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# Aerobic cellulolytic bacterial flora associated with decomposing *Phragmites* leaf litter in a seawater lake

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*Key words:* Cellulolytic bacteria, bacterial flora, cellulose decomposition, *Phragmites* litter, marine environment

## Abstract

A litter bag experiment was carried out in a eutrophic seawater lake from autumn to summer in order to determine which bacterial genera play an important role in decomposition of *Phragmites communis* leaf litter. The count of cellulolytic bacteria and decomposition rate of litter cellulose increased rapidly during the initial month. In contrast, the count of cellulolytic fungi was lowest in this period. *Pseudomonas* accounted for 65–90% of total isolates of cellulolytic bacteria up to 5 months. These results suggest that *Pseudomonas* plays an important role in at least the initial decomposition stage of the litter.

## Introduction

The importance of microorganisms as agents of the breakdown of plant litter in aquatic environments has long been recognized (Kaushik & Hynes, 1971; Fenchel & Jørgensen, 1977; Federle & Vestal, 1980 and 1982). Some reports concerning the relative importance of bacteria and fungi on the decomposition of the litter (Kaushik & Hynes, 1971; Mason, 1976; Benner *et al.*, 1984; Benner *et al.*, 1986; Tanaka, 1991) have suggested that the bacterial population plays an important role at least during certain stages of decomposition.

Among the various functional groups of bacteria, cellulolytic bacteria play an important role in the decomposition of emergent macrophyte litter, because cellulose is a major component of such litter. Few investigations, however, have been carried out on the flora of cellulolytic bacteria associated with decomposing litter.

This paper mainly focuses on the flora of cellulolytic bacteria associated with leaves of *Phragmites communis* submerged in eutrophic seawater by litter bag. In addition to the flora, dynamics of the cellulolytic bacteria and fungi and the decomposition rate of the litter cellulose were also examined in the present study to evaluate the contribution of bacteria to hydrolysis of the cellulose.

Lee (1990) pointed out that the cosmopolitan distribution, high resilience and extreme productivity of *Phragmites* spp. warrant more studies on its role in wetland ecosystems, and found that *P. communis* functions as an important detritus source in a marsh ecosystem. This suggests the importance for clarifying the microbial decomposition process of *Phragmites* litter in aquatic environments.

## Materials and methods

### *Litter type and incubation*

Dead, brown leaves of *P. communis* were collected from shoots standing in the margin of Lake Shinhama in October 1980. Seven hard plastic baskets (30 x 20 x 20 cm, 2 mm mesh), each with 100 air-dried and weighed leaves, were submerged 30 cm below the water surface near the shore (water depth of about 2 m) of eutrophic seawater lake, Lake Shinhama (35° 40′ N, 139° 56′ E), on 31 October 1980. Since the lake is connected to Tokyo Bay by two channels, its salinity of 13–17‰ (Furota, 1980) is almost equal to that of Tokyo Bay. Its shoreline and shallows are dominated by *P. communis*. Sessile organisms on the outer surface of the bags were scrubbed off several times a month to allow circulation of the water in the bags.

One bag was collected on each sampling date. The leaves in the bag were transferred to a polyethylene bag containing sterile seawater and transported to the laboratory. The leaves were rinsed gently with sterile seawater to remove silt and then subjected to the following analyses.

### *Chemical analyses*

Seventy leaves were air-dried, ground into a powder with a motor mill and used for determination of their ash-free dry weight (AFDW) and cellulose. Ashing was done in a muffle furnace at 450 °C for 1 h. Cellulose content was determined by the method of Allen *et al.* (1974).

### *Bacteriological analyses*

Thirty wet leaves were ground with a motor mill equipped with a sterilized blender jar (150 ml), and further homogenized with a sterilized Potter-Elvehjem- type glass homogenizer after addition of sterilized seawater (final volume of 400 ml). Cellulolytic bacteria were counted using the pour plate method of Kadota (1956), but with Avicel SF cellulose powder (Asahi Chemical Industry Co. Ltd.) instead of reprecipitated cellulose. The method is as follows: about 1 ml of the appropriately diluted inoculant and 8 ml of sterilized seawater had been placed in the plate, then 9 ml of the melted cellulose agar medium ($NaNO_3$, 0.5 g; $K_2HPO_4$, 1.0 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $FeSO_4 \cdot 7H_2O$, 0.01 g; Avicel SF powder, 10 g; Agar, 15 g; 1000 ml of pH 7.2 filtered seawater) was added to the plate and mixed thoroughly with the inoculant. The plate was incubated at 25 °C for 30 days in darkness.

Cellulolytic bacteria were picked up from each plate at a countable dilution. A total of 40 to 90 isolates was picked up from each sample. The colonies were purified by streaking on Avicel agar plates three or more times. The purity of the culture was checked by streaking on ZoBell 2216E agar plates (Oppenheimer & ZoBell, 1952) and by microscopy (magnification, × 1500).

The purified isolates were subjected to the following morphological and biochemical tests after growing on ZoBell 2216E agar plates at 20 °C. Motility and cell morphology were examined by phase-contrast microscopy on samples from 24- and 48-h cultures. Gram staining was done according to Hucker's modification (Hucker & Conn, 1923). Flagella were stained by the methods of Nishizawa-Sugawara and Toda (Institute of Medical Science, University of Tokyo, 1958) after 72 h incubation. The oxidase reaction was tested using Kovács' method (Kovács, 1956). Catalase activity was tested by the method described by Skerman (1967). The oxidation-fermentation (O-F) test for glucose was carried out by the method of Hendrie & Shewan (1979).

The hydrolyzing abilities of some polysaccharides and proteins were tested as follows. Filter paper hydrolytic ability was tested by inoculating the isolates into tubes of mineral medium ($NaNO_3$, 0.25 g; $K_2HPO_4$, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.25 g; $FeSO_4 \cdot 7H_2O$, 0.005 g in 1000 ml of filtered pH 7 seawater) containing a strip of filter paper, two-thirds immersed. Hydrolysis of the filter paper was checked by its collapse after gentle shaking. Xylanolytic ability was examined with a xylan agar medium ($NaNO_3$, 0.25 g; $K_2HPO_4$,

0.5 g; $MgSO_4 \cdot 7H_2O$, 0.25 g; $FeSO_4 \cdot 7H_2O$, 0.005 g; xylan purchased from Tokyo Chemical Industry Co., Ltd., 4 g; 1000 ml of filtered pH 7 seawater). Hydrolysis of xylan was checked by formation of a clear zone around the colonies. Tests for other hydrolases were carried out by streaking isolates on ZoBell 2216E agar plates containing an appropriate concentration of substrate: 0.8% for soluble starch, 1% for gelatin, 2.5% for skim milk, 1% for Tween 80. The inoculated plates were incubated at 20 °C for 7 days and examined for hydrolysis of the substrates using the methods described by Hendrie & Shewan (1979).

In order to test for nitrate-reducing ability, the isolates were inoculated into a nitrate broth (ZoBell 2216E medium containing 0.1% $KNO_3$). Production of nitrite was examined after 14 days incubation at 20 °C. When no nitrite was detected, remaining nitrate was further tested.

The generic identification was carried out according to Skinner & Lovelock (1979). Each genus was divided into groups mainly according to their hydrolase and nitrate-reducing ability.

*Determination of fungal biomass*

The total length of fungal hypha on the surface of the leaves was determined as follows. Ten leaf discs (1 cm diameter) were punched out with a cork borer. They were then stained with lactophenol cotton blue and safranin solutions successively, and observed by bright-field microscopy ($\times 400$). The number of the intersections between hypha and graticule lines on an ocular micrometer ($250 \times 250$ $\mu$m, 100 mesh) was counted for 20 microscope fields for each side of the leaf disc. The total length of fungal hypha was estimated from this count by the method 5 of Olson (1950).

In order to count the cellulolytic fungi, homogenized litter suspension was at first inoculated on the glucose-yeast extract agar medium (glucose 19 g $l^{-1}$, yeast extract 1 g $l^{-1}$) described by Tubaki (1974). Streptomycin sulfate and Na-penicillin G were added to this medium to inhibit bacterial growth at the concentrations described by Aaronson (1970) (both 500 mg $l^{-1}$). All fungal colonies on the agar medium were picked up after 7 days incubation (20 °C), and then purified by isolating single hypha. The cellulolytic ability of the purified isolates was examined by inoculating them on agar plates of the Avicel cellulose medium and observing the formation of a clear zone around the colony after one month incubation at 20 °C. The number of cellulolytic fungi per 1 gram AFDW was determined from this result.

*Gel chromatography of cellulases from purified bacterial isolates*

Gel chromatography of cellulases were carried out for two typical isolates belonging most dominant group and high *in vitro* cellulolytic activity possessing group, respectively. The *in vitro* activity was examined qualitatively by observing expansion speed of clear zone around colonies.

The choosed isolates were inoculated into 500 ml sterile medium ($NaNO_3$, 0.5 g; $K_2HPO_4$, 0.1 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $FeSO_4 \cdot 7H_2O$,

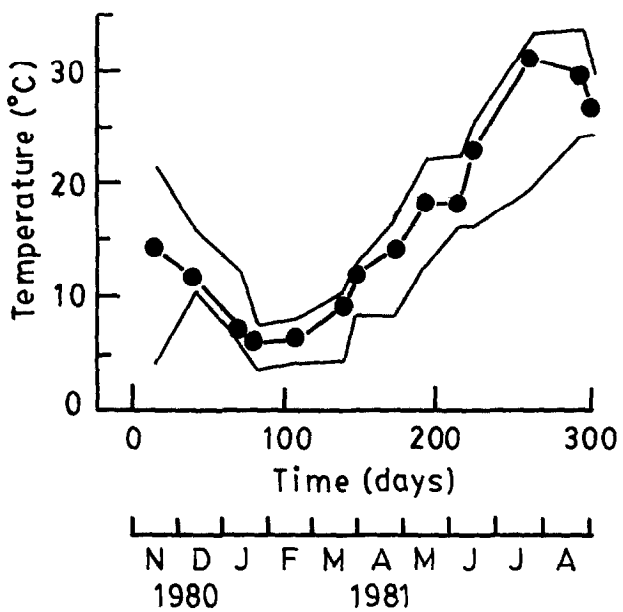


*Fig. 1.* Change in water temperature during the litter bag experiment. (Thin two lines represent maximum and minimum temperatures during recovering periods.)

0.01 g; cellulosic substance, 5 g; filtered and aged seawater, 1000 ml, pH 7.2), and incubated at 25 °C with stirring using a magnetic stirrer. When the cellulase activity reached maximum, the whole culture liquid was centrifuged at 5000 rpm for 5 minutes at 5 °C. The supernatant was then concentrated with an ultrafiltration membrane (Amicon YM10), and applied to a Bio-Gel P-200 column (1.7 x 40 cm) using filtered seawater (pH 8) as the elution medium. Successive 1.5 ml fractions were collected and mixed with 1.5 ml of a 1% seawater solution of carboxymethyl cellulose. After 3 hours incubation at 37 °C, the reducing sugars liberated were determined by the colorimetric method of Nelson (1944).

## Results

As shown in Fig. 1, the water temperature declined from 15 °C in early November to a minimum of 5 °C in February (day 100) and then increased gradually to a maximum of 30 °C in July (day 300). The percentage by weight of cellulose remaining in the litter dropped to about 50% on day 190 and to 5% at the last stage of the experimental period (Fig. 2A). Decomposition rate, expressed as 'specific rate' (Tanaka, 1991), of cellulose rose steeply during the initial 30 days, then dropped until about day 100, and gradually increased again afterwards (Fig. 2B).

The count of cellulolytic bacteria per gram of ash-free dry weight (AFDW) increased rapidly immediately after submergence, reached a maximum of $9 \times 10^8$ colony-forming-units (CFU) $\times$ $(\text{g AFDW})^{-1}$ on day 39, and then decreased gradually (Fig. 3).

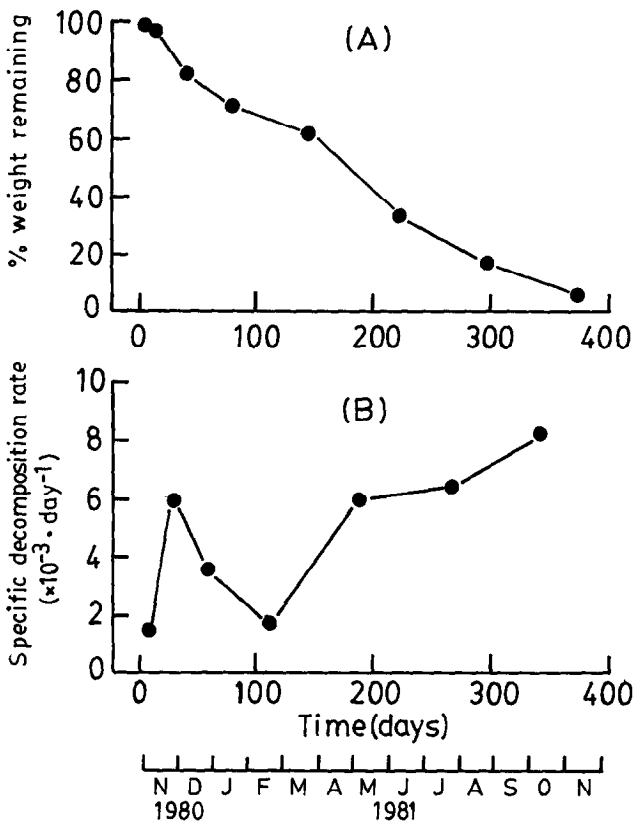


*Fig. 2.* Changes in amount of cellulose in percentage of starting quantities (A) and specific decomposition rate of cellulose (B) during the decomposition of *Phragmites* leaf litter.

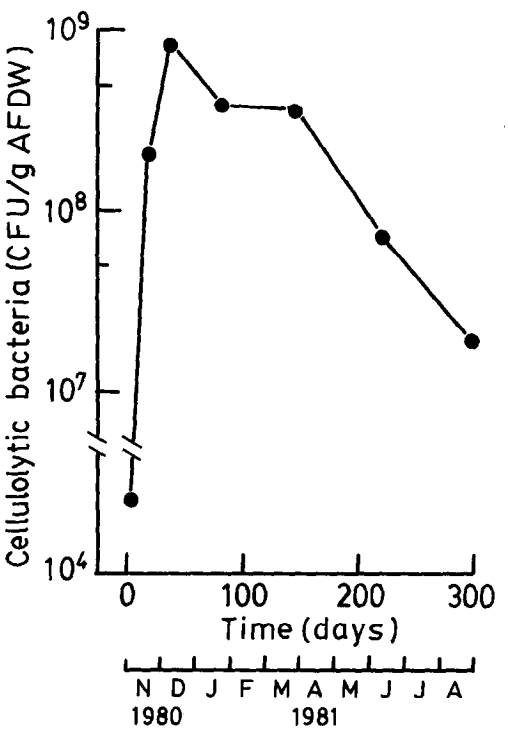


*Fig. 3.* Change in count of cellulolytic bacteria associated with *Phragmites* leaf litter. CFU, colony forming unit; AFDW, ash-free dry weight.

*Table 1.* Morphological and physiological characteristics of cellulolytic bacteria isolated from *Phragmites* leaf litter submerged in seawater.

| Character Group | 1 | 2 μm | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Pseudomonas* A | S | 1.5 | – | + | P | – | – | + | + | O | + | + | + | – | – | + | – | Y | Cr | Cr | Cr, CL | Y | CL |
| *Pseudomonas* B | S | 1.5 | – | + | P | – | – | + | + | O | + | + | + | – | – | – | – | Y | Cr | Cr | CL | Y | CL |
| *Pseudomonas* C | S | 1.3 | – | + | P | – | – | + | + | O | + | + | + | – | – | – | + | Y | Cr | Cr | CL | Y | CL |
| *Pseudomonas* D | S | 1.4 | – | + | P | – | – | + | + | O | + | + | + | – | – | + | + | Y | Cr | Cr | CL | Y | CL |
| *pseudomonas* E | C | 3 | – | + | P | – | – | + | + | O | + | + | + | – | + | + | + | Y | Cr | Cr | CL | Cr | Cr |
| *Vibrio* A | C | 3.8 | ? | + | ? | ? | ? | + | ? | F | + | + | + | + | + | + | + | Y | CL | Cr | Cr | Cr | Cr |
| *Vibrio* B | C | 4.5 | – | + | P | – | – | + weak | + weak | F | + | + | – | + | – | + | + | Or | Y | Cr, CL | CL | CL | CL |
| *Vibrio* C | C | 3.5 | ? | + | ? | ? | ? | + | ? | F | + | + | – | – | – | – | – | Or | Or | NG | NG | NG | CL |
| *Vibrio* D | C | 4.2 | ? | + | ? | ? | ? | + | ? | F | + | – | + | + | + | + | + | Y | CL | Cr | Cr | Cr | Cr |
| *Vibrio* E | C | 4.8 | – | + | P | ? | ? | + | + | F | + | – | + | + | + | + | – | CL | CL | Cr | Cr | Cr | Cr |
| *Vibrio* F | C | 3.8 | ? | + | ? | – | – | ? | ? | F | + | – | + | + | + | – | – | CL | CL | Cr | CL | Cr | Cr |
| *Vibrio* G | C | 3.8 | ? | + | ? | ? | ? | + | ? | F | + | – | – | – | – | – | + | Y | CL | NG | NG | NG | NG |
| *Vibrio* H | C | 4 | – | + | ? | – | – | + | + weak | F | – | – | + | + | + | + | – | CL | CL | Cr | Cr | Cr | Cr |
| *Vibrio* I | C | 3.5 | – | + | P | – | – | + | + weak | F | – | – | + | + | – | – | – | CL | CL | Cr | CL | Y | Cr |
| *Fla.-Cyt.-Fle.* A | C | 3.5 | – | – | – | – | – | + weak | + weak | F | + | + | + | – | – | + | + | Or | Cr | CL | CL | NG | CL |
| *Fla.-Cyt.-Fle.* B | C | 5.5 | – | – | ? | – | – | + weak | + weak | F | + | + | – | – | – | + | + | Or | Y | CL | NG | NG | CL |
| *Fla.-Cyt.-Fle.* C | C | 4 | – | + | – | ? | ? | + | – | F | + | + | – | – | – | – | + | Or | Or | NG | NG | NG | CL |
| *Fla.-Cyt.-Fle.* D | C | 5 | ? | – | ? | ? | ? | + | ? | N | – | + | + | – | – | + | + | NG | Or | Or | Or | Or | CL |
| *Pse.-Fla.* A | C | 5.5 | ? | + | ? | ? | ? | + | ? | N | – | + | – | – | – | – | + | NG | CL | NG | NG | NG | NG |
| *Pse.-Fla.* B | S | 1.8 | – | + | ? | ? | ? | + | + | N | – | – | + | – | – | – | + | NG | CL | Cr | Cr | Cr | Cr |
| *Pse.-Fla.* C | C | 3.2 | – | + | ? | – | – | + | + | O | + | + | + | – | – | + | + | Y | Cr | Cr | CL | Cr, Y | Cr |
| *Pse.-Fla.* D | S | 1.3 | ? | + | ? | ? | ? | + | ? | O | + | + | – | – | – | – | – | Y | Cr | NG | NG | NG | NG |
| *Pse.-Fla.* E | S | 1.4 | – | + | ? | – | – | + | + | O | – | + | + | – | – | + | – | CL | Cr | Cr | CL | Y | CL |

Groups – *Fla.-Cyt.-Fle.*: *Flavobacterium -Cytophaga-Flexibacter* group, *Pse.-Fla.*: *Pseudomonas-Flavobacterium* group.

Character – 1: morphology; 2: cell length; 3: Gram stain; 4: motility; 5: flagella; 6: fluorescence; 7: luminescence; 8: oxidase; 9: catalase; 10: O–F test; 11: filter paper hydrolysis; 12: xylan hydrolysis; 13: starch hydrolysis; 14: gelatin hydrolysis; 15: casein hydrolysis; 16: Tween 80 hydrolysis; 17: nitrate reduction; 18: pigment on filter paper medium; 19: pigment on xylan medium; 20: pigment on starch medium; 21: pigment on gelatin medium; 22: pigment on casein medium; 23: pigment on Tween 80 medium.

Symbols – +: positve; –: negative; S: straight rod; C: curved rod; P: polar; O: oxidation; F: fermentation; N: no reaction; Y: yellow; Cr: cream; Or: orange; CL: colorless; NG: no growth; ?: unknown.

All isolates of the cellulolytic bacteria were Gram-negative, oxidase-positive rods. Most motile, short- or medium-sized, O-F test oxidative rods were identified as *Pseudomonas*. O-F test fermentative, motile, and medium or long rods were identified as *Vibrio*. Non-motile or gliding long curved rods were classified to the *Flavobacterium-Cytophaga-Flexibacter* group. Other isolates, which were motile, oxidase-positive, O-F test oxidative or non-acid-forming, and impossible to identify the flagellation by staining, were classified to the *Pseudomonas-Flavobacterium* group for convenience. These genera and groups were further subdivided into 23 groups, whose characteristics are summarized in Table 1.

Figure 4 presents the percentage composition of the groups. *Pseudomonas* A group predominated up to day 145 (50 to 65%). *Pseudomonas* B group was second in dominance. The percentage of these two groups amounted to 65–90% up to day 145, but diminished markedly thereafter. Many groups with a low percentage appeared in place of *Pseudomonas* A and B on day 223. On day 299, most cultures picked up were lost after purification or impossible to purify, probably due to their weak cellulolytic activity and slow growth rate.

Figure 5 shows the ratio of isolates able to hydrolyze a specific substrate to the total isolates. Most strains possessed hydrolyzing ability for xylan, starch and Tween 80. In contrast, less than 20% of the strains possessed hydrolyzing ability for casein and gelatin. It seems that the cellulolytic bacteria isolated from the litter utilize carbohydrates in preference to proteinaceous substrates.

Changes in hyphal length and cellulolytic fungal count are shown in Fig. 6. Though a fairly high value of hyphal length was detected immediately after submergence, this decreased steeply during the initial few days, and gradually increased again after about 76 days. The count of cellulolytic fungi was less than $10^3$ CFU × (g AFDW)$^{-1}$ until about day 76, but increased steadily afterwards in accordance with the increase in specific decomposition rate of cellulose.

The gel chromatography of cellulases was carried out for the isolates belonging to the *Pseudomonas* A group and *Vibrio* B group. The former was incubated for 29 days using Avicel SF powder as substrate. On the other hand, the latter was incubated for 6 days using half immersed

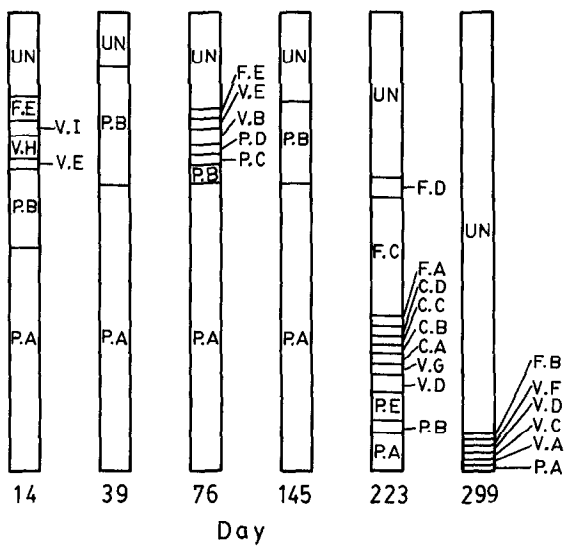


*Fig. 4.* Percentage composition of the taxonomic groups of cellulolytic bacteria isolated from *Phragmites* leaf litter. P., *Pseudomonas*; V., *Vibrio*; F., *Pseudomonas-Flavobacterium* group; C., *Flavobacterium-Cytophaga-Flexibacter* group; UN, lossed or unpurified.

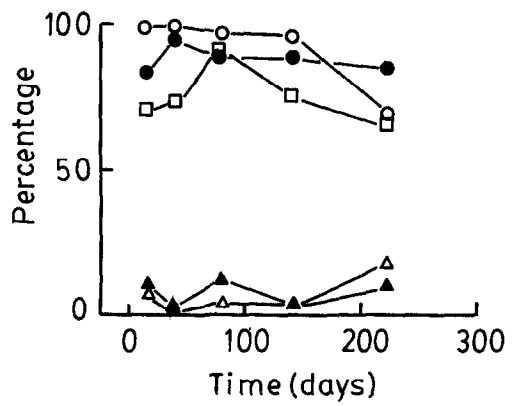


*Fig. 5.* Percentages of xylan, starch, gelatin, casein and Tween 80 hydrolyzing strains of cellulolytic bacteria in total isolates. ● xylan, ○ starch, ▲ gelatin, △ casein, □ Tween 80.

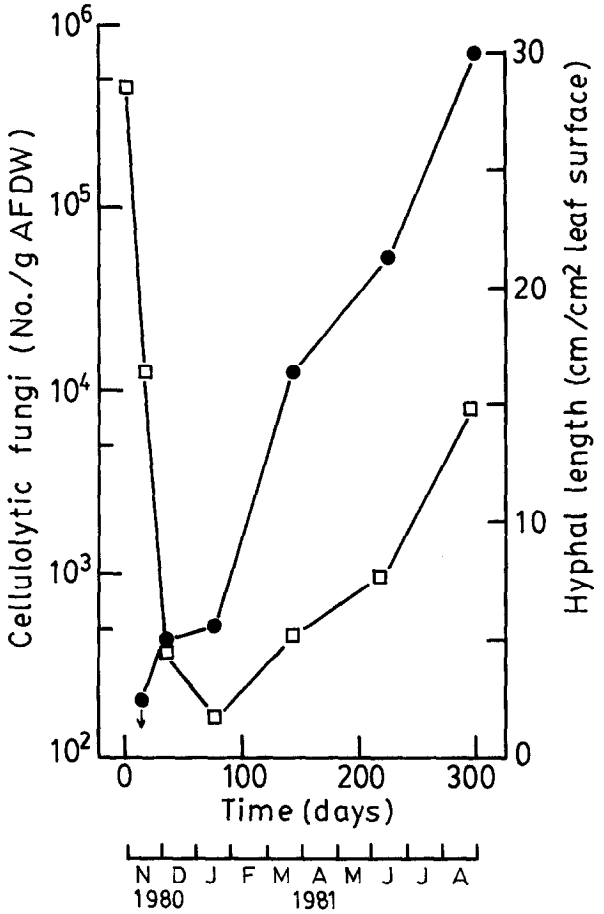


*Fig. 6.* Changes in hyphal length of fungi and count of cellulolytic fungi associated with *Phragmites* leaf litter. □ hyphal length, ● cellulolytic fungi, ↓ indicate less than that value.

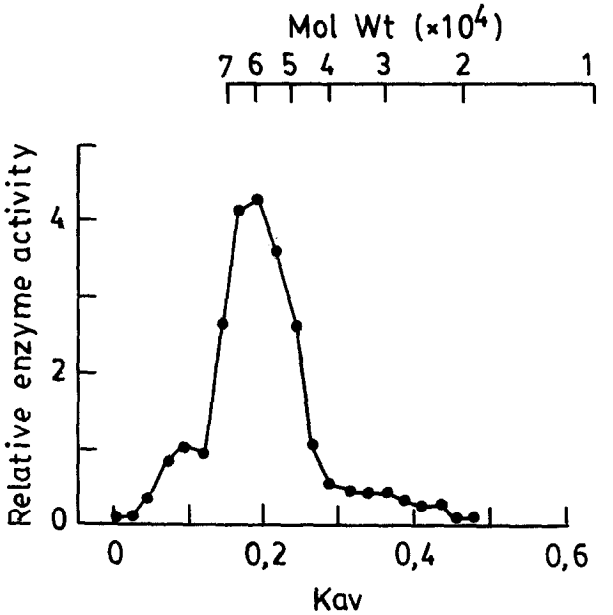


*Fig. 7.* Gel chromatography of cellulase derived from isolate of *Pseudomonas* A group. Mol Wt, molecular weight; Kav = (Elution vol. – Void vol.)/(Total bed vol. – Void vol.).

Whatman filter paper as substrate. As shown in Figs 7 and 8, the apparent molecular weight of the main peak were about 60000 (Kav = 0.2) for the isolate of *Pseudomonas* A group and more than 70000 (Kav = 0.1) for the isolate of *Vibrio* B group.

## Discussion

The count of cellulolytic bacteria and the decomposition rate of cellulose reached a maximum at about one month after submergence. In contrast, the count of cellulolytic fungi during the initial two months were one to three magnitudes lower than those of after day 145. These findings suggest the importance of the bacterial population on cellulolysis of the litter, at least during initial two months.

The previous paper (Tanaka, 1993), which concerned cellulases and xylanases associated with *Phragmites* litter in the same litter bag experiment, showed that a peak of cellulase activity occurred during the initial one month. This supports the suggestion above that cellulolytic bacteria actively hydrolyzed the litter cellulose during the initial stages.

The fact that *Pseudomonas* was the predominant genus of cellulolytic bacteria during the initial 5 months indicates its importance as a decomposer of the litter cellulose during at least the initial stages. Boulton & Boon (1991) pointed out that it is by no means certain that the bacteria isolated from decaying leaves are representative of those actually present. In the present study, however, it would be provable that *Pseudomonas* was actually one of the representative bacteria during the initial stages because of its high maximum percentage of about 23% to the viable count of total heterotrophic bacteria and of about 2%

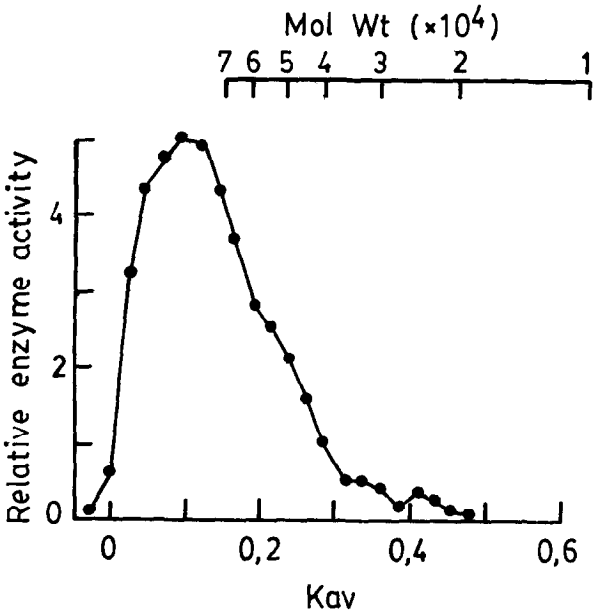


*Fig. 8.* Gel chromatography of cellulase derived from isolate of *Vibrio* B group. Mol Wt, molecular weight; Kav = (Elution vol. – Void vol.)/(Total bed vol. – Void vol.)

to the direct count of bacterial cells reported by the previous paper (Tanaka, 1991).

The apparent molecular weight of the cellulase from the isolate of *Pseudomonas* A group agreed with that of cellulases from decaying leaves reported by the previous paper (Tanaka, 1993). On the other hand, the apparent molecular weight of the cellulase from the isolate of *Vibrio* B group was differed from that of the cellulases from decaying leaves. The analyses by the gel chromatography were yet preliminary in the present study, and further extensive analyses will be required to obtain more decisive estimation on the relative importance of various bacterial groups. One thing, however, is certain that the present results of gel chromatography are compatible with above hypothesis supposing an exclusive contribution of *Pseudomonas* on cellulolysis during the initial stages.

Several investigators have also reported about the flora of aerobic cellulolytic bacteria in marine environments. Kadota (1956) isolated 3 genera, *Pseudomonas*, *Vibrio* and *Cytophaga*, from seawater and bottom deposits in temperate bays. According to Liston (1968), cellulolytic bacteria isolated from seawater and sediment have proved to be *Cytophaga* (most commonly) or aerobic *Pseudomonas*. These reports and the present study suggest that the population of aerobic cellulolytic bacteria in the marine environment generally includes *Pseudomonas*, *Vibrio* and *Cytophaga*.

There are also some reports on the flora of aerobic cellulolytic bacteria in freshwater environments. Ostertag (1950) stated that *Cytophaga* and *Sporocytophaga* species were responsible for almost the entire cellulose breakdown in the River Elbe. According to Suberkropp & Klug (1976), only two genera, *Cytophaga* and *Sporocytophaga*, had cellulolytic ability among the bacterial flora associated with decomposing leaves in a woodland stream. Güde (1978) investigated polysaccharide-degrading bacteria including a cellulolytic type in a eutrophic lake, and pointed out that the *Cytophaga-Flavobacterium* group was regularly found to consist of polysaccharide degraders. He further found by laboratory mixed culture experiments that cellulase was detected only in the presence of *Cytophaga*. These observations imply the importance of *Cytophaga* and *Sporocytophaga* as cellulose decomposers in freshwater environments.

The temperature change appears to be an important factor affecting the drastic change in the flora after day 145, because the temperature changed steeply in the latter half of the experimental period. Moreover, Kadota (1956) suggested from field studies and experiments that temperature is an important factor influencing the kinds and activities of cellulolytic bacteria in the sea. Besides the change in physical factors, biological factors such as the inhibitory effect of fungi, which increased markedly after day 145, might also affect the change in bacterial flora. Further precise studies by laboratory experiments will be necessary to assess the extent of the effect of these factors.

It is noteworthy that almost all the isolates of cellulolytic bacteria possessed xylanolytic ability (Fig. 5). The previous studies showed that the *Phragmites* leaf litter contained 20% of xylan (Tanaka, 1991), and that high xylanase activity

was detected throughout the experimental period (Tanaka, 1993). It is therefore suggested that the cellulolytic bacteria produced both cellulases and xylanases on the *Phragmites* leaf litter.

Bisaria & Ghose (1981) studied the role of xylanase action on the breakdown of bagase and pointed out that xylanase helped to create more accessible cellulosic regions, thereby resulting in higher sugar production. This implies that producing both cellulase and xylanase simultaneously would enhance efficient utilizing of structural carbohydrates in the litter by microorganisms.

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