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A New Method for the Intensive Isolation of Actinomycetes from Soil

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A new method was developed to isolate actinomycetes from soil more selectively and more thoroughly.

Elements of the procedure are (1) treatment of soil suspension with a solution containing yeast extract (YE) 6% and sodium dodecyl sulfate (SDS) 0.05%, at 40°C for 20 min, (2) subsequent dilution with water and (3) a few week's incubation on HV agar plates containing nalidixic acid (n.a.) 20 mg/L.

The YE and the heat shock at 40°C activated spore germination of a variety of actinomycete strains, the SDS acted as a germicide only on bacterial cells with rare exception, and the n.a. suppressed growth of bacterial spore formers without any effect on the actinomycetes, under the conditions used.

By the treatment of soil suspension with YE and SDS, the count of actinomycete cfu per g. of various soils (10 samples) was increased by 40% and the count of bacteria was decreased to 20%, on the averages. The bacterial count was further decreased to less than 10% by addition of n.a. into the isolation medium.

Actinomycetes are widely distributed in soil and constitute a significant part of soil microflora[1]). Selective isolation methods for these organisms are important for studying their ecology as well as for screening of industrially useful strains.

Various methods have been developed for the preferential isolation of general actinomycetes from soil. These methods include a variety of selective isolation media supplemented with certain antibiotics[2-6]), and the pretreatments of soil sample or soil suspension by enrichment[7]), heating[8,9]), membrane filtration[10]), or use of chemicals such as phenol[11]), benzalkonium chloride[12]), etc. Recently, the specific procedures for isolating particular genera or groups of actinomycetes have also been developed[13-24]).

As the actinomycetes are generally outnumbered by other bacteria in their habitat such as soil, many of the isolation methods have been designed to eliminate undesirable bacteria from inocula or to suppress the bacterial growth on isolation media, while preserving the viability of actinomycetes. However, following problems of the isolation procedures still remain: (i) any of the isolation media are not absolutely selective for actinomycetes[12,25]), and (ii) some of the pretreatments and incorporation of antibacterial antibiotics into isolation media cause the reduction in the number of actinomycetes[2,4,25,26]).

In this paper, we describe a new method for isolating general actinomycetes from soil, whereby the number of actinomycete colonies can be increased whereas bacterial colonies effectively decreased on the isolation plates. It includes the pretreatment of soil suspension with a spore activating agent (yeast extract) and a germicide (sodium dodecyl sulfate: detergent), and use of a selective isolation medium, HV agar[27]) containing nalidixic acid.

MATERIALS AND METHODS

Strains. Thirty-five strains of bacteria including 27 strains of actinomycetes (Table 2) were used. Sources of these strains are as follows: International Streptomyces Project (ISP); Institute for Fermentation, Osaka, Japan (IFO); Northern Utilization Research and Development Division, US Department of Agriculture, Peoria, Illinois (NRRL); Japan Collection of Microorganisms, RIKEN, Saitama, Japan (JCM); American Type Culture Collection, Rockville, Maryland (ATCC); Centraalbureau voor

Schimmelkultures, Baarn, Netherlands (CBS); Central Research Laboratories, Kaken Chemical Co., Ltd., Tokyo, Japan (KCC); Institute of Applied Microbiology, University of Tokyo, Japan (IAM); Laboratory strains (M6, Nonomura and Ohara, 1960[28]; S9, Nonomura and Ohara, 1960[29]; T80, Nonomura and Ohara, 1971[16]).

Actinomycete strains were stored on oatmeal agar YGG[44] or yeast starch agar[31] slants.

Soil samples. As shown in Table 1, fourteen samples were used. The samples were collected and preserved by the methods previously described[30]. A portion of the preserved soil was passed through a 2 mm-mesh sieve, air-dried at room temperature for one week and used for the isolation of actinomycetes as well as for chemical analysis[27].

Spore activating agents and germicide. The chemicals used are as follows: yeast extract (YE; Difco, U.S.A.), sodium dodecyl sulfate (SDS; Tokyo Kasei Co.), L-valine (Va; Wako Pure Chemical Ind. Ltd., Osaka), mercaptoethanol (ME; Wako), vitamin assay casamino acids (CA; Difco), and humic acid (HA; RF 21.3, $\Delta \log K$ 0.84) which was prepared from soil of mountain forest by the method previously described[27]. The humic acid was used also as a nutrient of HV agar[27].

These agents were dissolved in 5 mM-phosphate buffer (pH 7.0) and sterilized separately by autoclaving, except for ME which was added to a sterile phosphate buffer solution. The pH value of each solution was adjusted to 7.0 with 2N NaOH or 1N HCl before autoclaving.

Preparation of spore- or bacterial cell-suspensions. Actinomycete strains were inoculated on the plates of oatmeal agar Y[28] (pH 7.2, for the strains of *Streptomyces, Actinomadura, Microtetraspora niveoalba, Mit. glauca, Streptosporangium, Nocardia, Dactylosporangium* and *Actinoplanes*; pH 6.5, for the strains of *Microbispora*) or yeast starch agar[31] (pH 7.2, for the strains of *Micromonospora*; pH 8.0, for the strains of *Mit. viridis, Thermomonospora* and *Saccharomonospora*). The cultures were incubated at 30°C for 4 weeks, except for the strains of *Thermomonospora, Mit. viridis, Mit. niveoalba* and *Saccharomonospora* which were incubated at 35°C, subsequently stored at 5°C for about one month. Spores were scraped off the agar surface with an inoculating needle and collected in 5 ml of sterile tap water (15°C). The tube was vigorously stirred on a Thermo-mixer (Thermonics Co., Tokyo) and sonicated for 15 sec. in an ultrasonic bath (45Kz, 15W: Branson Ultrasonic Corp., U.S.A.). The spore suspension was then passed twice through a 5.0 μm pore size membrane (Gelman Science Inc., U.S.A.) to remove mycelial fragments and diluted with sterile tap water at a density of 1×10^7 spores/ml.

The strains of other bacteria were inoculated on nutrient agar[32] slants and incubated at 30°C for a day. Cell suspension was prepared by transferring the culture to sterile tap water at a density of 1×10^7/ml. Spores of the *Bacillus* strain were scraped off the nutrient agar slant culture incubated at 25°C for 10 days and collected in sterile tap water. The suspension was used after heating at 80°C for 15 min. to kill vegetative cells[33].

Recovery from spore- or bacterial cell-suspension after pretreatment with YE or SDS. To prepare an inoculum, 0.5 ml of spore- or bacterial cell-suspension was transferred to 4.5 ml of 5 mM-phosphate buffer (pH 7.0) containing either YE (at the final concentration of 6%, w/v) or SDS (at the final

Table 1. Soil samples used for isolation of actinomycetes.

Sample No.	Prefecture	Type*	Loss on ignition (%)	pH
323	Yamanashi	Fi	15.7	5.3
346	Nagano	mF	23.2	5.3
375	Yamanashi	Fi	7.2	6.1
377	Yamanashi	Fi	6.9	6.5
378	Yamanashi	lF	16.7	5.8
381	Yamanashi	P	12.7	5.8
384	Yamanashi	Fi	17.4	5.8
388	Yamanashi	Pa	22.3	6.3
389	Nagano	Fi	22.9	7.2
401	Yamanashi	P	6.0	5.1
403	Nagano	Fi	22.8	6.2
405	Yamanashi	Fi	16.1	5.8
406	Nagano	Fi	15.7	6.2
407	Nagano	Fi	21.1	6.0

* Fi: field (non paddy, cultivated),
mF: mountain forest,
lF: level-land forest,
P: paddy field, Pa: pasture.

Table 2. Recovery from actinomycete spore- and bacterial cell-suspensions after the pretreatment and dilution, and sensitivity to nalidixic acid (NA), on agar plates.

Species	Colony formation on HV agar, % to the control			Growth on HV-N agar[c)] plus NA 20 mg/L
	control (Ben)[a)]	Pretreatment[b)] YE	SDS	
(Actinomycetes)*				
Streptomyces viridochromogenes ISP 5110	100 (86)	138	113	+
Streptomyces gougeroti IFO 3198	100 (88)	118	89	+
Streptomyces coelicolor IFO 3504	100 (110)	121	126	+
Streptomyces antibioticus IFO 3126	100 (79)	111	35	+
Streptomyces flaveolus IFO 3408	100 (106)	105	61	+
Streptomyces flavus IFO 3359	100 (63)	124	61	+
Streptomyces albus IFO 3418	100 (230)	134	126	+
Streptomyces lavendulae IFO 3177	100 (98)	128	0**	+
Micromonospora carbonaceae NRRL 2972	100 (96)	118	121	+
Micromonospora chalcea JCM 3031	100 (50)	130	96	+
Micromonospora megalomicea IFO 14114	100 (73)	108	113	+
Microbispora amethystogenes M10	100 (83)	112	104	+
Microbispora diastatica M6	100 (56)	130	101	+
Microbispora rosea ATCC 12950	100 (51)	103	39	+
Microbispora chromogenes CBS 304.61	100 (60)	106	78	+
Actinomadura verrucosospora ATCC 27299	100 (105)	140	106	+
Actinomadura roseoviolaceae ATCC 27297	100 (70)	121	61	+
Actinomadura pussilla ATCC 27296	100 (64)	104	95	+
Microtetraspora viridis ATCC 27103	100 (59)		126	+
Microtetraspora niveoalba ATCC 27301	100 (84)	127	120	+
Microtetraspora glauca ATCC 27645	100 (89)	116	104	+
Streptosporangium roseum S9	100 (101)	120	74	+
Nocardia asteroides JCM 3384	100 (111)	132	74	+
Saccharomonospora viridis T80	100 (33)	115	80	+
Thermomonospora mesophila ATCC 27303	100 (87)	114	57	+
Dactylosporangium thailandense KCC A0084	100 (174)	111	113	+
Actinoplanes brasiliensis KCC A0196	100 (110)	90	0	+
(Bacteria)				
Bacillus subtilis IAM 1069	100		0	-
Bacillus cereus IAM 1072	100		0	⊥
Micrococcus lysoideikticus IAM 1056	100		0	+
Brevibacterium ammoniagenes IAM 1641	100		0	⊥
Pseudomonas putida IAM 1506	100		0	⊥
Pseudomonas saccharophila IAM 1504	100		0	⊥
Flavobacterium sewanense IAM 1014	100		0	+
Escherichia coli IFO 3044	100		84	-
Spores (*B. subtilis* IAM 1069)	100		136	

a) Bennett's agar as a control to HV agar.
b) YE (yeast extract) 6%, SDS (sodium dodecyl sulfate) 0.05%, in 5 mM phosphate buffer (pH 7.0) at 40°C for 20 min.
c) cf. text.
*Spores from stocked (5°C, one month) cultures, filtered to remove mycelial fragments.
**In combined use with YE, up to 30% survived.

concentration of 0.05%, w/v), and maintained at 40°C for 20 min with occasional stirring. A portion was then further diluted with sterile tap water (1:1,000). Inocula of 0.1 ml were spread over the 5 plates of HV agar with a sterile glass rod. The plates were incubated at 30°C (except for the strains of *Thermomonospora*, *Microtetraspora viridis*, *Mit. niveoalva* and *Saccharomonospora* which were incubated at 35°C) for 10-14 days, following which colonies appeared were counted. Untreated spore- or bacterial cell-suspension (control) was also diluted with sterile tap water, inoculated on the plates of HV agar and of Bennett's agar[45], and incubated, under the same conditions.

Sensitivity test to nalidixic acid. HV agar supplemented with 0.1% (w/v) beef extract, 0.1% (w/v) peptone and 0.05% (w/v) NaCl was used as basal medium (HV-N agar). Nalidixic acid (Sigma Chemical Co., U.S.A.) was dissolved in 0.1N NaOH (20 mg/2 ml, sterile) and added to the autoclaved HV-N agar.

Inocula of actinomycetes were prepared by suspending one loopful of spores from slant culture into 1 ml of sterile tap water. For other bacteria, the nutrient broth cultures incubated on a shaker at 30°C for overnight were used as the inocula. About 0.05 ml of the spore suspension or one loopful bacterial culture was streaked on the surface of HV-N agar containing 20 mg/L nalidixic acid. These plates were incubated and the growth was scored after 14 days (for actinomycetes) or 7 days (for other bacteria).

Selective isolation procedure for actinomycetes. One gram of air-dried soil sample was added to 10 ml of sterile tap water in a tube and stirred for 1 min. on a Thermo Mixer. After allowing the tube to stand for 1 min. to precipitate coarse sand, 0.5 ml of the supernatant was removed to 4.5 ml of sterile 5 mM-phosphate buffer (pH 7.0) containing spore activating agent and germicide separately or in combination. The mixture was maintained at 40°C for 20 min. with occasional stirring. A portion (1 ml) was then further diluted with sterile tap water (1:1,000) by the 10-fold dilution method[13]. Inocula of 0.1 or 0.2 ml were spread over the surface of 7-10 plates of HV agar[27] and incubated for 3 weeks at 30°C. Colonies of actinomycetes and other bacteria which appeared on the plates were examined with the naked eyes and a light microscope. The numbers of colonies were determined from the mean counts of the 7-10 plates and results were statistically analysed as previously described[30]. Untreated soil suspension (control) was also diluted with sterile tap water, inoculated on HV agar plates and incubated, under the same conditions.

RESULTS

Effects of each agent of the new method on spores or cells from pure cultures. The effect of the pretreatment (40°C, 20 min.) with yeast extract (YE) or sodium dodecyl sulfate (SDS) was confirmed using 35 species of actinomycetes and other bacteria. Details of the results are shown in Table 2. The pretreatment with YE increased the recovery% (colonies from spores) of many of the actinomycete strains tested. In contrast, the recovery% on Bennett's agar, which contains YE as a nutrient, were generally lower than those on HV agar. Agent SDS killed vegetative cells of the test soil bacteria including the strains of *Bacillus*, *Pseudomonas* and *Brevibacterium* genera, but this agent was harmless, with rare exception, to the spores of actinomycete strains and of *B. subtilis*. The recovery of several actinomycete strains tested was increased by the SDS treatment.

Results of the sensitivity test of actinomycetes and other bacteria against the antibiotic nalidixic acid are also given in Table 2. Nalidixic acid (20 mg/L) in HV-N agar had no effect on the growth of actinomycete strains examined, but suppressed the growth of the soil bacteria. The sporogenous species of *B. subtilis* was completely inhibited.

Mild heat treatment of soil suspension. Figure 1 shows the changes of the number of actinomycetes on isolation plates brought by the heating of a soil suspension in phosphate buffer at 40°C or 50°C for various periods. The treatment for proper time increased in number of actinomycetes on the plates, especially at 40°C for 20 min. resulted in the highest recovery of actinomycetes.

The effect of this treatment (40°C,

30 min.) was also examined for 6 other soil samples, and confirmed the increase in number of actinomycetes on isolation plates by 13% (0-28%) on an average (details not shown).

Effects of various spore activating agents on isolation of actinomycetes. Considering the results mentioned above, we investigated the effects of the pretreatments of soil suspension with these spore activating agents and with their relatives, such as casamino acid (CA), valine (Va), humic acid (HA) and mercaptoethanol (ME), on the isolation of actinomycetes.

The following concentrations (%, w/v) of the agents were used individually in soil pretreatment (40°C, 20 min.) for isolation of actinomycetes from 2 soil samples: YE (1, 2 and 4), HA (0.2, 1 and 2), CA (0.5, 1 and 2), Va (0.1, 0.2 and 0.5), ME (0.1, 0.2 and 0.4) and SDS (0.05 and 0.1). Table 3 shows the efficiencies of the pretreatments with individual agents at the optimal concentrations for isolating actinomycetes from a soil sample No. 405. The pretreatments clearly increased the number of actinomycete cfu (colony forming unit); the treatment with 2% YE gave the largest number of actinomycete cfu, followed by the treatment with 2% HA. The number of actinomycete cfu brought by the YE- or the HA-treatment was significantly ($P<0.01$) larger than the number obtained from untreated inocula (control). On the other hand, the treatments with SDS and HA significantly ($P<0.001$) decreased bacterial counts. For another soil sample (No. 406), the results obtained were similar to those in Table 3. Therefore it was planed to investigate the effects of the combined use of SDS (as a germicide to soil bacteria) with spore activating agents in the pretreatment of soil sample.

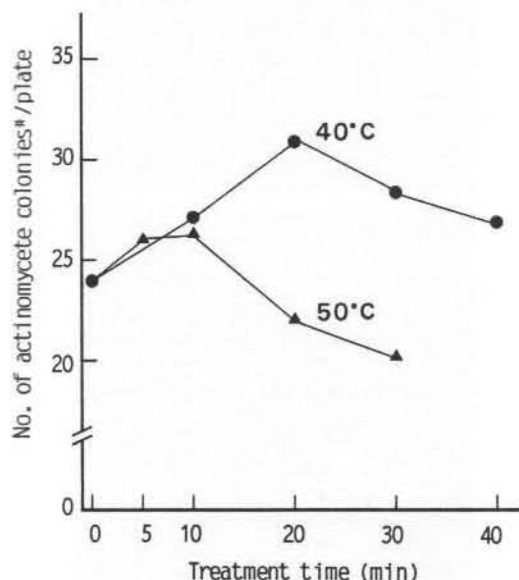

Fig. 1. Increase of actinomycete colonies by heat treatment at 40°C and 50°C in P-buffer (5 mM, pH 7.0) suspension. (Soil No. 407, HV agar)
* After 3 weeks at 30°C.

Effects of combined use of SDS with spore activating agents. In Figure 2, optimal concentrations of SDS and YE in combined use are shown, using a soil sample No. 405. In a combined use of 0.05% SDS and 6% YE, remarkable decrease in bacterial number and distinct increase in actinomycete number were observed.

Agents HA, Va, CA and ME were also combined individually with 0.05% SDS in treatment of soil suspension and investigated their optimal concentrations for isolating actinomycetes from a soil sample No. 405. The efficiencies of the combined uses of 0.05% SDS and individu-

Table 3. Effects of various spore-activating agents on isolation of actinomycetes from a soil sample (No. 405).

Treatment* 40°C, 20 min.	No. of colonies (cfu/g of soil**)	
	Actinomycetes $x10^5$ (%)	Bacteria $x10^5$ (%)
None (Control)	117 (100)	152 (100)
Heat shock (40°C)	136 (116)	155 (102)
YE 2%	192 (164)	169 (111)
HA 2%	183 (156)	92 (60)
CA 1%	170 (145)	165 (109)
Va 0.2%	159 (136)	178 (117)
ME 0.2%	163 (139)	136 (89)
SDS 0.1%	152 (130)	10 (7)

*YE: yeast extract, HA: humic acid, CA: casamino acid, Va: valine, ME: mercaptoethanol, SDS: sodium dodecyl sulfate, each in 5 mM-phosphate buffer(pH 7.0). HV agar was used for isolation.
**After 3 weeks at 30°C.

al agents at the optimal concentrations are compared in Table 4. Among the various combinations, a combination of 0.05% SDS and 6% YE produced the largest number of actinomycete cfu, while decreased the bacterial counts. The number of actinomycete cfu brought by the combined use of SDS and YE was significantly ($P<0.001$) larger than the number obtained from untreated inocula (control). Figure 3 shows the example of the isolation plates. On the isolation plate inoculated with the dilutant of soil suspension that had been treated with the pair of SDS and YE, the numbers of actinomycete colonies are larger than the numbers on control plate, while bacterial colonies are very few.

Figure 4 shows that the pretreatment of a soil suspension with a combination of SDS and YE gave rise to a great increase in the number of actinomycete colonies on isolation plates in a few days.

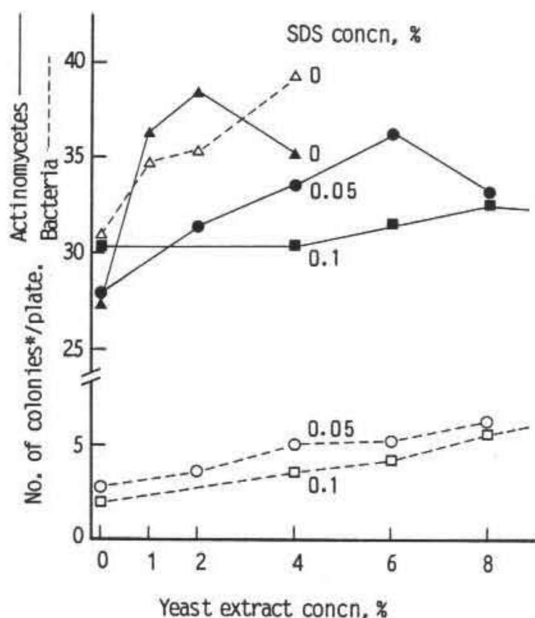

Fig. 2. Optimal concentrations of yeast extract and SDS in combined use, in the pretreatment (40°C, 20 min.) of soil in P-buffer (5 mM, pH 7.0) for selective isolation of actinomycetes. (Soil No. 405. HV agar).

* After 3 weeks at 30°C.

Table 4. Effects of the combined use of SDS with the other spore-activating agents on isolation of actinomycetes from a soil sample (No. 405).

Treatment* 40°C, 20 min.	No. of colonies (cfu/g of soil**)	
	Actinomycetes $x10^5$ (%)	Bacteria $x10^5$ (%)
None (Control)	117 (100)	160 (100)
SDS + YE 6%	183 (157)	22 (14)
" + HA 1%	173 (148)	9 (5)
" + CA 1.5%	153 (131)	15 (9)
" + Va 0.6%	147 (126)	19 (12)
" + ME 0.1%	158 (135)	14 (8)

* 0.05% SDS was dissolved in 5mM-phosphate buffer (pH 7.0). For abbreviations see Table 3. HV agar was used for isolation.

** After 3 weeks at 30°C.

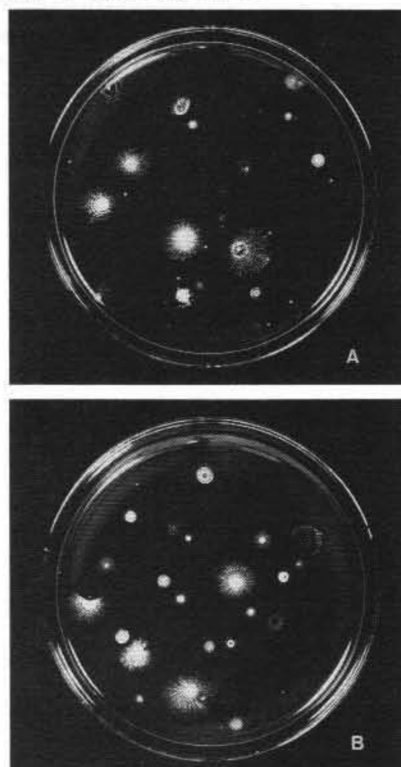

Fig. 3. Selective isolation of general actinomycetes from the soil No. 405. HV agar. 3 weeks at 30°C. Dilution $2x10^{-6}$). A: Control, B: Pre-treatment at 40°C for 20 min. with 6% YE and 0.05% SDS in 5 mM P-buffer (pH 7.0).

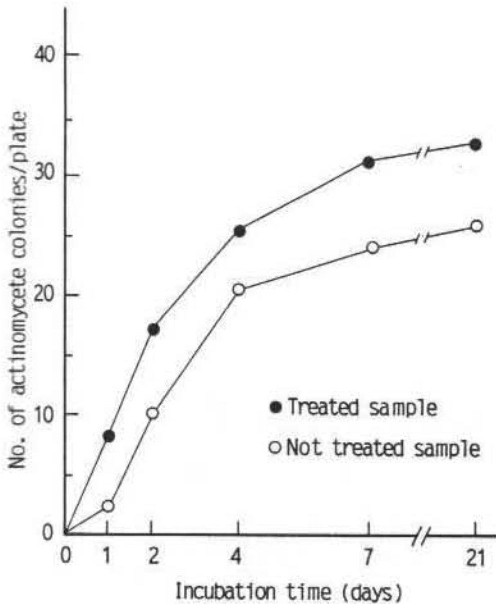

Fig. 4. Increase in actinomycetes number and colony formation rate by the pretreatment of soil (No.323) suspension. (see Fig. 3).

Application of the new method to various soil samples. The efficiency of the soil pretreatment with 6% YE and 0.05% SDS at 40°C for 20 min. was confirmed using 10 soil samples collected from cultivated fields, paddy rice fields, forests and pasture (Table 5). The treatment increased actinomycete cfu by about 40%, and decreased bacterial count to about 20%, on the averages. In 5 soil samples (No. 346, 381, 384, 389 and 403), the numbers of actinomycete cfu brought by the treatment were significantly larger ($P<0.01$) than the numbers on control plates. In all of the samples, bacterial counts were decreased significantly ($P<0.001$) by the treatment. Addition of nalidixic acid (20 mg/L) into the isolation medium, HV agar, further decreased the bacterial count to less than 10% on an average, while no decrease in the number of actinomycete cfu was observed in this case.

Procedure of the new method. From the results obtained above, the following

Table 5. Isolation of actinomycetes from various soils by the new method*.

Soil sample (type)	Untreated control on HV agar			Treated with YE and SDS* on HV agar		on HV+n.a.**
	Actino. $x10^5/g$	Actino. (control)	Bacteria %	Actino. %	Bacteria %	Bacteria %
(Field)						
No. 375	90	100	477	117	68	24
No. 377	149	100	320	114	36	17
No. 384	127	100	260	143	24	9
No. 389	134	100	921	146	271	128
No. 403	104	100	320	163	77	33
(Paddy field)						
No. 381	7	100	1905	168	397	170
No. 401	25	100	1180	104	204	90
(Forest)						
No. 346	11	100	137	157	79	25
No. 378	24	100	226	134	60	30
(Pasture)						
No. 388	91	100	1426	140	122	63
Average		100	717	139	134	59

*Pretreatment of soil suspension with yeast extract(YE) and sodium dodecyl sulfate(SDS), dilution, inoculation on HV agar plates containing nalidixic acid(n.a.) and a few week's incubation at 30°C. Increase of actinomycetes and decrease of bacteria). 6% YE and 0.05% SDS , in 5 mM P-buffer(pH 7.0) at 40°C for 20 min.

**No decrease in number of actinomycetes was observed by addition of nalidixic acid 20 mg/L.

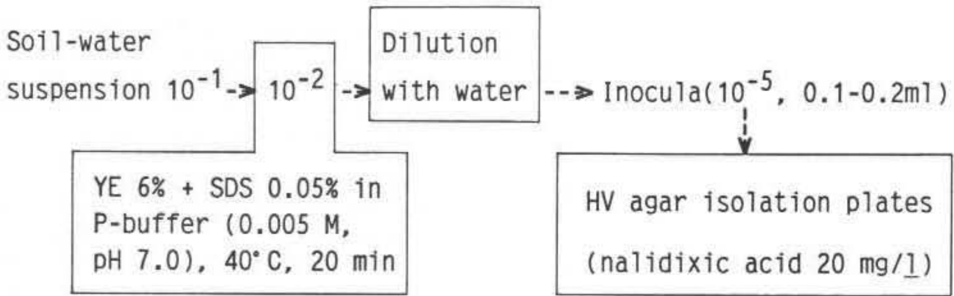

Fig. 5. Scheme for isolation of actinomycetes from soil.

procedure (Fig. 5) was set up as a new, useful method for the selective isolation of actinomycetes from soil. Sieved and air dried soil is used as the samples. The dilution after the pretreatment of soil suspension is necessary to remove the action of YE and SDS in isolation plates, because YE promotes bacterial growth, and SDS injures germinating spores of actinomycetes. To obtain a clear result, 2-3 weeks incubation and development of 20-40 colonies of actinomycetes per plate are desirable.

DISCUSSION

Several agents including YE[34], SDS[36] and heat shock[35] have been reported to stimulate the germination of actinomycete spores. However, none of these spore-activating agents have been applied to the isolation of actinomycetes. We found that the pretreatment of soil suspension with YE, SDS and heat shock (40°C, 20 min.) resulted in increase of both actinomycete recovery (Tables 4 and 5) and the rate of colony formation on isolation plates (Fig. 4). These effects are considered to be due to the form of actinomycetes in soil. Direct observation using scanning electron microscope[37], homogenization technique[37], the study of actinostasis by soil[38,39] and other experiments[40,41] have indicated that the actinomycetes in soil exist largely in the form of dormant spores in their life cycle.

Elimination of bacteria from actinomycete isolation plates is an important problem. We found that a detergent, SDS, acted not only as a spore activator of actinomycetes but also as a selective germicide to soil bacteria. The pretreatment of soil suspension with SDS killed most of bacteria, while increasing actinomycete recovery (Tables 3, 4 and 5). This is an advantage when compared with the phenol treatment[11] which has been reported to, as a result of restudying[2], cause reduction in the number of actinomycetes as well as bacteria. The germicidal action of SDS was removed easily by dilution with water. This is more advantageous than benzalkonium chloride treatment[12] which needs an inactivator. Bacterial spores were resistant to SDS (Table 2). However, HV agar limited bacterial growth and the addition of nalidixic acid (20 mg/L) into HV agar inhibited bacterial growth without affecting the actinomycete growth (Table 5). Barton and Hughes[42] used a selective medium containing nalidixic acid and other antibiotics for the isolation of *Rhodococcus* strains. Wakisaka[43] showed that the *Streptomyces* and *Micromonospora* strains tested were resistant to nalidixic acid. However, this antibiotic has not been used for the isolation of actinomycetes in general.

The pretreatment of soil suspension with spore activating agent and germicide at 40°C for 20 min. greatly increased the recovery of actinomycetes from various soil samples (Table 5). This pretreatment has been proved effective even when combined with agars other than the HV agar, such as starch-casein-nitrate agar[3], glycerol-arginine agar[2] and AV agar[13], supplemented with nalidixic acid 10-20 mg/L (data not shown). Thus, this pretreatment will be applicable to the study of the ecology of a variety of actinomycetes as well as to the search for useful strains of these organisms.

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