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ANTIBIOTIC SENSITIVITY OF OLIGOTROPHIC AND EUTROPHIC BACTERIA

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Forty-three isolates of oligotrophic bacteria and forty-three isolates of eutrophic bacteria were tested with the use of standard paper disks for their sensitivity to ten antibiotics, ampicillin, penicillin G, streptomycin, kanamycin, chloramphenicol, tetracycline, puromycin, mitomycin, rifampicin, and polymyxin B. Almost of the oligotrophic bacteria were multiple antibiotic resistant bacteria and were particularly resistant to ampicillin, penicillin G, streptomycin, chloramphenicol, puromycin, and mitomycin. Majority of the oligotrophic bacteria (83.7%) were resistant to more than 6 antibiotics. About 60% of the oligotrophic bacteria were sensitive to kanamycin, rifampicin, and polymyxin B. On the other hand, about more than 60% of the eutrophic bacteria were sensitive to the highest concentrarions of 10 antibiotics. 5 strains of the eutrophic bacteria (11.6%) were resistant to more than 6 antibiotics. Sensitivity to 10 antibiotics was highest (53.5%), followed by 9 antibiotics (14.0%).

Key words : oligotrophic bacteria, eutrophic bacteria, antibiotics, sensitivity.

Introduction

A large number of bacteria live and survive under various environments. Organisms capable of growing under very low nutrient broth media are called oligotrophic bacteria[1,2]. Various definitions of the oligotrophic bacteria have been suggested[1,3]; we define the oligotrophic bacteria as heterotrophic bacteria which are able to multiply at least 10-fold during 10-day cultures when the bacteria are inoculated at a concentration of 10^3 cells/ml on organic media containing less than 1 mg of organic carbon per liter. A 10^4-fold dilution of nutrient broth (NB/10^4) contains about 0.8 mg of organic carbon per liter, and was used as a selective medium for soil oligotrophs by Suwa and Hattori[4]. We isolated oligotrophic bacteria that grew in NB/10^4 with doubling times of 1.5 to 3 days and reached final yields of 5.2 to 8.6 x 10^6 colony-forming units (CFU) per ml[5]. During exponential growth in NB/10^4, the oligotrophic bacteria maintained a high level of adenylate energy charge.

Oligotrophic bacteria can utilize a wide variety of carbon compounds to take advantage of whatever substrates become available in low nutrient environments[6]. The oligotrophs, therefore, may play an important role in decomposing organic matter and recycling materials in low nutrient enviroments. The physiological properties of oligotrophs have been studied in bacteria isolated from the sea or from soil[7-9]. Many marine oligotrophs have both low and high affinity uptake systems at different substrate concentrations[10]. Characteristics of soil oligotrophs are thought to possess uptake systems of relatively high affinity and low substrate specificity for nutrient substances[7]. We isolated oligotrophs except for prosthecate bacteria from soil and investigated nutrient uptake of the oligotrophs. However, there were no significant differences of uptake kinetics between the three oligotrophic bacteria (*Aeromonas* sp. Y26, *Aeromonas* sp. Z06, and *Chromobacterium* sp. Y95) and *Escherichia coli*[5].

In natural world, bacteria obtained resistant-antibiotics is one of important means for survival. There is little known about antibiotic sensitivity of oligotrophic bacteria. In the present paper, sensitivity profiles against ten antibiotics were determined for 43 oligotrophic and 43 eutrophic bacteria isolated from soil.

Materials and methods

Media and culture conditions

Nutrient broth (NB) medium contained 10.0g each of polypeptone (Wako Pure Chemical Industries) and meat extract (Wako Pure Chemical Industries), 5.0g of NaCl per liter of Milli-Q water, pH 7.0. The NB was diluted with ultra pure water, which was prepared by treatments of reverse osmosis, distillation, and electric ion exchange, in the rations 1 : 50, 1 : 100, and 1 : 10000. Liquid cultures were incubated with gentle shaking at 28℃.

Isolation of bacteria

Oligotrophic and eutrophic bacteria were isolated from soil in Shikoku, Japan. The soil samples, each 0.5 g, were shaken in 10 ml of sterilized water and filtered with filter paper (No. 2, Toyo Co.). After suitable dilution, 0.2-ml aliquots of the filtrates were spread on NB or 100-fold dilution of NB (NB/10^2) agar plate containing 50 μg/ml of cycloheximide. After 5 to 10 days of incubation at 28℃, obtained colonies from the NB plates were isolated as eutrophic bacteria. Bacteria which are unable to grow in low-nutrient conditions but grow in high- nutrient condition such as NB medium have been called eutrophs. Oligotrophic bacteria were isolated according to the previously described[5]. The oligotrophic bacteria did not grow in NB medium. The colonies which have different colony morphology were selected. All isolates were confirmed to be bacteria by microscopic observations.

Sensitivity test against antibiotic

Antibiotic sensitivities were determined by the paper disc method. The isolate bacterium (about 10^4 cells) was mixed with melted NB/50 containing 1.5% of agar in plate. After the agar became solid, filter papers (10㎜ in diameter), saturated with the appropriate antibiotic solution, were placed upon the surface of the agar culture medium. The antibiotics used in the assay were the following : ampicillin, penicillin G, streptomycin, kanamycin, chloramphenicol, tetracycline, puromycin, mitomycin, rifampicin, and polymyxin B. The concentrations of antibiotics used are given in Table 1. Inhibition zone was observed after the plates were incubated at 28℃ for 3 - 7 days (depending on the rate of growth).

Results and discussion

When oligotrophic isolates were grown on NB/10^4 for 5 days, cell densities of the isolates reached 0.2 - 5 $\times$ 10^6 CFU/ml. Eutrophic isolates did not grow on NB/10^4.

The results of antibiotic sensitivity assay are shown in Fig. 1. About half the oligotrophic bacteria were sensitive to the highest concentrations of kanamycin, tetracycline, rifampicin, and polymixin B. However, almost oligotrophic bacteria (80-95%) were resistance to ampicillin, penicillin G, streptomycin, chloramphenicol, puromycin, and mitomycin. Although streptomycin and kanamycin are basic oligosaccharide group antibiotics, the both antibiotic actions were different on the oligotrophic bacteria. Misumi *et al.* reported that streptomycin acts on 30 S-subunit of ribosome, and kanamycin acts on both 30 S-and 50 S-subunits of ribo-

Table 1. Concentrations of antibiotics

Antibiotics	L. conc.	M. conc.	H. conc.
Ampicillin	12.5	31.3	62.5
Penicillin G	1.0	5.0	25.0
Streptomycin	5.0	12.5	25.0
Kanamycin	1.0	5.0	25.0
Chloramphenicol	15.0	37.5	75.0
Tetracycline	10.0	25.0	50.0
Puromycin	100.0	250.0	500.0
Mitomycin	1.0	2.5	5.0
Rifampicin	15.0	37.5	75.0
Polymyxin B	200.0	500.0	1000.0

Three steps (L ; low, M ; medium and H ; high) of antibiotic concentarations (μg/ml) was used in the paper disk tests.

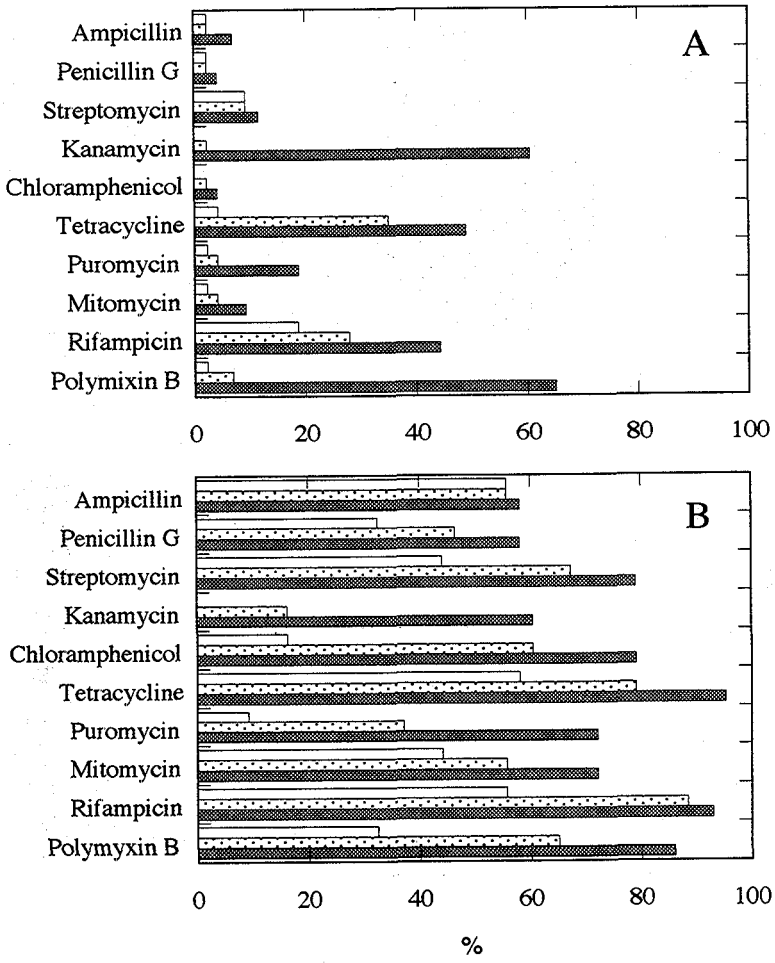

Fig 1. Sensibility of oligotrophic (A) and eutrophic bacteria (B) against antibiotics.
; low concentrations of antibiotics,
; medium concentrations of antibiotics,
; high concentrations of antibiotics

some [11].

On the other hand, more than 55 % of the eutrophic bacteria were sensitive to the highest concentrations of all antibiotics. The growth of the eutrophic bacteria were especially inhibited by streptomycin, chloramphenicol, tetracycline, rifampicin, and polymixin B. The sensitivity to kanamycin of the eutrophic bacteria showed similarity to that of the oligotrophic bacteria.

Fig. 2 shows number of sensitivity antibiotics per strain. On the highest concentrations of antibiotics, the number of sensitivity antibiotics was counted. Majority of the oligotrophic bacteria (83.7%) and 5

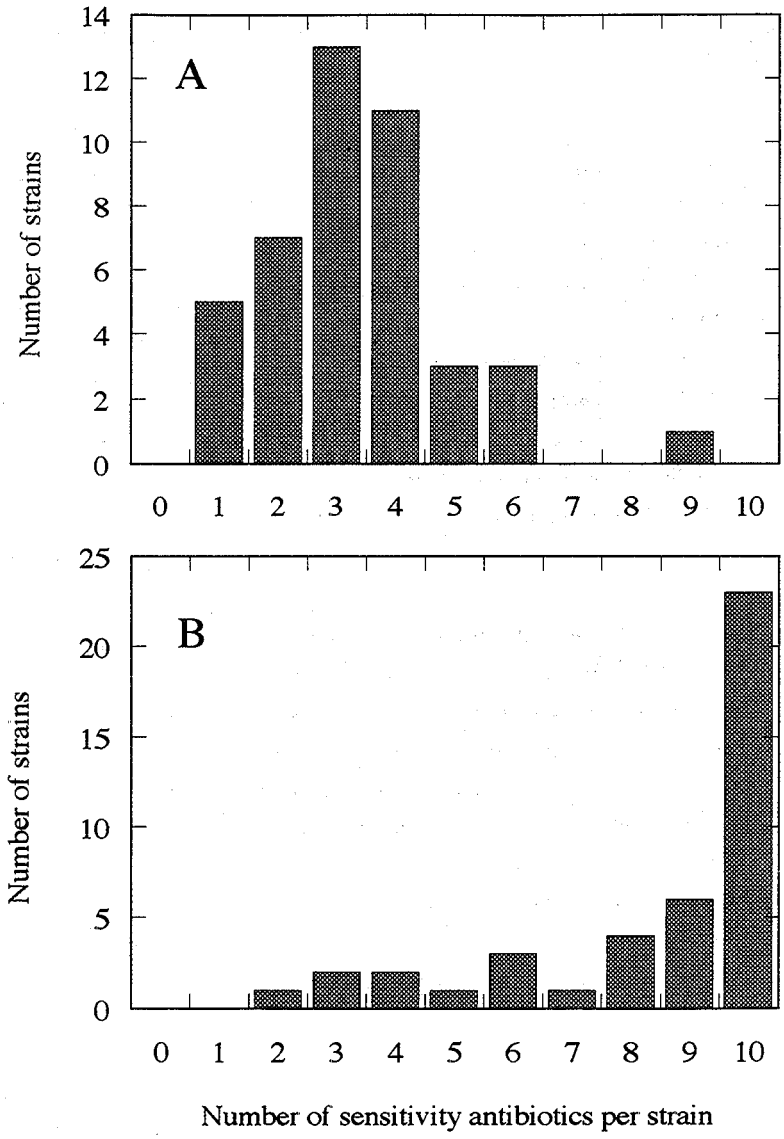

Fig 2. Number of sensitivity antibiotics per strain.
These graphs also show number of multiple-antibiotics resistance bacteria.
A, oligotrophic bacteria B, eutrophic bacteria

strains of the eutrophic bacteria (11.6%) were resistant to more than 6 antibiotics simultaneously. 53.5 % of the eutrophic bacteria were sensitive to 10 antibiotics. On the other hand, almost of the oligotrophic bacteria were multiple antibiotic resistant bacteria. Resistance to 7 antibiotics was highest (30.2%), followed closely by 6 antibiotics (25.6%). There are many reports on multiresistant bacteria. The multiresistant bacteria have become a clinical problem. Hatha *et al.* reported that 62 % of *E. coli*, which isolated from the Bhavai River, were resistant to more than four antibiotics[12].

The multiple antibiotic resistances are due to plasmids. It was suggested that perhaps the multiple-drug resistance was transferred from resistant organisms to sensitive ones. Many Gram-negative bacteria contain these resistance factors (R factors), and they transfer them to other Gram-negative bacteria[13]. They have not been found to occur in Gram-positive bacteria, nor has it been possible to transfer them to Gram-positive bacteria. On the other hand, Ohta and Hattori reported that about half oligotrophic bacteria are Gram-negative and 10 % Gram-positive (the remainder are irregularly Gram-stained organisms)[14]. In this situation, the oligotrophs may have capacity to grow under existence antibiotics, because most of the oligotrophic bacteria are Gram-negative bacteria.

Nikitin *et al.* also investigated antibiotic sensitivity of oligotrophic microorganisms[15]. They reported that the oligotrophic microorganisms were not multiple-antibiotics resistance microorganism, and the oligotrophic microorgamisms were resistant against antibiotics which affect membrane permeability (polymixin and gramicidin). Our results were different on those point. 65 % of our oligotrophic isolates were sensitivity to the highest concentration of polymixin B.

For survival in natural environment, the oligotrophic bacteria have multiple-antibiotics resistance is one of advantages. Further work is needed to determine the existence of R factors in the oligotrophic bacteria.

References

1) KUZNETSOV, S. I., DUBININA, G. A. and LAPTEVA, N. A. : Biology of oligotrophic bacteria. *Annu. Rev. Microbiol.* 33, 377－387 (1979).

2) FRY, J. C. : Oligotrophs. In C. Edwards (eds.), Microbiology of extreme environments. pp. 93－116. Open University Press, Milton Keynes. (1990).

3) ISHIDA, Y., and KADOTA, H. : Growth patterns and substrate requirements of naturally occurring obligate oligotrophs. *Microb. Ecol.* 7, 123－130 (1981).

4) SUWA, Y., and HATTORI, T. : Effects of nutrient concentration on the growth of soil bacteria. *Soil Sci. Plant Nutr.* 30, 397－403 (1983).

5) KIMURA, Y., UENO Y., KAJIKAWA, K., NAKAMURA, T. and SATO, M. : Physiological Characteristics of Three Oligotrophic Soil Bacteria, *Aeromonas* sp. Y 26, *Aeromonas* sp. Z06, and *Chromobacterium* sp. Y95, in Low Concentration of Nutrient Broth. *Bull. Jpn. Soc. Microb. Ecol.* 10, 21－29 (1995).

6) Van der KOOIJ, D., ORANJE, J. P., and HINJEN, W. A. M. : Growth of *Pseudomonas aeruginosa* in tap water in relation to utilization of substrates at concentrations of a few micrograms per liter. *Appl. Environ. Microbiol.* 44, 1086－1095 (1982).

7) POINDEXTER, S. J. : Oligotrophy. *Addv. Microb. Ecology.* 5, 63－89 (1981).

8) WHANG, K., and HATTORI, T. : Oligotrophic bacteria from rendzina forest soil. *Antonie Van Leeuwenhoek*, 54, 19－36 (1988).

9) KJELLEBERG, S., HERMANSSON, M., MARDEN, P., and LONES, G. W. : The transient phase between growth and nongrowth of heterotrophic bacteria, with emphasis on the marine environment. *Annu. Rev. Microbiol.* 41, 25－49 (1987).

10) ISHIDA, Y., IMAI, I., MIYAZAKI T., and KADOTA, H. : Growth and uptake kinetics of a facultatively oligotrophic bacterium at low nutrient concentrations. *Microb. Ecol.* 8, 23－32 (1982).

11) MISUMI, M., NISHIMURA, T., KOMAI, T., and TANAKA, N. *Biochem. Biophys. Res. Comm.* 84, 358 (1978).

12) HATHA, A. A. M., GOMATHINAYAGAM, P., and LAKSHANAPERUMALSAMPY, P. : Incidence of multiple antibiotic resistant *Escherichia coli* in the Bhavani river. *World J. Microbiol. Biotechnol.* 9,

609－610, (1993).
13) DAVIES, J., BRZEZINSKA, M., and BENZENISTE, M.: R factors: biochemical mechanisms of resistance to amino glycoside antibiotics. *Ann. N. Y. Acad. Sci.* 182, 226 (1971).
14) MITSUHASHI, S., HARADA, K., HASHIMOTO, H. *Japan. J. Exp. Med.* 30, 179 (1960).
15) NIKITIN, D. I., VISHNEVETSKAYA, O. Y., and ZLATKIN, I. V.: Grouping of oligotrophic microorganisms on the basis of their antibiotic resistance and dynamic membrane characteristics. *Microbiologiya.* 57, 210－215 (1988).

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低栄養及び高栄養細菌の抗生物質に対する感受性

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我々は土壌中より低栄養細菌43菌と高栄養細菌43菌を分離し，感受性ディスク試験により，10種の抗生物質に対する感受性試験を行なった．その結果，低栄養細菌の大部分は，複数の抗生物質に対して耐性を示した．

83.7％の低栄養細菌は6種以上の抗生物質に耐性を示した．特にアンピシリン，ペニシリン，ストレプトマイシン，クロラムフェニコール，ピューロマイシン及びマイトマイシンに対しては，ほとんどの低栄養細菌において耐性を示した．約60％の低栄養細菌はカナマイシン，テトラサイクリン，リファンピシン及びポリミキシンBに対して感受性を示した．一方，高栄養細菌は，60％以上の菌においてすべての高濃度の抗生物質に対して感受性を示し，6種以上の抗生物質に対して耐性を示した菌は5菌株（11.6％）しかなかった．高栄養細菌では10種全ての抗生物質に感受性である菌が最も多く，53.5％で，ついで9種の抗生物質に対する感受性菌が多く，14％であった．