45 µm. After the filtration, the turbidity and dry weight were less than 0.01 at 650 nm and 31.3 mg per ml, respectively. The solution thus obtained contained 350 mM Na⁺ and 10 mM K⁺ ions (Compact ion meters, C-122 for Na⁺ ions, C-131 for K⁺ ions, Horiba), and was kept in a refrigerator until use.

Brevibacterium sp. JCM 6894, which was isolated from seawater at Aburatsubo, Kanagawa, Japan, and *E. coli* ATCC 9637 were pre-incubated for one day at 30°C in the

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complex medium⁷⁾. Then 0.5 ml of cell culture was added to an Erlenmeyer flask containing 50 ml of the water-soluble fraction of jellyfish. At a given time, turbidity was monitored at 650 nm with the spectrophotometer. After 27 h of incubation at 30°C, cells were harvested by centrifugation (10,000×g, 5 min).

After 27 h, the protein contents of the cell-free supernatant and pellet were measured with a protein assay kit (Pierce Chemicals, IL, USA). The concentrations of free amino acids and NH₃ in the cell free supernatant were measured with a high performance liquid chromatography system (Waters, MA, USA). The value of chemical oxygen demand (COD) was measured three times independently by a standard procedure as described elsewhere. The measurement of NO₂⁻ was carried out with a kit (Ion selective pack test, WAK-NO₂, Kyoritsu).

Fig. 1 shows the growth curves of *Brevibacterium* sp. JCM 6894 and *E. coli* ATCC 9637 in the water-soluble fraction of jellyfish. After about 20 h of incubation, the growth reached a stationary phase. Turbidities at 27 h of incubation were about 1.5 and 1.0 for *Brevibacterium* sp. JCM 6894 and *E. coli* ATCC 9637, respectively. As a negative control, we carried out the incubation with the water-soluble frac-

Fig. 1. Growth curves of *Brevibacterium* sp. JCM 6894 and *Escherichia coli* ATCC 9637 in water-soluble fraction of jellyfish. *Brevibacterium* sp. JCM 6894 (○) and *E. coli* ATCC 9637 (●) were grown aerobically in the solution at 30°C. As a negative control, the solution alone was incubated without being inoculated (△). At given times, turbidities were measured photometrically at 650 nm.

tion alone. It was confirmed that there was no increase in turbidity.

We used plates containing nutrients⁷⁾, 0.5 M NaCl, and 1.5% agar to detect contamination in the solution without inoculation before and after 27 h of incubation. We also tried to detect microorganisms other than *Brevibacterium* sp. JCM 6894 in the solution where the strain had been incubated for 27 h. However, no other colonies of microorganisms were detected after 3 days of incubation at 30°C.

Bacillus sp. HR6, which was isolated from soil and could degrade organic waste efficiently, especially bean curd refuse^{4,5)}, and a halophilic isolate, which was isolated from the jellyfish solution, were used to compare the growth with that of *Brevibacterium* sp. JCM 6894 and *E. coli* ATCC 9637. The growth for the two strains, however, did not overcome that for *E. coli* ATCC 9637 even after 27 h of incubation (data not shown).

After microbial degradation, protein contents for both the pellet and the supernatant were measured (Table 1). The initial protein content of 1.97 (mg/ml) was reduced to 1.31 (mg/ml) with an increase in cell protein of 0.40 (mg/ml) for *Brevibacterium* sp. JCM 6894. For *E. coli* ATCC 9637, the protein content in the supernatant was reduced by 0.29 (mg/ml) and the cell protein increased by 0.26 (mg/ml). Protein contents of 0.26 and 0.03 (mg/ml) in the supernatant were missing for *Brevibacterium* sp. JCM 6894 and *E. coli* ATCC 9637, respectively.

Table 2 shows the changes in the pool of free amino acids and NH₃ in the supernatant of the water-soluble fraction of jellyfish after 27 h of incubation. The remaining amino acid pool of 2.91 μmol/ml for *Brevibacterium* sp. JCM 6894 was about two times higher than that for *E. coli* ATCC 9637. On the other hand, the NH₃ content of 4.57 μmol/ml produced by *Brevibacterium* sp. JCM 6894 was about three times that produced by *E. coli* ATCC 9637. The content of NH₃ increased to be more than ten times higher than the initial value, 0.38 μmol/ml.

Brevibacterium sp. JCM 6894 showed higher growth activity in the water-soluble fraction of jellyfish than *E. coli* ATCC 9637 (Fig. 1). By measuring the content of cell protein after 27 h of incubation (Table 1), we could confirm the superior growth ability of *Brevibacterium* sp. JCM 6894 in comparison with that of *E. coli* ATCC 9637.

When proteins, or amino acids, are degraded completely, the final products are NH₃, CO₂, and H₂O. After the growth of the *Brevibacterium* sp. JCM 6894 cells for 27 h, the total content of proteins and free amino acids in the supernatant was reduced to 66 and 37%, respectively. The amount of free amino acids, however, remained large in the superna-

Table 1. Change in protein content in water-soluble fraction of jellyfish after cell growth.

Strain		Change in protein content (mg/ml)		Missing protein content (mg/ml) ^a
		before	after 27 h	
<i>Brevibacterium</i> sp. JCM 6894	pellet	0.00	0.40	0.26
	supernatant	1.97	1.31	
<i>Escherichia coli</i> ATCC 9637	pellet	0.00	0.26	0.03
	supernatant	1.97	1.68	

^a Missing protein content (mg/ml) was defined as the difference of total protein content before and after cell growth.

Table 2. Changes in free amino acid pool and NH₃ in water-soluble fraction of jellyfish after cell growth.

$\mu\text{mol/ml}$	<i>Brevibacterium</i> sp. JCM 6894		<i>Escherichia coli</i> ATCC 9637
	before	after 27 h	after 27 h
Amino acids	7.91	2.91	1.54
NH ₃	0.38	4.57	1.69

tant in comparison with that for *E. coli* ATCC 9637. Extracellular proteases might contribute to an increase in free amino acids in the supernatant. We have confirmed that strain JCM 6894 secreted proteases when grown on plates containing nutrients⁷⁾, 0.5 M NaCl, 3% skim milk, and 1.5% agar. As for *E. coli* ATCC 9637, the missing protein content as well as the production of NH₃ were much smaller than for *Brevibacterium* sp. JCM 6894. These results indicate that *E. coli* ATCC 9637 is not suitable for degrading jellyfish.

The supernatant was also used to measure the value of chemical oxygen demand (COD) and the concentration of NO₂⁻. Before and after incubation of *Brevibacterium* sp. JCM 6894 in the water-soluble fraction, the COD was 890±30 and 431±24 (AV±SE, mg O₂/liter) (n=3), respectively. The quantified NO₂⁻ was 0.5 (mg/liter) initially, a value which did not change even after 27 h of incubation, indicating that the remaining organic compounds in the cell-free supernatant contribute to an increase in the COD.

We believe that the microbial degradation of organic wastes under aerobic conditions depends strongly on the growth rate of the microorganism used⁵⁾. A fast growth rate allows the microorganism to become dominant in the targeted organic waste. As *Brevibacterium* sp. JCM 6894 is halotolerant, we could add NaCl at up to 2 M^{6,7)} in the solution of jellyfish to prevent the growth of other microorganisms.

Since the COD value was still too high for the degraded solution to be released into the marine environment, an

effort to reduce the remaining organic compounds should be made. The pathogenicity of *Brevibacterium* sp. JCM 6894 is not yet known, however the risk of disease in marine organisms does not seem to be high even if the jellyfish solution degraded by the strain JCM 6894 is released into the sea.

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