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Studies on the microbial ecology of the Tansui Estuary

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Abstract. The Tansui River and the mangrove forests contains two types of microorgnaisms. The first are the native microorganisms that already exist in the mangrove forests. The second are microorganisms that come from pollution. The microflora in the Tansui Estuary and the mangrove forests is very complex due to the presence of the two types of microorganisms. The purpose of this study was to investigate the mangrove's microbial flora and the influence of ecological relationship on the population's health. We studied the river during the spring, summer, fall, and winter of 1990 and 1991. Samples of the river water and the mangrove soils were collected to isolate animal and human pathogenic organisms, fungus, and actinomycetes in order to understand the relationships of these organisms in the natural environment. In a series of studies, 215 strains of common bacteria were isolated, including *E. coli,* salmonella, shigella, pseudomonas, micrococcus, bacillus, staphyloccus, and streptococcus, *et al.* Forty-two strains and 25 genera of fungi were isolated, including the 8 genera *Aspergillus, Alternaria, Cephalosporium, Trichoderma, Geotrichium, Fusarium, Verticillium,* and *Penicillium.* We isolated 110 strains of Actinomycetes, including the 5 genera nocardia, rhodococcus, streptomyes, streptoverticillium, and micromonospora. Enzyme production tests indicated that most of the actinomycetes and fungi were able to produce useful enzymes such as pectinase, gelatinase, amlylase, protease, lipase, agarase, and cellulase. Antibiotic screening tests also showed that strains of streptomyces, bacilli, and micromonospora were capable of producing antifungal and/or antibacterial antibiotics which can inhibit the growth of other microorganisms. We would like to investigate these strains of microorganisms in more detail to determine if they can be used to benefit humanity. The above results indicate that the river and the mangroves have many types of microorganisms which can clean up the waste material in the river. This phenomenon helps the plants and animals living in this area obtain energy and resources. Recently, industry, hospital, and house sewage has increased rapidly, adding many pathogenic organisms which can adversely affect health. The relationship of microorganisms and mangroves is close and quite important to help maintain the natural ecosystems. We should control the pollution of the river, maintain its valuable ecosystem, and keep the river a valuable resource. From the Tansui River, several special microorganisms were isolated, especially actinomycetes, which can produce antibiotics and enzymes. We will investigate these strains of microorganisms in more depth to determine if they can be used for the benefit of mankind. If the results are quite significant, these organisms could be used to reduce pollution and clean up the Tansui River.

Key words: Microbial ecology; Mangrove forest; Microflora.

Introduction

The Tansui River is northern Taiwan's largest and formerly most important rvier. Besides providing this region with a sewer system, transportation and irrigation, it is also used to furnish abundant water resources. Before World War II, the river water not only was clean, but also yielded abundant food such as shrimp, fish and other types of seafood. The people living along the river were provided for by its resources. However, because of the rapidly expanding population

and increasing industrialization in around Taipei over the last few decades, the Tansui River has become very polluted and now is a lifeless body of water. The people can no longer drink the water or depend on the river for food and resources. If this problem is not solved soon, more people's health will be adversely affected. Thus the water of the Tansui River must be cleaned and life brought back to this important body of water.

The Tansui River and the surrounding mangrove area, contain both the native microorganisms already living in the river and mangrove forest and also microorganisms originating upstream due to people and pollution. Because of the existence of two types of microorganisms, the microflora in this area is very complex. Thus, the purpose of this study is pointed towards the identification of the mangrove microbial flora and determination of the ecological relationships among the microorganisms in this area. We determined the microbial flora in the river during the spring, summer, fall, and winter of 1990 and 1991. Samples of river water and mangrove soil were collected in order to isolate animal and human pathogenic organisms, and the useful organisms, including fungus and actinomycetes.

In this paper the microbial flora and the relationships among the microorganisms in the Tansui Estuary are described.

Materials and Methods

dred grams of each layer of soil was collected and was then cultivated for the isolation of microorganisms.

b. River water

A sterilized 250 ml Erlenmeyer flask was used to collect the water. The bottle was placed at a depth of 30 cm and the cap was opened to collect water. The cap was then replaced, and the water was taken back to the laboratory to cultivate and isolate the microorganisms.

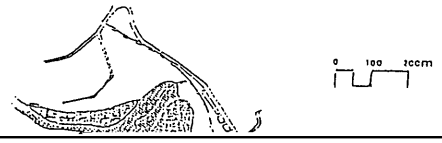
c. Plants

Collection of both green leaves and stems and old leaves and stems was conducted in the mangrove forest. All of the plants samples were collected and placed in sterilized paper envelopes.

Total Cell Count of Microorganisms

a. Spread plate method

According to the method established by Peltier *et al.* (1952), Pickett *et al.* (1973), Levine (1954), and Baker (1978), a phosphate buffer solution of pH 7 was used to dilute the samples 1x, 10x, 100x, and 1000x. A 0.1 ml



sample of each diluted solution was cultivated on nutrient agar, TYG agar (Tryptone yeast extract glucose agar), Endo agar, EMB agar (Eosin methylene blue agar) and cornmeal agar, respectively. The agar plates were incubated at 25°C, 30°C and 37°C, respectively, for 24 hours to 7 days. After cultivation, the colonies were counted. The number of bacteria in 1 gm of the original soil sample or in 1ml of original water was also calculated.

b. Millipore membrane filter method

According to the method established by Peltier *et al.* (1952), Pickett *et al.* (1973), Levine (1754) and Baker (1978), 1 ml of diluted solution was taken and added in 100 ml of sterilized pH 7 phosphate buffer solution. The solution was mixed and filtered through a 0.45 micrometer millipore membrane. The membrane was then placed on an agar plate and the plates were incubated at 25°C, 30°C, and 37°C respectively for 24 hours to 7 days. After incubation, the number of cells was counted to estimate the cell content in 1 ml of river water.

Isolation of microorganisms

a. Staphylococcus

To isolate and cultivate *Staphylococcus aureus,* Chapman agar plates (Difco) were used. The cultures were incubated at 37°C for 24 to 48 hours and formed gold-yellow colonies.

b. Enterobacteria

Endo agar, eosin methylene blue agar and Salmonella-Shigella agar were used for isolation of enteric bacteria. The cultures were cultivated at 37°C for 24 to 48 hours. The colonies of salmonella and shigella were colorless and clear. The larger colonies of *E. coli* were pinked-red, blue-black or violet-black.

c. Actinomycetes

Waksman agar, peptone yeast extract agar (PY), and tryptone yeast extract glucose agar (TYG) were used for isolation of actinomycetes. The cultures were cultivated at 28°C for 7 to 21 days. They had the typical actinomycetes colony appearance. Aerial mycelia and aerial spores had grown on the surface and the colonies were usually surrounded by inhibition zone.

d. Fungus

Czapek Dox agar (Difco), cornmeal agar (Difco) and potato sucrose agar (Difco) were used for isolation of fungi. The fungi on these media were cultivated at 25°C to 30°C for 2 to 7 days, typical fungi colonies were produced.

e. Yeast

Sabouraud agar (Difco) and malt extract agar were used for isolation of yeast. After incubation at 25°C to 30°C for 2 to 7 days, typical yeast colonies had grown.

f. Anaerobic Bacteria

Thioglycollate medium (Difco), nutrient agar, TYG agar, and Endo agar plates were used for isolation of anaerobic bacteria. BBL anaerobic jars and the Forma Scientific Anaerobic System Incubator (model 1021) were also used for cultivation of anaerobic bacteria.

Identification of Microorganisms

a. Common bacteria

Typical enteric bacteria colonies can be recognized in selective and differential media. Salmonella in these media appear as small colorless colonies. Finally, the organisms were classified according to Bergey's Manual classification method. (1984)

1. Morphological observations

A Nikon light microscope type 104 was used to observe the shape of each isolated strain after it was gram stained.

2. Growth characteristics

Each isolated strain cultivated on selective media was observed for growth characteristics.

3. Biochemical tests

Biochemical tests such as the indole test, MR test, VP test, citrate test, motility test, starch hydrolysis test, nitrate reduction test, serum liquefaction test, plasma coagulation test, and the hemolysis test were used. Pathogenic staphylococci were checked using the staphylockinase test and the mannitol fermentation test.

b. Fungus

Fungus was isolated from corn meal agar, Czapek Dox agar and potato sucrose agar. It was cocultivat

ed under light on 10% corn meal agar at 28°C for three to seven days to allow the growth of spores. A Nikon 104 light microscope was used according to the method described by Barnett (1960), Levine (1954), Baker (1978), and Bergey's Manual (1984) to classify the organisms.

c. Actinomycetes

The isolated actinomycetes were cultivated in water agar, 10% V-8 juice agar, ISP No. 4, and TYG agar at 28°C for 7, 14 and 21 days, respectively. The samples were fixed with 2.5% glutaraldehyde solution at pH 7.4 for 2 hours. They were then dehydrated with ethanol. The CPD method was used to dry the samples. After coating the samples using the gold-palladium method, a Zeiss DSM type 950 SEM was used to observe the morphology according to the method described by Arai (1976), Waksman (1976), Sykes (1973), Alexander (1961), and in Bergey's manual (1984).

Antibiotic Sensitivity Tests

According to the Finegeld *et al.* (1978), the isolated microogranisms were tested for sensitivity to clinically used antibiotics. The microorganisms were cultivated on antibiotics medium 1 agar plates. Sensitivity test paper discs (Difco) were then placed on these plates. Each disc contained the antibiotics shown below.

Cephalothin: 30 micrograms
Tetracycline: 30 micrograms
Penicillin G: 10 micrograms
Streptomycin: 10 micrograms
Colistin: 10 micrograms
Kanamycin: 30 microgarams
Gentamycin: 10 microgarams
Chlortetracycline: 30 micrograms
Erythromycin: 15 micrograms

Antimicrobial Spectrum Test

The isolated bacteria, fungi, and actinomycetes were cultivated in nutrient broth, Czapek-Dox broth, and TYG broth at 28°C 120 rpm for 7 days. The cylinder plate method and the paper disk method were then used to test for microbial activity. For these tests, *Aspergillus niger, Candida albicans* ATCC 10231, *Sarcina lutea* ATCC 9431. *S. aureus* ATCC 6538P. *Bacillus subtilis* PIC 219, *E. coli* ATCC 10536 and *Mycobacterium pheli* were used as the indicator bacteria.

Soft nutrient agar, Czapek-Dox agar, and Sabouraud agar were melted at 45°C to 50°C. The indicator bacteria were added to a concentration of 10^6/ml and mixed well. 4 ml samples of this agar were poured on hard agar plates. After cooling, 4 to 6 standard steel cylinders were put on each plate. The organism culture solution was then placed in these cylinders and the plate was incubated at 28°C, 30°C, and 37°C for 24 to 48 hours. The appearance of an inhibition zone around the steel cups showed that the tested organism produced antibiotics to inhibit the indicator bacteria. The paper disc method for the antibiotics sensitivity tests was also utilized. The diameter of the discs was 8.0 mm, and the discs were made by the Toyo Seisakusho Co. Japan.

Enzyme Screening Tests

The microorganisms isolated from selected samples were screened for agarase, α-amylase, gelatinase, lipase, pectinase and cellulase production according to the method of Hodgson and Chater (1981).

The organisms were cultivated on media with substrate and were incubated at 28°C for 4 days. The following staining reagents were used with the respective degradation tests: iodine for the starch test, acidic $HgCl_2$ for the gelatin test, and 10% copper acetate for the pectin test. If the enzyme test was positive, the colonies were surrounded by a clear zone.

Results

As Fig. 6 shows, the samples collected from the Tansui River and the soil collected from the mangrove forests were cultivated on NA, TYG, and Endo agar at 25°C, 30°C, and 37°C respectively for 3 to 5 days. Some of the colonies were surrounded by an inhibition zone.

This shows that microorganisms could produce bioactive substances such as antibiotics and/or enzymes to inhibit the growth of other organisms. Those bioactive substance producing organisms were gram positive bacillus or actinomycetes, especially those microorganism of the genus *Streptomyces* and *Micromonospora.*

Water collected from the Tansui River and the soil from the riverside were also cultivated on EMB and/or Endo agar at 37°C for 24 hours. In Fig. 7, the large, pink-red, smooth colonies are *E. coli.* The tiny, reflective colonies are either *Salmonella* or *Shigella.* As shown Fig. 7, in the summer time, 1ml of river water contaned over 10^5 of *E. coli.* This was far over the limit of 1 *E. coli* per ml. Sometimes, in the summertime, *Salmonella*

or *Shigella* could reach concentrations of over 100 CFU/ml.

To identify more specific enteric bacterial strains, tests such as the motility test, sugar fermentation test, H_2S production test, TSI test, and the IMViC test were used. These tests showed that the Tansui River contains *Salmonella paratyphi* A, *Shigella*, *Salmonella gallinarium*, and pathogenic *E. coli et al.*, *Salmonella* and *Shigella* were isolated in the river from spring to autumn.

There was a high concentration of *Salmonella gallinarium* in the upstream of the Tansui River. The upper part of the river has many farms with animals such as pigs and chickens. Everyday, untreated animal feces from those animals go into the river. *S. grllinarium* is pathogenic to chicken, ducks, and geese.

As shown in Fig. 1, which illustrates the microorganism count in each of the four seasons, the highest

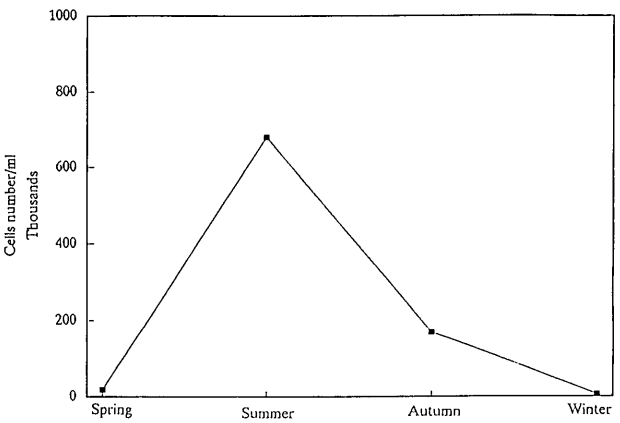


Fig. 1. Total bacteria cell count in different sampling seasons.

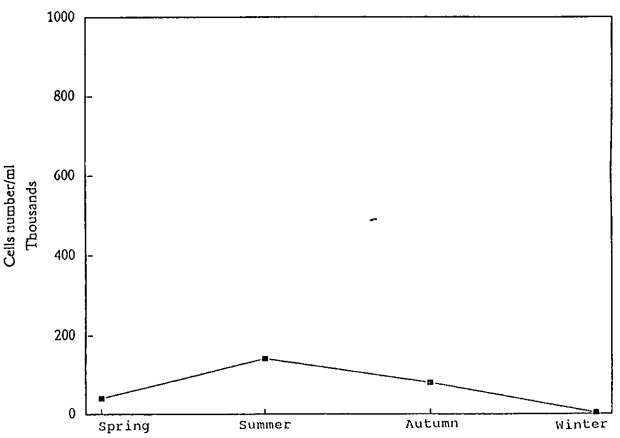


Fig. 2. Total *E. coli* cell count in different sampling seasons.

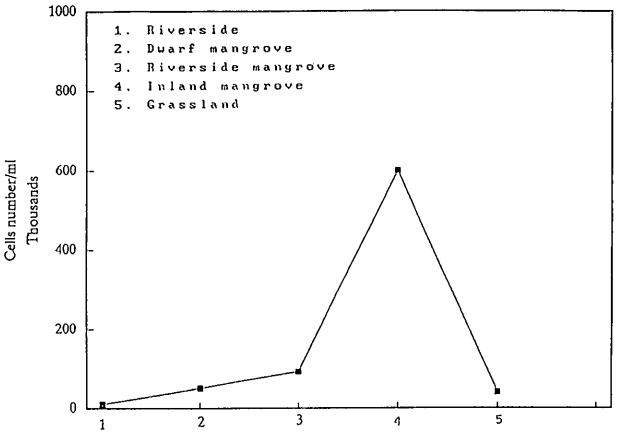


Fig. 3. Total cell count in different sampling areas.

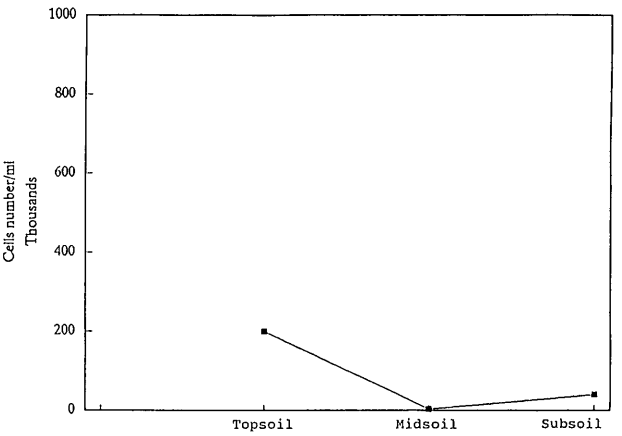


Fig. 4. Total cell count in different depths of soil under aerobic conditions.

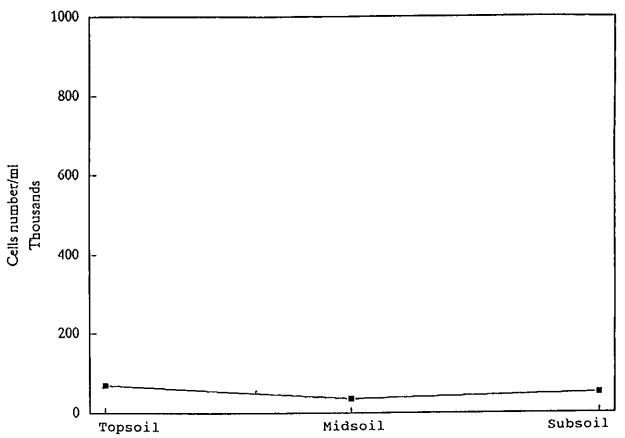


Fig. 5. Total cell count in different depths of soil under anaerobic conditions.

count of microorganisms was in the summer. Autumn was the season that had the next highest count of organisms. In winter time, the cell count was lowest. In the summer, the higher temperatures make conditions more suitable for the growth of microorganisms.

Figure 2 shows the *E. coli* cell count for each of the four seasons. Summer is the peak growing season for *E. coli*. The winter time is the worst time for the growth of *E. coli*, but even so, the concentration of *E. coli* is too high.

Figure 8 shows the cultivation of soil collected from around the mangroves on the banks of the Tansui River. There were both anaerobic and aerobic microorganisms in the soil samples. We cultivated the microorganisms in a Forma Scientific Anaerobic System Incubator (model 1024) under 30°C for 2 to 3 days. The picture shows that under aerobic conditions, many colonies producd antibiotics to inhibit other bacteria growth. Under aerobic conditions, the number of aerobic bacteria were greater than the number of anaerobic bacteria. In aerobic conditions, there were many actinomycetes and much fungus. In anaerobic conditions, there were no actinomycetes, only gram positive and gram negative bacterla.

Figures 4 and 5 show different depths of soil cultivated under aerobic and anaerobic conditions. Shown are the distributions of different bacteria in different conditions. There was 10 times more aerobic bacteria in top soil than in mid and sub soil.

In mild soil, there was more anaerobic bacteria than aerobic bacteria. In top soil, much of the aerobic bacteria's work is to digest lignin or organic material such as leaves and stem. After aerobic bacteria digests the lignin and leaves, the mid soil bacteria takes over digestion, where the anaerobic bacteria digest organic material or cellulose. In sub soil, the amount of aerobic and anarobic bacteria was about the same, as Figure 8 shows.

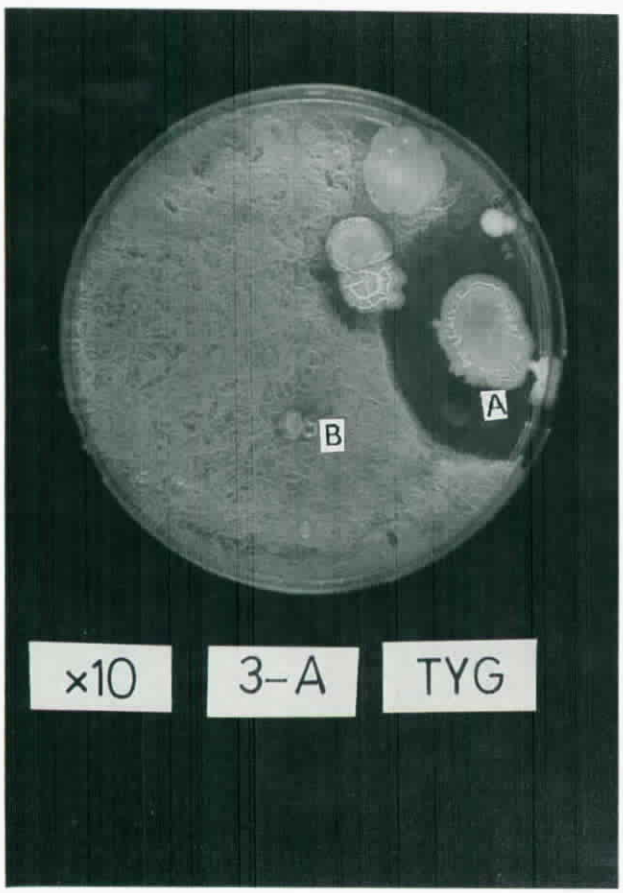


Fig. 6. Samples cultivated on TYG agar.

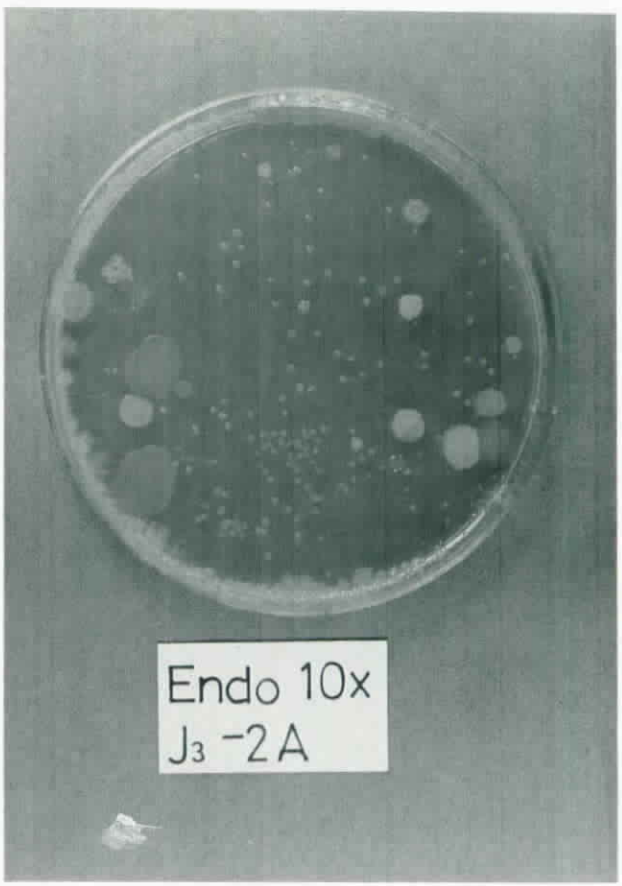


Fig. 7. *E. coli*, *Salmonella*, and *Shigella* cultivated on Endo agar.

Most of the fungus isolated on the mangroves was pathogenic to plants such as *Fusarium* and *Alternaria et al*. But some kinds of *Trichoderma* and *Aspergillus* have special enzymes to digest other organic material such as cellulase and/or agarase, etc. As shown in Table 1, the actinomycetes are members of the genera *Nocardia, Rhodococcus, Streptomyces, Streptoverticillium*, and *Micromonospora*. These microorganisms not only produce antibiotics, but are also able to produce more than one kind of enzyme as shown in Tables 5 and 6.

Thirty-three random strains of common bacteria isolated from the Tansui river and the mangrove forests were selected for the antibiotics sensitivity test. As shown in Table 2, pathogenic organsisms such as *S. aureus, E. coli*, salmonella, etc., are highly resistant to clinically used antibiotics such as penicillin, tetracycline, streptomycin, and chloramphenicol. But as Table 2, indicates, gram positive bacilli and gram positive cocci, which are none pathogenic organisms, are very sensitive to antibiotics. Results indicated that pathogenic staphylococci and enteric bacteria are often found in hospitals and/or sewage.

The results of enzyme screening tests of common bacteria isolated from the Tansui River and the mangrove forests are shown in Table 3. As Table 3 indicates, most gram positive bacillus are able to produce portease to digest proteins. These organisms are able to be utilized in food waste treatment.

As figure 3 shows, the total microorganism count of the moving river water had the least number of organisms of the regions surveyed. That is because the moving water washes the bacteria away. Area 4, the mangroves forest, was where the cell count was the highest. Area 2, grass, and Area 3, the riverside mangroves, were the next highest in total microorganism cell count. From what is indicated, We can draw a correlation between the total cell count and the number of trees in the forest. The more trees there were, the more bacteria cells there were. That is because the more trees that are in the forest, the more leaves, stems, and organic material there are for bacteria to digest.

In the water, much *S. aureus* was isolated with concentrations of 100 *Staphylococcus aureus* per 1 ml of river water. *S. albus, S. citreu, Sarcina, Diplococci*, and *Micrococcus* had the next highest concentrations. *S. aureus* is the most dangerous among those organisms because it is pathogenic to humans. That is why pathogenic *Staphylococcus* tests and antibiotic sensitivity tests were conducted on it. The results of antibiotic

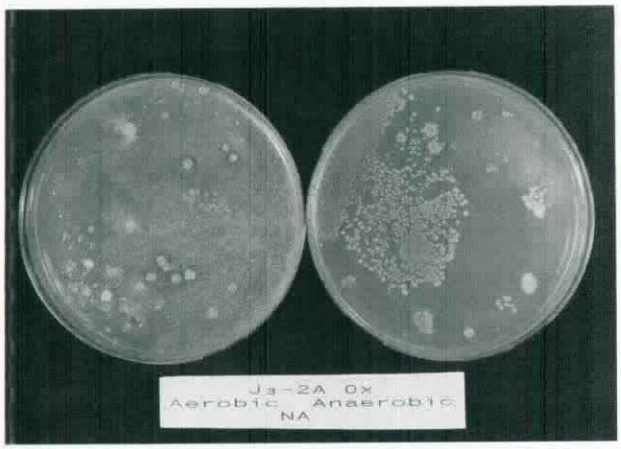


Fig. 8. Cultivation of soil.

Table 1. *Microorganisms isolated from the Tansui River and the mangrove forests*

Common bacteria	Fungi	Actinomycetes
Escherichia coli	*Mycotypha*	*Nocaridia*
Salmonella paratyphi A	*Spicaria*	*Rhodococcus*
	Penicillium	*Streptomyces*
Sallmonella gallinarum	*Aspergillus*	*Streptoverticillium*
	Cladosporium	*Micromonospora*
Shigella	*Gliocladium*	
Pseudomonas	*Fusarium*	
Hafnia	*Verticillium*	
Streptococci	*Hobsonia*	
Gram's (+) bacilli	*Rhizopus*	
Gram's (+) cocci	*Helicomina*	
Gram's (-) bacilli	*Alternaria*	
Staphylococci	*Bartilinia*	
	Polyscytalum	
	Geotrichum	
	Neurospora	
	Helicosporium	
	Botrytis	
	Cephalosporium	
	Trichoderma	
	Mucor	
	Catenularia	
	Monilia	
	Flagellospora	
	Yeast	

Table 2. *Results of antibiotics sensitivity tests of common bacteria isolated from the Tansui River and the mangrove forests*

Strain No.	C-30	A-30	CXM-30	E-15	S-10	TE-30	N-30	GM-10	P-10	K-30	CL-10	CR-30	TM-10
J1-A2-1 G(+)S.*	−	−	−	−	+	−	+	++	−	++	−	−	−
J1-A4-2 *E. coli*	−	−	+++	−	+	+	+	+	+	++	+	++	−
J1-5W6 P.*	−	−	+++	−	+	+	+	+	−	++	+	−	−
J2-2A4 *S.*#	−	−	+++	−	+	+	+++	+++	+	+++	+	−	−
J3-1A9 B.*	++	−	−	++	+	++	−	−	+	−	−	+	+
J3-1A15 B.	+	+++	+	+	+	++	+	+	++	+	−	+	+
J3-1A17 B.	+	+++	++	+++	++	+++	+	+	+++	++	−	+++	+
J3-1A20 B.	+	+++	++	+++	++	+++	+	+	+++	++	−	+++	+
J3-1B7 B.	++	−	−	++	+	++	−	−	+++	−	−	+++	+
J3-1B11 G(+)C.	+	−	−	++	+	+	−	−	+	−	−	+	+
J3-1C8 B.	+	−	−	++	+	++	−	−	+++	−	−	+++	+
J3-1C13 *E. coli*	+	−	−	++	−	+	−	−	+	−	−	+++	+
J3-2A4 B.	−	++	+	++	+	++	+	+	+++	+	−	−	−
J3-2C12 P.	−	−	−	+	−	−	−	−	+	−	−	+++	+
J3-3A9 B.	+	−	−	++	+	++	−	−	+++	−	−	+++	+
J3-3A14 S.@	−	−	−	+	−	−	−	−	−	−	−	−	−
J3-3A17 B.	++	−	−	++	+	+++	−	−	++	−	−	++	+
J3-3C3 B.	−	+++	++	++	+	+++	+	+	++	++	−	−	−
J3-3C13 B.	−	+++	++	+	−	++	+	+	++	+	−	−	−
J3-3W3 B.	+++	−	−	++	++	+++	−	−	+++	−	−	+++	+
J3-3W10 P.	−	−	−	−	+	−	−	−	−	−	+	−	++
J3-4B13 B.	−	+++	++	++	+	+++	+	+	+++	++	−	−	−
J3-4C23 B.	−	+++	++	++	++	+++	+	+	+++	++	−	−	−
J3-5A7 B.	−	+++	+++	+++	++	+++	+	+	+++	++	−	−	−
J3-5B4 B.	−	+++	+++	+++	+++	+++	+	+	+++	+++	−	−	−
J3-5B10 P.	−	−	−	−	−	−	−	−	−	−	−	−	−
J3-5C16 B.	−	++	+	+	−	++	+	+	++	+	−	−	−
J3-6C7 Hafnia	−	−	−	−	++	+++	++	+++	++	++	+	+	−
PA1-10 B.	−	+++	++	+++	++	+++	++	++	+++	+++	−	−	−
PA1-11 B.	−	++	−	+++	++	++	+	+	−	++	−	−	−
PA1-25 B.	−	++	−	+++	++	++	+	−	−	++	−	−	−
PA1-36 G(+)C.	−	+	−	+++	+	+	+	+	−	++	−	−	−
J4-2A5 S.#	−	−	++	−	+	+	+	+	+	++	+	++	−

S.* : Streptococci; P. : *Pseudomonas* sp.; B. : *Bacillus* sp.; C. : coccus sp.; S.@: *Staphylococcus aureus*; S. #: *Salmonella paratyphi A*.

C-30: Chloramphenicol 30 mcg; CXM: Cufuroxime 30 μg; S-10: Streptomycin 10 mcg; P-10: Penicillin G 10 units; A-30: Chlortetracycline 30 mcg; E-15: Erythromycin 15 mcg; TE-30: Tetracycline 30 mcg; N-30: Neomycin 30 mcg; K-30: Kanamycin 30 mcg; CL-10: Colistin 10 mcg; TM-10: Tobramycin 10 mcg; CR-30: Cephalothin 30 mcg; GM-10: Gentamycin 10 mcg.

−: Antibiotics resistant; +: Sensitive; ++: More sensitive; +++: Very sensitive.

sensitivty tests are shown in Figure 9. From the sensitivity tests, it was found that most strains of the *S. aureus* were highly resistant to antibiotics. Only a few strains of the organism were sensitive to erythromycin (15 mcg) and cephalothin (30 mcg). From these results, it is concluded that the *S. aureus* may have come from hospital waste or sewage.

Figure 10 shows the results of the sensitivity test of bacillus. From Figure 10, it is seen that gram positive bacteria are still sensitive to most antibiotics. This is indicated by the fact that the Tansui River has its own normal bacterial flora such as *B. subtilis* and *B. cereus*. Those bacteria are able to produce their own antibiotics and enzymes. As shown in Table 3, they is also able to produce amylase, pectinase and protease. Many strains of bacillus are able to produce amylase,

Table 3. *Results of enzyme screening tests of common bacteris isolated from the Tansui River and the mangrove forests*

Strain No.		Enzyme					
		Agarase	α-Amylase	Gelatinase	Lipas	Pectinase	Protease
J3-1A15	*Bacillus* sp.	−	+	−	−	−	−
J3-1A17	*Bacillus* sp.	−	−	−	−	+	+++
J3-1A20	*Bacillus* sp.	−	−	−	−	+	+++
J3-2A4	*Bacillus* sp.	−	−	−	−	−	++
J3-2A7	*Pseudomonas* sp.	−	−	−	+	−	+++
J3-2C6	*Pseudomonas* sp.	−	++	−	+	−	+++
J3-2C13	*Bacillus* sp.	−	−	−	−	+++	−
J3-3C3	*Bacillus* sp.	−	−	−	−	+	++
J3-3C13	*Bacillus* sp.	++	++	+	−	−	−
J3-3A9	*Bacillus* sp.	−	−	+	+	+++	−
J3-4A7	*Pseudomonas* sp.	−	+++	−	+	+	++
J3-4B13	*Bacillus* sp.	−	−	−	−	++	−
J3-4C23	*Bacillus* sp.	−	−	+	−	−	+++
J3-5A7	*Bacillus* sp.	−	−	−	−	−	+++
J3-5B4	*Bacillus* sp.	−	−	+	−	−	+++
J3-5B10	*Pseudomonas* sp.	+	+	−	−	++	++
J3-5C16	*Bacillus* sp.	+	+	+	−	−	−
PA1-10	*Bacillus* sp.	+++	+	+	−	−	+++
PA1-11	*Bacillus* sp.	++	+	−	−	−	−
PA1-25	*Bacillus* sp.	++	+	−	−	−	−
PA1-36	Gram(+) S.*	++	++	+	−	−	++
J4-2A3	*Bacillus* sp.	+	−	−	−	−	−
J4-2C1	*Bacillus* sp.	−	−	+	−	−	−
J4-2W1	*E. coli*	+++	++	−	−	−	−
J4-2W3	*E. coli*	+++	++	+++	−	−	−
J4-2W6	*E. coli*	−	−	+++	−	−	−

*Gram(+) Staphylococcus.

pectinase, and protease, but only a few strains can produce lipase. Each strain of bacillus can produce one or more enzymes. This indicates that microorganisms living in the mangroves and the Tansui River can produce antibiotics or enzymes to grow and sustain the microbial flora of the river.

Fungus

Samples collected from mangrove stems, leaves

which digests cellulose in the stem of wood. Thus, in mangrove forests, there are only few mangrove leaves and stems, because trichodermas has digested them. Figure 12 shows *Alternaria* sp. isolated from mangrove leaves. Table 4 shows the results of the enzyme screening test of the fungi isolated from the Tansui River. Many fungi are able to produce agarase and cellulase, but only a few of the isolated fungi that were found can produce lipase and pectinase.

Fig. 9. Antibiotic sensitivity tests of *Staphylococcus* sp.

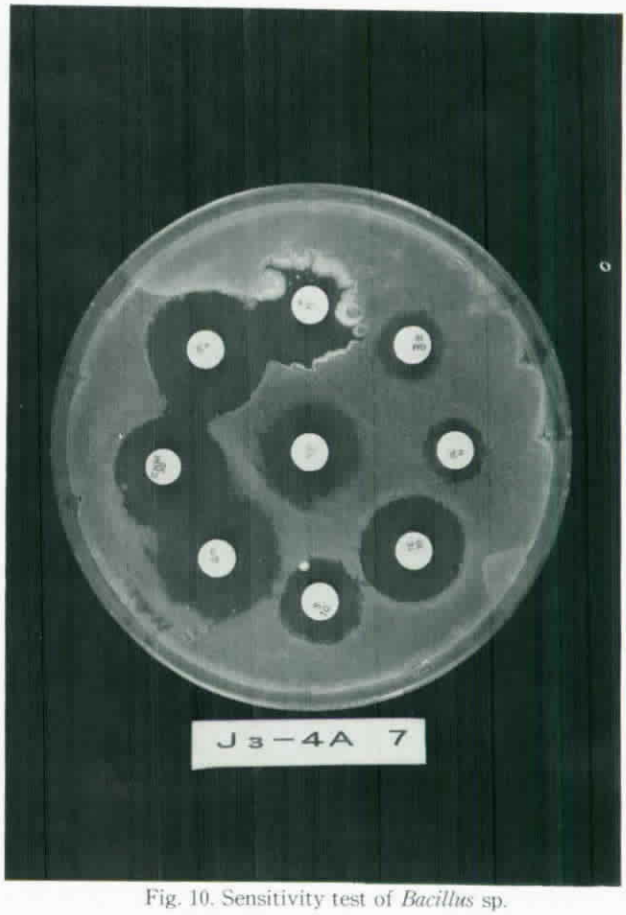


Fig. 10. Sensitivity test of *Bacillus* sp.

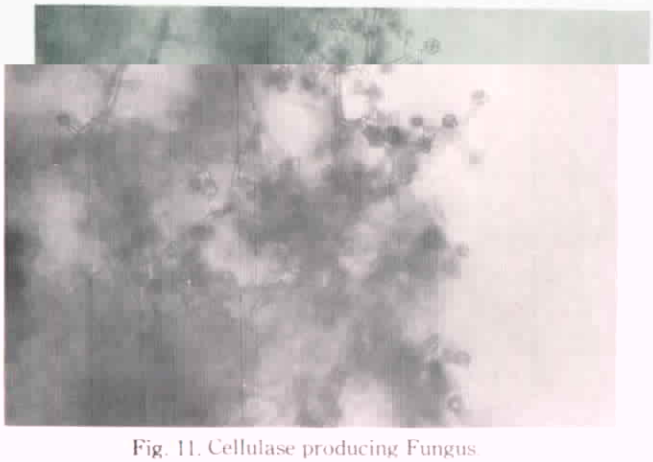


Fig. 11. Cellulase producing Fungus

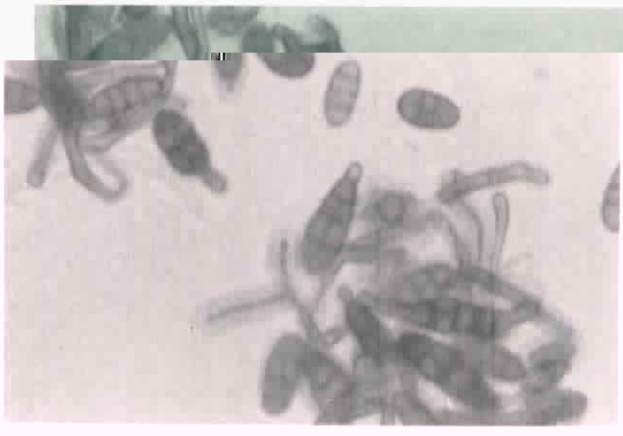


Fig. 12. *Alternaria* sp.

of the colony. Most of these colonies are streptomycetes. But some colonies on continued incubation turned black or borwn. These colonies were probably micromonosporas. An inhibition zone usually surrounded these colonies. Those colonies were antibiotics producing organisms. After purification, these

Table 4. *Results of enzyme screening tests of fungi isolated from the Tansui River and the mangrove forest*

Strain No.		Enzyme						
		Agarase	Amylase	Gelatinase	Lipase	Pectinase	Protease	Cellulase
J2-2B1	*Spicaria* sp.	+	−	−	−	+	−	−
J2-2B2	*Mycotypha* sp.	+	−	−	−	−	−	−
J2-2B4	*Penicillium* sp.	−	−	−	−	++	−	−
J2-2C2	*Aspergillus* sp.	++	−	++	−	+	+++	+
J2-3B11	*Gliocladium* sp.	−	−	−	−	+	−	−
J2-3C8	*Fusarium* sp.	+	−	−	−	−	−	+
J2-3D1	*Aspergillus* sp.	+	−	−	−	−	−	++
J2-3D3	*Aspergillus* sp.	+	−	−	−	+	−	++
J2-5B4	*Aspergillus* sp.	+	−	−	−	−	−	++
J2-5E9	*Mycotypha* sp.	+	−	−	−	−	−	−
J2-6D2-2	*Verticillium* sp.	++++	−	++	−	+	−	−
J3-2C24	*Hobsonia* sp.	+	−	−	−	−	+	−
J3-2C25	*Phoma* sp.	+	−	+	−	−	−	−
J3-GL1	*Fusarium* sp.	+++	−	−	−	−	−	+
J3-GL3	*Rhizopus* sp.	+++	−	−	−	−	−	−
J3-MB3	*Fusarium* sp.	−	−	−	−	−	+	+
J3-ML1	*Flagellospora* sp.	+++	−	−	++	−	−	−
J3-ML3	*Alternaria* sp.	+	−	−	−	−	−	−
J3-MW6	*Aspergillus* sp.	+++	−	−	−	−	+++	++
J3-MW7	*Aspergillus* sp.	+++	−	−	−	−	−	++
J3-MW8	*Aspergillus* sp.	+++	−	+++	−	−	++	++
J3-MW9	*Aspergillus* sp.	+++	−	++	−	−	+++	++
J3-MW11	*Fusarium* sp.	+++	−	+++	−	−	−	+
J3-MW15	*Fusarium* sp.	−	+++	+++	−	−	−	+
J3-MW17	*Fusarium* sp.	+++	−	+++	−	−	−	+
J3-MS4	*Fusarium* sp.	+++	−	−	−	−	−	+
J3-MS9	*Aspergillus* sp.	+	+++	−	+++	+++	−	+
J4-IW7	*Polyscytalum* sp.	−	−	−	−	+	−	−
J4-2A2	*Cladosporium* sp.	−	−	+	−	−	−	−
J4-2A4	*Geotrichum* sp.	+	−	−	−	−	−	−
J4-2A7	*Verticillium* sp.	−	−	−	−	−	+	−
J4-2A9	*Geotrichum* sp.	−	−	−	−	−	+	−
J4-2C6	*Cladosporium* sp.	+++	−	−	−	−	+	−
J4-4A4	*Botrytis* sp.	−	++	+	−	−	−	−
J4-4C8	*Cephalosporium* sp.	+	−	+	−	+	−	−
J4-5C5	*Botrytis* sp.	−	−	+	+	−	+	−
J4-6A3	*Trichoderma* sp.	++	−	−	−	−	−	+++
J4-6A4	*Cephalosporium* sp.	+	−	+	−	−	+	−
J4-GL1	*Fusarium* sp.	−	++	−	−	−	−	+
J4-MF2	*Trichoderma* sp.	−	+++	−	−	−	−	+++
J4-MB1	*Fusarium* sp.	++	−	−	−	+++	−	+
J4-MP3	*Aspergillus* sp.	+++	−	+++	−	−	-	++
J4-MP6	*Aspergillus* sp.	−	−	−	+	−	−	++

Fig. 13. *Nocardia* sp.

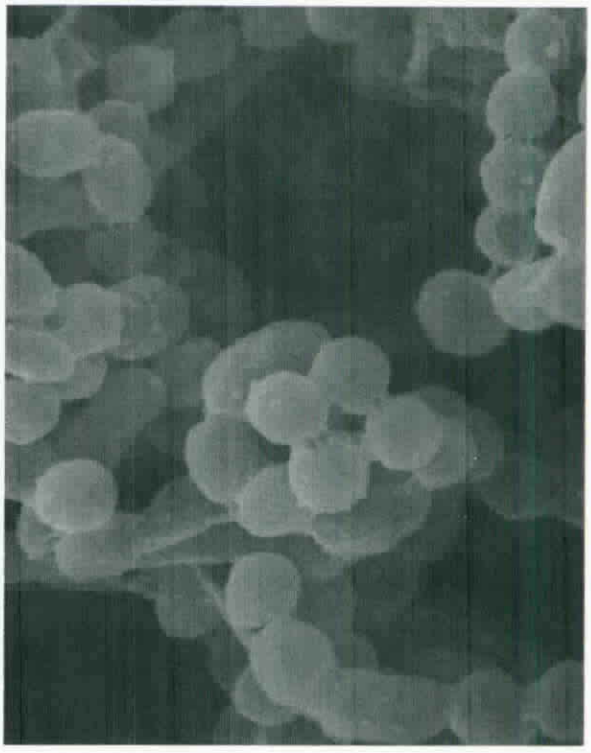
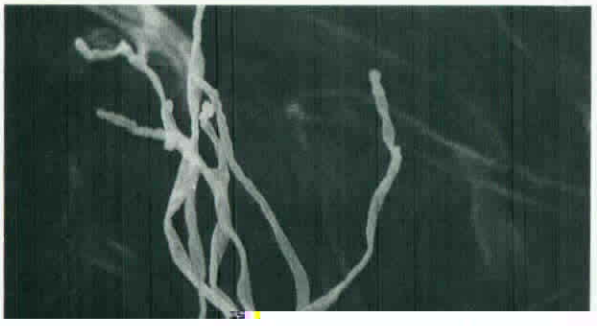
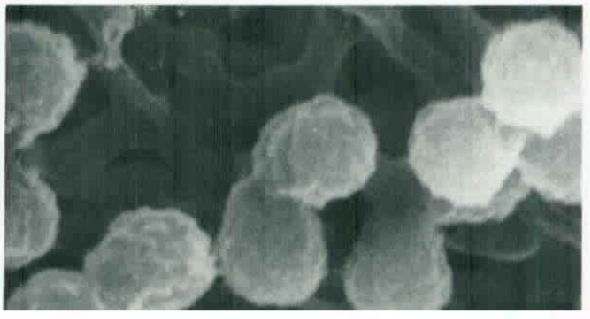


Fig. 14. *Streptomyces* sp.





actinomycete colonies were cultivated on ISP # 1 to #9 mediums to study their growth characteristics and to conduct cell wall amino acid analysis. Table 1 shows that the actinomycetes isolated from mangroves belong to 5 genera. These genera are *Nocardia, Rhodococcus, Streptomyces, Streptoverticillium,* and *Micromonospora.* Among the actinomycetes, about 80% were of the genus *Micromonospora.* About 15% were of the genus *Streptomyces.* The remaining genera, *Streptoverticillium, Nocardia,* and *Rhodococcus,* each constituted about 2%, respectively. Figure 13 shows *Nocardia* sp. observed by TEM and SEM. Figure 14 shows *Streptomyces* sp. Fig. 15 shows *Streptoverticillium* sp. Finally, Fig. 16 shows *Micromonospora* sp.

Towety nine strains of actinomycetes were selected to conduct the antibiotics producing test. The results are shown in Table 5. As Table 5 indicates, each actinomycetes can produce antibiotics to inhibit one or more kinds of indicator bacteria. From Table 5, it is seen that the actinomycetes in the mangrove forests mainly produce antifungal antibiotics to inhibit *Aspergillus* and *Candida albicans.* Also, from the chart, many actinomycetes can produce antibiotics to inhibit gram positive bacilli and gram positive cocci. In this experiment, 2 strains of actinomycetes (J-11A13 and J-11B1) were found which were able to produce antibiotics to inhibit *M. phlei.* However, of the the actinomycetes isolated from the Tansui River, only a few strains were

Table 5. *Antimicrobial activities of actinomycetes isolated from the Tansui River and the mangrove forests*

Strain No.		Indicator bacteria (cm)						
		A. niger	*C. albicans* ATCC 10231	*S. aureus* 209P	*E. coli* ATCC 10536	*M. lutea* ATCC 9341	*M. phlei*	*B. subtili* PIC 219
J1-1A21	S.	1.94	1.51	+	−	2.09	−	1.69
J1-2A10	S.	1.04	0.91	+	−	1.60	−	1.92
J1-2A15	S.	0.78	+	−	−	1.88	−	1.57
J1-3A7W	S.	1.92	1.13	+	−	1.50	−	1.48
J1-4B8	S.	1.25	2.17	−	−	1.23	−	−
J1-4B8-2	S.	1.69	1.59	0.98	−	1.02	−	+
J1-4B8-3	S.	1.59	2.03	−	−	1.25	−	1.48
J1-6A4	S.	1.68	−	−	−	2.98	−	1.84
J1-6B2	S.	−	−	−	−	1.58	−	−
J1-9B13	S.	1.35	1.41	−	−	+	+	1.42
J1-11A10	S.	3.37	2.11	−	−	1.05	−	−
J1-11A11	S.	+	1.58	−	−	−	+	1.42
J1-11A12	S.	+	1.48	−	−	−	−	−
J1-11A13	S.	0.86	1.44	−	−	+	0.79	2.51
J1-11A14	S.	1.22	1.19	−	−	+	−	1.36
J1-11B1	S.	1.75	1.6	−	−	1.65	−	1.21
J1-11B3	S.	0.98	2.67	−	−	−	−	−
J1-11B4	S.	−	−	1.82	−	3.86	1.11	2.85
J2-3B2	M.	2.89	−	−	−	−	−	−
J3-3A3	S.	+	−	−	−	1.56	−	−
J4-4A5	M.	−	−	1.66	−	2.50	−	+
J4-4C3	M.	0.94	1.57	−	−	1.81	−	−
J4-4C5	M.	−	−	2.64	1.52	2.18	−	1.15
J4-4C6	M.	−	−	3.15	1.26	2.21	−	1.05
J4-4C10	M.	1.28	−	−	−	1.35	−	+
J4-4C11	M.	2.87	+	−	−	−	−	1.04
PA2-1	S.	1.79	1.72	−	−	1.34	−	1.66
PC1-2	S.	1.18	2.00	−	−	1.22	−	−
PD1-2	S.	−	+	−	−	1.31	−	1.48

*S. : *Streptomyces* sp.; M. : *Micromonospora* sp.

able to produce anti-gram negative antibiotics. Only strains J4-4C5 and J4-4C6 were able to produce antibiotics to inhibit *E. coli* growth. The results of the enzyme screening tests on the actinomycetes are shown in Table 6. the actinomycetes isolated from the mangroves were able to produce one or more types of enzymes such as agarase, amylase, gelatinase, and protease. However, only a few strains were able to produce lipase or cellulase. Only strain J4-4C9 was able to produce lipase, and only strain J1-3A7 was able to produce cellulase. Figures 17 thru 20 show the alpha -amylase, agarase, gelatinase, and proteinase producing organisms.

Discussion

In the Tansui Esturary, many microorganisms were isolated that could produce substances important to the environment. They are discussed below.

Tago (1975, 1977, 1985) isolated the floc-forming bacterium, *Pseudomonas* sp. No. 12, from bioactivated sludge. This organism is able to promote floc formation and the aggregation of polypeptides and cellulose. These substances then precipitate and result in clearer waste water. Among the microorganisms isolated form the Tansui River, *Pseudomonas* spp. accounted for 2% of the strains.

According to Fukuda (1985), the organisms *Pseudomonas putida, Bacillus licheniformis, Aspergillus clavatus, Monifria greophita, Penicillium digitatum,* and *Rhizopus japonicus* are able to produce ethylene and bio -olefin. These organisms were also isolated from the mangroves and the soil on the river banks. By digesting organic substances in the soil, humus and leaves, these organisms are able to produce important substances, such as bio-olefin, that are helpful to the estuary's ecology.

According to Ohmori (1985), Senez *et al.* (1961),

Fig. 17. Alpha-amylase producing microorganism.

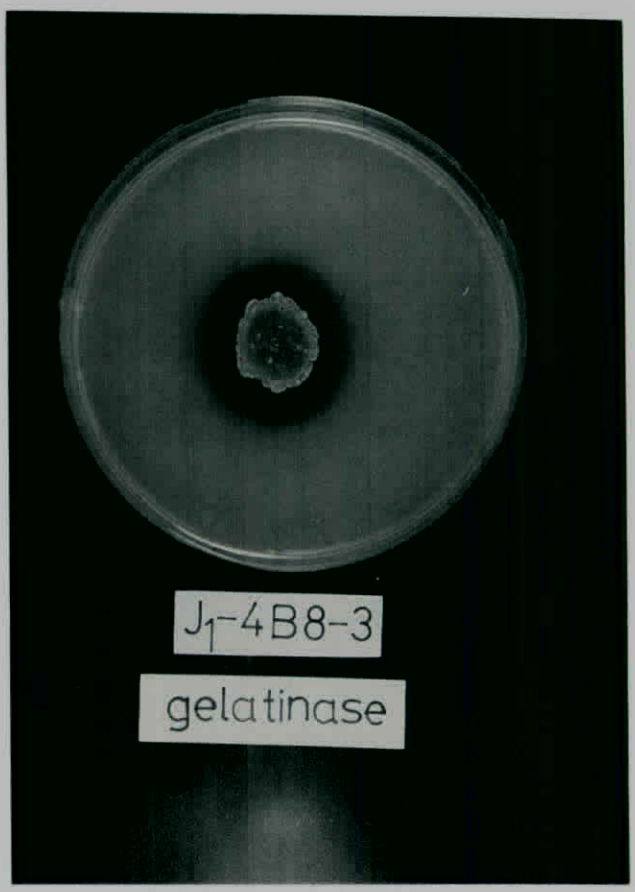


Fig. 18. Gelatinase producing microorganism.

Table 6. *Reuslts of enzyme screening tests of actinomycetes isolated from the Tansui River and the mangrove forests*

Strain No.		Enzymes					
		Agarase	Amylase	Gelatinase	Lipase	Pectinase	Protease
J1-1A7	Micro.	++	+	+	−	−	+
J1-1A8	Micro.	+	−	+	−	−	−
J1-1A15	Micro.	+	−	−	−	−	+
J1-1A17	Micro.	++	+	+	−	−	+
J1-1A18	Micro.	−	+	+	−	−	+
J1-1A21	Strep.	+	+	+	−	−	+
J1-1B4	Micro.	−	−	+	−	−	+
J1-1B5	Micro.	+	−	+	−	−	+
J1-1B6	Micro.	+++	+	+	−	−	+
J1-2A9	Micro.	+	−	−	−	−	−
J1-2A10	Strep.	++	−	++	−	−	++
J1-2A11	Micro.	+	−	+	−	−	+
J1-2A12	Micro.	++	+	+	−	−	+
J1-2A15	Strep.	+	+	+	−	+	+
J1-2A16	Strep.	−	+	+	−	−	++
J1-2B2	Micro.	++	+	−	−	−	+
J1-3A7o	Micro.	+++	+	+	−	−	+
J1-3A7W	Strep.	+	+	+	−	−	+
J1-3A8	Micro.	++	+	+	−	−	+
J1-3A10	Micro.	+++	+	+	−	+	+
J1-4A6	Micro.	+++	+	+	−	−	+
J1-4A8	Micro.	+	−	+	−	−	+
J1-4B7	Micro.	++	+	+	−	−	+
J1-4B8-2	Strep.	+++	+	+	−	−	++
J1-4B8-3	Strep.	+++	+	+	−	−	++
J1-4B12	Micro.	++	−	+	−	−	+
J1-6A4	Strep.	−	+	+	−	+	−
J1-6B2	Strep.	+	−	+	−	−	+
J1-9A5	Micro.	+++	+	+	−	−	+
J1-9A6	Micro.	+	−	+	−	−	+
J1-9A7	Micro.	+	+	+	−	−	+
J1-9A8	Micro.	+++	+	+	−	−	+
J1-9B11	Micro.	+	−	+	−	−	+
J1-9B13	Strep.	+++	+++	+++	−	−	++
J1-9B17	Micro.	+	−	+	−	−	+
J1-10A8	Micro.	−	+	+	−	−	+
J1-11A9W	Strep.	+	−	−	−	−	+++
J1-11A10	Strep.	+	+	+	−	+	+
J1-11A11	Strep.	++++	+++	+++	−	−	++
J1-11A12	Strep.	++++	+++	+++	−	−	++
J1-11A13	Strep.	++++	+++	+++	−	−	++
J1-11A14	Strep.	+++	+	++	−	−	+
J1-11B1	Strep.	+++	++	+	−	−	++
J1-11B3	Strep.	+	+	+	−	−	+
J1-11-3	Micro.	++	+	++	−	+	+
J1-12-4	Micro.	++	+	+	−	−	+
J3-3A3	Strep.	+++	+	++	−	−	+
J3-3C20	Strep.	+++	++	++	+	+	+
PA2-7	Strep.	++	+	+	−	+++	+
PC1-1	Strep.	++	+	+	−	−	+
PD1-2	Strep.	−	+	+	−	−	+
PD2-5	Strep.	+++	+	+	−	+	−

*Micro.: *Micromonospora* sp.; Strep.: *Streptomyces* sp.

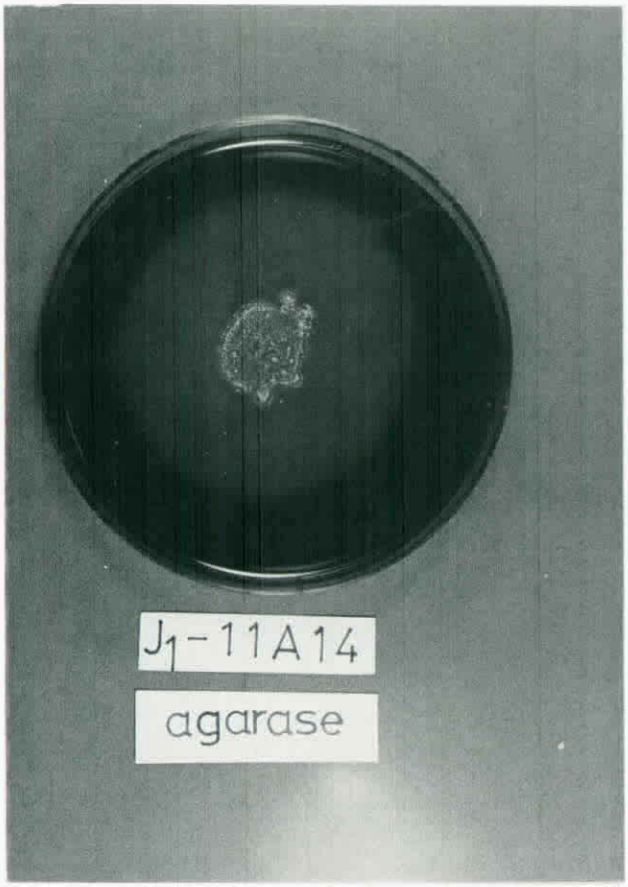


Fig. 19. Agarase producing microorganism.

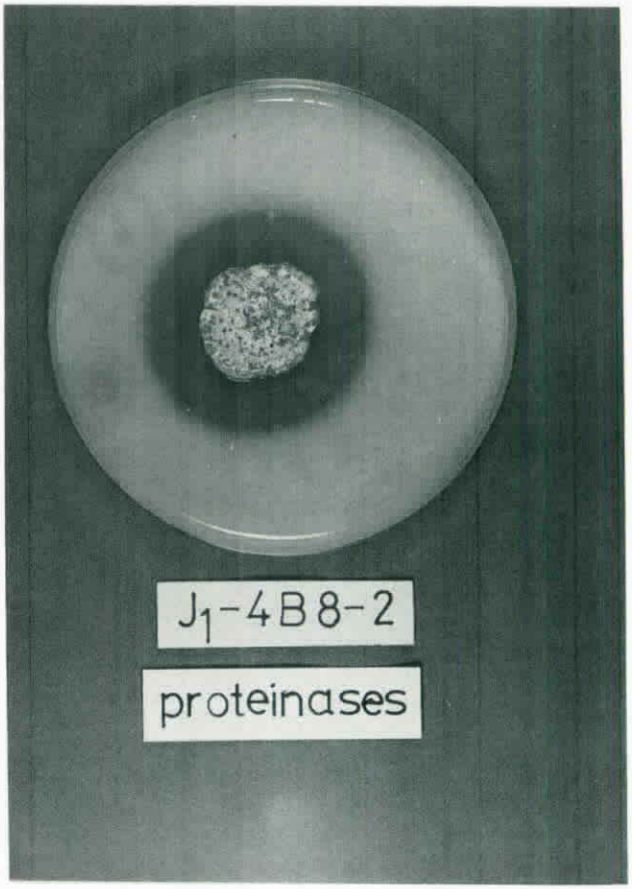


Fig. 20. Proteinase producing microorganism.

Duppel *et al.* (1973), Mckenna *et al.* (1970), Klug *et al.* (1968), Pirnik *et al. (1974), Yamada et al.* (1977), and Beam *et al.* (1974), microorganisms belonging to the genera of *Bacillus, Nocardia, Pseudomonas, Rhizopus, Aspergillus, Penicillium, Botrytis, Candida,* and *Cryptococcus* are able to perform degradation of hydrocarbons and dehydrogenation of n-alkanes in soil. These organisms were also found in the Tansui River.

In natural conditions, polycyclic aromatic hydrocarbons (PAHS) are widely distributed in soil and water. These substances are carcinogens and are very toxic to humans. In nature, only microorgnaisms can digest these materials. According to Nakano (1985), Badger (1962), El-Bayoumy *et al.* (1983), Cerniglia *et al.* (1979), and Gibson *et al.* (1982), *Cunninghamella elegans* is the best known microorganism able to digest PAH's. *Pseudomonas, Flavobacterium, Bacillus* and *Lactobacillus* are other microorganisms that also can digest PAH's. In the Tansui River, many strains of *Bacillus* were isolated.

According to Kato *et al.* (1985), Gunner *et al.* (1968), Sakazawa (1981), and Shimao (1983), in nature, there are usually two or more microorganisms that have a mutualistic relationship. They help each other by supplying CO_2, O_2, or energy, or by reducing toxins such as the diazinon compound. Two or more types of organisms are needed to digest those toxic compounds. These organisms were found in the Tansui River; examples are *Streptomyces* spp..

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淡水河口沼澤地微生物生態系之研究

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淡水河之紅樹林沼澤地帶，根據調查，含有兩大類微生物，其一類爲紅樹林內原本存在之原有微生物，另一類爲由於污染而混入生態系之微生物。因此其菌相較其他地方來得複雜；本研究針對此一地區生態系及微生物相間之關係，以及所產生之影響加以研討。自 1990 及 1991 年間的春、夏、秋、冬四季裏，採集紅樹林沼澤地帶及河水之土壤及樹林之莖、葉等爲材料，分離培養細菌、黴菌、放線菌及對人畜有害之病原菌等，並加以探討此微生物羣在自然界之相互關係。從一系列的研究中，已分離出 215 株細菌，其中包括大腸桿菌、沙門氏副傷寒桿菌、志賀桿菌、綠膿桿菌、單球菌、桿菌、葡萄球菌及鏈球菌等。眞菌方面，共有 42 株，分別屬於 25 屬，包括 *Aspergillus*, *Alternaria*, *Cephalosporium*, *Trichoderma*, *Geotrichum*, *Fusarium*, *Verticillium* 及 *Penicillium* 等。在放線菌方面共有 110 株，包括 *Rhodococcus*, *Nocardia*, *Streptomyces*, *Streptoverticillum* 及 *Micromonospora* 之五屬等。在酵素產生試驗中得知大部份之桿菌、放線菌，及眞菌皆能產生工業及污染物質處理上有用之酵素，如 pectinase, gelatinase, α-amylase, protease, lipase, agarase 及 cellulase 等。從抗生素篩選試驗中顯示，許多鏈黴菌、桿菌及單胞放線菌均能產生抗黴抗生素，及抗細菌抗生素來抑制部分微生物之生長。本研究室擬做更深入之研究，來探討這些微生物是否能產生對人類有益之抗生素、酵素等，以便開發來造福人類。淡水河紅樹林區域生長許多能清除河內污染物之微生物，對於生活在此區域之人類、動物及植物是有益的，並爲自然界中寶貴的資源。但近來由於工業、醫院、畜牧場及家庭廢棄物等快速增加並流入河中，因而滋生許多病原菌，影響人類之健康及環境衛生。紅樹林區內微生物及紅樹林之關係是密不可分的，因其相互依靠、保護，幫助使生態維持平衡並得以循環，因此若要整治淡水河之污染，應考慮到維持微生物相之生態平衡，保護此寶貴資源，並可於日後善加開發利用。