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## Isolation of *Pseudomonas aeruginosa* from Ushubetsu River water in Hokkaido, Japan

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### Abstract

To provide information on the ecology of *Pseudomonas aeruginosa* in nature, bacteriological surveillance was performed in the defined area in Hokkaido, Japan. *P. aeruginosa* was isolated from water samples of Ushubetsu River in the down stream from the urban area of Asahikawa. *P. aeruginosa* was isolated from fecal samples of pigs but not from samples of soil of a tomato field, sand of sandboxes in vest-pocket parks, fresh vegetables, or feces of wild deer. The present results indicate that *P. aeruginosa* strains isolated from the river water is originated from the environment of human activity and not from wild life or domestic animals.

Key words : *P. aeruginosa* , natural environment, Hokkaido.

### Introduction

After the introduction of antibiotics into the medical and veterinary fields, *P. aeruginosa* has been widely recognized as a potential pathogen<sup>2,4,8,16-19,21,22,28)</sup>, and now is one of the most important pathogenic bacteria causing opportunistic and nosocomial infections<sup>6,14-16,19,25,26)</sup>. Apparently healthy humans and animals carry *P. aeruginosa* in their intestines and shed in the stools<sup>12,19-21,24,27)</sup>. *P. aeruginosa* widely distributes in natural environment such as soil, plant surfaces, fresh vegetables, sewage, waste water, sink, moist

environment, and river water<sup>3,5-7,9-13,23,24)</sup>. In a case of nosocomial *Pseudomonas* infection with fresh vegetable salad, the pathogen was suspected to originate from soil<sup>3,7,23)</sup>.

Hokkaido is one of the four major islands of Japan, where population density is comparatively low with a large number of domestic and wild animals in relatively preserved natural environment. To provide information on the distribution of *P. aeruginosa* in environment, a bacteriological surveillance study was made in a area along Ushubetsu River in Hokkaido in the present study.

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## Materials and methods

### Samples

Soil samples were collected around the tomato stalks in the field of Hokkaido University of Education, Asahikawa. Wet sand samples were collected from sandboxes in 20 different vest-pocket parks in Asahikawa City. Packages of vegetable salad mainly containing sliced cabbage and lettuces were purchased in a market in Asahikawa. Fecal samples of pigs were collected from the rectum in the slaughterhouse. Fresh fecal samples of deer were collected on the bank at Hakuyodaira at the upstream of Ishikari River. Those were collected in spring and summer in 1987.

Water samples were collected from

Ushubetsu River, one of the tributaries of Ishikari River, at the bridges of Pepan, Mizuho, Oiwake, Katori, Isao, Hinode, Shin, and Kojin (Fig. 1). Pepan is located in forest area, near by the riverhead. Mizuho and Oiwake are located in paddy field, and there are small pastures upper stream of these two sampling sites. Katori and Isao are located in residential area of Asahikawa, and Hinode and Shin in central Asahikawa where population density is relatively high and a paper mill runs at 2 km upper of Hinode. Kojin, where Ushubetsu River joins Ishikari River in Asahikawa. Sampling of the river water was carried out 6 times between 12 October 1984 and 30 July 1985.

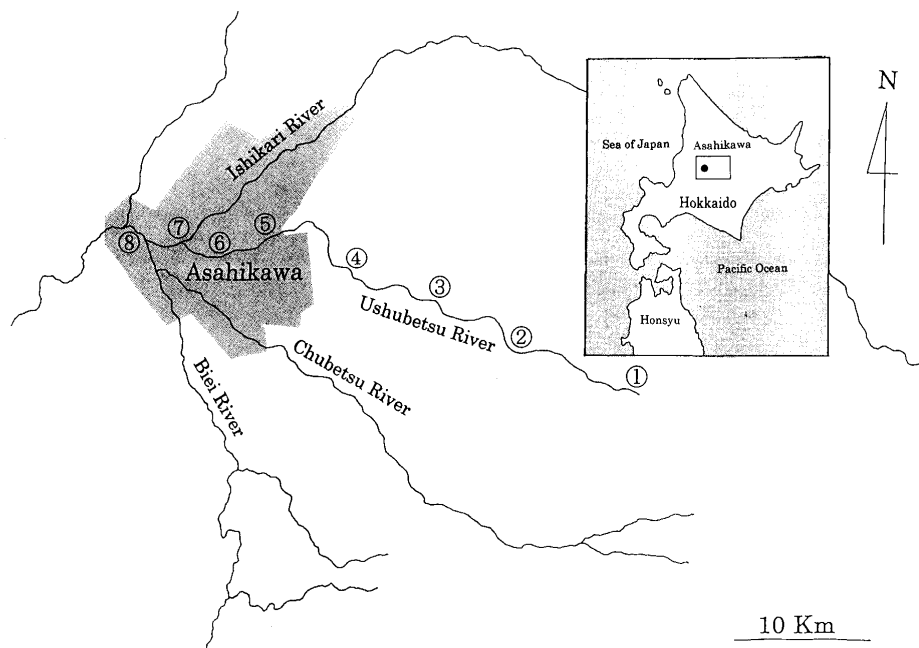


Fig. 1. Location of sampling sites of Ushubetsu River water, one of the tributaries of Ishikari River, Hokkaido, Japan. Sampling sites are upper to downer along the stream, Pepan (①), Mizuho (②), Oiwake (③), Katori (④), Isao (⑤), Hinode (⑥), Shin (⑦), and Kojin (⑧). Pepan located in forest area, near by the river head, Mizuho and Oiwake located in paddy fields, Katori and Isao are in the residential area of Asahikawa City, Hinode and Shin are located in central Asahikawa where densely populated and there is a paper mill 2 km upper of Hinode. Kojin is located at the south end of Asahikawa. The bar indicates 10 km.

### Isolation and identification of bacteria

Each one g of soil, sand, fresh vegetables, and fecal samples of animals was inoculated into 10 ml of nalidixic acid-cetrimide (NAC) broth (Eiken Chemical Co., Ltd. Tokyo, Japan) and incubated at 37°C. After incubation for 24 to 96 hours, a loopful of the culture was spread on a NAC agar plate and incubated at 37°C.

Each ten fold dilution of river water samples was inoculated into 5 tubes of equal volumes of 2 times concentrated NAC broth. After incubation for 24 to 144 hours, a loopful culture was inoculated on NAC agar and incubated at 37°C. Enumeration of *Pseudomonas aeruginosa* isolates was done by the most probable number procedure<sup>1)</sup>.

Isolated bacterial cells were Gram-stained, tested for the growth at 42°C, oxidative versus fermentative glucose metabolism, cytochrome oxidase, oxidation of gluconate, and production of pyocyanin on King's A medium (Eiken Chemical Co., Ltd. Tokyo, Japan). These were then identified as *P. aeruginosa* by ID test NF-18 (Nissui Pharmaceutical Co., Ltd. Tokyo, Japan) or Biotest No 2 (Eiken Chemical Co., Ltd. Tokyo, Japan). The characteristics used for identification of isolates included dehydration of arginine, acylamidase test, mobility, gelatin liquefaction, fermentation of lactose, saccharose, xylose, maltose, arabinose, and mannitol, reduction of citrate, production of indole, and utilization of urea.

For the isolation of fecal coliforms from samples other than river water, one g of each sample was inoculated into 10 ml of brilliant green-lactose bile broth (BGLB; Eiken Chemical Co., Ltd. Tokyo, Japan) and incubated at 44°C. In each river water sample, numbers of total and fecal coliforms were determined by the presumptive, confirmed, and complete tests<sup>1)</sup> using lactose broth (Eiken Chemical

methylene blue (EMB; Eiken Chemical Co., Ltd. Tokyo, Japan) agar, respectively. Isolated bacteria were subcultured on EMB agar and colonies were examined by Gram-staining and IMViC, and identified by ID-test EB-9 (Nissui Pharmaceutical Co., Ltd. Tokyo, Japan) or Biotest No-1 (Eiken Chemical Co., Ltd. Tokyo, Japan).

### Results

#### Isolation of *P. aeruginosa* from river water

*P. aeruginosa* isolation from river water samples collected in Pepan, Mizuho, and Oiwake, located in the forest and field areas, was negative in 6 independent tests. *P. aeruginosa* was consistently isolated from the river water samples collected at the sites with large population and downer stream (Fig. 2). At Hinode where a paper mill factory runs 1 km upper stream, *P. aeruginosa* was isolated from the water samples. At Shin, 5 *P. aeruginosa* were isolated per 100 ml water at 5 cases out of 6 examinations. *P. aeruginosa* was not isolated from the samples collected on 5 November 1984. At Kojin, 2 to 17 *P. aeruginosa* were isolated at 5 cases out of 6 examinations. *P. aeruginosa* isolation was negative for the samples collected on 10 July 1985. At Katori, *P. aeruginosa* isolation was positive in 3 out of 6 examinations, of which enumerated numbers were 2, 5, and 6 per 100 ml. At Isao, 5 *P. aeruginosa* per 100 ml water sample was isolated once collected on 22 October 1984.

Fecal and total coliforms were isolated from water samples collected at Pepan and the number increased with the downer stream along the river.

In six independent examinations, *P. aeruginosa* was isolated from water samples collected at densely populated area and downer stream, but not isolated at the area of forest or field with little or no population. The

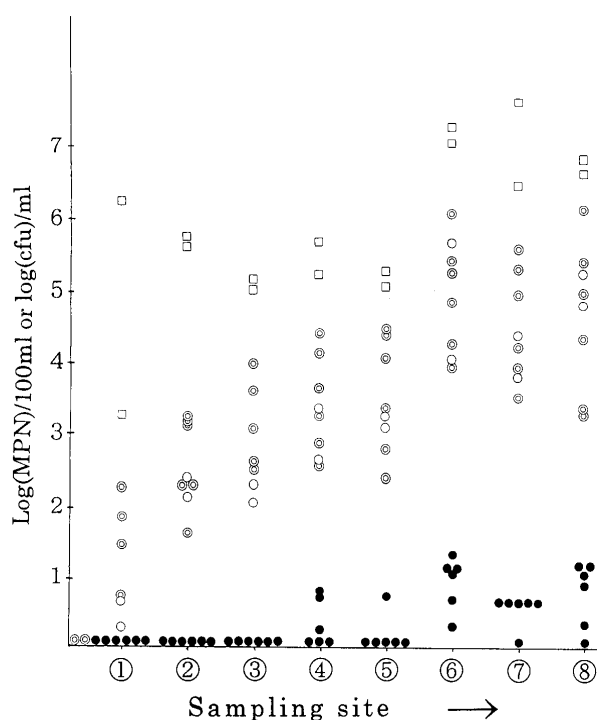


Fig. 2. Number of *Pseudomonas aeruginosa* (●), fecal coliforms (○) and total coliforms (◎) per 100 ml, and CFU of plate counts (□) per ml in Ushubetsu River, one of the tributaries of Ishikari River, Hokkaido, Japan. Sampling was done on, 12, 22 and 30 October, and 6 November, 1984, and 10 and 30 July, 1985. Sampling sites are ①: Pepan, ②: Mizuho, ③: Oiwake, ④: Katori, ⑤: Isao, ⑥: Hinode, ⑦: Shin and ⑧: Kojin. The river flows ① to ⑧ as indicated by the arrow.

currence of *P. aeruginosa* in river water.

#### *Isolation of Pseudomonas aeruginosa from feces, soil, sand, and vegetables.*

*P. aeruginosa* was isolated from fecal samples of pigs and not from those of deer (Table 1). No *P. aeruginosa* was isolated from soil samples of tomato field, sand of vest-pocket parks in Asahikawa City, or sliced fresh vegetables. Fecal coliforms were isolated from 7 of 65 soil samples and 9 of 20 sand samples.

#### Discussion

To provide information on the distribution of *Pseudomonas aeruginosa* in natural environment, especially in river water in the

defined area of Hokkaido, bacteriological surveillance was carried out in the present study. *P. aeruginosa* was isolated from water samples of Ushubetsu River and the feces of pigs, and not from soil, sand, fresh vegetables, or feces of deer. It has been known the presence of *P. aeruginosa* in water environment, such as river, pond and waste water<sup>5,9-11,13,24</sup>. *P. aeruginosa* isolated from water samples of Ushubetsu River may have derived from human activity in the urban area of the populated City Asahikawa.

*P. aeruginosa* is distributed in the intestines and fecal samples of human and animals<sup>12,16,19-21,24,26,27</sup>. In the present study, fecal coliforms was isolated from fecal samples of wild deer or from sand of vest-pocket parks where dogs and cats often visit, while *P. aeruginosa* was not isolated from these samples. The origin of these *P. aeruginosa* strains isolated from the water samples of Ushubetsu River could be in humans and domestic animals. *P. aeruginosa* was isolated from wild animals<sup>12</sup>. Further epidemiological studies are required to provide information on the distribution of *P. aeruginosa* among wild animals, such as rats, rabbits and foxes. *P. aeruginosa* was not isolated from the river water samples at Pepan in the area of forest. *P. aeruginosa* was not either isolated from the river water samples collected at Mizuho and Oiwake where small number of cattle and pigs were bred. *P. aeruginosa* was isolated from the river water samples collected at Hinode and Shin, residential and industrial areas with no livestock.

The present results, thus, support the notion that *P. aeruginosa* in the river water originates from the feces of domestic animals and humans<sup>12,13,24</sup>. To provide information on the relationship between environmental and clinical isolates of *P. aeruginosa*, genotypes and biological characteristics of the isolates

Table 1. Isolation of *Pseudomonas aeruginosa* and fecal coliforms from environment

Sample <sup>a)</sup>	<i>P. aeruginosa</i>	Fecal coliforms
Soil of tomato field	0/65 <sup>b)</sup>	7/65
Sand from vest-pocket parks	0/20	9/20
Feces of pigs	5/35	35/35
Feces of wild deer	0/19	19/19
Fresh vegetable salads	0/10	0/10

<sup>a)</sup>Samples were collected from the tomato field of Hokkaido University of Education, sand boxes of vest-pocket parks in Asahikawa City, feces of the recta of slaughtered pigs, fresh feces of wild deer collected at Hakuyo-daira located on upper Ishikari River, and vegetable salads, purchased in a market in Asahikawa.

<sup>b)</sup>Number of positive cultures per samples tested.

are under investigation.

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