KOR. JOUR. MICROBIOL August, 1992 p. 265~268 Vol. 30, No. 4

Bacteriocins in Purple Nonsulfur Bacteria

Lee, Sang-Seob,* Tae-Jeong Oh and Hyun-Soon Lee

Department of Biology, College of Natural Science, Sung Kyun Kwan University, Suwon 440-760, Korea
**Department of Biology, College of Natural Science, Kyuong-Ki University, Suwon 440-760, Korea*

In this study, we want to detect bacteriocin production in purple nonsulfur bacteria. As a results, it was showed that bacteriocin produced between some strains of *Rhodobacter capsulatus*, *Rhodobacter sphaeroides* and *Rhodocyclus gelatinosus*. In particular, it was appeared that cell membrane-bound bacteriocin was also produced by *Rhodobacter capsulatus* ATCC 17016.

KEY WORDS □ *Rhodobacter capsulatus*, *Rhodopseudomonas palustris*, Bacteriocins

Bacteriocins are proteins or protein complexes which showed bacteriocidal activity towards closely related species (5, 8, 9, 12, 15, 18, 20, 23). It differs from most of the previously known antibiotics, which have a much wider activity spectrum (21). Bacteriocidal effect of bacteriocins have been recognized as an important subject in food fermentation and food preservation(6, 17). Plasmid-encoded bacteriocins are commonly observed among both Gram-negative and Gram-positive bacteria(1, 26, 27), but there are also DNA-encoded bacteriocins(11).

Productivity of depends on the growth stage and culture conditions(11, 16).

There are two types of bacteriocins, secrete and cell bounded(2, 10, 22).

Purple nonsulfur bacteria produce also bacteriocins, but only *Rhodobacter capsulatus*, *Rhodobacter sphaeroides* and *Rhodopseudomonas palustris* have been reported to produce bacteriocin(7, 24, 25). In this study, we want to detect bacteriocin production in purple nonsulfur photosynthetic bacteria. These studies could be the basis of molecular level studies of bacteriocins on purple nonsulfur photosynthetic bacteria.

MATERIALS AND METHODS

Bacteria and growth conditions

Bacterial species listed in table 1 were obtained from ATCC(American Type Culture Collection) and DSM(Deutsches Sammlung für Mikroorganismen).

Precultures were grown on a medium 27(DSM) containing trace element SL-6.

Anaerobic photosynthetic growth was achieved in 10 m*l* cap tubes completely filled with media and illuminated with incandescent lamps(ca.3,000 lux) at 28±2°C.

Bacteriocin producer and indicator were incubated always in a medium of Lascells's(1956) instead of medium 27(DSM). The pH of all media was adjusted to pH 6.8.

Secreted bacteriocin

Producer species were cultured under same light and temperature conditions as preculture. Log phase cultures of strains were inoculated by loop onto the center of surface, about 3-4 cm wide, of agar plate, which contained Lascell's medium, and 1.5% agar and cultured aerobically in sterillized metal tube for 3-4 days. When they became fully grown and dark brown, producers were exposured to chloroform vapor for 2-3 min in order to kill producer strains and dead cells were removed with cotton soaked with 75% alcohol.

The producer plates were overlaid with soft agar (0.5%) seeded with a culture of 2 m*l* indicator organism (ca. 4.8×10^8 cells/m*l*) and incubated in anaerobic jars(CO_2+H_2 gas pack, BBL) under light at 28±2°C for 5 days.

Clear zones of inhibition surrounded the colonies, indicating bacteriocin activity.

Cell membrane bounded bacteriocin detection

Liquid cultures were grown in 5000 m*l* bottles containing 3000ml medium inoculated with 5 m*l* of preculture(O.D. 1.5). Cells were shaken(50 rpm) at 28±2°C for 4 days in darkness, harvested, and centrifuged for 10 min(Sorvall GS-S rotor 8,000 rpm). Cell residues were washed with D.W. 2 times, sonicated for 21 min(ultrasonic 19 mm probe, 230 Hz, 3 min×7 times) and centrifuged(15, 000 rpm). Supernatant was filtered with Amicon TC-10 ultrafiltration membrane and separated into three fractions of molecular weight <1000 dalton, 1000−10000, and >10000 dalton. They

Table 1. *The list of strains used for this experiment*

Species	Source
Rhodobacter capsultus	DSM 17016
Rhodobacter capsultus	DSM 155
Rhodobacter capsultus	DSM 938
Rhodobacter capsultus	DSM 1710
Rhodobacter capsultus	ATCC 17016
Rhodobacter sphaeroides	ATCC 17023
Rhodobacter sulfidophilus	DSM 1374
Rhodopseudomonas palustris	DSM 123
Rhodopseudomonas palustris	ATCC 17003
Rhodopsudomonas sulfoviridis	DSM 729
Rhodocyclus gelatinosus	ATCC 17011

Table 2. *Screening for bacteriocin production from purple nonsulfur photosynthetic bacteria.*

Producer strains / Indicator strains	C_1	C_2	C_3	C_4	C_5	Sph	Gel
C_1	·	+	−	−	+	−	−
C_2	−	·	−	−	+	−	
C_3	−	−	·	−	−	−	
C_4	−	−	−	·	−	−	
C_5	−	−	−	−	·	−	
Pal′	+	−	−	+	−	+	−
Pal	−	−	−	−	−	−	−
Sul	−		−		−	−	+
Sph	+	−	−	−	−	·	−
Sulfi	−	−				−	·

\+ : produced - : not produced
C_1 : *Rhodobacter capsulatus* ATCC 17016
C_2 : *Rhodobacter capsulatus* DSM 152
C_3 : *Rhodobacter capsulatus* DSM 155
C_4 : *Rhodobacter capsulatus* DSM 938
C_5 : *Rhodobacter capsulatus* DSM 1710
Sph : *Rhodobacter sphaeroides* ATCC 17023
Gel : *Rhodocyclus gelatinosus* ATCC 17011
Pal′ : *Rhodopseudomonas palustris* ATCC 17003
Pal : *Rhodopseudomonas palustris* DSM 123
Sul : *Rhodopseudomonas sulfoviridis* DSM 729
Sulfi: *Rhodobacter sulfidophilus* DSM 1374

were lyophilized and tested for bacteriocin activity as in test for the secreted bacteriocin.

RESULT

Result Production of bacteriocins secreted by purple nonsulfur photosynthetic bacteria are shown in Table 2. Several strains of α-group and β-group were tested for bacteriocin production against 12 indicators, which were selected from α-group.

Diffusable or secreted bacteriocin were produced by *R. capsulatus* ATCC 17016, DSM 938, 1710 and *R. sphaeroides* ATCC 17023. But *R. capsulatus* DSM 155 didn't show any inhibition zone around either its own species or other species.

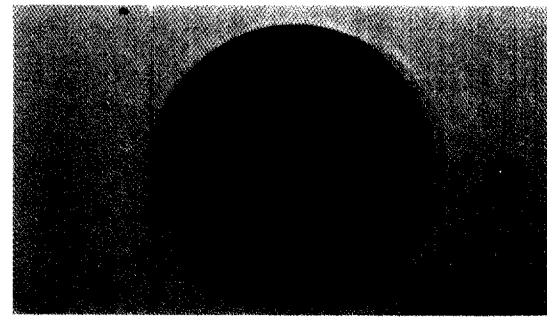

Fig. 1. *Bacteriocin inhibition of Rhodopseudomonas palustris ATCC 17003 indicator lawn on Lascells medium by producer strain of Rhodobacter capsulatus ATCC 17016.*

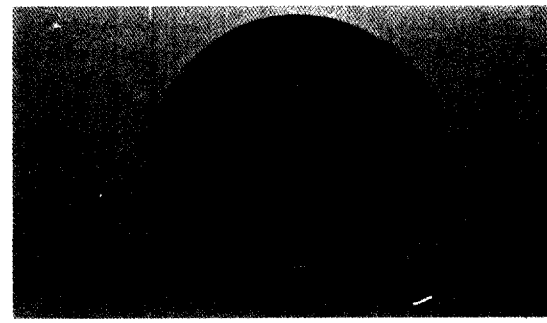

Fig. 2. *Cell-bound bacteriocin inhibition of Rhodopseudomonas palustris ATCC 17003 indicator lawn on Lascells medium by producer strain of Rhodobacter capsulatus* ATCC 17016.

R. capsulatus ATCC 17016 didn't show intraspecific inhibitory interactions but showed bacteriocin activity against two different species, *R. palustris* ATCC 17003 and *R. sphaeroides* ATCC 17023. Even *R. gelatinosus* belonging to β-group inhibits *R. sulfoviridis* DSM 729 of β-group.

Cell-bound bacteriocin activity was also noticed for *R. capsulatus* ATCC 17016 against *R. palustris* ATCC 17003 as indicator (Fig. 2).

DISCUSSION

Bacteriocin from purple nonsulfur photosynthetic bacteria was reported in *R. capsulatus*, *R. sphaeroides* and *R. palustris* by Guest(1974) and

studied only in *R. capsulatus* by Guest(1974) and Willison *et al.* (1987). We confirmed that *R. capsulatus* ATCC 17016 produced bacteriocin against *R. palustris* ATCC 17003(Fig. 1, Table 2) and *R. sphaeroides* ATCC 17023.

It was suggested that bacteriocins shown narrow range of inhibitory activity toward closely related species, but do not affect the producing strains(5, 8, 15, 19, 23). However, it was also noticed that bacteriocins inhibited remotely related species(7). Different strains of *R. capsulatus* have different types of activity of bacteriocin, with *R. capsulatus* DSM 152 active against only *R. capsulatus* ATCC 17016 and *R. capsulatus* ATCC 17016 inhibited both *R. sphaeroides* ATCC 17023 and *R. palustris* ATCC 17003, while *R. capsulatus* DSM 938 inhibited only *R. palustris* ATCC 17003. *R. capsulatus* DSM 155 didn't show any activity. However it is interesting that -group of *R. gelatinosus* ATCC 17011 inhibited -group of *R. sulfoviridis* DSM 729.

Why does bacteriocin act so differently between the same species? Reeves noticed that many different colicins existed(21). Studies of colicins of *E. coli* have included amino acid sequencing, location of the gene and receptor binding sites (Colter and Klaenhammer, unpublished). However, these studies failed to answer this question. We studied the effect of pH on bacteriocin production. At pH 6, there was no bacteriocin activity, while between pH 6.8 and pH 8.0 no difference was observed in activity.

Reeves(1972) reported when a producer was exposed UV light about 6 hours after growth, bacteriocin productivity was increased. But UV light didn't show any influence on bactericin produced by purple nonsulfur photosynthetic bacteria. Similar results were seen with *Lactobacillus casei*, which was not affected by UV light exposure(20). It was reported that production of bacteriocins depends on culture condition(11, 16). We tested for its activity from cultures grown in aerobic- and anaerobic in the darkness and anaerobic cultures grown in the light, but bacteriocin activity was found cultures only in aerobic cultures grown in darkness.

Bacteriocin production depends on composition of culture medium. We varied amounts of yeast extract from 0.1 to 1.0% in serial dilutions, but we didn't find any large difference in activity. If any ingredient was omitted from the medium used (Lascells's medium containing yeast extract and trace element solution), no activity was observed. According to the method Guest(1974) we tested bacteriophage contamination. Producer was cultured aerobically in the darkness and filtered(pore size. 0.45 μm). Filtrate was cultured, but did not cause plaque formation. These results agree with those of Guest(1974) and Wall(1975).

We recognized both types of bacteriocins, secreted and bounded ones. Until now, bacteriocins have simply been reported to exist in purple nonsulfur photosynthetic bacteria, little has been studied about them. More information is needed about these substances, including molecular composition of the purified bacteriocins.

ACKNOWLEDGEMENT

This work is in part supported by a grant from the Korea Research Foundation, 1987.

REFERENCES

1. **Ahn, C. and M.E. Stiles**, 1990. Plasmid-associated bacteriocin production by a strain of *Carnobacterium piscicola* from meat. *Appl. Environ. Microbiol.*, **56**, 2503-2510.
2. **Austin-Prather, S.L. and S.J. Booth**, 1983. Evidence for a membrane-bound bacteriocin of *Bacteroides uniformis* TI-1. *Can. J. Microbiol.*, **30**, 268-272.
3. **Brock, T.D., B. Peacher and D. Pieson**, 1963. Survey of the bacteriocins of a2enterococci. *J. Bacteriol.*, **86**, 702-707.
4. **Chhibber, S. and D.V. Vadehra**, 1986. Purification and characterization of a bacteriocin from *Klebsiella pneumoniae 158*. *J. Gen. Microbiol.* **132**, 1051-1054.
5. **Flores, E. and C.P. Wolk**, 1986. Production, by filamentous, nitrogen-fixing cyanobacteria, of a bacteriocin and of other antibiotics that kill related strains. *Arch. Microbiol.*, **145**, 215-219.
6. **Gonzalez, C.F. and B.S. Kunka**, 1987. Plasmid-associated bacteriocin production and sucrose fermentation in *Pediococcus acidilactici*. *Appl. Environ. Microbiol.*, **53**, 2534-2538.
7. **Guest, J.R.**, 1974. Bacteriocinogeny in the *Athiorhodaceae*. *J. Gen. Microbiol.*, **81**, 513-515.
8. **Herschman, H.R. and D.R. Helinski**, 1967. Purification and characterization of colicin E_2 and colicin E_3. *J. Biol. Chem.*, **25**, 5360-5368.
9. **Holland, I.B.**, 1975. Physiology of colicin action. *Adv. Micro. Physiol.*, **12**, 56-139.
10. **Hu, P.C. and R.R. Brubaker**, 1974. Characterization of pesticin. *J. Biol. Chem.*, **249**, 4749-4753.
11. **Jorger, M.C. and T.R. Klaenhammer**, 1986. Characterization and purification helveticin J and evidence for a chromosomally determined bacteriocin produced by *Lactobacillus helveticus* 481. *J. Bacteriol.*, **167**, 439-446.
12. **Konisky, J.**, 1982. Colicin and other bacteriocins with established mode of action. *Ann. Rev. Microbiol.*, **36**, 125-144.
13. **Landolo, J.J.**, 1989. Genetic analysis of extracellular toxins of *Staphylococcus aureus*. *Ann. Rev. Microbiol.*, **43**, 375-402.
14. **Jascells, J.**, 1956. Synthesis of porphyrins and bac-

teriochlorophyll by cell suspensions of *Rhodopseudomonas sphaeroides*. *Biochem. J.*, **62**, 78-93.
15. **Lyon, W.J. and B.A. Glatz**, 1991. Partial purification and characterization of a bacteriocin produced by *Propionibacterium thoenii*. *Appl. Environ. Microbiol.*, **57**, 701-706.
16. **Mayr-Harting, A., A.J. Hedges and R.C.W. Berkley**, 1972. Methods for studing bacteriocins. In: Methods in microbiology(eds.J.R.Norris and D.W.Ribbons)., **7A**, 315-422.
17. **Meghrous, J., P. Euloge, A.M. Junelles, J. Ballongue and H. Petitdemange**, 1990. Screening for *Bifidobacterium* strains for bacteriocin production. *Biotechnology Letters.*, **12**, 575-580.
18. **M rtvedt, C.I. and I.F. Nes**, 1990. Plasmid-associated bacteriocin production by a *Lactobacillus sake* strain. *J. Gen. Microbiol.*, **136**, 1601-1607.
19. **Muriana, P.M. and T.R. Klaenhammer**, 1987. Conjugal transfer of plasmid-encoded determinants for bacteriocin production and immunity in *Lactobacillus acidophilus 88*. *Appl. Environ. Microbiol.*, **53**, 553-560.
20. **Rammelsberg, M., E. Muller and F. Radler**, 1990. Casein 80:purification of a new bacteriocin from *Lactobacillus casei*. *Arch. Microbiol.*, **154**, 249-252.
21. **Reeves, P.**, 1972. The bacteriocins. Springer-Verlag Berlin, Heidelberg, New York.
22. **Southern, J.R., W. Katz and D.R. Woods**, 1984. Purification and properties of a cell-bound bacteriocin from a *Bacteroides fragilis* strain. Antimicrobial Agents and Chemotheraphy, **21**, 253-257.
23. **Tagg, J.R., A.S. Dazani and W. Wannamaker**, 1976. Bacteriocins of gram positive bacteria. *Bacteriol. Rev.*, **49**, 725-756.
24. **Wall, J.D., P.F. Weaver and H. Guest**, 1975. Gene transfer agents, bacteriophages and bacteriocins of *Rhodopseudomonas capsulata*. *Arch. Microbiol.*, **105**, 217-224.
25. **Willison, J.C., J.P. Magnin and P.M. Vignais**, 1987. Isolation and characterization of *Rhodobacter capsulatus* strains lacking endogenous plasmids. *Arch. Microbiol.*, **147**, 134-142.
26. **Yasuyoshi, I., D.B. Clewell, R.A. Segarra and M.S. Gilmore**, 1990. Genetic analysis of the pAD1 hemolysin/bacteriocin determinant in *Enterococcus faecalis*: Insertional mutagenesis and cloning. *J. Bacteriol.*, **172**, 155-163.
27. **Yule, R. and B.D. Barridge**, 1976. Isolation and characterization of a bacteriocin production by *Bacillus stearothermophillus* strain NU-10. *Can. J. Microbiol.*, **22**, 1743-1759.

(Received May 24, 1992)
(Accepted June 3, 1992)

초 록: 홍색 비유황 광합성세균에서의 Bacteriocins에 관한 연구

이상섭 · 오태정 · 이현순 (성균관대학교 이과대학 생물학과, *경기대학교 이과대학 생물학과)

홍색 비유황 광합성세균들중에 몇종을 선택하여 bacteriocin 생산실험을 실시하였다. 그 결과, *Rhodobacter capsulatus*, *Rhodobacter sphaeroides* 그리고 *Rhodocyclus gelatinosus*의 세균들이 bacteriocin을 분비하는 것으로 나타났으며, 특히 *Rhodobacter capsulatus* ATCC 17016은 cell-bound bacteriocin도 생산하는 것으로 나타났다. 대부분의 경우, 근연종들간에 bacteriocin이 분비되는 것으로 나타났으나, 비교적 유연관계가 먼 *Rhodocyclus gelatinosus*와 *Rhodopseudomonas sulfoviridis*간에도 bacteiocin이 분비되는 것으로 나타났다.