Short Communication

EUBACTERIAL STRAINS SENSITIVE TO APHIDICOLIN ARE DISCOVERED AMONG MARINE BACTERIA

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APHIDICOLIN, known as a highly specific inhibitor of DNA replication by eukaryotes' and eukaryote-like replicative enzymes, has been considered to have no effect on DNA replication system of eubacteria (8,12). When aphidicolin was applied to a group of bacteria of marine origin, some members of this group were found sensitive to this drug. This group of marine bacteria is the first example of aphidicolin-sensitive eubacteria, and their DNA replication system and phylogenetic implications are highly interesting from the standpoint of evolution.

We have been conducting experiments using marine bacteria in order to accumulate their fundamental biological features, since they have not been studied as extensively as terrestrial bacteria. During the course of experiment, we have found restriction enzymes, *Fsp* I 1604 (1) and *Fsp* I 1611 (2,3), from *Flavobacterium* sp. I 1604 and I 1611. These bacteria showed extraordinarily strong resistance to streptomycin (minimum inhibitory concentration, MIC: 20 mg/ml <) (1-3). These strains are classified by means of rRNA homology (11) in the rRNA superfamily IV sensu De Ley (5), class *Proteobacteria*, and, incidentally possess ubiquinone 10 (Q10) as the major component of coenzyme Q (4). (M. Akagawa, personal communication). According to Dr. Akagawa, both of these strains belong to a group of marine bacteria of which characteristic features are Gram-negative, rod-shaped, strictly aerobic eubacteria. The Q10-possessing marine bacteria of rRNA superfamily IV are the first findings, and proved to be taxonomically so diverse that at least three undescribed genera would be necessary to represent them. Since Q10 usually distributes among higher animals, we attempted to apply aphidicolin to this group of marine bacteria, including *F. sp.* I 1604 and I

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1611, to obtain one of the criteria to get a better understanding of their phylogenetic relationships.

The aphidicolin sensitivity of these Q10-possessioning marine bacteria was examined on the solid medium (Table 1). Aphidicolin inhibited or restricted the growth of nine strains out of the 15 strains so far examined. Several strains were chosen from this group and their growth profiles in a liquid culture in the presence of aphidicolin was examined using the method applied to halophilic archaeobacteria (6), together with aphidicolin-insensitive controls: *Alcaligenes* sp. MD1 (this strain is not classified in superfamily IV) and *Escherichia coli* DH5 α . Growth of Alc 204 was inhibited by aphidicolin (Fig. 1). Alc 203 showed a similar growth profile to that of Alc 204 (data not shown). *Flavobacterium* sp. I 1604 and 1611 were less affected by the drug. About 12 h after the addition of the drug, the increment of turbidity at 660 nm (A_{660}) in the presence of aphidicolin was observed as 0.03 for Alc 203, 0.07 and 0.3 for *F.* sp. I 1604 and 1611, respectively, while, during the same time span, the control run, namely without aphidicolin, reached full growth (A_{660} = 1.2–1.4). *A.* sp. MD1 and *E. coli* DH5 α were insensitive to aphidicolin (Fig. 1). Addition of the drug at any growth phase of the sensitive bacteria brought about inhibition and/or restriction of cell growth. The enlargement of the cell shape during incubation with aphidicolin was observed under a light microscope, and resembles the observation in the case of *Halobacterium* (13). Cell growth was

Table 1. Growth of Q10-possessioning marine bacterial strains in the presence of aphidicolin.

Strain name	Aphidicolin (μ g/ml)				
	0	100	150	200	250
Alc 204	++++	+	—	—	—
Alc 203	++++	+++	+	—	—
Alc 252	++++	++++	+	+	—
Alc 100	++++	+++	+++	++	—
Alc 255	++++	++++	+++	++	—
<i>Flavobacterium</i> sp. I 1604	++++	+++	+++	++	+
Alc 096	++++	++++	+++	++++	+
<i>Flavobacterium</i> sp. I 1611	++++	++++	+++	+++	++
Alc 242	++++	++++	++++	++++	++
<i>Alcaligenes</i> sp. MD1	++++	++++	++++	++++	++++
<i>Escherichia coli</i> DH5 α	++++	++++	++++	++++	++++

The marine bacteria listed were incubated at 30°C on a PPES II agar plate containing the indicated concentration of aphidicolin (0–250 μ g/ml), and the extent of growth was observed. Growth scored from no growth (—) to plus 4 (++++), with plus 4 equivalent to growth on PPES II agar plates without aphidicolin. Optimum growth was observed when the cells were inoculated to PPES II Broth [Modified Sea Water Broth: 0.1% Proteose Peptone No. 3 (Difco Laboratories), 0.2% Polypeptone (Daigo Eiyo Co. Ltd.), 0.1% Bacto Yeast Extract (Difco Laboratories), 0.1% Bacto Soytone (Difco Laboratories) in Jamarin S “Artificial Sea Water” (Jamarin Laboratory) pH 7.5] and PPES II Plates [1.5% Agar in PPES II Broth], and incubated at 25°C–35°C. Strains denoted “Alc,” *Flavobacterium* species and *Alcaligenes* sp. MD1 were kindly donated by Dr. M. Akagawa and Dr. U. Simidu, respectively.

Fig. 1. Growth profile of the Q10-possessing marine bacterium (Alc 204) in the presence of aphidicolin (aph). The method used for halophilic archaeobacteria (6) was applied to the strain Alc 204, *A. sp. MD1* and *E. coli* DH5 α . Arrows indicate the moment of aph addition. The change in growth was determined by reading turbidity at 660 nm (A_{660}). Strains were incubated up to the logarithmic phase (A_{660} =0.2–0.4) and divided into two portions. The drug was added to one portion, and dimethyl sulfoxide (DMSO: the solvent for aph) was added to another portion. Alc 204 was inoculated in PPES II broth containing 150 $\mu\text{g/ml}$ aph, and *A. sp. MD1* (negative control) was examined using PPES II broth containing 250 $\mu\text{g/ml}$ aph. In the case of *E. coli*, cell growth was examined in LB broth with/without 250 $\mu\text{g/ml}$ drug. For Alc 204, viable counts during the incubation were determined on PPES II using appropriate dilution with artificial sea water (Jamarin S).

(A) Growth profile of Alc 204 monitored by turbidity, $\circ$: without aph; $\bullet$: with aph, 150 $\mu\text{g/ml}$; (B) Growth profile of Alc 204 monitored by viable counts, $\circ$: without aph; $\bullet$: with aph, 150 $\mu\text{g/ml}$; (C) Growth profile of *A. sp. MD1* and *E. coli* DH5 α monitored by turbidity, Δ : *Alcaligenes sp. MD1* without aph; $\blacktriangle$: *Alcaligenes sp. MD1* with aph, 250 $\mu\text{g/ml}$; $\square$: *E. coli* DH5 α without aph; $\blacklozenge$: *E. coli* DH5 α with aph, 250 $\mu\text{g/ml}$.

reversibly regenerated when the drug was removed from the broth (data not shown).

The different sensitivities of the Q10-possessing bacteria toward aphidicolin is still uncertain. However, when those insensitive strains were treated to make cells permeable with dimethyl sulfoxide, and examined on the solid media, they showed some degree of restricted growth on a 250 $\mu\text{g/ml}$ drug-containing plate (data not shown). One possibility is a difference in cell envelopes which may produce diffusion barriers which inhibit the drug from reaching the target. This is demonstrated by yeast (8) and *Methanococcus* (14), an archaeobacterium.

The working concentration of aphidicolin for this group of bacteria is comparable to *Bacillus* phages $\phi 29$ and M2 (7,10). Though a much higher concentration contained in the medium (100 to 250 $\mu\text{g/ml}$) compared to that of eukaryotes

(1 $\mu\text{g/ml}$) (8) and archaeobacterial α -like polymerase (20 $\mu\text{g/ml}$) (13) was required, the experiments suggested that some marine eubacterial strains used in this study, whose phylogenetic position is totally different from that of archaeobacteria, share a similar type of replicative enzyme as a common characteristic.

Past studies demonstrated that the target enzyme of aphidicolin *in vivo* is DNA polymerase α , and recently DNA polymerase δ has further been found to be sensitive to this drug. These two types of polymerases are considered to play the significant role in DNA replication (9). The existence of aphidicolin-sensitive eubacterial strains among marine bacteria may reflect certain evolutionary intermediate of the eukaryotic type DNA replicative enzyme.

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