Bacterial Abundance and Heterotrophic Activity in Sudong Stream

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수동천에서의 세균의 분포와 생리적 활성도

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ABSTRACT: The density of heterotrophic bacterial population and heterotrophic activity were measured at monthly interval from March, 1986 to March, 1987 at four sites in the Sudong Stream, a tributary of North Han River. Total bacterial numbers and maximum uptake velocity (V_{max}) of glucose ranged as 0.8 – 25.2 × 10^5 cells/m*l*, 0.0006-24.39 μgC/1/hr, respectively. V_{max} of glucose showed marked seasonal periodicity, with highest values in summer. But density of viable bacteria varied considerably, with no definite seasonal pattern. At the uncontaminated site which located in upstream, heterotrophic bacterial population and activities were relatively low, and small variations were observed downstream flowing except the site where animal originated sewage inputs occurred. And this large input of allochthonous materials and bacteria was an important factor for the stream condition.
KEYWORDS □ Sudong stream, heterotrophic bacteria, distribution, heterotrophic activity.

Suspended bacteria are abundant in running freshwaters and potentially important as heterotraphic degrader. Including recycling activity of inorganic matter, bacterial role of organic matter degradation contributed to biological self-purification process. Same studies reported to figure out the distribution pattern of suspended bacteria in stream water. Bacterial abundance and heterotrophic activity is mainly influenced by physicochemical variables in streams, for example, temperature, water discharge and concentration of suspended solids (Baker and Farr, 1977; Bell *et al.*, 1980; Nuttall, 1982). Seasonal variation of total bacterial number (Geessey and Costerton, 1979; Marxsen, 1980), and total viable counts (Starzecka, 1979; Morikawa, 1984) were reported. But they found that there was no obvious seasonal pattern. Seasonal variations of heterotrophic activity in temperate rivers, however, shows similar summer maxima (Bell *et al.*, 1980; Goulder, 1980).

Previously we investigated taxonomic composition of bacterial population in mountain headstream (Choi and Kim, 1987). In this study, we reported seasonal variation of bacterial population and heterotrophic activity in the same area. Also its regional distribution, specially concerned with animal-originated fecal contamination, was included.

MATERIALS & METHODS

Site description

Four samples were taken from the surface water of the Sudong stream, a tributary of North Han River (Fig. 1). The principal source rises from the Mt. Sori and several subsidiary streams

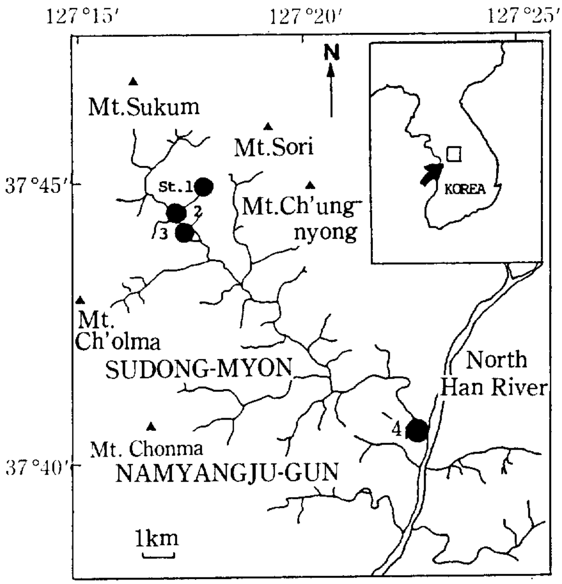

Fig. 1. *Map of the research area and sampling sites.*

increase stream discharge and organic input which contains domestic, farmland and animal-originated sewage. Especially, fecal discharges from farm animals (3500 pigs) were present between the principal source and the Site 2. The stream bed was mostly gravel or sand except the principal source which shows some accumulation of fallen leaves. From the principal source to the joining site with main river, water retention time was about 20 hrs (estimated on June 1986).

Sample Collection

Sample were collected in sterile 2L screwcapped polypropylene bottles (Nalgene, USA) between 9:00 a.m. and 11:00 a.m. at about monthly intervals from March 1986 to March 1987. Concentrations of inorganic ions were measured according to the "Standard Methods for the examination of water & wastewater" (APHA, 1985).

Total bacterial numbers were determined with the AODC method of Hobbie *et al.* (1977), which uses Nuclepore membrane filter (0.2 μm pore size, ϕ 25 mm) counterstained with Sudan black B (Merck). Epifluorescence microscope (Nikon, Labophot) was used and the bateria in 30 randomly selected fields were counted until 100 to 1000 cells were accumulated in the tally. Biovolume was determined from size spectrum of cocci and rod-forms. Using conversion factor, 0.121 pgCμm^{-3}, bacterial biovolume was converted to biomass (Watson *et al.*, 1977; Fuhrman, 1981; Krambeck *et al.*, 1981).

Colony-forming bacteria were counted on ZoBell's 2216e medium with distilled water. Incubation was carried out with pour plate method and incubated at 25°C in dark incubator for 14 days. In case of fecal coliforms, EC medium (Difco) was used and counted after incubation for 44 hrs at 44°C.

Maximum uptake velocity (V_{max}) and turnover time (T_t) for glucose assimilation was used as an indicator of heterotrophic activity (Gocke, 1977). Radioactive tracer, U-D-[^{14}C]-glucose (Amersham), was added to each smaple as a final concentration of 0.5, 1.0, 1.5, 2.5, 5.0 and 10.0μg-Cl^{-1}. Following to shaking incubation for 2 hours at the *in situ* temperature, reaction was stopped with prefiltered formalin as a final conc. of 0.4% (v/v). Fixed samples were filtered through 0.2μm millipore filter and the membrane was added into scintillation vial with 5m*l* of Dioxane-based scintillation cocktail solution. Average cpm values were counted for 2 min with the aid of a Packard Tri-Carb Liquid Scintillation Spectrometer (model 3385, Packard Co., USA).

All variables were tested for significant relationship using VAX-11. SPSS was used to obtain the Nonparametric Spearman Rank correlation coefficients.

RESULTS

Seasonal variation of physicochemical factors are given in Table 1. Mean values of water discharge ranged with 10.6-70.6m^{3}min^{-1}. Because of great precipitation, maximum discharge was recorded in September 1986. From June to October 1986, mean water temperatures were measured above 10°C. And the stream was covered with ice during January and February, 1987. Hydrogen ion concentrations of water

Table 1. *Seasonal variations of mean values of physicochemical factors. Ranges of the measured values are represented in the parenthesis.*

parameters Date	Temp. (°C)	pH	Discharge (m^3/min)
'86. 3	6.0 (2.0- 7.0)	6.2 (5.1-6.8)	14.9 (0.2- 38.3)
4	7.0 (3.0- 9.5)	6.8 (6.5-7.3)	16.5 (0.1- 53.6)
5	7.3 (5.5-12.5)	6.5 (5.9-7.0)	24.8 (0.7- 76.6)
6	10.3 (4.0-22.0)	6.7 (6.3-7.0)	10.6 (0.2- 23.0)
7	18.0 (14.5-21.5)	6.9 (6.5-7.3)	20.1 (0.2- 47.9)
8	19.3 (17.0-21.5)	7.1 (7.0-7.3)	35.0 (0.5- 76.9)
9	13.2 (12.0-14.2)	7.1 (6.8-7.4)	70.6 (0.2-191.5)
10	12.0 (8.6-14.4)	7.1 (6.9-7.1)	45.7 (0.5-114.9)
11	3.9 (3.0- 5.0)	7.0 (6.9-7.1)	46.9 (0.1-143.6)
12	3.0 (2.5-3.0)	7.0 (6.8-7.1)	22.3 (0.1- 76.6)
'87. 3	4.9 (3.5-6.5)	6.7 (6.6-6.8)	30.0 (0.4- 96.5)

parameters Date	Ammonia (μg/L)	Nitrite (μg/L)	Nitrate (μg/L)	Phosphate (μg/L)
'86. 3	38.76 (10.34-103.76)	39.48 (16.28- 93.79)	50.81 (19.97- 72.17)	233.32 (28.62- 757.47)
4	22.97 (9.03- 50.08)	35.26 (10.48- 88.51)	79.81 (32.10-143.88)	129.32 (59.98- 233.32)
5	17.71 (12.18- 27.97)	73.75 (36.05-159.69)	72.70 (36.05-116.99)	702.17 (50.90-1583.73)
6	14.55 (9.55- 16.66)	22.08 (9.95- 37.37)	151.26 (25.77-295.20)	116.11 (40.17- 261.39)
7	20.08 (13.76- 23.50)	21.29 (11.53- 38.42)	190.80 (41.85-365.33)	326.60 (36.87-1063.71)
8	13.50 (11.92- 14.82)	66.10 (9.95-195.29)	43.43 (27.88- 70.06)	264.69 (62.46- 617.97)
9	22.71 (13.50- 43.76)	93.26 (9.16-323.15)	161.28 (30.25-280.97)	286.98 (31.09- 931.64)
10	34.03 (14.29- 81.66)	43.96 (9.42-118.30)	29.20 (13.91- 42.64)	226.72 (31.92- 675.75)
11	12.97 (10.08- 19.29)	26.03 (10.74- 57.67)	34.47 (22.87- 50.55)	307.61 (84.75- 704.64)
12	29.82 (10.08- 76.13)	20.50 (8.37- 45.28)	37.63 (11.53- 76.39)	173.89 (26.96- 479.30)
'87.3	19.82 (1.39- 67.97)	35.26 (1.25-117.51)	260.93 (74.28-418.06)	173.89 (9.63- 556.07)

samples were relatively unchanged, but slightly higher value was observed in summer and autumn. In the forest and grassland soil samples around the research area, pH was considerably low, measured as 4.8-5.2 and 5.3-5.4, respectively. Though seasonal variations of inorganic ions fluctuated irregularly, minimal and maximal concentrations were always measured at the principal source and site 2 (Spatial variation data were not shown in Table 1).

Total bacterial number(TBN) and saprophytic number at the principal source were always lower than downstream sites (Fig. 2). And the range of seasonal variation of TBNs were greater at Site 2 than other sampling sites. Also this is obvious in case of distribution pattern of saprophytes. Seasonal distribution Patterns of bacterial numbers varied irregularly, but they were positively correlate with stream water discharge, especially at Site 2. Distribution pattern of fecal coliform bacteria showed typical summer high, winter low pattern, because rising of water temperature enhanced the bacterial survivability. Also in principal source, relatively uncontaminated site, fecal coliform bacteria remained as constant level (0-6 CFUs/m*l*).

Bacterial biomass distributions were similar as the pattern of TBNs. Especially in December 1986 at Site 2 and 3, showed maximum values of their annual periodicity.

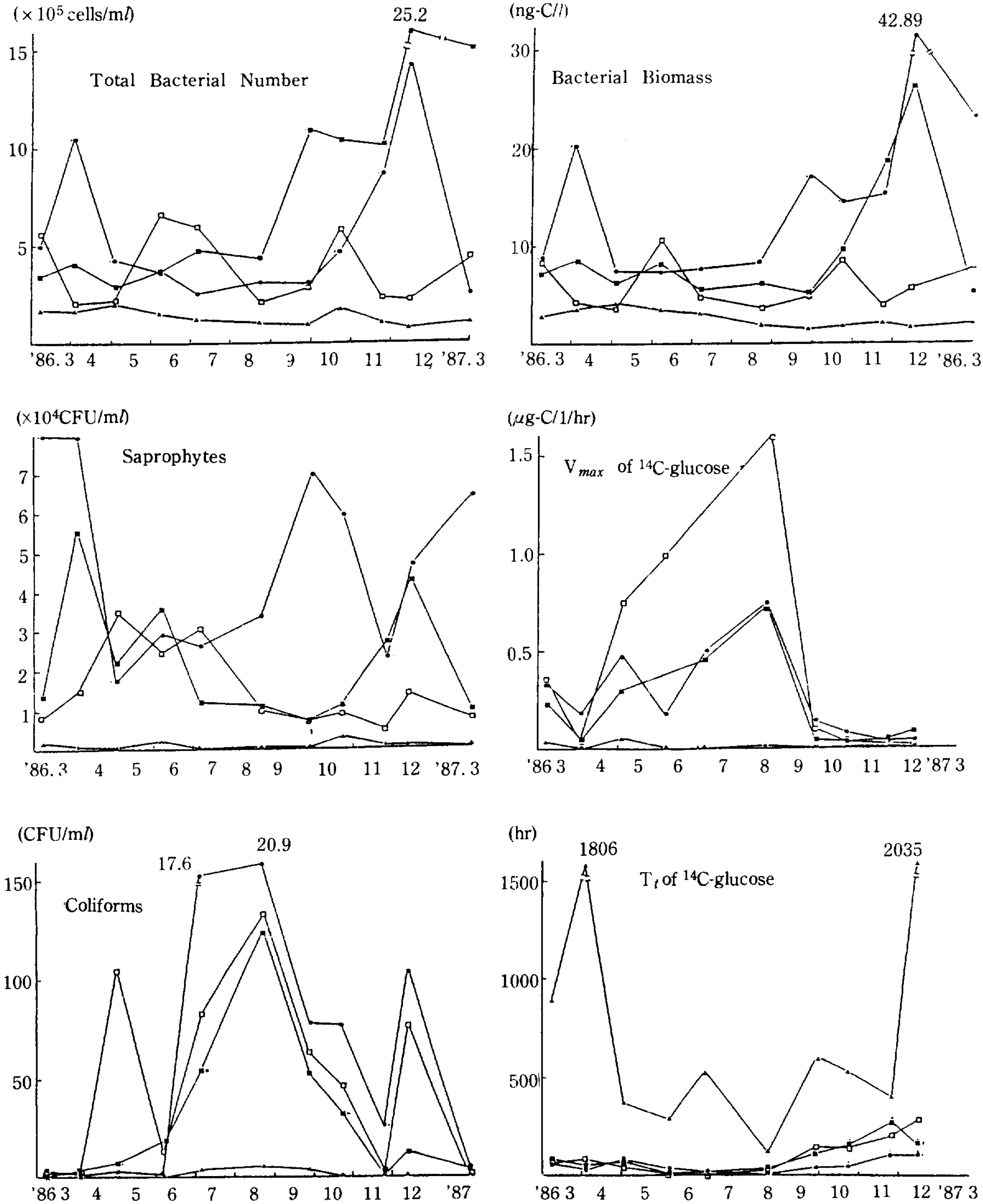

Fig. 2. *Seasonal variations of bacterial number, biomass and heterotropnic activity in Sedong Stream.*
(▲; site 1, ●; site 2, ■; site 3, □; site 4)

Glucose assimillation rates as bacterial heterotrophic activity parameters showed obvious positive correlation with water temperature. In summer, though TBN and saprophytic bacterial numbers at Site 4 were lower than Site 2, they were reversed in case of maximum uptake velocity (V_{max}) of glucose. Turnover times of glucose were ranged as 4-2035 hr, and somewhat smaller fluctuations were observed in downstrean sites. Also they were not exceed 300 hrs of turnover of natural quantities of glucose.

Distribution patterns of glucose assimilation and the density of coliform bacterial populations showed highly temperature- coliform aspects, but total bacterial number and saprophytes fluctuated irregularly. At the principal source, all variables were observed as relatively uncontaminated features. Greatly increased biological variables at the Site 2 level off to relatively constant in the downstream sites (St. 3 & 4).

DISCUSSION

Concentrations of directly-counted bacteria in our research area ($0.8 - 25.2 \times 10^5$ cells/ml) similar to the range of River Ouse, Derwent (Goulder, 1986) and slightly abundant compared to the headwaters of Mill Beck, a calcareous stream in North Humberside (Goulder, 1984). They observed that downstream increase of TBNs were gradual, not a stepwise process. Because release from aquatic vegetation and the stream bed was major source of bacteria (Baker and Farr, 1977). In this study, including this source, animal-originated sewage outfall at Site 2 caused drastic bacteria-rich inputs. Nearly all around a year higher concentrations of TBNs were measured at the Site 2. And its seasonal variation was more fluctuated that other sites. This, presumably, is due to the sudden change of animal farm outfalls.

Maximum uptake velocity and turnover time of glucose correlated well with water temperature (V_{max}; $r = 0.4544$, $P<0.001$, $n = 149$: Tt; $r = -0.5199$, $P<0.001$, $n = 150$). Total bacterial number and saprophytes, however, showed no correlation with water temperature above 95.0% significance level. It might be true that the density of bacteria which are capable of assimilating labelled organic substrates confined to a portion of metabolically active bacteria. And these group also participated as a small group in total or saprophytic bacteria. Studies using autoradiography have shown that there is not necessarily a relationship between the density of viable bacteria and metabolically active bacteria (Ramsay, 1974; Ramsay & Fry, 1976).

Principal source (St. 1) originated from Mt. Sori, and flowed down to the animal farm which located at just upstream of Site 2 (Fig. 1) This subsidiary stream channeled into the main stream which originated from Mt. Sukum. Joining to the main stream caused sharp decrease of annual averages of inorganic ion concentration (St. 3:St. 2≅1:2), TBN (1:2), saprophytes (1:2.5) coliform bacteria (1:6), and V_{max} of glucose (1:20). Presumably these sharp decreases were explained by following reasons; (1) Dilution effect happened with the joining of main stream water mass. Just before the joining, main stream site measured extra at 400m apart from the joining point contains only 1/24 numbers of saprophytes and 1/2 of total bacteria compared to the subsidiary stream. Also the amount of stream discharge was measured at the main stream about 3.5 folds greater. (2) Sedimentation through adhesion to the suspended solids, and/or attachement to aquatic vegetations. (3) Predation by possible bacteriovores. That two reasons (2 & 3) above mentioned are not favorable, presumably, because the Site 3 located at a distance of only 0.3 km from Site 2. Another reason for the decreasing of bacterial density is (4) Deaths of fecal coliform bacteria which exposed suddenly to the natural environments. This explains why coliform bacterial group dramatically decreased compared to TBNs or saprophytes.

In this study area, allochthonous inputs of farmland sewages including animal feces were major source of bacteria. Introduced bacterial group, however, showed sharp decrease in population density and heterotrophic activities. It is likely that these bacteria did not thrive in stream water as a free-living state, and possibly, therefore, attached to particles. Goulder (1986) found that particle-bound bacteria appeared to contribute little to the overall heterotrophic activity of suspended bacteria, and he suggested that particle-bound bacteria were principally allochthonous. Using ^{3}H-methyl-Thymidine, doubling time of bacteria was estimated as 58.6-137.4 hrs (estimated on August 1986). Considering that the water retention time was too short (20 hrs) in the Sudong Stream, cell division of the bacterioplankton was not possible except attachment of bacteria (Hossell & Baker, 1979).

Although the density and heterotrophic activities of allochthonous bacterial population from the animal farm sewage was decreased rapidly by dilution and inactivation, bacterial density were maintained as increased values at the sites of downstream (St. 3-St. 4) than the principal site. Marxen (1980) found that allochthonous bacterial inputs were unimportant in small streams. In contrast to this observation, bacterial density and heterotrophic activities in Sudong Stream were significantly affected by allochthonous input.

적 요

북한강 수계의 지류인 수동천에서 1986년 7월부터 1987년 3월까지 4개의 정점으로부터 종속영양 세균의 크기와 생리적 활성도를 조사하였다. 총 세균수는 0.8−2.5×10^5 cells/ml의 범위를 나타냈으며, 글루코오즈 흡수능(V_{max})은 0.0006−24.39 μg-C/l/hr의 변화폭을 보여주었다. 계절적인 분포에 있어서 V_{max}값은 여름에 높고, 겨울에 낮은 뚜렷한 계절적 양상이 관찰되었으나 콜로니 생성균 수의 변화는 특징적 양상을 볼 수 없었다. 지역적으로는 인간활동의 영향을 거의 받지 않는 상류 정점에서 세균수와 생리적 활성도가 가장 낮으며, 유기 오염물질이 대량 유입되는 정점을 제외하고는 유사한 수준을 나타내어 이러한 유입 현상이 전 수동천 생태계의 세균 군집의 크기 및 생리적 활성도의 변화에 가장 중요한 요인으로 작용함이 관찰되었다.

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