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Note

Antifungal Activity of *Streptomyces griseofuscus* 200401 against Pathogens causing Late Blight and Anthracnose on Pepper

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Abstract : To select microorganisms that exhibit antifungal activity against the fungal pathogens of pepper, *Phytophthora capsici* and *Colletotrichum acutatum*, soil samples from a forest and natural fields of Gajang-Dong, Sangju-city were tested *in vitro* and *in vivo*. *Streptomyces griseofuscus* 200401 was finally selected throughout antifungal activity test with dual culture, culture broth, and fruits. For the identification of the strain, nucleotide sequences of 16S rDNA and whole cell fatty acids were analyzed. It is like that the strain 200401 may be a novel biological control agent that can reduce application of chemical fungicides to control late blight and anthracnose on pepper.(Received January 17, 2005; accepted March 23, 2005)

Key Words : anthracnose, late blight, pepper, Streptomyces griesofuscus.

Late blight and anthracnose which are caused by *Phytophthora* sp. and *Colletotrichum* sp., respectively, are major threats on production of pepper (Korsten and Jeffries, 2000; Mao *et al.*, 1998). Major control strategy for the diseases is an application of chemical fungicides such as metalaxyl, dimethomorph, benomyl, and tebuconazole during growing season. However, the control efficacy is limited with biological property of pathogen such as sporulation, life cycle and inhabitant, development of fungicide resistant isolates, increasing in disease pressure, and risk to food and environment (Ames, 1979; Hausbeck and Lamour, 2004; Lim *et al.*, 1998). Even though growers prefer to the method because of efficacy and cost, requirement for a new method or agents to control the diseases is continually increasing.

Biocontrol, the use of living organisms as pest control agents, is now an important alternative to chemical pesticide (Baker *et al.*, 1983; Becker, 1993; Powel and Fox, 1993). The mechanisms of biocontrol are mycoparasitism, antibiosis and competition (Baker *et al.*, 1983, Mao *et al*, 1998, Postermaster *et al.*, 1997; Xio *et al.*, 2002). Trejo-Estrada, SR *et al.*(1998) noted that *Streptomyces violaceusniger* YCED-9 producing antibiotics(nigericin, geldanamycin, and guanidylfungin) exhibited antifungal activity against several plant pathogenic fungi with *Phytophthora* spp. Biocontrol of plant diseases has been achieved using microorganisms containing *Agrobacterium* sp., *Bacillus* spp., *Penicillium* spp., *Pseudomonas* spp., *Streptomyces* spp., and *Trichoderma* spp. (Baker *et al.*, 1983; Becker, 1993, Lim *et al.*, 2000; Sivan and Chet, 1989; Skujus *et al.*, 1965). Actinomyces has been described as potent biocontrol agents of phytopathogenic fungi under several conditions (Harindran *et al.*, 1999; Hausbeck and Lamour, 2004; Samac *et al.*, 2003; Skujus *et al.*, 1965; Trejo-Estrada SR *et al.*, 1998; Xio *et al.*, 2002). The purpose of this study is to detect biocontrol agents which have ability to reduce pressure of late blight and anthracnose on pepper.

Soil containing antifungal microorganisms were collected from several sites of a forest and natural fields near Sangju during August and September 2003. Soil samples were air-dried under shade for 24 h at room temperature and incubated for 4 h at 60℃ to improve isolation rate of beneficial microorganisms. Then, soil was diluted with sterilized dH2O. The suspension (200 $\mu\ell$) was dropped on DifcoTM Actinomycetes Isolation Agar (DAIA, Lot 2136900) plates amended with PCNB (500 $\mu g/\mu\ell$) to inhibit fungal growth. The suspension

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Table 1. Effect of *Streptomyces* sp. 200401 against late blight and anthracnose on peppers

Treatment	Late blight		Anthracnose	
	No, of peppers with lesion	Control Value (%)[a]	No, of peppers with lesion	Control Value (%)
Pathogen	55	-	58	-
Pathogen+ 200401[b]	8	85.5	6	89.7
Control(H2O)	0	100	0	100

[a]Control value = (1- No. of peppers with lesion on treatment/No. of peppers with lesion on pathogen)× 100.
[b]The strain 200401 was inoculated on paper disk onto pathogen-mycelium plug with 50 ㎕ of culture broth(108 cfu/㎖) incubated for 7 days at 30℃.

was spread with a glass-rod and the plates were incubated at 30℃. The emerged colonies were transferred to the new DAIA plate.

To bioassay, the isolated bacterial strains were inoculated by streaking single line on PDA(Potato extract 4 g ,Dextrose 20 g, Agar 15 g, and dH2O 1 L) for C. acutatum and P. capsici . The plates were incubated for 7 days at 30℃. After incubation, the plates were fumigated with chloroform for 1 h and incubated again under clean bench for 1 h to diffuse chloroform. The inoculum-mycelium disks (diameter; 5 ㎜) pre-incubated for 5 days at 25℃ and 28℃ on PDA for C. acutatum and Oatmeal agar (Oatmeal 60 g, Agar 12.5 g, and dH2O 1 L) for P. capsici were inoculated on opposite point to bacterial colony. The first antifungal activity of tested strains was detected by fungal growth to the strain after incubation for 6 days. The strain 200401 was finally selected for advanced studies.

To test antifungal activity of culture broth without bacterial cells on pathogens causing late blight and anthracnose on pepper, the strain 200401 was incubated on YMB (Yeast extract 3 g, Malt extract 3g, Peptone 5 g, Dextrose 10 g) for 7 days with condition of 150 rpm shaking and 30℃. After incubation, culture broth was dried with vacuum freeze dryer (Samwon, SFDSM24L, Korea). The dried powder containing bacteria was treated with MeOH to make sub-antifungal powder without bacteria and concentrated with vacuum rotary evaporator (EYELA, N-1000-W, Japan). The activity of sub-antifungal powder was tested on PDA amended with 1% and 0.5% of sub-antifungal powders dissolved in MeOH. The inhibitory effect (%) was evaluated as following formula; [1-fungal growth of treatment/fungal growth of control] × 100. The activity was checked daily for 5 days. Both 1% and 0.5% of sub-extraction inhibited fungal growth of P. capsici to 76.2% and 61%, respectively. The inhibitory effect (%) to fungal growth of *C. acutatum* was 88% and 75.3%, respectively (Fig. 1).

To test with pepper fruits, peppers were taken from the market and sterilized with 70% EtOH for 1 min to remove pre-infected pathogens and other microorganisms. The mycelium plugs were prepared from 5-day-old mycelium as the first bioassay. The strain 200401 was inoculated on paper disk onto pathogen-mycelium plug with 50 ㎕ of culture broth(108 cfu/㎖) incubated for 7 days at 30℃. The inoculated peppers (20 peppers/replicate/3replicate/trial) were transferred on plastic container(H 80 ㎜ × W 220 ㎜ × L 290 ㎜) with wet paper towel to keep high relative humidity (> 95%). The symptoms by pathogen were checked daily. As shown in Fig. 2 and Table 1, on peppers treated with only *P. capsici*, the symptom appeared after 3 days. After 7 days, symptom was extended to whole peppers. However, on peppers treated with *P. capsici* and strain 200401, any symptom did not appear throughout the test. The activity to *C. acutatum* showed pattern similar to *P. capsici*. Morphological characteristics of strain 200401 were investigated by scanning electron microscopy (LEO, LEO1530, Germany). Under the microscope, spores showed smooth surface and consisted spiral chains (Fig. 2).

For identification of strain 200401, 16S rDNA sequence and whole cell fatty acids were analyzed. Target DNA was amplified with a PCR kit using universal primers (27F 5'-AGA GTT TGA TCA TGG CTC AG-3', 1492R 5'-GGA TAC CTT ACG ACT

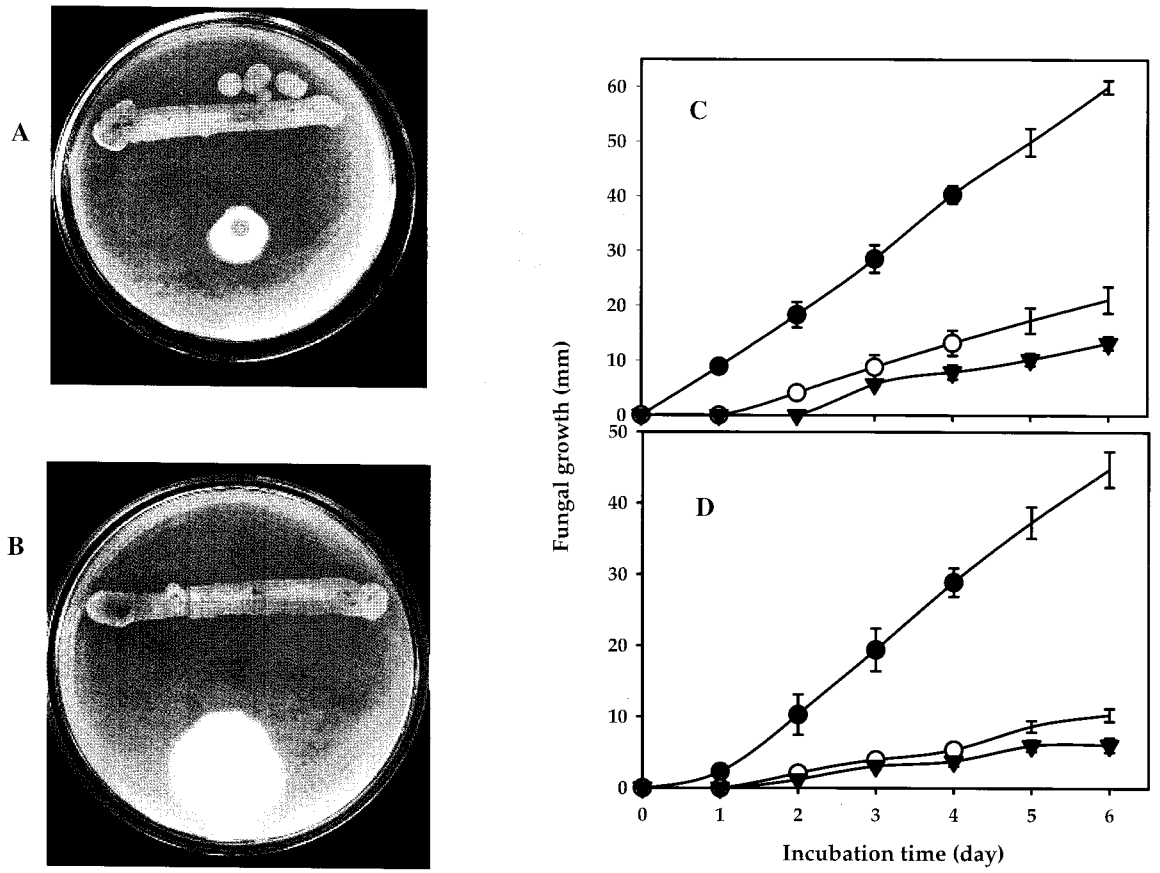

Fig. 1. Inhibition of the growth of *P. capsici* (A) and *C. acutatum* (B) by strain 200401 and antifungal activity of sub-extraction of the strain 200401 culture broth to *P. capsici* (C) and *C. acutatum* (D). Bars represent standard deviations of the mean responses. ●: Control, ▼: Sub-extraction 1%, ○: Sub-extraction 0.5%.

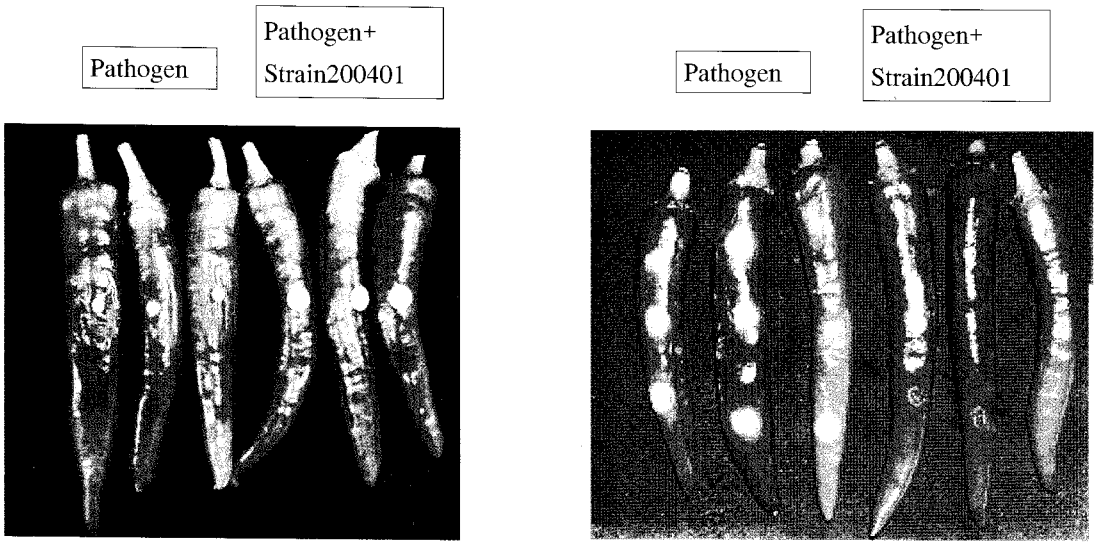

Fig. 2. Effects of strain 200401 to late blight (left) and anthracnose(right) on peppers caused by P. capsici and C. acutatum. The photos were taken after 7days incubation at 25℃.

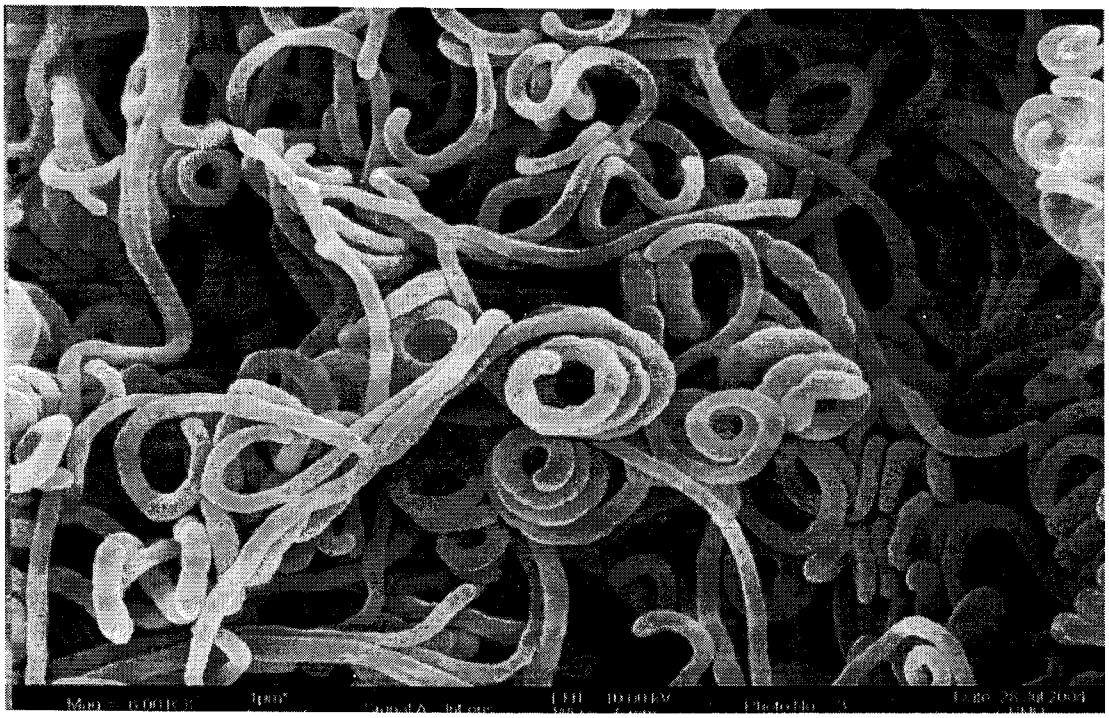

Fig. 3. Scanning electron micrograph of strain 200401 incubated on YM medium for 7 days at 30℃.

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L TGCAAGTCGAACGATGAAGCCCTTCGGGGTGGATTA-GTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATATGACCATCTTGGGCATCC  135
A TGCAAGTCGAACGATGAAGCCCTTCGGGGTGGATTANGTGGCGAACGGGTGAGTAATACGTGGGCAATCTGCCCTGCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATATGACCATCTTGGGCATCC  136
L TGATGGTGTAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCC  271
A TGATGGTGTAAAGCTCCGGCCGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCC  272
L AGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTG  407
A AGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTG  408
L CAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGATTGTGAAAGCTCGGGGCTTAACCCCG  543
A CAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCAOGTCGATTGTGAAAGCTCGGGGCTTAACCCCG  544
L AGTCTGCAGTCGATACGGGCTAGCTAGAGTGTGGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCCATTACTGACGCTGAGGAG  679
A AGTCTGCAGTCGATACGGGCTAGCTAGAGTGTGGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCCATTACTGACGCTGAGGAG  680
L CGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGAACTAGGTGTTGGCGACATTCCACGTCGTCGGTGCCGCAGCTAACGCATTAAGTTCCCCGCCTGGGGAGTACGGCC  815
A CGAAAGCGTAGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGAACTAGGTGTTGGCGACATTCCACGTCGTCGGTGCCGCAGCTAACGCATTAAGTTCCCCGCCTGGGGAGTACGGCC  816
L GCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAGCATTAGAGATAGTGCCCCCCTTGTG  951
A GCAAGGCTAAAACTCAGAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAGCATTAGAGATAGTGCCCCCCTTGTG  952
L GTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCCAGCAGGCCCTTGTGGTGCTGGGGACTCACGGGAGACCGCCGG 1087
A GTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCCAGCAGGCCCTTGTGGTGCTGGGGACTCACGGGAGACCGCCGG 1088
L GGTCAACTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTACAATGGCCGGTACAATGAGCTGCGATACCGTGAGGTGGAGCGAATCTCAAAAAGCCGGTCTCAG 1223
A GGTCAACTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTACAATGGCCGGTACAATGAGCTGCGATACCGTGAGGTGGAGCGAATCTCAAAAAGCCGGTCTCAG 1244
L TTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCACGAAAGTCGGTAACACCCGAAG 1359
A TTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCACGAAAGTCGGTAACACCCGAAG 1360
L CCGGTGGCCCAACCCCTTGTGGGAGGGAGCT-TCGAAGTGAC 1400
A CCGGTGGCCCAACCCCTTGTGGGAGGGAGCTGTCGAAGGTGG 1402
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Fig. 4. Comparison DNA sequences between strain 200401(L) and *S. griesofuscus* AY2076(A) by BLAST. Bold letters represent different DNA sequences.

T-3'). The conditions of the thermal cycles were as follow: pre-denaturation at 94℃ for 5 min; 40 cycles of denaturation for 1 min at 94℃, annealing at 60℃ for 1 min, extension at 72℃ for 1.5 min, further extension at 72℃ for 10 min, and cooled to 4℃. After purification of PCR product, 1400 bp were sequenced with DNA analyzer (ABI PRISM 3700). The determined sequences were analyzed by a BLASTN program. The analysis indicates that the strain 200401 has a 99% homology with *Streptomyces griseofuscus* AY2076 (Fig. 4).

Whole cell fatty acids were analyzed by Gas chromatography (HP 6890, Hewlett-Packard, USA) using calibration standard kit bought from Hewlett-Packard Co. and Sherlock program of MIDI Co. Major fatty acids were 17:0 ANTE(57.9%) and 17:0 ISO(29.5%) (Fig. 5).

Streptomycetes are typically soil-inhabitant microorganisms but they have shown potential for biocontrol of plant pathogenic fungi on foliar and soil because of producing some antibiotics and lytic enzymes (Harindran *et al.*, 1999; Sivan and chet, 1989; Skujus *et al.*, 1965;

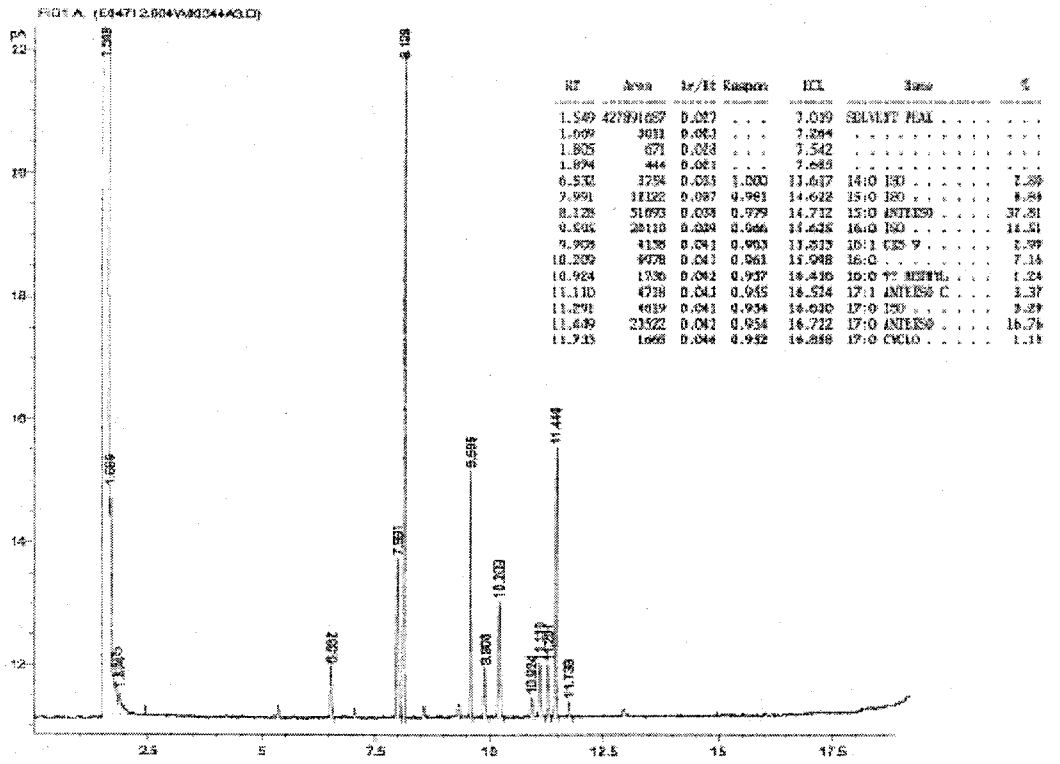

Fig. 5. The analysis of whole cell fatty acids of strain 200401 with Gas chromatography (HP 6890).

Trejo-Estrada SR *et al.*, 1998). The strain 200401 selected throughout this research is like that product some antibiotics (Fig. 1 & 2). Based on other reports (Ahmed *et al.*, 2003; Harindran *et al.*, 1999; Samac *et al.*, 2003; ; Xio *et al.*, 2002) and this results, the strain 200401 may be used as biocontrol agent to late blight and anthracnose by *P. capsici* and *C. acutatum*. Postmaster Author *et al.* (1997) and Hiradate *et al.* (2002) suggested that yeast (Rhodototrula glutinis), fungi (*Trichoderma* spp.), and bacterium (*Bacillus amyloliquefaciens*) exhibited antifungal activity to *Colletotrichum* spp. through producing antibiotics and competition for nutrient on leaf surface.

This result indicates that the strain 200401 has higher antifungal activity to *C. acutatum* than *P. capsici* (Fig. 1 and 2). It can be developed as an agent for diseases by *P. capsici* and *C. acutatum* on pepper after many works such as identification of antibiotics, colonization onto soil and foliar, and other mechanism to confide in ability and development of proper formulation and delivery system (Korsten and Jffries, 2000). Even though several works should be necessary to confide as biocontrol agent of the strain, applying to soil and foliar of culture broth with bacterial cells will reduce inoculum density of by *P. capsici* and *C. acutatum* and disease of late blight and anthracnose by *P. capsici* and *C. acutatum* on pepper.

Acknowledgments

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Literature cited

Ahmed, A. S., M. Ezziyyani, P. Sánchez and M. E. Candela (2003) Effect of chitin on biological control activity *Bacillus* spp. and *Trichoderma harzianum* against root rot disease in pepper (*Capsicum annuum*) plant. European J. Plant Pathology 109:633~637.

Ames, B. N. (1979) Identifying environmental chemicals causing mutations and cancer. Science 204:587~593.

Baker, C. J., J. R., Stavely, C. A., Thomas, M. Saser and J. S. MacFall (1983) Inhibitory effect of Bacillus subtilis on *Uromyces phaseoli* and on development of

rust pustules on bean leaves. Phytopathology 73:1148~1152.

Becker, J. O. (1993) Control of soil-born pathogens with living bacteria and fungi: status and outlook. Pestic. Sci. 37:355~363.

Harindran, J., T. E. Gupte and S. R. Naik (1999) HA-1-92, A new antifungal antibiotic produced by *Streptomyces* CDIL-312: Fermentation, isolation, purification and biological activity. World J. Microbiology & Biochemistry 15:425~430.

Hausbeck, M. K. (2004) Phytophthora capsici on vegetable crops: Research progress and management challenges. Plant Disease 88:1292~1303.

Hiradate, S., S. Yoshida, Y. H. Sugie and Y. Fujii (2002) Muberry anthracnose antagonists (iturins) produced by *Bacillus amyloliquefaciens* RC-2. Phytochemistry 61:693~698.

Korsten, L. and P. Jeffries (2000) Potential for Biological control of diseases caused by *Colletotrichum*. pp.266~291. In *Colletotrichum* (ed. Prusky, D., S. Freeman and M. B. P. Dickman), APS Press, Minnesota, U.S.A.

Lim, T. H., T. H. Chang and B. J. Cha (1998) Incidence of benzimidazole- and dicarboximide-resistant isolates of *Monilinia fructicola* from overwintering mummies and peduncles on peach trees. Korean J. Plant Pathol. 14:367~370.

Lim, T. H., J. M. Lee, T. H. Chang and B. J. Cha (2000) Antifungal activity and identification of an Actinomycetes strain isolated from mummified peaches. Kor. J. Microbiol. Biotechnol. 28:161~166.

Mao, W., J. A. Lewis, R. D. Lumsden and K. P. Hebbar (1998) Biocontrol of selected soil borne diseases of tomato and pepper plant. Crop Protection 17:535~542

Postermaster, A., J. Kuo, K. Sivasithamparam (1997) Interaction between *Colletotrichum musae* and antagonistic microorganisms on the surface of banana leaf discs. Scientia Horticulturae 71:113~125.

Powel, K. A. and F. M. Fox (1993) Technical and commercial aspects of biocontrol produts. Pestic. Sci. 37:315~321.

Samac, D. A., A. M. Willert, M. J. McBride and L. L. Kinkel (2003) Effects of antibiotic-producing Streptomyces on nodulation and leaf spot in alfalfa. Applied Soil Ecology 22:55~66.

Sivan, A. and I. Chet (1989) Degradation of fungal cell wall by lytic enzymes of *Trichoderma harzianum*. J. Gen. Microbiol. 135:675~682.

Skujus, J. J., H. J. Potgieter and M. Alexander (1965) Dissolution of fungal cell walls by a Streptomycete chitinase and β-(1→3) glucanase. Arch. Biochem. Biophys. 111:358~364.

Taso, P. H. and J. J. Oster (1981) Relation of ammonia and nitrous acid to suppression of Phytophthora in soils amended with nitrogenous organic substances. Phytopathology 71:53~59.

Trejo-Estrada, S. R., A. Paszczynski and D. L. Crawford (1998) Antibiotics and enzymes produced by biocontrol agent *Streptomyces vioaceusniger* YCED-9. J. Industrial Microbiology & Biotechnology 21:81~90.

Xiao, K., L. L. Kinkel and D. A. Samac (2002) Biological control of Phytophthora root rots on alfalfa and soybean with *Streptomyces*. Biological Control 23:285~295.

역병균과 고추 탄저병에 대한 Streptomyces griseofuscus 200401의 항균활성

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요약 : 고추 탄저병과 역병균에 항균활성을 보이는 미생물을 산림과 비 경작지 토양으로부터 분리하였다. 대치배양, 배양액 및 열매를 이용한 간이 검정을 통하여 *Streptomyces* sp. 200401 균주가 최종 선발되었다. 선발 균주는 16S rDNA 염기서열과 지방산 분석을 통하여 *Streptomyces griseofuscus*으로 동정되었다.

색인어 : 생물적 방제, 스트렙토마이세스, 역병, 탄저병.

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