J. Microbiol. Biotechnol. (2007), **17**(10), 1700–1703

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Soybean Oil-degrading Bacterial Cultures as a Potential for Control of Green Peach Aphids (*Myzus persicae*)

KIM, SEUL KI[1], SEO RI KIM[1], MIN SEOK CHOI[1], CHANG EON PARK[1], YOUNG CHEOL KIM[1], KIL YONG KIM[1], KYUNG SOOK WHANG[2], KYUNG-TAEK OH[1], AND IN SEON KIM[1]*

[1]*Division of Applied Bioscience and Biotechnology, Environmentally-friendly Agricultural Research Center, Institute of Agricultural Science and Technology, College of Agriculture and Life Sciences, Chonnam National University, Gwangju 500-757, Korea*
[2]*Department of Microbiology, Mokwon University, Daejeon 302-729, Korea*

Received: March 8, 2007 Accepted: May 21, 2007

Abstract Microorganisms capable of degrading crude oil were isolated and grown in soybean oil as a sole carbon source. The microbial cultures were used to control green peach aphids *in vitro*. Approximately 60% mortality of aphids was observed when the cultures were applied alone onto aphids. To examine the cultures as a pesticide formulation mixture, the cultures were combined with a low dose of the insecticide imidacloprid (one-fourth dose of recommended field-application rate) and applied onto aphids. The cultures enhanced significantly the insecticidal effectiveness of imidacloprid, which was higher than imidacloprid alone applied at the low dose. The isolated microorganisms exhibited high emulsifying index values and decreased surface tension values after being grown in soybean oil media. GC/MS analyses showed that microorganisms degraded soybean oil to fatty acids. The cultures were suggested to play the roles of wetting, spreading, and sticking agents to improve the effectiveness of imidacloprid. This is the first report on the control of aphids by using oil-degrading microbial cultures.

Keywords: Aphids, biocontrol, biosurfactant, emulsifying agent, insecticide

Organic farming toward the use of environmentally safe chemicals in agriculture has triggered scientific activities into developing new pesticide formulations in order to reduce the use of synthetic pesticides. Microbiological approaches for crop protection have been generally accepted as being environmentally safe methods [4, 8], alternative to conventional methods. For example, oil-degrading microbial cultures have received great attention from the agricultural business because of their complex molecules, which exhibit antimicrobial activities against pathogens [1, 13, 14, 23].

*Corresponding author
Phone: 82-62-530-2131; Fax: 82-62-530-2139;
E-mail: mindzero@chonnam.ac.kr

Studies of diverse oil-degrading microorganisms have demonstrated that microbial growth in the presence of plant oils results in the production of complex molecules consisting of glycolipids, fatty acids, peptides, polysaccharide-protein complexes, neutral lipids, lipopeptides, and phospholipids [5, 12, 17, 18]. The complex molecules are biosurfactant-type chemicals with emulsification properties.

Considering that oil-degrading microorganisms degrade the oils into saturated and unsaturated fatty acids and also produce emulsifying agents, the microbial cultures may potentially be used in pesticide formulation mixtures. The synthetic pesticide formulations generally consist of an active ingredient, an organic solvent, and an emulsifying agent. Fatty acids and emulsifying agents produced by oil-degrading microorganisms are expected to play the roles of wetting, spreading, emulsifying, and sticking agents to control pest insect. To date, little study has been done on the insecticidal activity of the cultures. The present study examined soybean oil-degrading microbial cultures to control green peach aphids. Soybean oil-degrading microbial cultures in the presence and absence of a synthetic insecticide were sprayed onto aphids, and the mortalities of the insects were investigated *in vitro*.

Microorganisms were isolated by enrichment culture techniques using soils that had been contaminated with crude oils. The two different isolates that showed the best growth among the 12 isolates on diesel and soybean oils were selected for further study. Identification of the isolates was performed by 16S rRNA sequence analyses by comparing their sequences to other 16S rRNA sequences available from a BLAST search of the DDBJ database, as described previously [11].

Chinese cabbage seeds (Heungnong Seed Co., Korea) were surface-disinfected with 70% ethanol for 1 min and rinsed several times with sterile distilled water. The seeds were planted one per cell in square plastic insect breeding dishes (80×80×100 mm) containing commercial nutrient soil (Nongwoo Bio Co., Korea). The seeds were then grown in a greenhouse at 25±2°C and 60±5% relative humidity. Green peach aphids, *Myzus persicae*, were kindly supplied by the Systemic Entomology Laboratory, Agricultural Research Services (ARS), Chonnam, Korea. For bioassays of aphids, the isolates were grown in LB overnight and inoculated at 0.5% (v/v) to mineral salt medium (MSM) containing 0.2% (v/v) soybean oil as the sole carbon source. The cultures were then incubated for 3 days and used to control the aphids. Aphid treatments were performed with soybean oil-degrading microbial cultures in the presence and absence of Conido, a commercial formulation of the insecticide imidacloprid. Imidacloprid was applied onto the aphids at a dose recommended (50 mg/kg) by the manufacturer for vegetables. When the cultures combined with imidacloprid were applied onto the aphids, the Conido was diluted four times from the recommended dose to reach 12.5 mg/kg. Twenty aphids were reared on the leaves of Chinese cabbage in the square plastic insect breeding dishes (80×80×100 mm). The aphids were allowed to settle onto the leaves for 4 h prior to spray application. All plants were checked for the presence of 20 aphids. Subsequently, the plants were sprayed uniformly to run-off using small hand-held sprayers. The plants were dried and then held in the growth chamber at 25±2°C, 65±5% relative humidity, and a photoperiod of 16:8 h L:D. Plants that received only the MSM were the control samples. The mortalities of aphids were investigated 24 h after spray applications. All experiments were repeated five times, unless otherwise stated.

Biosurfactant activity was indirectly investigated by measuring the surface-interfacial tension value using a Du Nouy model 3010 tensiometer (Du Nouy, Japan) as described previously [19]. Biosurfactant measurements were performed on cell-free supernatants obtained by centrifugation at 12,000 ×*g* for 10 min. The instrument was calibrated with air and water to a reading of 72.75 mN/m. The surface tension values were averaged from three replicate measurements. Emulsifying activity was determined by measuring the emulsifying index as described previously [2]. The emulsifying index values were calculated as [height of emulsion layer/(height of oil+height of emulsion layer)]×100. All experiments were repeated three times. For fatty acid analyses, the cultures were centrifuged at 12,000 ×*g* for 10 min and the resulting supernatant was extracted twice with 50 ml of *n*-hexane to remove residual soybean oil in the supernatant. The supernatant was extracted again with 100 ml and 50 ml of a solvent mixture of $CHCl_3$ and CH_3OH (2:1, v/v) at pH 2. The extract was dehydrated on anhydrous sodium sulfate and the solvent was evaporated at 40°C. The extract was methyl-esterified in a mixture of 1 N HCl and methanol at 80°C for 10 min. The methyl esters were extracted twice with two times the extract's volume of a mixture of *n*-hexane and methyl *tert*-butyl ether (1:1, v/v). The organic extracts were then dehydrated on anhydrous sodium sulfate and vacuum-evaporated at 50°C. The dried extracts were dissolved again in *n*-hexane and subjected to GC/MS analysis. GC/MS analyses were performed using a Shimadzu model QP2010 system with a DB-5 capillary column (0·25 mm i.d.×30 m, 1·0 µM film thickness). The carrier gas was helium with a flow rate of 1 ml/min. Injections were done in a split mode of 50:1. The column temperature was programmed for 2 min at 100°C, followed by a ramp rate of 10°C min/ml to 280°C. The injector temperature was 280°C. Mass spectroscopy was performed in the electron impact ionization (EI) mode at 70 eV and was further confirmed in the chemical ionization (CI) mode with isobutane as a chemical gas. The source and inlet temperatures were held at 200°C and mass values were scanned from 50 to 500 amu.

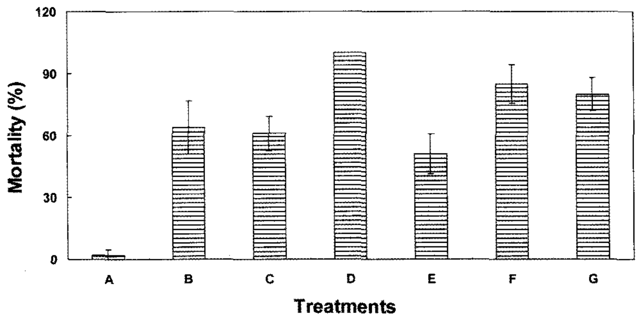

Fig. 1. Mortalities of aphids treated with soybean oil-degrading cultures in the presence and absence of the insecticide imidacloprid. The data are means of five replicates.
A. Control; **B.** *Pseudomonas* MC83 (MC83); **C.** *Rhodococcus* sp. CF2-5 (CF2-5); **D.** Imidacloprid at the recommended dose; **E.** Imidacloprid at a low dose diluted four times from the recommended dose (1/4imidacloprid); **F.** MC83+1/4imidacloprid; **G.** CF2-5+1/4imidacloprid.

The cultures grown in the soybean oil showed aphid mortalities higher than 60% (Fig. 1), which was comparable to the mortalities of the aphids treated with only MSM. No significant differences in the mortalities of the aphids were observed between the cultures, suggesting that microbial species were not the main factor resulting in their mortalities. Imidacloprid treated at the recommended dose showed 100% mortality of the aphids. In Korea, this insecticide has been used largely to control aphids in variable crops. When imidacloprid was diluted four times from the recommended dose (hereinafter "1/4imidaclorpid") and then was sprayed onto the aphids, approximately 50% mortality of the aphids was observed, which was lower than the mortality by culture treatments alone. The mixture of cultures and 1/4imidacloprid significantly increased the toxicity over that of 1/4imidacloprid alone, giving mortalities higher than 80%. The culture treatments did not cause symptoms of phytotoxicity on the cabbage plants, as judged by visual observation. Thus, side effects on crops of culture treatments would likely be negligible.

Table 1. Surface tension and emulsion index values of soybean oil-degrading cultures.*

Isolate	Surface tension value (mN/m)	Emulsion index value (%)	
		E-24	E-48
Pseudomonas sp. MC83	44.0	93	91
Rhodococcus sp. CF2-5	51.4	78	74

*The data are means of three determinations with standard error less than 5%.

To investigate whether the microorganisms isolated in this study produce the emulsifying chemicals, biosurfactant and emulsifying activities were assayed using the supernatants of cultures grown in soybean oil. The surface tension values and emulsifying activities are presented in Table 1. The surface tension values were 44.0 and 51.4 for the isolates MC83 and CF2-5, respectively. Emulsion index values were measured 24 h (E24) and 48 h (E48) after mixing the culture supernatants with soybean oil. The isolate MC83 showed 93% of E24 and 91% of E48, and the isolate CF2-5 showed 78% of E24 and 74% of E48. Oil-degrading microorganisms synthesize a wide variety of bioemulsifiers with high and low molecular masses [22]. The low molecular mass bioemulsifiers are generally known to decrease the surface-interfacial tension values, lower than 30 mN/m. The high molecular mass bioemulsifiers are effective at stabilizing oil-water emulsions. GC/MS analysis identified palmitate ($C_{16:0}$), stearate ($C_{18:0}$), oleate ($C_{18:1}$), and linoleate ($C_{18:2}$) as the main fatty acids from the cultures grown on soybean oil (Table 2). Linoleate was detected as the major fatty acid in the cultures, giving the percentages of total fatty acids ranged from approximately 47% to 60%. No detectable fatty acids were observed when soybean oil was incubated in the MSM without microorganisms.

The intensive use of synthetic pesticides in agriculture has become an environmental concern owing their toxicities and persistence. Microbiological approaches have been generally accepted as alternatives to conventional methods using synthetic pesticides. In the present study, we examined soybean oil-degrading microbial cultures to control green peach aphids. Mortalities of the aphids treated with the insecticide imidacloprid at a low dose (one-fourth dose of the recommended application rate) were significantly enhanced by microbial culture treatments, suggesting a synergistic effect of the cultures on the aphid mortalities. Fatty acids and emulsifying agents produced by oil-degrading microorganisms are expected to play the roles of wetting, spreading, emulsifying, and sticking agents to control aphids. The main mode of action of the cultures to control aphids may be their asphyxial effects on the aphids, as described previously being a synergistic effect of oils on insect control [16]. Aphids would be covered with a thin film of oily materials such as fatty acids and emulsifying agents produced in the cultures. Many studies have demonstrated the synergistic activity of mixtures of oils and synthetic pesticides for the control of pest insects [6, 9, 15, 20, 21]. However, oils sometimes result in serious phytotoxicity and in yield reduction of crops. Soybean oil-degrading microbial cultures may be used as an alternative to oils to control pest insects.

Table 2. Typical fatty acid compositions of soybean-oil degrading cultures.

Fatty acid	% of total fatty acid	
	Pseudomonas sp. MC83	*Rhodococcus* sp. CF2-5
$C_{14:0}$	tr*	tr
$C_{16:0}$	10.51	18.73
$C_{18:0}$	3.87	8.11
$C_{18:1}$	25.95	26.68
$C_{18:2}$	59.67	46.48

*% of total fatty acid: <0.01%.

Korea has strongly driven the Environment-friendly Organic Farming (ENOF) program in agriculture since 1995. The ENOF program has launched an agricultural plan decreasing the use of synthetic pesticides up to 40% by 2010. These significant decreases in the use of synthetic pesticides require that a new pesticide type be available in organic farming. Botanical insecticides, such as nicotine from tobacco leaves, rotenone from derris tree roots, pyrethrum from chrysanthemum flowers, and azadirachitin from neem tree, have been accepted as alternatives to conventional synthetic insecticides [3, 7, 10]. Soybean oil-degrading microbial cultures may be used in formulations of botanical insecticides, because the cultures contain fatty acids and emulsifying agents that enhance the insecticidal effectiveness. Because microorganisms capable of degrading oils are not commonly found in agricultural waters, they can be incubated selectively in waters containing soybean oil. The cultures are then mixed directly with the insect chemicals prior to spraying onto the pest insects. The cultures can decrease the use of synthetic insecticides to control pest insects, which may play an important role in organic farming. This is the first study to report the control of aphids by using oil-degrading microbial cultures. Further study to identify and characterize the emulsifying agent(s) in the cultures is required.

Acknowledgments

This work was supported by a grant from the Environment-friendly Agricultural Research Program of the Ministry of Agriculture and Forestry, Republic of Korea.

REFERENCES

1. Baek, S., X. Sun, Y. Lee, S. Wang, K. Han, J. Choi, and E. Kim. 2003. Mitigation of harmful algal blooms by sophorolipid. *J. Microbiol. Biotechnol.* **13:** 651–659.
2. Bodour, A. A., C. Guerrero-Barajas, B. V. Jiorle, M. E. Malcomson, A. K. Paull, A. Somogyi, L. N. Trinh, R. B. Bates, and R. M. Maier. 2004. Structures and characterization of flavolipids, a novel class of biosurfactants produced by *Flavobacterium* sp. strain MTN11. *Appl. Environ. Microbiol.* **70:** 114–120.
3. Casanova, H., P. Araque, and C. Ortiz. 2005. Nicotine carboxylate insecticide: Effect of the fatty acid chain length. *J. Agric. Food Chem.* **53:** 9949–9953.
4. Chae, D. H., R. D. Jin, H. Hwangbo, Y. W. Kim, Y. C. Kim, R. D. Park, H. B. Krishnan, and K. Y. Kim. 2006. Control of late blight (*Phytophthora capsici*) in pepper plant with a compost containing multitude of chitinase-producing bacteria. *Biocontrol* **51:** 339–351.
5. Costa, S. G. V. A. O., M. Nitschke, R. Haddad, M. N. Eberlin, and J. Contiero. 2006. Production of *Pseudomonas aeroginosa* LB1 rhamnolipids following growth on Brazilian native oils. *Process Biochem.* **41:** 483–488.
6. Dhingra, S. 1996. Effect of different vegetable oils on the toxicity of cypermethrin in mixed formulations on the adults of *Mylabris pustulata* Thunb. *J. Entomol. Res.* **20:** 19–22.
7. Edelson, J. V., J. Duthie, and W. Roberts. 2002. Toxicity of biorational insecticides: Activity against the green peach aphid, *Myzus persicae* (Sulzer). *Pest Manag. Sci.* **58:** 255–260.
8. Folman, L. B., M. J. E. M. De Klein, J. Postma, and J. A. van Veen. 2004. Production of antifungal compound in relation to its efficacy as a biocontrol agent of *Pythium aphanidermatum* in cucumber. *Biol. Control* **31:** 145–154.
9. Horowitz, A. R., Z. Mendelson, and I. Ishaava. 1997. Effect of abamectin mixed with mineral oil on the sweet potato whitefly (*Homoptera: Aleyrodidae*). *J. Econ. Entomol.* **90:** 349–353.
10. Isman, M. B. 1997. Neem and other botanical insecticides: Barriers to commercialization. *Phytoparastica* **25:** 339–344.
11. Kim, I. S., J. Y. Ryu, H. G. Hur, M. B. Gu, S. D. Kim, and J. H. Shim. 2004. *Sphingomonas* sp. strain SB5 degrades carbofuran to a new metabolite by hydrolysis at the furanyl ring. *J. Agric. Food Chem.* **52:** 2309–2314.
12. Kim, H. S., Y. Kim, B. Lee, and E. Kim. 2005. Sophorolipid production by *Candida bombicola* ATCC 22214 from a corn oil processing byproducts. *J. Microbiol. Biotechnol.* **15:** 55–58.
13. Kim, K., D. Yoo, Y. Kim, B. Lee, S. Shin, and E. Kim. 2002. Characteristics of sophorolipid as antimicrobial agent. *J. Microbiol. Biotechnol.* **12:** 235–241.
14. Lee, B., H. Lee, S. Choi, H. Yun, and E. Kim. 2005. Effective screening of antagonist for the biological control of soilborne infectious disease (damping off). *J. Microbiol. Biotechnol.* **15:** 701–709.
15. Liu, T. X. and P. A. Stansly. 2000. Insecticidal activity of surfactants and oils against silverleaf whitefly (*Bemisia argentifolii*) nymphs (*Homoptera: Aleyrodidae*) on collards and tomato. *Pest Manag. Sci.* **56:** 861–866.
16. Martín-López, B., I. Varela, S. Marnotes, and C. Cabaleiro. 2006. Use of oils combined with low doses of insecticide for the control of *Myzus persicae* and PVY epidemics. *Pest Manag. Sci.* **62:** 372–378.
17. Mata-Sandoval, J. C., J. Karns, and A. Torrents. 1999. High-performance chromatography method for the characterization of rhamnolipids mixtures produced by *Pseudomonas aeruginosa* UG2 on corn oil. *J. Chromatogr. A* **864:** 211–220.
18. Moon, H., Y. Kim, H. Kim, D. Kwon, and W. Chung. 2002. Glycolipid biosurfactants produced by *Pseudomonas aeruginosa* D2 from diesel-contaminated soil. *J. Microbiol. Biotechnol.* **12:** 371–376.
19. Oh, K., C. Kang, M. Kubo, and S. Chung. 2003. Cultural characteristics of a biosurfactant-producing microorganism *Pseudomonas aeruginosa* F722. *Kor. J. Microbiol. Biotechnol.* **31:** 171–176.
20. Puri, S. N., B. B. Bhosle, M. Ilyas, G. D. Butler, and T. J. Henneberry. 1994. Detergents and plant-derived oils for control of the sweet potato whitefly on cotton. *Crop Protect.* **13:** 45–48.
21. Rao, G. R. and S. Dhingra. 1997. Synergistic activity of some vegetable oils in mixed formulations with cypermethrin against different instars of *Spodoptera litura* (Fabricius). *J. Econ. Entomol. Res.* **21:** 153–160.
22. Rosenberg, E. and E. Z. Ron. 1999. High- and low-molecular-mass microbial surfactants. *Appl. Microbiol. Biotechnol.* **52:** 154–162.
23. Yoo, D., B. Lee, and E. Kim. 2005. Chracterization of microbial biosurfactants as an antifungal agent against plant pathogenic fungus. *J. Microbiol. Biotechnol.* **15:** 1164–1169.