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Bacterial Chemotaxis to Extracts, Exudates, Solutions in Vitro and Soil

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In vitro 및 土壤에서 抽出物, 滲出物, 溶液에 대한 細菌의 化學走性

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要　　約

土壤抽出物, 人蔘滲出物과 溶液이 *Pseudomonas* sp. *P. fluorescens* 및 *Erwinia carotovora*에 미치는 化學走性은 土壤抽出物處理, 滲出物 및 溶液은 蒸溜水 보다 이들 細菌移動에 영향을 나타냈다. 고무관내 흙으로 細菌의 移動性은 *P. fluorescens* 가 土壤抽出物處理 滲出物에서 走性을 나타냈으나, *P.* sp와 *E. carotovora*는 走性이 없었다. 細菌走性에 미치는 眞菌의 滲出液은 移動에 영향력을 나타내지 못하였다. 고무관내 흙을 眞菌으로 接種한 후에 細菌의 走性은 *Pseudomonas* sp.에서는 走性이 나타나지 않았으나, *P. fluorescens*와 *E. carotovora*는 *F. solani*, *F. oxysporum* 및 混合菌處理에서는 走性이 나타났다. *Alternaria panax*는 이들 細菌에 대해 走性이 없었으나, *E. carotovora*는 *A. panax*에 대해 주성이 있었다. 土壤有機物含量은 金浦·江華의 罹病地와 健全地에는 낮았고, K함량은 金浦의 健全地에 높았다. 細菌分布는 槐山의 罹病 및 健全地와 江華健全地에 眞菌은 錦山 槐山의 罹病 및 健全地에 많이 분포되었다. 放線菌은 槐山健全地에 특히 많이 分布하였고 다음이 豊基罹病地였다.

ABSTRACT

Accumulation of *Pseudomonas* sp., *P. fluorescens* and *Erwinia carotovora* in 60 min treatment was greater in extracts from soil, exudate from ginseng root and solutions than distilled water. In bacterial movement toward rubber tube soil from chamber, accumulation of *P. fluorescens* in response to soil supplemented with soil extracts, exudate and solutions was generally greater in soil extracts compared to control and other solutions, but *Pseudomonas* sp. and *E. carotovora* were not much response to supplemented extracts, exudate and

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solutions. Accumulation of the bacteria in capillaries containing various exudates from fungal propagules w not attracted to the exudates. For an accumulation of bacteria in rubber tubes containing soil inoculated wi fungal propagules, the *Pseudomonas* sp. was not attracted in soil inoculated by the organisms as attractant but *P. fluorescens* and *E. carotovora* to fungi were attracted to *F. solani*, *F. oxysporum* and mixed organism *Alternaria panax* did not affect on bacterial movement except *E. carotovora*. The organic matter conten in Kangwha and Kimpo soil were low in diseased and healthy soil. The K content was especially high in Kim healthy soil. Bacterial population in Goesan and Kangwha were more abundant than other soil. The number actinomycetes was populated abundant in healthy soil of Goesan and diseased soil of Poonggi.

Key words: Bacteria, chemotaxis, ginseng disease.

INTRODUCTION

Most of the motile bacteria are attracted by certain chemicals and repelled by others. Bacteria may swim in water-filled pores in soil at a sufficiently high matric potential, depending on soil texture (16. 17. 33), but little is known about bacterial chemotaxis to potential substrates in soil. Motile bacteria were attracted to a wide variety of organic chemicals in vitro(2.7), and *Rhizobium* spp. were attracted to exudates of leguminous and nonleguminous plants, which contained sugars, amino acids, alcohols and glycoproteins (9.14). *Rhizobium* spp. are also known to accumulate around plant roots in vitro, however it is not clear whether this is due to chemotaxis or to multiplication of bacterium (10). Fungi appear to play a role in bacterial movement and growth in soil, through hyphae serving as migration pathways, and hyphal and spore exudates as growth substrate (23. 30. 34). Cell wall components of *Pythium ultimum* were reported to be attractants to a *Pseudomonas* sp. in vitro (7) and *Bacillus pumilus* was attracted to germinating conidia of *Cladosporium cladosporioides* in vitro and on barley leaves (12). Fungal propagules and their exudates in soil may act as attractants for motile bacteria in soil (3.8). However, there is apparently no information concerning the attraction of motile bacteria to soil extracts and fungal propagules in soil. In the present study, the chemotactic responses of three motile bacteria to various soil extracts, solutions and exudates were studied in capillaries and soil.

MATERIALS AND METHODS

Bacterial culture and cell suspensions. Th following isolates of motile bacteria from tw genera of bacteria were used: *Pseudomonas* sp (strain EP8, isolated from diseased ginseng root i 1985), *P. fluorescens* (obtained from Dept. o Microbiology, Seoul National University) an *Erwinia carotovora (22).* These bacteria wer maintained on nutrient agar. Bacteria in 20m of nutrient broth (Difco) were grown at 27° with rotary shaking incubator (120 rpm) for 12 hr One ml of this culture was transfered to 10ml o nutrient broth and grown to optical density of 0. at 550nm. At this density the bacterial populatio was in exponential growth phase. Cells were washe three times with 50mM phosphate buffer by cer trifugation at 5000 rpm for 8 min at 4°C, and th cell number was adjusted to 10^9/ml as determine by optical density with 10mM phosphate buffe containing 10^{-4} disodium ethylenedimethyltetraac tic acid (Na_2 EDTA,)(2). Dilutions of this suspe sion of this suspenion were used for chemotacti experiment. Motility was determined by observin a bacterial cells suspended in phosphate buffe under microscope.

Fungal culture and spore suspensions. Cultur of *Alternaria panax* Whetzel isolated from ginsen (19), *Fusarium oxysporum* Scholect, *F. monil forme* Scholdon (obtained from Dept. of Pla Pathology, Taiwan Agricultural Res. Inst. Wafen Taiwan), *F. solani (Mart.)* Appel, Wr. isolated fro ginseng (20) and *Cylindrocarpon destructa*

Zins) Scholten (obtained from Dr. Sung, Dept. of Forest, Kangwon National University, Chunchon, Kangwon-do) were maintained on potato dextose agar. Culture transfers were routinely derived from one parent culture of each fungus. Conidia of those fungi were obtained by flooding cultures with sterile 50 mM phosphate buffer (pH 6.8) and gently dislodging the propagules with sterile glass rod. Propagules were washed three times with 50 mM phosphate solution by centrifugation of 5000 rpm for 5 min at 4°C. Fungal exudate was prepared by incubating washed propagules in 50 mM phosphate buffer (26°C) for a period less than about three hours required for germination. The exudate then was collected by passing through a sterile membrane filter (0.4μm pore size, Millipore Corp. U. S. A.). The exudate was stored at 4°C until used.

Preparation of various extracts, exudate and solutions. A dilute soil solution was obtained by a method of Parker(26). Soil samples were collected from the ginseng cultivated field of root rot diseased soil (diseased soil) and root healthy soil (healthy soil) in Gumsan, Kimpo, Poonggi and Kangwha respectively. The mixture was stirred in a reciprocal shaker for three hours and was left to settle for five hours. The suspension was centrifuged for 10 min in 3000 rpm. The supernatants were then filtered through Millipore membrane (0.4 μm) aseptically. The extracts were also stored at 4°C until used. Preparation of fresh root exudate of three year old ginseng was done by adding the root to water at the ratio of 1:2 (w/v). The mixture was stirred in elenmayer flask for three hours in shaker. The suspension was filtered through filter paper (Watman No. 3) and the filterate was again filtered through Millipore membrane filter (0.4 μm) aseptically. Distilled water, buffer solution (0.05 M potassium phosphate pH 6.8) (4), and dilute nutrient solution of glucose(6) were used for the study.

Measurement of chemotaxis in vitro. Chemotaxis was assayed by modification of the method used by Arora et al(3). A 0.5cm (internal diameter) x 2cm (long) glass test tube was centered near one end and parallel to the axis of a glass slide (7.5cm

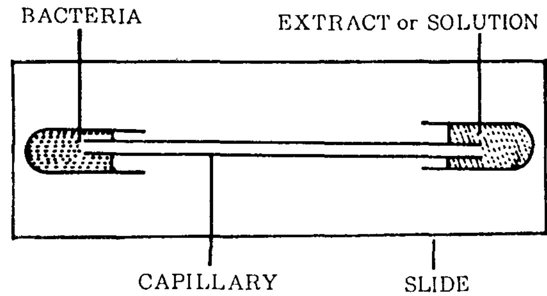

Fig. 1. Apparatus used in chemotaxis assays in cappilary.

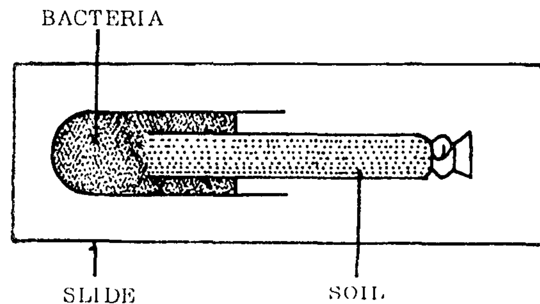

Fig. 2. Apparatus used in chemotaxis assays in soil.

x 2.5cm). The tube held 0.2ml of a bacterial suspension containing about 10^7 cells/ml (Fig. 1). A 3.3 μl precision disposable capillary tube (Coultronics, France S.A.) with open at both ends was inserted into the test tube. Capillaries were filled with a small volume of extracts, fungal exudate, exudates and solutions. One end of the capillary was inserted into the bacterial suspension and the other end into exudates or media held in a second test tube which was fixed to the slide by white vaseline. Chemotaxis assay was done at 27°C, and there were three replicates per assay. After incubation for 60 min the capillaries were removed and the exterior briefly washed by a jet of sterile water. The capillaries were crushed with sterile forceps in 50ml of 0.9% NaCl solution. Dilution was made and 0.2ml of each dilution was spread over four plates containing nutrient agar, using 0.3cm glass bead. Numbers of bacteria which had accumulated in capillaries were transformed to log value for statistical analysis.

Measurement of chemotaxis in soil. A sandy loam soil with the following characteristics was

used: pH 5.5, 2.6% organic matter, 12% clay, 32.1% silt and 55.9% sand. The soil was stored at room temperature until used. Air-dried soil was ground in a mortar to pass through a 400 mesh sieve. Washed latex rubber tubes (0.3cm i.d. x 3cm long) knotted at one end were autoclaved and then packed with 0.3-0.4g of unsterilized sample soil saturated with different soil, extracts, exudates and solutions. The open end of the tube was placed into 0.2ml of bacterial suspension or buffer held within the glass chamber of a chemotaxis apparatus (Fig. 2). After tubes were incubated aseptically for 90 min at 26°C, they were cut into three pieces of equal length, and placed together into 50ml of 0.9% NaCl solution in sterile glass vials. Soil in the tubes was shaken free by agitating the flask for 1 min on a vortex mixer at high speed. Dilution and bacterial enumeration were the same as method described above. Experiments were repeated twice.

Microbial populations in soils. Microbial populations in soils were estimated by dilution plate counts on the following media; chitin medium(18) for actinomycetes, Martin's rose bengal medium(24) for fungal propagules, and Hutchinson medium(5) for total bacteria. Plates were streaked with 0.2ml of soil dilution and incubated at 26°C for three days for bacteria, five days for fungi and seven days for actinomycetes.

Soil characteristics. Samples of soil were analyzed by a method of following; organic matter contents by Tyurin(32), P_2O_5 by Lancaster(28), K, Ca, Mg and NH_4-N by atomic absorption spectrometer (Hitachi, 303), and sand, silt, clay and texture by hydrometer method of Day(11).

RESULTS

Accumulation of *Pseudomonas* sp. in 60 min sample was greater in extract from healthy soil of Poonggi accumulated greater number of bacteria than other extracts. Accumulation of these bacteria was lower in exudate from fresh root of ginseng and distilled water (Table 1). The accumulation phenomenon in capillaries was to time dependent; the shorter he incubation in capillaries generally resulted in the fewer bacterial accumulation. Accumulation of *P. fluorescens* was a statistitical difference among the treatments (P = 0.01) (Table 1). Especially greater numbers of bacterial cells were accumulated in the extract of Kimpo diseased soil. Greater number of bacterial accumulation was marked in various soil extracts than other treatment of solutions or exudate(Table 2). In number of accumulation of *E. carotovora* in capillaries in

Table 1. Accumulation of bacteria in capillaries containing soil extrats, exudate, solutions and buffer as attractants in 60 minutes

Treatment	Log no. of bacteria		
	Pseudomonas sp.	*P. fluorescens*	*Erwinia carotovora*
Diseased soil of			
Gumsan	7.21 b[x]	6.09 a	7.30 b
Kimpo	7.17 b	6.60 bc	7.53 b
Poonggi	6.83 b	6.25 ab	7.24 b
Kangwha	6.78 b	6.40 ab	7.14 b
Healthy soil of			
Gumsan	7.17 b	6.43 ab	6.96 b
Kimpo	6.64 b	6.26 ab	6.20 a
Poonggi	7.56 bc	6.45 ab	6.45 b
Kangwha	7.35 b	6.48 ab	7.29 b
Exudate from ginseng root	5.14 a	6.35 ab	6.30 b
Dextrose solution	6.65 b	5.71 a	7.06 b
Buffer solution	6.18 b	6.21 ab	3.88 a
Distilled water	4.65 a	6.08 a	4.40 a

[x]Means in a column followed by the same letter are not significantly different (P=0.01) according to Duncan's new multiple range test.

Table 2. Accumulation of bacteria in soil containing different extracts, exudate, solutions and buffer as attractants in 90 minutes

Treatment	Log no. of bacteria		
	Pseudomonas sp.	*P. fluorescens*	*Erwinia carotovora*
Diseased soil of			
Gumsan	1.16 a[x]	0.63 a	2.16 a
Kimpo	1.66 a	1.73 ab	2.30 a
Poonggi	1.63 a	1.53 ab	2.20 a
Kangwha	2.16 a	1.20 a	2.10 a
Healthy soil of			
Gumsan	2.23 a	1.40 ab	2.56 a
Kimpo	1.73 a	1.03 a	2.13 a
Poonggi	2.03 a	1.70 ab	2.06 a
Kangwha	2.10 a	1.26 a	2.33 a
Exudate from ginseng root	2.16 a	1.53 ab	2.16 a
Dextrose solution	1.93 a	1.13 a	2.33 a
Buffer solution	2.00 a	1.20 a	2.20 a
Distilled water	2.23 a	1.30 a	2.03 a
Control	0.80 a	0.73 a	0.93 a

[x]Means in a column followed by the same letter are not significantly different (P=0.01) according to Duncan's new multiple range test.

treatment of 60 min, extract from diseased soil plot accumulated in greater numbers than those in containing healthy soil extrct or other solutions. Especially lower number was observed in treatment of distilled water and buffer solution. The accumulation phenomenon in capillaries was also considered to time interval (Table 1).

Bacterial movement toward rubber tube soil nearly similar to natural soil conditions was observed (Table 2). Accumulation of *P.* sp. in response to soil supplemented with soil extract and solutions was generally higher number in order of Kangwha soil, Gumsan soil, distilled water, Kimpo soil and exudate from fresh root of ginseng, but there was not significant difference in the treatment. Accumulation of *P. fluorescens* in response to extracts, exudate and solutions was generally greater in soil extracts than other solutions except exudate of fresh ginseng root. The accumulation of *E. carotovora* was not significantly different in treatment, but the bacteria moved greater in number to treatments of extracts, exudates and solutions compared to the control. In latex rubber tube of soil supplemented with fungal exudates, the three trested organisms were not significantly attracted to the exudates (Table 3). In an accumulation of bacteria in rubber tube soil inoculated with fungal propagules, the response of *P.* sp. to propagules in 60 min resulted in the higher bacterial accumulation in chambers containing propagules, but the numbers of

Table 3. Accumulation of bacteria in capillaries containing various exudates from fungal propagules as attractants

Bacteria / Organisms	Log no. of bacteria		
	Pseudomonas sp.	*P. fluorescens*	*Erwinia carotovora*
Fusarium solani	6.47 a[x]	6.17 a	6.63 a
F. oxysporum	6.51 a	6.63 a	6.61 a
Alternaria panax	6.60 a	6.12 a	6.50 a
Cylindrocarpon destructans	6.57 a	5.95 a	6.76 a
Streptomyces sp	6.42 a	6.24 a	6.30 a
Mixed organisms	6.30 a	6.36 a	6.36 a

[x]Means in a column followed by the same letter are not significantly different (P=0.01) according to Duncan's new multiple range test.

Table 4. Accumulation of bacteria from chambers into soil inoculated with organisms as attractants in 60 minutes

Bacteria / Organisms	Log no. of bacteria		
	Pseudomonas sp.	*P. fluorescens*	*Erwinia carotovora*
Fusarium solani	4.26 a[x]	4.12 ab	4.03 a
F. oxysporum	4.36 a	4.14 b	4.54 c
Alternaria panax	4.48 a	3.97 a	4.15 ab
Cylindrocarpon destructans	4.74 a	4.32 a	4.04 a
Streptomyces sp.	4.78 a	3.84 a	3.90 a
Mixed organisms	4.87 a	4.05 ab	4.26 ab

[x]Means in a column followed by the same letter are not significantly different (P=0.01) according to Duncan's new multiple range test.

Table 5. Soil characteristics of various soil samples

Soil	Location	O.M.	pH	P_2O_5	Ex (me/100g) K	Ca	Mg	sand	Silt	Clay	Textural class
Diseased	Kangwha	1.8	5.0	172.0	0.58	2.3	0.5	36.6	42.0	21.4	loam
	Kimpo	1.5	5.3	121.0	0.24	1.4	0.6	39.7	47.9	12.4	loam
	Kumsan	2.3	4.5	159.0	0.33	2.8	0.7	27.3	59.1	13.6	silt loam
	Goesan	1.0	5.7	57.0	0.25	20.1	4.7	17.5	66.5	16.0	silt loam
	Poonggi	3.2	4.8	138.0	0.65	3.7	0.9	17.5	59.9	22.6	silt loam
Healthy	Kangwha	1.7	5.3	159.0	0.61	2.3	0.6	61.3	29.7	9.0	silt loam
	Kimpo	1.8	5.8	201.0	1.29	4.6	1.7	59.0	30.2	10.8	silt loam
	Kumsan	3.2	5.2	186.0	0.24	3.5	0.5	24.0	64.4	11.6	silt loam
	Goesan	3.0	4.9	70.0	0.36	2.9	1.4	6.5	65.5	28.0	silt clay loam
	Poonggi	3.9	5.7	167.0	0.17	3.9	0.7	17.5	65.8	13.0	silt loam

bacteria were not significantly different (P = 0.05) (Table 4). The response of *P. fluorescens* to soil inoculated with different orgnisms in 60 min was significantly different (P = 0.01); *F. solani* and *F. oxysporum* attracted more bacterial cells compared to others, and also *streptomyces* sp. and mixed culture of organisms showed greater accumulation of the bacteria (Table 4). Response of *E. carotovora* to soil in 30 min was statistically significant (P = 0.01): *F. oxysporum*, mixed organisms and *streptomyces* sp. attracted more bacteria. A more accumulation of bacteria was observed in soil inoculated with *F. oxysporum*, mixed organisms and *A. panax*. The organic matter contents of Kangwha and Kimpo were lower in diseased and healthy soil than other soil conditions. The contents of P were low in two soil conditions in Goesan. The contents of K was especially high in Kimpo healthy soil, but Ca and Mg contents were high in Goesan diseased field. Sand contents was generally high in healthy soil. The textural class of the soil was silt loam except loam in Kangwha and Kimpo diseased soil, and silt clay loam in Goesan healthy soil (Table 5). Bacterial populations in Goesan and Kangwha were more abundant than other field soils. The relatively abundant numbers of fungal propagules were found in both soil fields of Gumsan and Goesan. The number of *Actinomycetes* was populated much abundant in healthy soil of Goesan and diseased soil of Poonggi (Table 6).

DISCUSSION

The results of this study showed that extracts from soils or exudate could attract more cells of motile bacteria in general. In comparison of bacterial movement, the accumulation of bacteria was more increased in the capillaries tube than in soil condition or inoculated soils by fungal propagules or others. The exdates from living organisms in vitro can also attract larger motile bacteria than soil extracts, exudate and solutions.

Table 6. Populations of microorganisms present in ginseng cultivated under different soil conditions

	No. of bacteria (10^5/g soil)	No. of fungal propagules (10^4/g soil)	No. of actinomycetes (10^5/g soil)
Diseased soil of			
Gumsan	4.3 a[x]	27.3 e	5.5 a
Kimpo	6.4 a	0.9 a	0.8 a
Poonggi	3.5 a	8.7 b	16.3 ab
Kangwha	4.5 a	1.2 a	5.2 a
Goesan	10.0 ab	21.7 d	8.5 a
Healthy soil of			
Gumsan	2.7 a	73.8 f	5.7 a
Kimpo	9.8 a	0.9 a	1.6 a
Poonggi	3.7 a	7.5 b	6.5 a
Kangwha	10.7 ab	1.3 a	8.0 a
Goesan	11.7 a	14.0 c	57.2 c

[x]Means in a column followed by same letters are not significantly different (P=0.01) according to Duncan's new multiple range test.

The soil water contents in this test were saturated, which was sufficiently high to allow bacterial movement in water filled pores. Goring and Hamaker(15) reported that motility of bacteria would be expected to depend on soil texture and adsorption of the exudate of colloids, soil moisture contents(34) and continuity of soil pores(17). Stozky and Post(31) observed that the rate of bacterial spread was independent of bacterial characteristics such as morphology and presence of flagella, but Wong and Griffin(33) proved that the rate of bacterial spread was mainly due to active flagella movement rather than to cell increase. Palleroni(25) observed that no distinct migratory band was seen to form in capillaries filled with media containing an oxidizable carbon sources and the numbers of organic compounds that can be used for growth substances are ineffective or very weak chemotactic attractants of the motile spores of *Actinoplanes*. Fradkin and Patrick(13) have recently shown extensive colonization of conidia of *Cochliobolus sativus* and propagules of other root infecting fungi by bacteria in two soils. The extent to which the accumulation was the results of bacterial growth on fungal exudate or of chemotaxis was unknown, but recently Arora et al(3) mentioned that the increased numbers of bacteria associated with fungal propagules were results of chemotaxis and not of multiplication. Rai and Strobel(27) in their test proved that maximum chemotatic response among the exudates was histidine among the amino acids, and glucose and gluconic acid among the sugars(29).

At this work, glucose or exudate of fresh root of ginseng could be used as carbon sources for growth and energy was not so much effective on the accumulation of *P.* sp and *E. carotovora* among the bacteria, or weak chemotactic attractants of the bacteria. Thus it is tempting to speculate that motile bacteria may be attracted to some specific compounds which might be different in chemical stimuli. Chemotactic responses of bacteria to organic compounds may be independent of their capacity to serve as carbon or energy sources(1). Accumulation of a lot of bacteria in extracts from soils was especially notable facts in this experiments.

The motile phase gives the bacteria the opportunity for migration, and it is therefore likely that chemotactic attraction plays an important role in the search for appropriate ecological niche in nature. The contents of k was higher in healthy plot in Kangwha and the contents of Ca and Mg were higher in diseased plot of soil in Goesan. These results were coincident with the report of Lee(21) that incidence of disease or reduction of ginseng root rot was much concerned to contents of mineral and microorganisms in soil.

REFERENCES

1. ADLER, J. (1969). Chemoreceptors in bacteria.

Science 166: 1588-1597.

2. ADLER, J. (1973). A method for measuring chemotaxis and use of the method to determine optimum condition for chemotaxis by *E. coli*. *J. gen. Microbiol* 4:77-91.
3. ARORA, D. K., FILONOW, A. B. & LOCKWOOD, J. L. (1983). Bacterial chemotaxis to fungal propagules in vitro and in soil. *Can. J. Microbiol.* 29:1104-1109.
4. ARORA, D. K., FILONOW, A. B. & LOCKWOOD, J. L. (1983). Exudation from ^{14}C-labeled fungal propagules in the presence of specific microorganisms. *Can. J. Microbiol.* 29: 1487-1492.
5. BHAT, J. V. & SHETTY, M. V. (1942). A suitable media for the enumerating of microorganisms. *J. Univ. Bombay Seat. B.*: 13-15.
6. BRISTOW, P. R. & LOCKWOOD, J. L. (1975). Soil fungistasis; role of microbial nutrient sink and of fungistatic substance in two soils. *J. gen. Microbiol.* 90: 147-156.
7. CHET, I., FOGEL, S. & MITCHELL, R. (1971). Chemical detection of microbial prey by bacterial predators. *J. Bacteriol.* 106: 863-967.
8. COLEY-SMITH, J. R. & DICKINSON, D. J. (1970). Effects of sclerotia of *Sclerotium cepivorum* Berk. on soil bacteria; the nature of substance exuded by sclerotia. *Soil Biol. Biochem.* 3: 27-32.
9. CURRIER, W. W. & STROBEL, G. A. (1976). Chemotaxis of *Rhizobium* spp. to a glycoprotein produced by birdsfoot trefoil roots. *Science* 176: 434-435.
10. DAZZO, F. B. (1980). Adsorption of microorganisms to roots and other plant surfaces. Ed. by J. Witton, & K. C. Marshall, pp. 253-316, John Wiley & Sons, New York.
11. DAY, R. R. (1965). Methods of soil analysis, Ed. by C. A., Blacks D. D. Evans, J. L. White, L. E. Ensminger, & F. E. Clark, pp. 545-565 Amer. Soc. Agronomy.
12. DIEM, H. G. (1975). Attraction et repulsion des bacteries per les spores de *Cladosporium cladosporioides* en cours de germination. *Can. J. Bot.* 53: 1092-1096.
13. FRADKIN, A. & PATRICK, Z. A. (1982). Fluorescence microscopy to study colonization of conidia and hyphae of *Cochliobolus sativus* by soil microorganisms. *Soil Biol. Biochem.* 14: 543-548.
14. GITTE, R. R., RAI. P. V. & PATIL, R. B. (1978). Chemotaxis of *Rhizobium* sp. toward root exudate of *Cicer arietnum* L. *Plant abd Soil* 50: 553-566.
15. GORING, C. A. I. & HAMAKER, J. W. (1972). *Organic chemicals in the soil environment.* Vol. II. Marcel Dekker, New York.
16. GRIFFIN, D. M. & QUAIL, G. (1968). Movement of bacteria in moist particulate system. *Aust. J. Biol. Sci.* 21: 579-582.
17. HAMADI, Y. A. (1971). Soil water tension and movement of *Rhizobia*. *Soil Biol. Biochem.* 3: 121-126.
18. HSU, S. C. & LOCKWOOD, J. L. (1975). Powdered chitin agar as a selective medium for enumeration of actinomycetes in water and soil. *Appl. Microbiol.* 29: 422-426.
19. KIM, J. H. (1967). A study of nutritional physiology on *Alternaria panax*. *Dongguk Univ. Jour.* 3, 4: 507-516.
20. KIM, J. H. & LEE, M. W. (1974). On the root rot of ginseng (I), –isolation and identification of *Fusarium* sp. *Kor. Jour. Microbiol.* 12: 94-98.
21. LEE, M. W. (1977). Studies on the root rot of ginseng(VII), - on pathogenic microbial population in continuous culture and first time culture of ginseng. *Kor. Jour. Microbiol.* 15:20-30.
22. LEE, M. W. (1979). Studies in the etiology of red rot of ginseng. *Kor. Jour. Microbiol.* 17: 179-186.
23. LOCKWOOD, J. L. (1968). The fungal environment of soil bacteria. In *the ecology of soil bacteria*, Ed. by T. R. G. Gray & D. Parkinson pp. 44-65, Liverpool Univ. Press, Liverpool.
24. MARTIN, J. P. (1950). Use of acid, rose bengal and streptomycin in the plate method for estimating soil fungi. *Soil Sci.* 69: 215-232.

25. PALLERONI, N. J. (1976). Chemotaxis in *Actinoplanes*. *Arch. Microbiol.* 110: 13-18.
26. PARKER, F. W. (1921). Methods of studying the concentration and composition of soil solution. *Soil Sci.* 12: 209-232.
27. RAI, P. V. & STEROL, G. A. (1966). Chemotaxis of zoospores of *Aphanomyces cochlioides* to sugar beet seedlings. *Phytopathology* 56: 1356-1369.
28. RURAL DEVELOPMENT ADMINISTRATION. (1983). A method of analysis of soil chemistry, Suweon. pp. 103-106.
29. SEYMOUR, F. W. K. & DOETSCH, N. (1973). Chemostatic response by motile bacteria. *J. gen. Microbiol.* 78: 287-296.
30. SIALA, A. & GRAY, T. R. G. (1974). Growth of *Bacillus subtilis* and spore germination in soil observed by a fluorescent antibody technique. *J. gen. Microbiol.* 81: 191-198.
31. STOZKY, G. & POST, A. H. (1965). Growth rates of microorganisms in soil. *Argon. Abstr.* 89.
32. TYURIN, A. T. (1938). The composition and structure of soil organo-mineral gels and soil fertility. *Soil Sci.* 45: 343-357.
33. WONG, P. T. W. & GRIFFIN, D. M. (1976). Bacterial movement at higher matric potentials, in artificial and natural soils. *Soil Biol. Biochem.* 8: 215-218.
34. WONG, P. T. W. & GRIFFIN, D. M. (1976). Bacterial movement at higher matric potentials, I. in fungal colonies. *Soil Biol. Biochem.* 8: 219-223.