ISSN 0026-2617, Microbiology, 2013, Vol. 82, No. 1, pp. 102–110. © Pleiades Publishing, Ltd., 2013.

EXPERIMENTAL ARTICLES

Microwave Irradiation is a Useful Tool for Improving Isolation of Actinomycetes from Soil[1]

D. S. Wang[a], Q. H. Xue[a, 2], W. J. Zhu[a], J. Zhao[b], J. L. Duan[a], and G. H. Shen[b]

[a] *College of Resource and Environment, Northwest A & F University, Tangling, Shaanxi 712100, China*

[b] *College of Life Science, Northwest A & F University, Yangling, Shaanxi 712100, China*

Received February 23, 2012

Abstract—Actinomycetes are an important source of novel, biologically active compounds. New methods need to be developed for isolating previously unknown actinomycetes from soil. The objective of this experiment was to study microwave irradiation of soil as a means for isolating previously unknown actinomycetes. Soil samples were collected at ten elevations between 800 m and 3670 m on Taibai Mountain, Shaanxi Province, China. Moistened soil samples were irradiated at 120 W heating power (2450 MHz) for 3 min using a household microwave oven. Irradiation increased total actinomycete, streptomycete, and antagonistic actinomycete counts on three types of culture media. Irradiation also increased the number of culturable actinomycete isolates. Some actinomycete isolates were culturable only after the soil was irradiated, whereas other isolates could not be cultured after irradiation. Irradiation of soil from elevations >3000 m increased actinomycete counts significantly but had little effect on the number of culturable actinomycete isolates. In contrast, irradiation of samples from elevations <3000 m had relatively little effect on actinomycete counts, but significantly increased the number of culturable actinomycete isolates. We used 16S rDNA sequence analysis to identity 14 actinomycete isolates that were only culturable after irradiation. Microwave irradiation of soil was helpful for isolating *Streptomyces* spp., *Nocardia* spp., *Streptosporangium* spp., and *Lentzea* spp. Slightly more than 90% of the identified actinomycete species were biologically active. In conclusion, microwave irradiation is a useful tool for isolating biologically active actinomycetes from soil.

Keywords: Gause's synthetic medium, antagonistic actinomycetes, Taibai Mountain, 16S rDNA

DOI: 10.1134/S0026261712060161

It is estimated that about 45% of all biologically active microbial metabolites are produced by actinomycetes [1]. Many actinomycete species that produce biologically active compounds have been isolated from soil. However, more than 90% of soil microorganisms can not be cultured using current isolation methods and techniques [2]. Scientists are searching for improved methods for isolating actinomycetes from soil, including new methods of treating the soil, new ways of inhibiting the growth of unwanted microorganisms, and new types of culture media [3–7]. As a result of these efforts, it has been reported that the addition of yeast extract to soil can activate actinomycetes spores [8]. One research group selectively isolated *Micromonospora* spp., *Dactylosporangium* spp., *Microbispora* spp., *Microtetraspora* spp., *Actinomadura* spp., and *Streptosporangium* spp. by adding 0.05% SDS and 6% yeast extract to a soil suspension and then shaking at 40°C for 20 min [9]. Another research group found that treating soil with calcium carbonate increased the number of colony forming units (CFU) of *Actinokineospora* spp. [4]. In terms of physical treatments, heating the soil helped with the isolation of rare actinomycetes [10] and ultrasonication increased the culturability of some actinomycete species but decreased the culturability of others [11].

Microwaves are part of the electromagnetic spectrum, ranging in frequency from 300 million cycles per second (300 MHz, $\lambda = 1000$ mm) to 300 billion cycles per second (300 GHz, $\lambda = 1$ mm). Many studies have examined the use of microwaves for sterilization of soil. For example, Ferriss [12] reported that microwave irradiation of soil reduced total fungal and total prokaryote counts in soil extracts. The same author found that the effects of microwave irradiation increased with treatment time, decreased with the amount of soil, and decreased as the soil water content increased from 16 to 37% (w w^{-1}). There are few reports about the effect of microwave irradiation on the culturability of microorganisms, and especially the culturability of actinomycetes [13–15]. Bulina et al. [13] reported that microwave irradiation significantly increased the number of culturable rare actinomycete taxa in soil, including *Micromonospora*, *Micropolyspora*, *Norcardia,* and *Actinomadura*. Yang et al. [14] reported that short periods of microwave irradiation increased culturable actinomycete counts and the num-

[1] The article is published in the original.

[2] Corresponding author; e-mail: xuequanhong@nwsuaf.edu.cn

Table 1. Selected chemical properties of the soil samples

Sample	Elevation (m)	Soil type	Organic matter (g kg^{-1})	pH	$CaCO_3$ (g kg^{-1})
1	800	Alpine cinnamonic soil	24.4	7.24	8.23
2	1200	Alpine cinnamonic soil	27.7	6.73	5.96
3	1850	Alpine brown soil	57.9	6.58	1.84
4	2270	Alpine brown soil	40.6	5.76	0.98
5	3490	Paramo soil	32.5	5.7	0.55
6	3530	Paramo soil	72.0	5.57	0.72
7	3600	Paramo soil	40.8	5.86	0.47
8	3640	Paramo soil	50.5	6.29	0.52
9	3660	Paramo soil	32.0	6.34	1.03
10	3670	Paramo soil	42.6	6.61	0.82

ber of culturable actinomycete isolates in a sandy aeolian soil. The same author also found that irradiation increased the number of antagonistic actinomycete isolates as a percentage of the total number of cultural actinomycete isolates. Xue et al. [15] reported that microwave irradiation of a calcareous soil increased the total counts of culturable actinomycetes, *Streptomyces* spp., and *Micromonospora* spp. in soil extracts. The increases were greater when moist soil was irradiated, rather than dry soil. These studies demonstrated that microwave irradiation can increase the total counts and isolates number of culturable actinomycetes in sandy aeolian or calcareous soils, however the novel actinomycete isolates isolated from microwave irradiated soil were not identified.

The objective of this research was to investigate the use of microwave irradiation as a possible means for isolating previously unknown actinomycete species from soil. We compared the actinomycetes population, isolates number, and the number of antagonistic actinomycete isolates in irradiated and non-irradiated soil samples. Isolates which were unique to irradiated samples were identified by 16S rDNA sequence analysis.

MATERIAL AND METHODS

Soil sampling. Soil samples (5–20 cm depth) were collected at ten elevations on the north side of Taibai Mountain, Shaanxi Province, China (33°57′~ 34°58′ N, 107°45′~107°53′ E) (Table 1). The samples were placed in sterile polyethylene bags, sealed, and stored in the dark at 4°C until use.

Soil chemical analyses. Soil organic matter was measured by the Walkley and Black method. Calcium carbonate was determined by the vacuum-gasometric method. Soil pH was measured with a pH meter (Leici PHS-3D, Shanghai, China) using a soil : water ratio of 1 : 2.5. Selected soil properties are shown in Table 1.

Soil sample pretreatment. Soil samples were air-dried for two weeks and then passed through a 1 mm sieve to remove gravel and large organic material. Soil samples (5.0 g) were put into 10 mL centrifuge tubes and then moistened with 2 mL sterile water to help absorb microwave irradiation. The tubes with moistened samples were put into a 1000 mL breaker with 900 mL room-temperature water to reduce heating. The breaker was placed in the center of a 2450 MHz microwave oven (Galanz P7021TP-6, Guangdong, China) and irradiated at 120 W power for 3 min. Change in the water temperature in the breaker was monitored with a mercury thermometer. The change was less that 1°C. These soil samples will be referred to as the microwave irradiated samples. Non-irradiated soil samples were the controls.

Isolation. We used the soil dilution plate technique to isolate actinomycetes from the soil samples. Serial dilutions were prepared by adding 5.0 g of irradiated or non-irradiated soil to 45.0 mL sterile distilled water (10^{-1}), followed by shaking and further dilution to 10^{-5}. Three agar media were tested: Gause's synthetic agar (G) (Gause et al., 1983), Gause's synthetic calcium agar (5 g $CaCl_2$ added to 1000 mL Gause's synthetic agar, GCa), and Gause's synthetic nutrient-poor agar (Gause's synthetic agar at one tenth the recommended concentration, GP). All media were supplemented with potassium dichromate (80 mg L^{-1}) to inhibit the growth of bacteria and fungi. All plates were incubated at 28°C for 15 days. Colonies were identified by their cultural and morphological characteristics, and by mi-

croscopic observation if needed. One colony of each type was transferred onto Gause's synthetic agar slants, incubated at 28°C for seven days, and then stored in the dark at 4°C.

All experiments were performed in triplicate. The average actinomycete and streptomyces colony counts on each plate were calculated. Data are reported as CFU g^{-1} dry soil. Differences in colony numbers between the corresponding irradiated and non-irradiated samples were analyzed using *t*-tests with SAS 9.0 statistical software.

Antimicrobial activity. Antimicrobial activity was analyzed using the agar block method and fifteen target microorganisms (2 bacterial and 13 fungal strains), provided by the Microbiology Resource Laboratory at the College of Natural Resources and Environment, Northwest A&F University. The bacterial strains were *Escherichia coli* and *Staphylococcus aureus*. The fungal strains were *Penicillium* sp., *Candida tropicalis* and 11 plant pathogens: *Fusarium* sp. and *F. solani*, which cause potato dry rot; *F. equiseti*, which causes fusarium wilt of melon; *F. oxysporum* f. sp. *niveum*, which causes fusarium wilt of watermelon; *F. oxysporum* f. sp. *cucumerinum*, which causes fusarium wilt of cucumber; *Pestalotiopsis* sp., which causes strawberry root rot; *Verticillium dahliae*, which causes cotton verticillium wilt; *Didymella bryoniae*, which causes ascochyta blight; *Cylindrocarpon* sp., which cause rust rot in American ginseng; and two strains of *Cylindrocarpon destruction*, which cause ginseng rot.

Identification. Comparisons were made between corresponding irradiated and non-irradiated samples plated on G medium. Actinomycete isolates unique to the irradiated samples were divided into 14 groups according to colony morphology, color of aerial and substrate mycelium, and sporophore characteristics. A representative of each group was identified by 16S rDNA sequence analysis.

The DNA extracts were made from pure isolates using the method described by Saito and Miura [16]. Partial 16S rDNA gene fragments were amplified from these extracts by polymerase chain reaction (PCR) using the bacterial primers 27F: 5'-AGAGTTTGATCCTGGCTCAG-3' and 1541R: 5'-AAGGAGGTGATCCAGCCGCA-3'. Amplification was carried out in a DNA Engine thermal cycler (BIO-RAD, United States), using a 50 μL reaction mixture containing 4 μL *Taq* DNA polymerase (2.5 U μL^{-1}, Tiangen, Beijing), 5 μL 10× buffer (Tiangen), 1 μL 20 mM deoxynucleoside triphosphate (Tiangen), 37 μL of sterile distilled water, 1 μL of each primer (50 μM), and 1 μL of template. The PCR thermo cycling conditions were initial denaturation at 94°C for 4 min, 30 cycles at 94°C for 1 min, 56°C for 1 min, 72°C for 2 min, and a final elongation at 72°C for 10 min. The PCR reactions were purified and sequenced by Sangon Biotech (Shanghai) Co., Ltd. The sequences from all reactions were compared with the published sequences of reference strains available from the EMBL/GenBank/DDBJ databases.

RESULTS

Actinomycete and streptomycete counts. Microwave irradiation increased the total actinomycete and streptomycete counts on all three culture media in this study. The exception was sample 8 on GCa medium (Table 2–4). Irradiation increased total actinomycete counts on G medium by 17 to 248% compared with the corresponding non-irradiated treatment. The increases due to irradiation were significant ($P < 0.05$) for samples 3, 6, 7, 9 and 10. Irradiation also increased the total streptomycete counts on G medium by 7 to 239% compared with the corresponding non-irradiated treatment. The increases were significant ($P < 0.05$) for samples 5, 6, 7 and 10. Irradiation significantly ($P < 0.05$) increased the total actinomycete or streptomycete counts on G medium in five of six samples from elevations >3000 m versus in one of four samples from elevations <3000 m (Table 2).

Irradiation increased the total actinomycete counts on GCa medium by 41 to 353% compared with non-irradiated samples (Table 3). The increases were significant ($P < 0.05$) in samples 1, 2, 3, 4, 6, 9 and 10. Irradiation increased the total streptomycete counts on GCa medium by 15 to 422%. The increases were significant ($P < 0.05$) for samples 1, 2, 6, 7, 9 and 10. Irradiation significantly ($P < 0.05$) increased the total actinomycete or streptomycete counts on GP medium in four of six samples from elevations >3000 m versus in four of four samples from elevations <3000 m (Table 3).

Irradiation increased the total actinomycete counts on GP medium by 41 to 353%. The increases were significant ($P < 0.05$) in samples 1, 2, 3, 4, 6, 9 and 10. Total streptomycete counts were 15 to 422% greater in irradiated samples than in non-irradiated samples. The increases were significant ($P < 0.05$) for samples 1, 2, 6, 7, 9 and 10. Irradiation significantly ($P < 0.05$) increased the total actinomycete or streptomycete counts on GP medium in five of six samples from elevations >3000 m versus in two of four samples from elevations <3000 m (Table 4).

Actinomycete isolates number. Irradiation increased the number of culturable actinomycete isolates. The number of actinomycete isolates isolated from irradiated samples was equal to or greater than the number isolated from non-irradiated samples (Table 5). Furthermore, each irradiated sample had culturable actinomycete isolates which were not culturable in the corresponding non-irradiated sample. This observation was true for all three culture media in this study. The number of actinomycete isolates that were only culturable in irradiated samples ranged from 3 to 19 on G medium, 2 to 12 on GCa medium, and 1 to 13 on GP medium. Compared with samples from higher elevations, samples from elevations <3000 m

Table 2. Soil actinomycete population ($\times 10^4$ CFU g^{-1} dry soil) on Gause's synthetic agar

Soil	Microbe type	Control	Irradiated samples	Soil	Microbe type	Control	Irradiated samples
1	Total	98.8 ± 27.4	147.0 ± 29.4	6	Total	3.4 ± 0.4	11.9 ± 1.5*
	Strep[a]	48.1 ± 2.2	56.0 ± 21.6		Strep	1.4 ± 0.3	4.6 ± 0.6*
	Other	50.7 ± 29.5	91.0 ± 29.5		Other	2.0 ± 0.4	7.4 ± 1.1*
2	Total	214.0 ± 38.1	259.0 ± 12.8	7	Total	5.4 ± 0.3	11.9 ± 1.5*
	Strep	92.5 ± 24.7	105.0 ± 21.8		Strep	2.4 ± 0.2	5.1 ± 0.7*
	Other	121.6 ± 53.2	154.0 ± 32.6		Other	3.0 ± 0.4	6.9 ± 1.1*
3	Total	277.4 ± 24.9	366.8 ± 35.7*[b]	8	Total	16.1 ± 1.3	19.4 ± 1.8
	Strep	73.5 ± 11.6	100.8 ± 25.5		Strep	8.3 ± 1.0	8.9 ± 1.1
	Other	203.9 ± 36.5	266.0 ± 10.6*		Other	7.8 ± 0.3	10.5 ± 1.3
4	Total	45.6 ± 23.1	134.4 ± 80.1	9	Total	1.3 ± 0.4	3.4 ± 0.4*
	Strep	19.0 ± 65.8	64.4 ± 35.7		Strep	0.3 ± 0.1	0.6 ± 0.1
	Other	26.6 ± 21.2	70.0 ± 46.5		Other	1.0 ± 0.3	2.8 ± 0.5*
5	Total	1.6 ± 0.4	2.1 ± 0.7	10	Total	82.3 ± 24.7	243.6 ± 70.7*
	Strep	0.1 ± 0.02	0.3 ± 0.06*		Strep	27.9 ± 9.6	81.2 ± 25.7*
	Other	1.5 ± 0.5	1.8 ± 0.6		Other	54.5 ± 22.3	162.4 ± 45.3*

Notes: [a] Total, total actinomycetes; Strep, streptomycetes; Other, total actinomycetes minus streptomycetes.
[b] * Significant difference at $P < 0.05$.

had more actinomycete isolates which were culturable only after irradiation. Overall, we infer that irradiation caused the germination of some actinomycete spores which could not germinate under regular conditions.

Some actinomycete isolates became unculturable after irradiation (Table 5). This was true for all soil samples and culture media except sample 7 on G medium. The number of actinomycete isolates that were unculturable after irradiation ranged from 0 to 16 on G medium, 2 to 10 on GCa medium, and 1 to 13 on GP medium. Compared with samples from higher elevations, samples from elevations <3000 m had more actinomycete isolates that became unculturable after irradiation.

Irradiation also changed the dominant actinomycete isolates isolated from soil samples, especially in soil samples from elevations <3000 m. The change was more obvious on G and GP media than that on GCa medium.

Antagonistic actinomycetes. Microwave irradiation increased the number of antagonistic actinomycete isolates, especially in samples from elevations <3000 m (Table 6). Irradiation increased the number of antagonistic isolates cultured on G medium by 57% for sample 1, by 69% for sample 2, by 20% for sample 3, and by 100% for sample 4. For GCa medium, irradiation increased the number of antagonistic isolates by 17% for sample 1, 67% for sample 2, 8% for sample 3, and 100% for sample 4. For GP medium, irradiation increased the number of antagonistic isolates by 50% for sample 1, 83% for sample 2, 50% for sample 3, and 700% for sample 4. Irradiation had less effect on the number of antagonistic actinomycete isolates in samples from high elevations.

Microwave irradiation generally increased the number of culturable antagonistic actinomycete isolates when expressed as a percentage of the total number of actinomycete isolates. Specifically, irradiation increased the percentage in seven samples when cultured on G medium, in five samples when cultured on GCa medium, and in six samples when cultured on GP medium (Table 6). The percentages decreased slightly or did not change in the other samples. Averaged across all ten samples, the percentage of antagonistic isolates culturable on G medium increased from 48% in the non-irradiated treatment to 60%) in the irradiated treatment. On GCa medium, the percentage of culturable antagonistic isolates increased from 41% in the non-irradiated treatment to 49% in the irradiated treatment. On GP medium the percentage of culturable antagonistic isolates increased from 44% in the non-irradiated treatment to 51% in the irradiated treatment. Among actinomycete isolates that were culturable only after irradiation, 65% of those on

Table 3. Soil actinomycete population ($\times 10^4$ CFU g^{-1} dry soil) on Gause's synthetic calcium agar

Soil	Microbe type	Control	Irradiated samples	Soil	Microbe type	Control	Irradiated samples
1	Total	54.5 ± 14.4	141.4 ± 17.0*[b]	6	Total	5.0 ± 1.4	10.0 ± 1.8*
	Strep [a]	10.1 ± 7.9	28.0 ± 4.8*		Strep	1.7 ± 0.3	3.7 ± 0.5*
	Other	44.3 ± 1.3	113.4 ± 15.1*		Other	3.3 ± 1.1	6.3 ± 1.3*
2	Total	121.6 ± 30.4	254.8 ± 46.8*	7	Total	4.9 ± 1.1	8.0 ± 1.8
	Strep	40.5 ± 2.2	64.4 ± 2.4*		Strep	0.5 ± 0.0	2.8 ± 0.4*
	Other	81.1 ± 32.3	190.4 ± 45.3*		Other	4.4 ± 1.0	5.2 ± 1.6
3	Total	168.5 ± 11.0	263.2 ± 43.7*	8	Total	11.9 ± 0.7	11.6 ± 0.8
	Strep	29.1 ± 8.8	33.6 ± 8.4		Strep	6.6 ± 1.1	5.0 ± 0.9
	Other	139.3 ± 2.2	229.6 ± 38.1		Other	5.3 ± 0.6	6.6 ± 0.7
4	Total	20.3 ± 2.2	84.0 ± 7.3*	9	Total	3.5 ± 0.4	5.0 ± 0.7*
	Strep	7.6 ± 3.8	22.4 ± 10.6		Strep	0.8 ± 0.2	1.3 ± 0.1*
	Other	12.7 ± 4.4	61.6 ± 4.8*		Other	2.7 ± 0.3	3.6 ± 0.6
5	Total	0.5 ± 0.3	1.6 ± 0.8	10	Total	38.0 ± 13.7	172.2 ± 58.3*
	Strep	0.1 ± 0.1	0.3 ± 0.1		Strep	6.3 ± 2.2	23.8 ± 6.4*
	Other	0.4 ± 0.4	1.3 ± 0.7		Other	31.7 ± 13.3	148.4 ± 63.0*

Notes: [a] Total, total actinomycetes; Strep, streptomycetes; Other, total actinomycetes minus streptomycetes.
[b]* Significant difference at $P < 0.05$.

Table 4. Actinomycetes counts ($\times 10^4$ CFU g^{-1} dry soil) on Gause's synthetic nutrient-poor agar

Soil	Microbe type	Control	Irradiated samples	Soil	Microbetype	Control	Irradiated samples
1	Total	81.1 ± 17.6	114.8 ± 28.0	6	Total	2.4 ± 1.1	4.8 ± 0.5*
	Strep[a]	39.3 ± 8.8	54.6 ± 12.6		Strep	0.7 ± 0.4	1.9 ± 0.2*
	Other	41.8 ± 15.2	60.2 ± 15.9		Other	1.7 ± 0.6	2.8 ± 0.3*
2	Total	163.4 ± 1.0	204.4 ± 72.1	7	Total	2.2 ± 0.7	4.9 ± 1.3*
	Strep	79.8 ± 10.1	117.6 ± 33.3		Strep	0.5 ± 0.0	1.1 ± 0.3*
	Other	83.6 ± 3.8	86.8 ± 44.9		Other	1.8 ± 0.7	3.8 ± 1.2
3	Total	159.6 ± 17.4	275.8 ± 47.2*[b]	8	Total	5.0 ± 0.5	11.7 ± 0.8*
	Strep	54.5 ± 15.8	98.0 ± 14.7*		Strep	2.6 ± 0.5	4.4 ± 0.3*
	Other	105.1 ± 32.3	177.8 ± 32.6		Other	2.3 ± 0.0	7.3 ± 0.9*
4	Total	19.0 ± 3.8	70.0 ± 21.1*	9	Total	1.3 ± 0.6	2.8 ± 0.4*
	Strep	7.6 ± 6.6	32.2 ± 6.4*		Strep	0.5 ± 0.2	1.4 ± 0.3*
	Other	11.4 ± 3.8	37.8 ± 15.1*		Other	0.7 ± 0.4	1.4 ± 0.2
5	Total	0.5 ± 0.1	1.2 ± 0.0*	10	Total	48.1 ± 8.8	77.0 ± 24.6
	Strep	0.1 ± 0.0	0.3 ± 0.2		Strep	25.3 ± 9.6	33.6 ± 0.0
	Other	0.4 ± 0.0	0.9 ± 0.1*		Other	22.8 ± 3.8	43.4 ± 24.6

Notes: [a] Total, total actinomycetes; Strep, streptomycetes; Other, total actinomycetes minus streptomycetes.
[b]* Significant difference at $P < 0.05$.

Table 5. Number of culturable actinomycete isolates isolated from irradiated and non-irradiated soil samples

Soil	Gause's synthetic agar				Gause's calcium agar				Gause's nutrient-poor agar			
	NI [a]	MI	MO	DA	NI	MI	MO	DA	NI	MI	MO	DA
1	19	21	16	14	10	11	6	5	11	16	12	7
2	25	28	19	16	16	17	11	10	17	15	10	12
3	19	21	12	10	18	17	7	8	13	13	13	13
4	7	14	12	5	9	14	12	7	4	10	8	2
5	10	11	3	2	10	12	6	4	7	7	4	4
6	5	7	4	2	11	14	6	3	9	11	6	4
7	8	12	4	0	10	10	7	7	7	9	5	3
8	14	15	8	7	10	11	5	4	11	16	7	2
9	11	14	8	5	12	12	2	2	11	11	1	1
10	15	14	6	7	8	8	4	4	10	11	8	7

Note: [a] NI, non-irriadiated control; MI, microwave irradiated samples; MO, actinomycete isolates isolated from MI only; DA, actinomycetes which were isolated from NI but not observed in MI.

Table 6. Number of culturable antagonistic actinomycete isolates isolated from irradiated and non-irradiated soil samples

Soil	Gause's synthetic agar			Gause's calcium agar			Gause's nutrient-poor agar		
	NI[a]	MI	MO	NI	MI	MO	NI	MI	MO
1	7	11	7	6	7	4	8	12	8
2	16	27	17	6	10	10	6	11	9
3	12	15	10	13	14	6	10	15	11
4	4	8	7	3	6	5	1	8	7
5	5	6	3	6	6	3	0	0	0
6	2	3	2	3	3	2	6	6	3
7	2	2	0	1	1	1	1	3	3
8	7	7	2	3	3	1	1	2	2
9	6	9	6	7	6	1	8	8	1
10	8	10	6	2	6	5	6	6	2
Total	69	98	60	50	64	40	48	72	47

Note: [a] NI, non-irriadiated control; MI, microwave irradiated samples; MO, actinomycete isolates isolated from MI only.

Table 7. Fourteen actinomycete strains that were only culturable after microwave irradiation of the soil samples were identified by 16S rDNA sequence analysis

Isolate	Nearest database match				Antagonistic to
	Name	Accession number	Bioactivity	Similarity (%)	
MG202	*Streptomyces zaomyceticus*	AB184346	Zaomycin [17]	100.0	S, Ct
MG418	*Streptomyces violarus*	AB184316	Antagonism [18]	100.0	S, Fe, D, Foc, C
MG414	*Streptomyces aureus*	AB249976	Luteomycin, Fungicidine, Aureomycin, [19–21]	99.9	S
MG203	*Streptomyces glauciniger*	AB249964	Antagonism [22]	99.8	–
MG401	*Streptomyces xanthophaeus*	AB184177	Geomycin [23]	99.7	–
MG211	*Streptomyces bungoensis*	AB184696	Antibiotic [24]	99.6	–
MG207	*Streptomyces rubiginosohelvolus*	AB184240	Daunomycin [25]	99.2	S
MG306	*Streptosporangium amethystogenes* subsp. *amethystogenes*	X89935	–	99.2	S
MG314	*Streptomyces goshikiensis*	AB184204	Bandamycin [26]	99.2	S, Pe, D, Fe, V, P, C, CS
MG606	*Streptomyces olivochromogenes*	AB184737	Ferulic acid esterase [27]	99.0	E
MG411	*Streptomyces phaeofaciens*	AB184360	PAF antagonist [9]	98.8	S, Fe, D, V, P, F, C
MGa06	*Lentzea flaviverrucosa*	AF183957	–	98.5	S, CS
MG210	*Nocardia soli*	AF430051	–	98.0	–
MG308	*Streptomyces hygroscopicus* subsp. *ossamyceticus*	AB184560	Antagonism [28]	97.8	–

Note: [a] E, *Escherichia coli*; S, *Staphylococcus aureus*; Pe, *Penicillium* sp.; Ct, *Candida tropicalis*; F, *Fusarium* sp.; Fe, *F. equiseti*; Foc, *F. oxysporum* f. sp. *cucumerinum*; P, *Pestalotiopsis* sp.; V, *Verticillium dahliae*; D, *Didymella bryoniae*; C, *Cylindrocarpon* sp.; CS, *Cylindrocarpon destruction*.

G medium showed antagonistic activity versus 59% of those on GCa medium, and 57% of those on GP medium. These percentages were higher than the percentages of antagonistic actinomycete isolates isolated from irradiated and non-irradiated soil.

Soils from elevations <3000 m generally had a greater number of antagonistic actinomycete isolates that were culturable only after irradiation than soils from elevations >3000 m (Table 6). For example, sample 1 had 7 actinomycete isolates that were culturable on G medium after irradiation, sample 2 had 17, sample 3 had 10, and sample 4 had 7. These numbers were all greater than those for higher elevations.

Identification. A total 92 actinomycete isolates were culturable only after irradiation of these 10 soil samples. The strains were tentatively classified on the basis of the morphological characteristics, then 14 isolates were selected for identification by 16S rDNA sequence analysis. The 14 isolates mainly belong to four genera: *Streptomyces* (79%), *Nocardia* (7%), *Streptosporangium* (7%) and *Lentzea* (7%) (Table 7). Thirteen of the fourteen strains are known to show bioactivity.

DISCUSSION

The results showed that irradiation with a 120 W, 2450 MHz microwave oven increased the culturable actinomycete population and isolates number, especially increasing the number of isolates that were not culturable using conventional methods. Microwave irradiation had a particularly significant effect on samples from elevations <3000 m. It is also noteworthy that irradiation increased the number of culturable antagonistic isolates. This could be helpful for the discovery of new antibiotic producers and the exploitation and utilization of new, biologically active compounds.

Ferriss [12] found that treatment with 625 W reduced total fungi and total prokaryotes counts. In comparison, Bulina et al. [13] used an 80 W microwave in their study and reported that microwave irradiation significantly increased *Micromonospora* spp., *Micropolyspora* spp., *Norcardia* spp. and *Actinomadura* spp. counts. Our results showed different results using 120 W microwave, and the difference indicated that the effect of microwave irradiation on soil was vary due to the differences of power of microwave. The results of this research, which studied the effects of microwave irradiation on this calcium deficient mountain soil, were similar to the studies of Aeolian sandy soil [14] and calcareous soil [15]. Therefore, we conclude that 120 W microwave irradiation could improve the isolation efficiency of actinomycetes from various types of soil on wide range of media.

To our knowledge, this study is the first to report that some actinomycetes became culturable after microwave irradiation of soil samples whereas other actinomycetes became unculturable after irradiation. The mechanism for these changes in actinomycetes culturability is unknown.

ACKNOWLEDGMENTS

The authors are grateful for Dr. Shuo-bi Li, College of Food Science and Engineering at Northwest A & F University, for the use of his laboratory and laboratory equipment. The authors thanks for Dr. William Jeff Gale for the revision of the paper. This research was supported by the Program for Changjiang Scholars and Innovative Research Team in University (PCSIRT, no. IRT748).

REFERENCES

1. Berdy, J., Bioactive Microb1.Berdy, J., Bioactive Microbial Metabolites, a Personal Review, *J. Antibiot.*, 2005, vol. 1, pp. 1–26.
2. Pachter, L., Interpreting the Unculturable Majority, *Nat. Methods*, 2007, vol. 4, pp. 479–480.
3. Tabacchioni, S., Chiarini, L., Bevivino, A., Cantale, C., and Dalmastri, C., Bias Caused by Using Different Isolation Media for Assessing the Genetic Diversity of a Natural Microbial Population, *Microbiol. Ecol.*, 2000, vol. 40, pp. 169–176.
4. Otoguro, M., Hayakawa, M., Yamazaki, T., and Iimura, Y., An Integrated Method for the Enrichment and Selective Isolation of *Actinokineospora* spp. in Soil and Plant Litter, *J. Appl. Microbiol.*, 2001, vol. 91, pp. 118–130.
5. Khaled, E.T. and Krishnapillai, S., Non-Streptomycete Actinomycetes as Biocontrol Agents of Soil-Borne Fungal Plant Pathogens and as Plant Growth Promoters, *Soil Biol. Biochem.*, 2006, vol. 38, pp. 1505–1520.
6. Pelletier, S., Tremblay, G.F., Tremblay, A., Bélanger, G., Castonguay, Y., and Michaud R., Drying Procedures Affect Non-Structural Carbohydrates and Other Nutritive Value Attributes in Forage Samples, *Anim. Feed Sci. Technol.*, 2010, vol. 157, pp.139–150.
7. Pudjiraharti, S., Takesue, N., Katayama, T., Lisdiyanti, P., Hanafi, M., Tanaka, M., Sone, T., and Asano, K., Actinomycete *Nonomuraea* sp. Isolated from Indonesian Soil Is a New Producer of Inulin Fructotransferase, *J. Biosci. Bioeng.*, 2011, vol. 6, pp. 671–674.
8. Okami, Y., Concepts and Techniques for Isolation and Characterization of Actinomycetes, Madison: University of Wisconsin, 1991.
9. Okamoto, M., Yoshida, K., Nishikawa, M., Ando, T., Iwami, M., Kohsaka, M., and Aoki, H., FR-900452, a Specific Antagonist of Platelet Activating Factor (PAF) Produced by *Streptomyces phaeofaciens*, *J. Antibiot.*, 1986, vol. 2, pp. 198–204.
10. Jiang, Y., Duan, S.R., Tang, S.K., Cheng, H.H., Li, W.J., and Xu, L.H., Isolation Methods of Rare Actinomycetes, *Chin. Microbiol.*, 2006, vol. 1, pp. 181–183.
11. Jiang, Y., Cao, Y.R., Zhao, L.X., Wang, Q., Jin, R.X., He, W.X., and Xue, Q.H., Ultrasonic Treatment of Soil Samples for Actinomycete Isolation, *Acta Microbiol. Sinica.*, 2010, vol. 8, pp. 1094–1097.
12. Ferriss, R.S., Effects of Microwave Oven Treatment on Microorganisms in Soil, *Phytopathology*, 1984, vol. 74, pp. 121–126.
13. Bulina, T.I., Alferova, I.V., and Terekhova, L.P., A Novel Approach to Isolation of Actinomycetes Involving Irradiation of Soil Samples with Microwaves, *Microbiology*, 1997, vol. 66, pp. 231–234.
14. Yang, B. Xue, Q.H., Chen, Z.Q., Zhou, Y.Q., Zhang, X.L. Xu, Y.J., and Guo, Z.Y., Effects of Microwave Irradiation on Isolation of Soil Actinomycetes, *Chin. J. Appl. Ecol.*, 2008, vol. 5, pp. 1091–1098.
15. Xue, Q., Dua, C.M., Wang, L.N., and Lin, Y.B., The Influence of Microwave Irradiation to the Isolation Effect of Soil Actinomycetes, *Chin. J. Microbiol.*, 2010, vol. 3, pp. 19–24.
16. Saito, H. and Miura, K., Preparation of Transforming Deoxyribonucleic Acid by Phenol Treatment, *Biochim. Biophys. Acta*, 1963, vol. 72, pp. 619–629.
17. Motoo, S., US Patent 3160561, 1964.
18. *IEDA* (2009) *Streptomyces violarus*. Portal of Chinese Science and Technology Resource. http://www.cdcm.net/search/searchexcel/show.asp?jun_id=1511C0009IEDA00710, Accessed 26 June 2011.

19. Duggar, B.M., Aureomycin: a Product of the Continuing Search for New Antibiotics, *Ann. N.Y. Acad. Sci.*, 1948, vol. 51, pp. 177–181.
20. Nawata, Y., Adno, K., and Iitaka, Y., Crystal Data of Macrotetrolide Antibiotics Tetranactin and its Homologues, *Acta. Crystallogr.*, 1971, vol. 27, pp. 1680–1682.
21. Renato, C. and Glovanni, G., US Patent 3093543, 1963.
22. *IEDA* (2008) *Streptomyces glauciniger*. Portal of Chinese Science and Technology Resource. http://www.cdcm.net/search/searchexcel/show.asp?jun_id=1535C00 01000003677, Accessed 26 June 2011.
23. Brockmann, H. and Musso H., Geomycin, a New Antibiotic Effective Against Gram-Negative Bacteria, *Naturwissenschaften*, 1954, vol. 19, pp. 451–452.
24. Eguchi, T., Takada, N., and Nakamura, S. *Streptomyces bungoensis* sp. nov, *Int. J. Syst. Bacteriol.*, 1993, vol. 43, pp. 794–798.
25. Huk, J. and Blumauerova, M., Streptomycetes Producing Daunomycin and Related Compounds: Do We Know Enough about Them after 25 Years?, *Folia Microbiol.*, 1989, vol. 4, pp. 324–349.
26. Stuart, A., Kuhstoss, R., and Nagaraja, R., US Patent 4766066, 1988.
27. Faulds, C.B. and Williamson, G., The Purification and Characterization of 4-Hydroxyl-3-Methoxycinnamic (Ferulic) Acid Esterase from *Streptomyces olivochromogenes*, *J. Gen. Microbiol.*, 1991, vol. 137, pp. 2339–2345.
28. Selvameenal, L. Radhakrishnan, M., and Balagurunathan, R. Antibiotic Pigment from Desert Soil Actinomycetes; Biological Activity, Purification and Chemical Screening, *Indian J. Pharmaceut. Sci.*, 2009, vol. 5, pp. 499–504.