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Numerical Classification of Actinomycetes Isolated from Volcanic Soil

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Of actinomycetes isolated from volcanic compost soils, 115 representative strains which showed distinctive morphological features were numerically classified, compared with reference strains of *Streptomyces*. One hundred and twenty unit characters were tested and the average probability of error was 4.27%. The cluster analysis resulted in two groups: group A included strains of streptomycete which covered 80% of the total strains, whereas group B included various actinomycetes except streptomycetes. Group A was divided into 2 major clusters (over 5 strains), 10 minor clusters (2-4 strains), and 14 single member clusters, and clusters which contained LL-diaminopimelic acid. Group B was divided into 5 clusters, of which 4 clusters contained *meso*-diaminopimelic acid and 1 cluster LL-diaminopimelic acid. The major clusters of group A showed higher abilities of substrate utilization and degradation, and higher resistance to inhibitors, whereas the minor and single member clusters of group A showed relatively higher antimicrobial activities. On the other hand, all clusters of group B showed relatively lower abilities of substrate utilization and degradation and lower resistance to inhibitors.

Key words: Actinomycetes, *Streptomyces*, cluster analysis, diaminopimelic acid.

The importance of actinomycetes has been well recognized as producers of antibiotics and useful secondary metabolites, causal agents of some diseases, decomposers of a wide range of substances, and materials utilized in genetic studies (6, 9).

One genus of actinomycestes, *Streptomyces* has been intensively focussed, due to the interests on the unique morphological changes during their life cycle and their industrial importance. The extensive numerical classifications on *Streptomyces* type cultures were performed by Williams *et al.* (36) and Kämpfer *et al.* (11), giving reasonable criteria for the classification, although the criteria given by them revealed minor differences from each other. Numerical classification does not rely upon unique characters for each organism or cluster, rather is based on a large number of characters, and does not emphasize any specific unit character, improving stability and disregarding undesirable effects from erroneous experimental results (2, 30). The taxonomic groups defined by numerical classification are thus polythetic where there is no single character that is essential for the recognition of taxa (11), and the result is a set or matrix of probabilistic responses to tested characters of all clusters, which can be used in identification of unknown strains (12, 37).

Morphology and pigmentation are very important keys in the taxonomy of *Streptomyces* in spite of some difficulties in determining each character (19, 26, 35). They are phenetic characters and some of the apparent same characters, especially for pigment color, can be originated from chemically different bases (15), and visual observation includes almost unavoidable test errors, which restrict the availability of such highly potential characters in the taxonomy of streptomycetes, together with the genetic instability of streptomycetes that can lead to the change of phenetic characters (18). With all of difficulties these characters can generally explain the results of numerical classification, and morphology of spore chain, ornamentation of spore, pigmentation of spore mass and substrate mycelium, and excretion of diffusible pigment which are considered to be highly diagnostic characters.

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In the present study actinomycetes were isolated from volcanic soils and grouped firstly according to morphology and pigmentation properties and classified numerically using various morphological and physiological characters, compared with *Streptomyces* type strains as reference strains. And the generic composition of the isolates was determined by diaminopimelic acid analysis and morphological observations.

Materials and Methods

Isolation of actinomycetes

Soil samples treated as described by Labeda (16) were diluted with 1/4 strength Ringer's solution (26) and inoculated on starch casein agar plates (14) supplemented with cycloheximide and nystatin (50 μg/ml each). Single colonies were taken and subcultured repeatedly on ISP 2 medium (26), and suspensions of spores and mycelial fragments were kept in 20% glycerol (v/v) at -4°C.

Numerical classification

Each strain was tested for 120 unit characters, and all the test procedures including carbon source utilization tests, degradation tests, resistance to inhibitors, enzyme activities, and antimicrobial activities were performed as described by Shirling & Gottlieb (26), and Williams *et al.* (36). Pigmentation of spore mass and substrate mycelium as well as soluble pigment production were determined on ISP 3 agar plates whereas morphology of spore chain was observed on the coverslips grown on ISP 4 medium for two weeks. The patterns of spore chains and the fine structures of the spore surfaces were observed by scanning electron microscope (Stereoscan 260, Cambridge). Inoculated media were incubated at 28°C. Duplicate strains were used to test the reproducibility of the results.

Data analysis

The binary data were recorded with X program (4), and the cluster analysis were performed using CLUSTAN 2.0. Test reproducibility, average probability of test errors and percent positive tables of the clusters were calculated from the data matrix by the X program. The most diagnostic characters were calculated from DIACHAR by Sneath (28).

Diaminopimelic acid (DAP) analysis

DAP isomers from the whole cell hydrolyzates were obtained according to the method proposed by Staneck and Roberts (33), and the thin-layer chromatography was performed with the HPTLC-5787 cellulose plate (10×10 cm, Merck) and a mixture of methanol: water : 6 N HCl: pyridine (40 : 13 : 2 : 5) as a developing system (13).

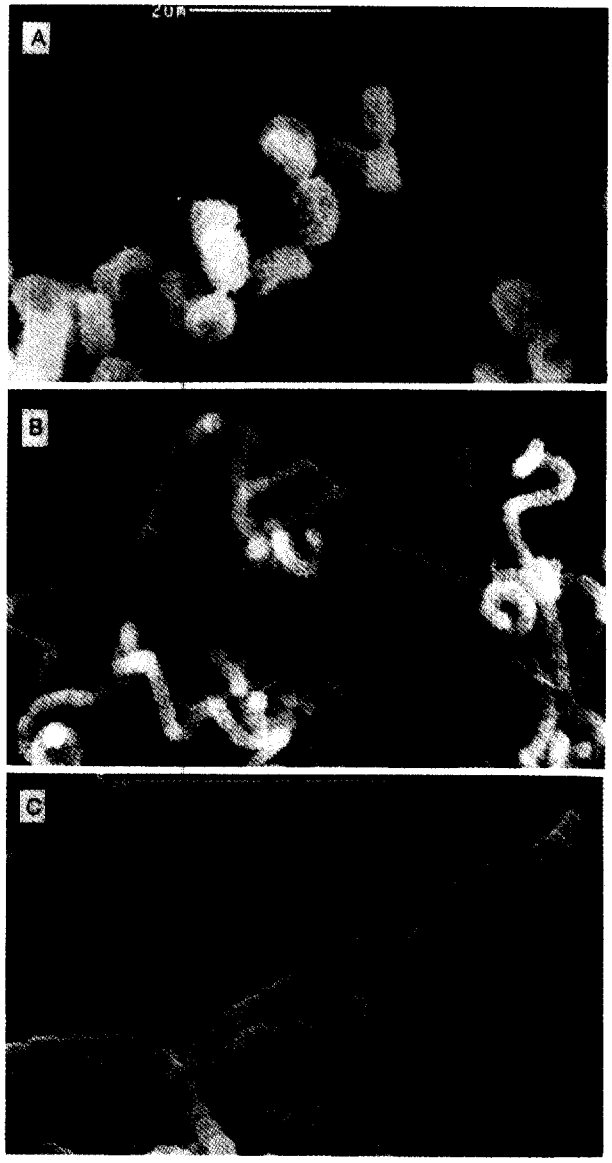

Fig. 1. Scanning electron micrograph images of spore chains. (A) Spiral (*Spirales*) spore chains with spiny surface of strain SB266. (B) Looped (*Reticuliaperti*) spore chains with hairy surface of strain SB283. (C) Flexuous (*Rectiflexibiles*) spore chains with smooth surface of strain SB672.

Results

Isolation of actinomycetes

Two hundred and twenty nine strains were isolated from three volcanic composite soils with weakly acidic pH (6.3~6.4). Most of the isolated strains produced aerial and substrate mycelia, and formed aerial spores, which were unique properties of actinomycetes. They produced

Table 1. Test errors estimated in numerical classification

Characters	# Tests	Variance[a]	Probability of error (%)[a]
1. Morphology	19	0.016	1.60
2. Carbon source	65	0.041	4.23
3. Resistance to inhibitors	21	0.051	5.37
4. Degradation	10	0.073	7.97
5. Enzyme activity	2	0.050	2.27
6. Antimicrobial activity	3	0.022	5.28
Total	120	0.041	4.27

[a] Variance and probability of error by Sneath & Johnson (29).

retinaculiaperti (RA), rectiflexibile (RF), or spiral (SP) spore chains, and the spores of smooth, hairy or spiny surface ornamentations (Fig. 1). The isolates were grouped according to their colors, and 115 strains that had different colors with one another were selected for numerical classification.

Test reproducibility

Fifteen duplicate cultures among isolate strains were used to evaluate experimental test error. The average probability of error was 4.27% which was calculated from the pooled variance (s^2=0.041) of 120 unit characters (29), and the average reproducibility between the pairs of 15 duplicates was 91.8%. Morphology and pigmentation properties showed low average probability of error, while degradation activities showed higher values of error than the other character sets (Table 1). The growth on D-glucuronolactone, melibiose, raffinose, sorbitol, asparagine, and dextran as sole carbon sources, and the growth in the presence of 9% sodium chloride, and the degradation of starch and arbutin were excluded from the final data matrix for their high test variances (variance above 0.1). And since all the strains did not grow on L-galactonolactone, D-gulonolactone, fumaric acid, furoic acid, glucuronic acid, glycolic acid, maleic acid, malic acid, oxalic acid, and sorbic acid as sole carbon sources, and were inhibited by 0.1% cupric sulfate, those tests were also excluded. Thus the final data matrix contained 151 strains and 15 duplicate strains against 100 unit characters.

Cluster analysis

The cluster analysis using simple matching coefficient (S_{SM}; 31) and unweighted pair group with arithmetic averages (UPGMA; 30) algorithm resulted in 2 major groups defined at 66.3% similarity level, and an abridged dendrogram showing the relationship between clusters is given in Fig. 2.

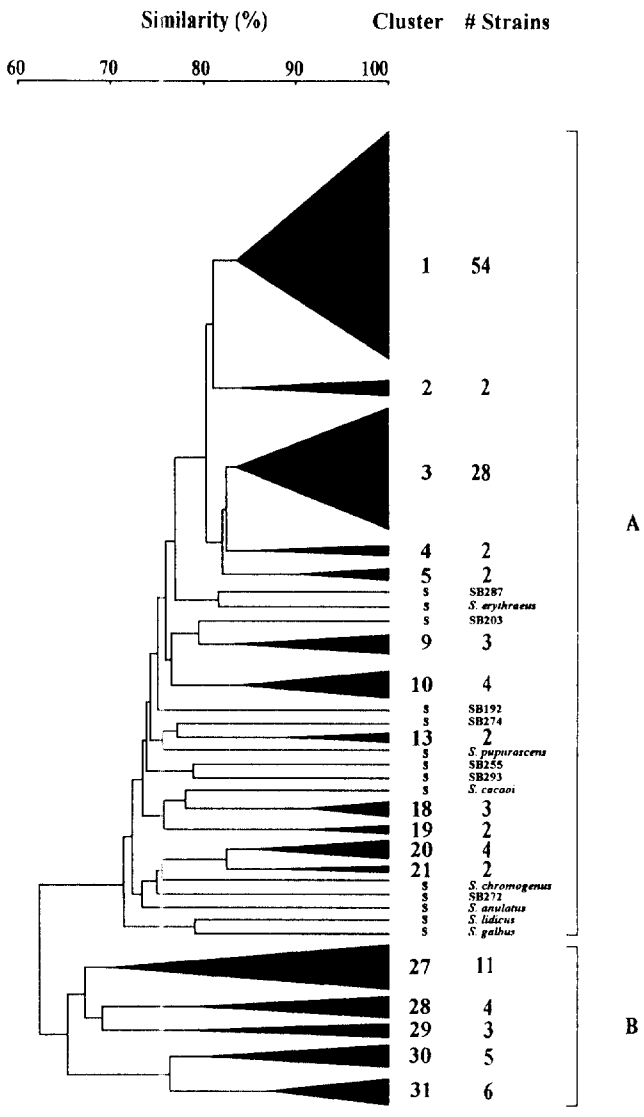

Fig. 2. An abridged dendrogram showing relationships between clusters defined by S_{SM} and UPGMA (Clustan 2.0). The cophenetic correlation coefficient was 0.86.

Of the 151 test strains, 122 fell into group A in which all of the *Streptomyces* reference strains were included (Table 2). Most strains in this group showed morphological properties of characteristic streptomycetes. They had LL-DAP as their diagnostic amino acid. Group B contained 29 strains, which consisted of diverse groups of actinomycetes except streptomycetes, mostly having *meso*-DAP except for cluster 28. The distribution of positive characters among the clusters is given in Tables 3 and 4.

Group A contained 2 major, 10 minor (having 2 to 4 strains), and 14 single member clusters, which were defined at 82.5% similarity level. About two thirds of total strains fell into the two major clusters, and the re-

Table 2. List of clusters and their characteristics

Cluster	Strains	Spore mass	Spore chain	Reference strains
		Group A		
1A	39	Grey	RA	*S. parvullus*
				S. violaceolatus
1B	5	Blue	RA/SP	-
1C	8	White	RA	*S. pilosus*
2	2	Grey	RF	*S. platensis*
3A	10	Ivory	RF	*S. fluorescens*
				S. vanaceus
3B	18	Grey	RF	*S. griseolus*
				S. halstedii
				S. atrolivaceus
				S. rimosus
				S. humidus
				S. diastaticus
				S. kanamyceticus
				S. naraensis
4	2	Grey/white	RF	*S. daghestanicus*
5	2	Pink/white	RF	*S. californicus*
				S. phaeopurpureus
9	3	Grey/ivory/blue	RF	*S. narbonensis*
				S. venezuelae
10	4	Grey/white	RA	*S. janthinus*
13	2	White/ivory	RF/RA	*S. xantholiticus*
18	3	Grey	SP	*S. endus*
				S. violaceusniger
				S. hygroscopicus
19	2	Grey	RA/SP	*S. murinus*
				S. misionensis
20	4	Grey	RF	*S. exfoliatus*
21	2	Pink/iviory	RF	*S. roseosporus*
		Group B		
27	11	Orange/maroon	-	-
28	4	Grey	-	-
29	3	Pink	-	-
30	5	Pink	-	-
31	6	White	-	-

mainders formed small clusters or single member clusters. Cluster 1 was the largest, containing 53 strains, which could be divided into five subclusters, 1A to 1E. Subcluster 1A contained 39 strains that formed mostly grey spores and RA or RF spore chains, and about half of the strains produced brown soluble pigment. This cluster included two reference strains, ISP 5048 and 5438. Subcluster 1B contained 5 strains that formed mainly blue spores and RA or SP spore chains. All strains produced brown soluble pigment. Subcluster 1C contained 8 strains that formed white spores and RA or RF spore chains. Cluster 3 contained 28 organisms, and could be divided into two subclusters, 3A and 3B. The former contained 10 strains that formed ivory or white spores and RF spore chains. The latter subcluster contained 18 strains that formed mainly grey spores and RF spore chains. Most strains produced brown soluble pigment.

Group B could be divided into 5 clusters according to their morphological characteristics (Table 2). Cluster 27 contained 11 strains, most of which showed characteristic features of micromonospora. Most strains did not produce visible aerial mycelium, their surface was orange or black, and usually became mucoid. Cluster 28 contained 4 strains that solely had LL-DAP among the subgroups in group B. They formed grey spores and produced brown soluble pigment, and poor or no growth was observed in oatmeal agar and ISP 4 agar medium. Cluster 29 contained 3 strains, all formed pink spores and red-orange substrate mycelia, and the formation of aerial hyphae was poor. Cluster 30 contained 31 strains that formed pink spores and RF or RA spores with abundant aerial hyphae. Cluster 31, a nocardioform group, contained 6 strains that formed mostly white spores and all RF spore chains.

Grouping with reference strains

With 45 reference strains representing major clusters defined by Williams et al. (36), the isolates were grouped, and tentatively identified. Cluster 1 with grey spore masses, RA or RF spore chains, and without melanin production, was clustered to *S. rochei* group. Cluster 2 with yellow, green or white spore masses and RF spore chains was clustered to *S. anulatus* group. Cluster 3 with green spore masses and RF spore chains was clustered to *S. rimosus* group. And cluster 4 and 5 were clustered to *S. exfoliatus* group. The members of each cluster shared common morphological and physiological features with the corresponding reference clusters.

Physiological properties of clusters

From the percent positive table of major, minor, and single member clusters (Table 3 and 4), the average response of the clusters to given sets of characters were calculated (Table 5). The subclusters in cluster 1 utilized up to 70% of carbon sources, thus showed highest ability to use a variety of substrates among the major and minor clusters, though some single member clusters showed the responses over 70%. Cluster 19 showed the highest resistance against inhibitors. The clusters 3A and 3B showed relatively high resistance; and the strains in group B showed low resistance again. The degradation activities were highest in clusters 5 and 21, and lowest in the clusters 30 and 31 in group B. The clusters 18 and 19 as well as the single member clusters 24 and 25, showed high antimicrobial activities while few strains in other clusters showed antimicrobial activities. The an-

Table 3. Distribution of positive characters to major and minor clusters

Clkuster	1A	1B	1C	2	3A	3B	4	5	9	10	13	18	19	20	21	27	28	29	30	31
# Organisms	39	5	8	2	10	18	2	2	3	4	2	3	2	4	2	11	4	3	5	6
Spore mass color																				
Grey	90	20	0	100	0	56	50	0	33	50	0	67	100	100	0	0	100	0	0	0
Pink	0	0	0	0	0	0	0	50	0	0	0	0	0	0	50	9	0	100	100	17
White	10	20	100	0	20	17	50	50	33	50	50	33	0	0	0	0	0	0	0	83
Ivory	0	0	0	0	70	22	0	0	0	50	50	0	0	0	50	0	0	0	0	0
Blue	0	60	0	0	0	0	0	0	33	0	0	0	0	0	0	0	0	0	0	0
Orange	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	45	0	0	0	0
Maroon	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	45	0	0	0	0
Substrate mycelium color																				
Yellow	10	0	50	0	20	0	0	0	0	0	100	0	0	0	50	0	0	0	0	0
Brown	82	100	38	100	70	100	100	50	100	100	0	100	100	100	50	18	25	0	100	100
Orange	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	73	0	100	0	0
Purple	0	0	12	0	0	0	0	50	0	0	0	0	0	0	0	0	0	0	0	0
Black	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	75	0	0	0
Pigment Color																				
Yellow	5	0	12	0	20	0	0	0	0	0	0	0	50	0	0	0	0	0	0	17
Brown	46	100	38	100	40	89	100	100	100	100	0	33	50	50	100	27	100	0	80	33
Purple	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Presence of aerial mycelium	00	100	100	100	90	100	100	100	100	100	100	100	100	100	100	9	100	100	100	100
Spore chain morphology																				
RF	38	0	62	50	90	100	100	100	100	0	50	0	0	0	100	0	0	0	80	100
RA	72	80	75	0	0	0	0	0	0	75	50	33	50	100	0	0	0	0	60	0
SP	8	60	0	0	0	0	0	0	0	25	0	67	50	0	0	0	0	0	0	0
Utilization of C source																				
N-Ac-glucosamine	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	45	50	100	100	100
Adonitol	0	60	12	0	70	6	0	50	0	75	0	100	0	0	0	9	100	100	0	0
D-Arabinose	18	20	12	0	10	6	0	0	333	0	0	0	0	0	0	9	0	100	0	0
L-Arabinose	100	100	100	50	70	100	100	0	67	100	100	67	50	50	100	64	100	100	60	0
Cellobiose	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	73	100	100	20	67
2-deoxyglucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0
Fructose	100	100	100	100	100	100	50	100	100	100	100	100	100	0	0	64	100	67	0	50
D-Galactonolactone	8	0	0	0	0	0	50	0	0	0	0	100	0	0	0	0	0	0	0	0
Galactose	100	100	100	100	100	100	100	100	100	100	100	100	100	75	75	55	50	100	0	7
D-Glucosamine	13	20	0	0	0	11	0	0	0	0	0	0	000	0	0	0	0	0	0	0
Glucosamine	97	100	88	100	100	94	100	100	67	75	100	100	100	100	100	18	25	33	100	50
Glycerol	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	27	75	67	100	100
L-Gulonolactone	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Inositol	97	100	100	100	0	0	0	0	0	100	100	0	0	0	0	9	0	0	0	17

Table 3. Continued

Cluster	1A	1B	1C	2	3A	3B	4	5	9	10	13	18	19	20	21	27	28	29	30	31
# Organisms	39	5	8	2	10	18	2	2	3	4	2	3	2	4	2	11	4	3	5	6
Lactose	100	100	100	100	90	100	100	100	100	100	100	100	100	50	100	82	50	100	0	17
D-Lyxose	87	100	100	50	10	0	50	0	0	75	100	0	100	0	0	18	0	67	0	0
Maltose	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	73	100	100	100	17
Mannitol	97	100	100	100	100	89	100	100	33	100	100	100	100	0	0	55	100	100	0	17
Mannose	100	100	100	100	100	100	100	100	100	100	100	100	100	75	100	64	75	100	40	17
Melezitose	5	0	0	50	10	0	0	0	0	0	0	0	0	0	0	27	0	0	0	0
1-*O*-Methylgucose	100	100	100	50	90	89	0	100	33	25	100	67	0	0	50	9	100	67	0	0
α-methyl-D-mannose	92	100	75	0	100	67	50	100	0	25	100	100	0	0	0	9	100	67	0	0
Rhamnose	95	100	100	0	90	6	50	0	100	100	100	100	0	0	100	27	100	67	0	0
Ribose	97	100	100	100	100	100	100	100	100	75	100	100	100	25	0	9	25	67	20	67
Sorbose	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sucrose	95	100	100	100	100	100	100	100	100	100	100	100	50	25	50	73	50	100	60	67
Trehalose	100	100	100	100	100	100	100	100	67	100	100	100	100	75	100	64	75	100	100	33
Xylitol	0	0	0	50	10	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0
D-Xylose	100	100	100	100	100	100	100	100	100	100	100	100	100	75	100	73	75	67	0	0
L-Xylose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33	0	0
Gluconic acid	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	64	100	67	0	33
Sodium malonate	46	0	62	0	10	83	0	0	33	0	50	0	0	0	0	0	100	0	0	0
α-Alanine	100	100	100	100	100	89	100	0	67	100	100	33	100	25	0	9	25	67	0	0
β-Alanine	100	100	100	100	70	78	50	0	100	100	100	33	100	75	100	18	0	0	40	0
Arginine	62	100	88	100	100	83	0	100	100	100	100	0	100	75	0	9	75	33	40	0
Glutamine	97	100	100	100	100	28	50	50	100	100	100	100	100	25	50	0	50	33	60	0
Glutamic acid	100	100	100	50	100	100	100	100	100	100	100	100	100	100	100	27	75	67	100	0
Glycine	64	80	75	50	80	22	0	0	100	100	100	0	0	0	0	0	0	0	40	0
Histidine	97	100	100	100	100	94	50	100	100	50	100	67	50	100	100	9	0	0	40	0
Isoleucine	8	0	50	50	0	6	0	0	67	0	50	0	0	75	0	18	25	0	0	100
Lysine	26	60	50	100	30	0	0	0	100	100	0	67	100	25	100	9	25	100	60	0
Methionine	0	0	0	0	0	33	0	0	33	0	0	0	0	0	0	0	0	0	0	0
Ornithine	97	100	88	100	50	0	50	0	67	75	50	67	100	0	0	27	0	100	0	0
Proline	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	36	75	100	100	83
Dextran	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0
Dextrin	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	45	100	100	00	50
Inulin	100	10	100	100	100	100	50	50	100	100	100	100	50	0	50	45	100	100	40	50
Dulcitol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33	0	0
Salicin	92	80	88	0	70	83	0	0	33	75	100	0	50	100	100	9	0	67	80	0
Growth in the presence of																				
NaCl 5%	97	100	100	100	100	100	100	50	33	50	0	100	100	50	50	0	75	0	17	17
7%	95	80	88	50	90	89	100	50	0	0	0	0	100	0	0	0	50	0	0	0

Table 3. Continued

Cluster		1A	1B	1C	2	3A	3B	4	5	9	10	13	18	19	20	21	27	28	29	30	31
# Organisms		39	5	8	2	110	18	2	2	3	4	2	3	2	4	2	11	4	3	5	6
Sodium azide	0.01%	46	20	25	0	20	78	0	100	0	0	0	100	100	0	100	0	0	0	0	0
	0.02%	15	0	0	0	0	33	0	100	0	0	0	100	100	0	0	0	0	0	0	0
Phenol	0.1%	97	100	88	50	70	94	100	100	67	25	50	0	100	100	100	27	0	0	20	33
	0.2%	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thallous	0.001%	100	40	88	100	100	100	50	0	67	50	50	100	100	75	50	73	75	33	60	67
acetate	0.002%	92	20	38	100	8	100	0	0	100	25	100	0	100	25	50	18	25	0	40	67
Phenylethanol	0.1%	100	100	100	100	100	100	100	100	100	75	50	100	100	100	100	64	75	33	80	100
	0.2%	100	100	100	100	100	100	100	100	100	50	50	100	100	100	100	45	25	0	80	33
Potassium	0.001%	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	82	75	100	80	100
tellurite	0.002%	100	100	100	100	100	100	100	100	100	75	100	100	100	100	100	82	100	100	80	100
Crystal	0.0001%	85	80	12	100	100	83	100	100	67	50	0	100	100	75	100	0	0	0	40	50
violet	0.0002%	33	40	0	100	90	33	100	100	0	25	0	100	100	0	50	9	0	0	0	0
Cupric	0.1%	3	20	0	50	0	0	0	0	0	25	0	0	100	50	100	0	0	0	0	0
sulfate	0.05%	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Methylene	0.0001%	100	100	100	100	100	100	100	100	100	100	0	100	100	100	100	36	50	33	80	100
blue	0.0002%	100	100	100	100	100	100	100	100	100	100	0	100	100	100	100	27	50	33	80	100
	0.0005%	100	80	100	100	100	100	100	100	100	100	0	100	100	100	100	0	25	33	100	100
Degradation of																					
Hypoxanthine		85	60	75	100	100	100	100	100	67	25	100	0	0	100	100	9	75	0	60	0
Xanthine		69	40	62	50	50	83	0	100	33	25	50	0	0	75	100	9	0	33	0	17
Xylan		95	100	75	50	0	39	0	0	67	0	100	33	0	0	100	73	50	100	0	0
Esculin		31	80	25	0	90	61	100	100	100	100	0	67	50	100	100	64	0	0	0	33
Urea		23	20	12	0	30	83	50	100	33	25	0	0	0	0	0	9	25	0	0	0
Tween 40		87	60	62	50	100	100	100	100	100	0	50	100	50	75	100	64	100	67	40	0
Tween 80		95	60	75	50	100	100	100	100	100	0	100	100	100	100	100	55	00	67	20	17
Allantoin		38	0	62	100	60	33	50	50	0	75	100	33	50	0	0	18	25	67	0	0
Antimicrobial activity against																					
Aspergillus nidulans		0	0	0	0	0	17	0	0	0	0	0	100	100	0	0	9	0	0	40	0
Candida albicans		0	0	0	0	10	44	0	0	0	0	0	100	100	0	0	0	0	0	60	0
Bacillus subtilis		5	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Enzyme activity of																					
Nitrate reduction		56	0	50	0	80	33	0	100	67	50	50	100	0	100	100	55	0	100	0	83
H_2S production		87	80	100	5	100	100	100	100	67	25	0	100	0	100	50	45	75	0	60	100

Table 4. Distribution of positive characters to single member clusters

Cluster	1D	1E	6	7	8	11	12	14	15	16	17	22	23	24	25	26
Spore mass color Grey	-	+	+	-	-	-	-	-	+	-	-	+	-	-	+	+
Pink	-	-	-	-	-	-	-	+	-	+	-	-	+	-	-	-
White	+	-	-	+	-	+	+	-	-	-	+	-	-	-	-	-
Ivory	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Blue	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Orange	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Maroon	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Substrate mycelium color																
Yellow	+	-	-	-	-	+	-	-	-	-	+	-	-	-	+	-
Brown	-	+	+	+	+	-	+	-	-	-	-	+	-	+	-	+
Orange	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Purple	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-
Black	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Pigment color																
Yellow	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Brown	-	-	-	+	-	-	+	-	+	+	-	+	+	-	-	-
Purple	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Presence of aerial mycelium	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spore chain morphology																
RF	+	-	+	+	-	-	-	+	-	-	-	-	+	+	+	+
RA	+	+	-	-	+	+	+	-	-	+	+	+	-	-	-	-
SP	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Utilization of C source																
N-Ac-glucosamine	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
Adonitol	-	-	+	+	-	-	+	+	-	-	+	-	-	-	+	-
D-Arabinose	-	-	+	+	-	-	+	-	+	-	+	-	-	-	-	-
L-Arabinose	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
Cellobiose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2-Deoxyglucose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fructose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-galactonolactone	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
Galactose	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
D-Gluconolactone	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glucosamine	+	+	+	-	-	+	+	-	+	+	-	-	+	-	-	-
Glycerol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L-Gulonolactone	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Inositol	+	+	+	+	-	-	-	+	-	-	-	-	-	-	+	+
Lactose	+	+	+	+	-	+	+	+	+	+	+	-	+	-	+	+
D-Lyxose	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-
Maltose	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+
Mannitol	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+
Mannose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Melezitose	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-
1-o-Methylglucose	+	+	+	+	+	-	+	+	-	-	+	-	+	-	+	-
α-Methyl-D-mannose	+	-	+	+	-	-	+	+	-	-	+	-	+	-	-	-
Rhamnose	+	+	+	+	+	-	+	+	+	+	-	-	-	-	-	-
Ribose	+	+	+	-	-	+	+	+	+	+	-	+	+	-	-	+
Sorbose	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Sucrose	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
Trehalose	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+
Xylitol	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	-
D-Xylose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L-Xylose	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-

Table 4. Continued

Cluster		1D	1E	6	7	8	11	12	14	15	16	17	22	23	24	25	26
Gluconic acid		-	+	+	+	+	-	+	+	+	+	+	+	+	-	+	-
Sodium malonate		-	-	+	+	-	+	+	+	-	-	-	-	-	-	-	-
α-Alanine		+	+	+	-	+	+	+	+	-	-	-	-	-	-	+	-
β-Alanine		+	+	+	-	+	+	+	+	-	-	+	-	-	+	-	-
Arginine		+	+	+	+	+	-	+	+	-	+	-	+	-	+	+	+
Glutamine		+	-	-	+	-	+	+	+	+	+	+	-	-	+	-	-
Glutamic acid		+	+	+	+	-	+	+	+	+	+	+	-	+	+	-	+
Glycine		+	-	+	+	+	+	-	+	-	-	+	-	-	+	+	-
Histidine		+	+	+	+	+	-	+	+	-	-	+	+	+	-	-	-
Isoleucine		-	-	-	+	-	+	-	-	-	-	-	-	-	+	-	-
Lysine		-	-	+	+	+	+	+	+	-	-	+	-	-	+	-	-
Methionine		-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Ornithine		+	-	+	+	+	+	+	+	-	-	-	-	+	-	-	+
Proline		+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
Dextran		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dextrin		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Inulin		+	+	+	+	+	-	+	+	-	-	+	+	-	+	+	+
Dulcitol		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Salicin		+	-	-	+	-	+	-	+	+	-	-	+	+	+	-	-
Growth in the presence of																	
NaCl	5%	+	+	+	+	-	+	+	-	+	+	+	+	+	-	+	-
	7%	+	+	+	+	-	+	-	-	-	+	+	+	-	-	-	-
Sodium azide	0.01%	+	+	+	-	-	-	-	-	-	-	+	-	-	-	-	+
	0.02%	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
Phenol	0.1%	+	+	+	+	-	+	-	-	+	+	-	+	-	+	-	+
	0.2%	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Thallous	0.001%	+	+	+	-	-	+	+	-	+	-	+	+	+	+	+	-
acetate	0.002%	+	-	-	-	-	+	-	-	+	-	-	+	-	+	-	-
Phenylethanol	0.1%	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	0.2%	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+
Potassium	0.001%	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Crystal	0.0001%	+	-	+	+	-	-	-	-	+	+	+	-	-	+	+	+
violet	0.0002%	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-
Cupric	0.01%	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
sulfate	0.05%	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Methylene	0.0001%	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+
blue	0.0002%	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+
	0.0005%	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+
Degradation of																	
Hypoxanthine		-	-	+	+	-	-	-	+	-	+	-	+	+	+	-	-
Xanthine		+	-	-	+	+	+	-	-	-	-	+	+	+	+	-	-
Xylan		+	+	-	-	+	+	+	-	-	+	-	+	-	+	-	-
Esculin		+	+	+	+	-	-	-	+	+	-	-	+	+	-	-	-
Urea		+	-	+	-	-	-	+	-	+	-	+	+	-	-	-	-
Tween 40		+	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+
Tween 80		+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+
Allantoin		-	+	+	+	-	+	-	+	+	-	+	+	+	+	-	-
Antimicrobial activity against																	
Aspergillus nidulans		-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
Candida albicans		-	-	-	-	-	-	-	-	-	-	+	-	+	+	+	-
Bacillus subtilis		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Enzyme activity of																	
Nitrate reduction		+	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+
Sufide production		-	+	+	-	-	+	+	+	-	-	-	+	+	-	-	+

Table 5. Average positive response of the clusters to the character sets (major and minor clusters)

Cluster	# Strains	Character sets[a]				
		C(49)	I(19)	D(8)	M(3)	E(2)
1A	39	67	73	65	2	72
1B	5	70	62	53	0	40
1C	8	69	60	56	0	75
2	2	61	71	50	0	25
3A	10	62	71	66	7	90
3B	18	56	74	75	20	67
4	2	49	66	63	0	50
5	2	48	68	81	0	100
9	3	59	54	63	0	67
10	4	64	45	31	0	38
13	2	68	26	63	0	25
18	3	57	68	42	67	100
19	2	56	89	31	67	0
20	4	38	57	56	0	100
21	2	44	68	75	0	75
27	11	27	24	38	3	50
28	4	57	33	47	0	38
29	3	45	19	42	0	50
30	5	29	39	15	33	30
31	6	19	46	8	0	92

[a] C, carbon source utilization, I, resistance to inhibitors, D, degradation, M, antimicrobial activities, and E, enzyme activities. In ( ) are the numbers of characters.

timicrobial activities were relatively high in some minor and single member clusters, most of which consisted of reference strains.

From the above results, especially given in Table 5, the major clusters showed high positive responses for all the character sets except for the antimicrobial activity, while the clusters in group B showed lowest positive responses for all the character sets except for antimicrobial activity. Little difference in responses was observed between minor and single member clusters.

Discussion

The actinomycetes are characterized by their morphological diversity and pigmentation (19, 36). And their numerical and chemical taxonomic studies were generally in good agreement with the morphological descriptions (10, 11, 24, 25, 36). The microscopic observation of the morphology of spore chains and the ornamentation of spore surface gives stable characters, though there are some other features such as sclerotia, sporangia, and synemata-like structures (19, 20) that were hardly found in the isolates. The pigmentation properties of spore masses, mycelia and diffusible pigments, despite their possible variations according to the culture conditions or mutations, enabled the distinction between the clusters defined by numerical methods in the present study. Together with this, their inclusion in numerical data did not affect significantly the taxonomic structure of the numerical classification with the physiological characters, which implied the stability of the present study.

Numerical data may contain erroneous readings which are probable to affect in wrong ways the determination of taxonomic structure of test organisms (29), so the test error should be considered and reduced if possible. The average test error estimated in this experiment was 4.27%, which was considered slightly high as compared to the other experimental errors of 3.36% by Williams *et al.* (36), and 3.11% by Kämpfer *et al.* (11), but low enough for the suggested limit of 10% by Sneath and Johnson (29). The clusters defined by S_{SM} was not altered significantly by Jaccard coefficients (S_j; 27) and pattern difference coefficients (D_p; 1) (data not shown).

Streptomycetes and other actinomycete groups were separated effectively by numerical classification, and each group of actinomycetes was recovered well as distinctive groups again. The cluster composition may reflect the original distribution of actinomycetes in soil samples, even though there could be some selective forces for or against some specific groups of organisms. Streptomycetes resided about 75% of the total isolates, micromonosporas about 10%, and nocardioforms about 5%. respectively. Streptomycetes showed relatively higher abilities of utilizing substrates and higher resistance to inhibitory substances than other actinomycete groups. In streptomycete clusters, the two major clusters showed higher responses than the minor or single member clusters, which implied their higher adaptability or survivability than the others in the environments. The cluster composition of the isolate strains may reflect the population distribution of the isolate strains constituting major clusters which are competitive for a wider range of organic substrates rather than those of minor or single member clusters. High antimicrobial activities were found in some of the minor and single member clusters, but most of the antibiotic producing clusters are composed of the reference strains except for a single member cluster, cluster 23. It revealed that the actinomycete population in the sampling area had low antimicrobial activities in general. The low responses in group B may be partly due to the experimental conditions, for those tests were employed for streptomycetes in the previous studies, but not for the other actinomycetes. In practice the two among the three duplicates in group B showed lowest reproducibilities among the whole duplicates. Many of the actinomycete genera may require different

basal media, growth temperatures or prolonged incubation periods.

The numerical taxonomy employs the unit phenetic characters that can be clearly observed and defined. However, one must consider the experimental conditions as an important factors. for the results may be influenced greatly by these conditions, which can interfere with having a good comparison between laboratories. Chemical methods such as pyrolysis mass spectrometry or fatty acid profile analysis proved their usefulness with actinomycete groups (3, 5, 21, 24, 25, 34) and these will enable as to provide evaluation of views from the numerical classification. Molecular taxonomy is different from numerical or chemical methods in that the genetic materials to be studied do not change in accordance with environmental or conditional variations. Even though it does not reveal phenetic characters, the molecular taxonomy became one of the popular methods in the classification of actinomycetes, providing new views with a number of techniques (7, 8, 17, 23, 32), Thus the classification of actinomycetes should be a polyphasic taxonomy which includes numerical, chemical, and molecular based works altogether.

There have been a few works on the taxonomic study of actinomycetes isolated from Korean soils by the numerical and chemical classification which have revealed the distribution and the taxonomic relationship among the isolated strains and the reference strains of actinomycetes (13, 22, 25). Actinomycetes are one of the important members in comprising microbial diversity in soil ecosystems, and are also well recognised for their industrial significance, and it might be essential to study the taxonomic basis for the further study of actinomycetes in nature.

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