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Identification of *Streptomyces* species antagonistic to *Fusarium solani* causing Ginseng root rot

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人蔘 뿌리썩음 病菌, *Fusarium solani*에 拮抗的인 *Streptomyces species*의 同定

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ABSTRACT

Among 131 isolates of *Streptomyces* obtained from ginseng cultivating soil, the two isolates ST 59 and ST 129 showing high antagonistic activity to *Fusarium solani* (Mart.) Appel & Wr. causing ginseng root rot were identified. The two isolates were identified *Streptomyces alboniger* Porter, *et al.* and *Streptomyces reseolilacinus* Pridham, *et al.*, respectively, based on morphology, cultural, and physiological characteristics on various culture media. Spore chains of ST 59 and ST 129 were flexuous(RF) and coiled(S). Spore surfaces of two isolates were all smooth. Aerial mass color of ST 59 was white series and ST 129 red series.

INTRODUCTION

Most species of *Streptomyces* produce antimicrobial agents known as antibiotics(Gottlieb, *et al.* 1952, Knauss, 1976, Rangaswami, 1962, Shinoda, 1979) and exocellular enzymes which lyse fungi in soil(Baker, 1968, Baker, *et al.* 1974). The possession of enzymes of this type may be important in the soil microbiological equilibrium with the action of antibiotics.

The major antagonistic mechanism of *Streptomyces* against *Fusarium* species generally recognized was lysis caused by antibiotics or exocellular enzymes (Baker, 1968, Baker, *et al.* 1974, Chung, et al. 1981). Repeated observations indicated that lysis of fungi was a common phenomena in soils (Chinn, *et al.* 1961, Huber, *et al.* 1966, Lockwood, 1960, Sequeira, 1962). Lysis of propagules is a logically satisfying method of biological control of soil born plant pathogen since it could reduce inoculum density.

Ginseng root rot caused by *Fusarium solani* (Chung, *et al.* 1977) and *Cylindrocarpon destructans* (Chung, 1975) is known to be main

limiting factor in increasing yield. In 6-year-old ginseng cultivating soil, a highly significant negative correlation coefficient ($r=-0.3742^{**}$) was obtained between population of *Streptomyces* and *Fusarium* (Ohh, *et al.* 1980.) Therefore, many attempts were made to control root rot by stimulation of the natural population of streptomycete that suppresses *Fusarium* species (Chung, 1976, Chung, *et al.* 1978).

Chung, *et al.* (Chung, 1976, Chung, *et al.* 1978) suggested that the growth inhibition of *C. destructans* in vitro was due to mycolytic activity and/or antibiotic production by *Streptomyces*. Lysis of conidia, mycelium and chlamydospore of *F. solani* by *Streptomyces* was reported (Chung, *et al.* 1981, Lloyd, *et al.* 1965). It was also suggested that the lysis of *F. solani* might be caused by *Streptomyces* producing chitinase affected by antibiotics or nutrient deficiency and senescence.

The purpose of the present study was to identify two isolates of *Streptomyces* antagonistic to *F. solani* obtained from diseased ginseng root. An abstract of the paper on antagonism of these two isolates have been reported (Chung, *et al.* 1981).

MATERIALS AND METHODS

1. ISOLATION OF *STREPTOMYCES*

Two isolates showing antagonism to *F. solani* were used among 131 isolates of *Streptomyces* obtained from ginseng cultivating soil. For the isolation of *Streptomyces*, the chitin agar medium was used (Lingappa, *et al.* 1962). The chitin agar medium contained 1.5g of colloidal chitin and 20g agar per *l* of distilled water. Colloidal chitin was prepared as follows; 5g of the cleaned chitin (Kanto Chemical Co., Tokyo, Japan) were moistened with acetone and dissolved in 100 m*l* of cold concentrated HCl, then filtered through glass wool pads. The colloidal chitin was washed with distilled water several times, and then remaining acid was neutralized with 1 N NaOH.

2. IDENTIFICATION OF *STREPTOMYCES* ISOLATES

For identification of isolates of *Streptomyces*, taxonomic characterization was done according to the International Streptomyces Project (I.S.P.) Method (Shirling, *et al.* 1966) and Bergey's Manual of Determinative Bacteriology (Pridham, *et al.*).

1) Morphological characteristics

The morphology of Isolates ST 59 and ST 129 was examined microscopically grown on glycerol-glycine agar and oatmeal agar, respectively, after 14 days incubation at 28°C. Spore wall ornamentation was determined with Electron Microscope (Hitachi, HU-11E).

2) Cultural and physiological characteristics

Growth, aerial mycelium, and soluble pigment formation were observed on the 7th, 14th and 21st day after incubation on various media described by International Streptomycs Project Method at 28°C. Utilization of carbon sources was tested by growth on the Pridham and Gottlieb's medium (Shirling, *et al.* 1966) containing 1% of various carbon sources.

In the test of physiological characteristics, peptone-yeast extract iron agar, tyrosine agar, and tryptone-yeast extract broth media were used to examine the formation of melanin pigment.

RESULTS AND DISCUSSION

Spore chains of ST 59 and ST 129 were flexuous (RF) and coiled (S), respectively (Fig. 1). Spore surfaces of two isolates were all smooth (Fig. 2). Growth and formation of aerial mycelium were good on glycerol asparagine agar, nutrient agar, tyrosine agar, glucose peptone agar, glycerol glycine agar, and potato dextrose agar in *Streptomyces* ST 59 and inorganic salts starch agar, oatmeal agar, yeast

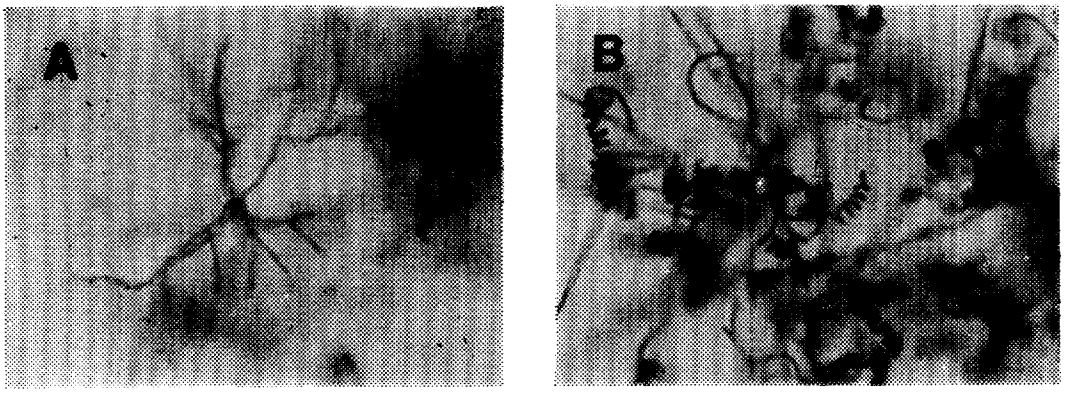

Fig. 1. Spore chains of Isolate ST 59(*Streptomyces alboniger* Porter, *et al.*) on glycerol glycine agar(A)and Isolate ST 129 (*Streptomyces roseolilacinus* Pridham, *et al.*) on oatmeal agar(B) after 14days incubation at 28°C(x200).

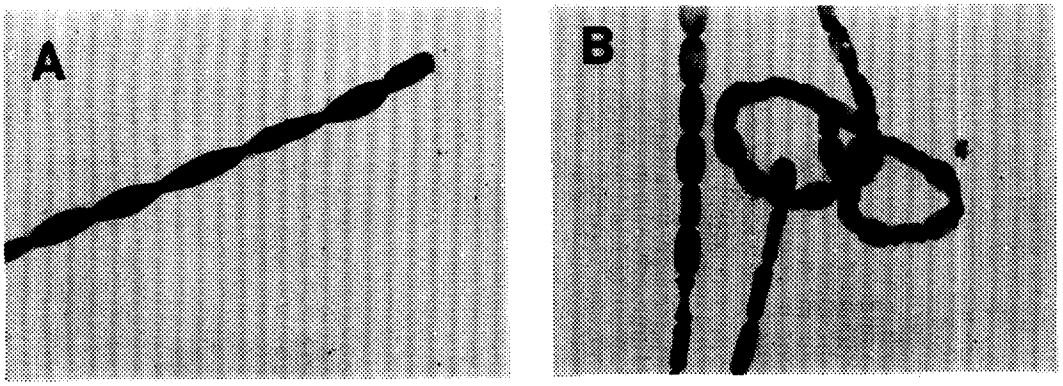

Fig. 2. Electron micrograph of smooth spores of Isolate ST 59 (*S. alboniger* Porter, *et al.*) on glycerol glycine agar(A) and Isolate ST 129 (*S. roseoliacinus* Pridham, *et al.*) on oatmeal agar(B) after 14days(x6,500)

Table 1. Cultural characteristics of *Streptomyces alboniger* Porter, *et al.* ST 59 and *Streptomyces roseolilacinus* Pridham, *et al.* ST 129

Medium		*Streptomyces alboniger* ST 59	*Streptomyces roseolilacinus* ST 129
Czapek's agar	G*	poor	poor
	A	poor, oyster gray,	moderate, pinkish gray
	S	yellow	yellow
Glycerol asparagine	G	good	moderate
	A	moderate, yellowish white	poor, pinkish gray

agar(I.S.P. No.5)**	S	none	none
Inorganic salt-starch agar	G	moderate	good
(I.S.P. No. 4)	A	moderate, yellowish white	abundant, pale brown
	S	none	none
Nutrient agar	G	good	poor
	A	abundant, yellowish white	poor
	S	yellow	orange
Oatmeal agar (I.S.P. No. 3)	G	poor	good
	A	poor, white	abundant, pale brown
	S	none	none
Tyrosine agar (I.S.P. No. 7)	G	good	poor
	A	abundant, yellowish white	poor
	S	orange	none
Glucose peptone agar	G	good	moderate
	A	abundant, yellowish white	poor
	S	yellow	orange
Glycerol glycine agar	G	good	poor
	A	abundant, yellowish white	poor, pinkish gray
	S	yellow	none
Yeast extract-malt extract	G	moderate	good
agar(I.S.P. No. 2)	A	poor, white	abundant, pale brown
	S	orange	yellow
Poptone-yeast extract iron	G	poor	poor
agar(I.S.P. No. 6)	A	poor, white	poor, pinkish gray
	S	orange	orange
Potato dextrose agar	G	good	good
	A	abundant, yellowish white	abundant, purplish white
	S	yellow	none
Starch agar	G	poor	moderate
	A	moderate, yellowish white	moderate, pale grown
	S	none	none

* G: growth, A: aerial mycelium, S: soluble pigment
** Medium employed by International Streptomyces Project.

extract malt extract agar, and potato dextrose agar in *Streptomyces* ST 129(Table 1)

Aerial mass color of ST 59 was white series at 14 days after inoculation and ST 129 red series. Soluble pigments were all yellow to orange on some media (Table 2).

Physiological properties of two isolates are shown in Table 2. ST 59 showed a positive reaction in gelatin liquefaction, starch hydrolysis, milk peptonization, and H_2S production, a negative reaction in melanin formation, nitrate reduction and cellulose decomposition. Starch hydrolysis, melanin formation, and nitrate reduction were positive, while gelatin liq-

Table 2. Physiological characteristics of *Streptomyces alboniger* Porter, *et al.* ST 59 and *Streptomyces roseolilacinus* Pridham, *et al.* ST 129

Characteristics	*Streptomyces alboniger* ST 59	*Streptomyces roseolilacinus* ST 129
Gelatin liquefaction	+	−
Starch hydrolysis	+	+
Melanin formation[a)]	−	+
Nitrate reduction	−	+
Peptonization of milk	+	−
Cellulolytic activity	−	−
H_2S production	+	−

a) Peptone-yeast extract iron agar (I.S.P. No. 2), Tyrosine agar (I.S.P. No. 7) ann Tryptone-yeast extract broth (I.S.P.No. 1) media were all used for this test.

uefaction, milk peptonization, cellulyoltic ability and H_2S production were negative in the ST 129.

D-glucose, L-arabinose, D-xylose, D-fructose, and D-mannitol were utilized well but L-rhamnose, I-inositol, sucrose, and. raffinose were not used for growth in ST 59. Isolate ST 129 used D-glucose, D-fructose, and sucrose as c arbon sources, but did not utilize the other sugars mentioned above (Table 3).

In comparison of ST 59 and ST 129 with

Table 3. The utilization of carbon sources by *Streptomyces alboniger* Porter, *et al.* ST 59 and *Streptomyces roseolilacinus* Pridham, *et al.* ST 129

Carbon source	*Streptomyces alboniger* ST 59	*Streptomyces roseolilacinus* ST 129
D-glucose	+	+
L-arabinose	+	−
D-xylose	+	−
D-fructose	+	±
L-rhamnose	−	−
Inositol	−	−
D-mannitol	+	−
Sucrose	−	+
Raffinose	−	−
No carbon control	−	−

+: Positivee ±: Doubtful −: Negative

Table 4. Comparison of ST 59 and ST 129 with *Streptomyces alboniger* Porter, *et al.* ISP 5043 and *Streptomyces roseolilacinus* Pridham, *et al.* ATCC 19922

Characteristics	Isolate ST 59 *S. alboniger* ISP 5043	Isolate ST 129 *S. roseolilacinus* ATCC 19922
Morphology		
Spore chain	Rectus-flexibilis	Spira
	Rectus-flexibilis	Spira
Spore surface	Smooth	Smooth
	Smooth	Smooth
Cultural characteristics		
Aerial mass color	White series	Red series
	White series	Red series
Melanoid pigment	Negative	Positive
	Negative	Positive
Soluble pigment	Variable	Variable
	Variable	Variable
Carbon utilization		
D-glucose	+	+
	+	+
D-xylose	+	−
	+	−
L-arabinose	+	−
	+	+
L-rhamnose	−	−
	−	−
D-fructose	+	±
	+	±
Raffinose	−	−
	−	−
D-mannitol	+	−
	+	−
I-inositol	−	−
	+	−
Sucrose	−	+
	−	−

Streptomyces alboniger Porter, *et al.* ISP 5043 (Shirling, *et al.* 1968) and *Streptomyces reseolilacinus* Pridham, *et al.* ATCC 19922(Pridham, *et al.* 1974), respectively, all of the morphological and cultural characteristics and almost physiological properties were in agreement with those of the latter (Table 4). In physiolo-

gical characteristics only a little difference was recognized, Isolate ST 59 did not utilize inosital, but *S. alboniger* Porter, *et al.* ISP 5043 did. The ability of Isolate ST 129 to utilize L-arabinose and sucrose as carbon sources was different from that of *S. roseolilacinus* Pridham, *et al.* ATCC 19922. Therefore, the Isolate ST 59 and ST 129 were identified as *S. alboniger* Porter, *et al.* and *S. roseolilacinus* Pridham, *et al.*, respectively.

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摘 要

人蔘栽培土壤에서 分離한 131個 *Streptomyces* 菌株中에서 뿌리썩음病菌 *Fusarium solani* (Mart.) Appel & Wr.에 拮抗的인 分離菌 ST 59와 ST 129를 同定하였다. 2菌株의 胞子사슬, 胞子表面의 형태, 여러 培地上에서의 培養特性 및 生理的 特性 등을 調査한 結果 前者는 *Streptomyces alboniger* Porter, *et al.*, 後者는 *Streptomyces roseolilacinus* Pridham, *et al.*로 同定되었다. 胞子사슬의 모양은 ST 59는 꾸불꾸불(RF)하였고 ST 129는 감긴(S) 형태이었다. 胞子의 表面은 2菌株 모두 smooth였으며 菌體色은 ST 59가 白色, ST 129가 赤色계통이었다.

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