

タバコ培養細胞との共存培養による *Pseudomonas syringae* pv. *atropurpurea* の pCOR1 プラスミドの高頻度離脱

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High Frequency Loss of pCOR1 Plasmid from *Pseudomonas syringae* pv. *atropurpurea* by Co-cultivation with Tobacco Cells

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Key words : *Pseudomonas syringae* pv. *atropurpurea*, tobacco cell, co-cultivation, plasmid, curing.

Pseudomonas syringae pv. *atropurpurea* (abbrev., pv. *atropurpurea*) is the causal agent of halo blight of Italian ryegrass (*Lolium multiflorum* Lam.). The bacterium produces the phytotoxin 'coronatine' which is responsible for developing symptoms of halo blight¹⁾. Coronatine producing ability of the bacterium is associated at least partly with 58 Mdal plasmid named pCOR1³⁾. Sato (1986) have reported that pCOR1 plasmid is lost from the host bacterium pv. *atropurpurea* at a high frequency, accompanying with loss of bacterial virulence, when the bacterium was inoculated into the leaves of tobacco or Italian ryegrass⁴⁾. Here, we report on the preliminary result that pCOR1 plasmid was also lost at a high frequency when the bacterium was cultured with tobacco cells.

Bacterial strain used in this experiment was pv. *atropurpurea* NIAES 1309 (furnished from Dr. K. Nishiyama, Natl. Inst. Agro-Environment. Sci.). Tobacco (*Nicotiana tabacum* L. cv. Xanthi nc and cv. Xanthi) cells were derived from mesophyll protoplasts culturing in the Murashige and Skoog's (MS) medium⁵⁾ supplemented with α -naphthalene acetic acid (NAA, 1 ppm) and benzyl adenine (BA, 1 ppm). Nine ml of plant cell suspension (2×10^4 cell colony/ml) and one ml of bacterial suspension (usually 1×10^8 cell/ml) were mixed and cultured in the MS medium containing mannitol (0.2 M usually, 0.5 M for protoplast culture) at 28 C in a stationary condition. After 1 to 2 weeks' co-cultivation⁶⁾, the suspension was appropriately diluted in sterile water, 0.2 ml of it was spread on a King's B (KB)⁷⁾ plate, and incubated at 28 C. Viable bacteria were counted and a single colony isolation was carried out. Coronatine productivity of the isolates thus obtained was examined by the potato tuber assay described by Nishiyama¹⁾. The

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test was repeated twice when negative reaction was observed. Furthermore, coronatine-minus mutants (Cor⁻) were tested for the presence of pCOR1 plasmid according to the procedures described by Birnboim and Doly⁸⁾. Plasmid DNA was analyzed by electrophoresis at 100 volts in 0.8% agarose gel with Tris-borate buffer (89 mM Tris base, 2.5 mM disodium EDTA and 8.9 mM boric acid, pH 8.0) according to the procedures described by Sato *et al.*³⁾.

At first, pv. *atropurpurea* NIAES 1309 was cultured for 7 days with tobacco cells of 2 or 17 days after protoplast isolation. When the bacterium was cultured with 17 days old tobacco cells at 28 C in the stationary condition, pCOR1 plasmid was lost at the frequency of 2.8% and the total bacteria fairly increased in the medium during 7 days (Table 1). Our recent data show that pv. *atropurpurea* tends to increase until 7 days after inoculation and then, to decrease gradually in co-cultivation (unpublished data). In contrast, when this bacterium was cultured with 2 days old tobacco cells, the loss of pCOR1 plasmid was not detected in tested colonies and the viable count of bacteria decreased drastically. Without plant cells it decreased also drastically in number, and none of survived bacteria lost their plasmid. Similar result was obtained in co-cultivation experiment at high temperature (37 C) beyond the range of growth temperature of the bacterium, although it has been reported that Ti plasmid of *Agrobacterium tumefaciens*

Table 1. Loss of pCOR1 plasmid in *P. syringae* pv. *atropurpurea* NIAES 1309 by co-cultivation for 7 days with tobacco (Xanthi nc) cells

Tobacco cell (growth stage)	Incubation temp.	Frequency of loss ^{b)}	Bacterial multiplication
17 days ^{a)} cell	28 C	3/108(2.8%)	+(ca. 20 ^{c)})
2 days cell	28 C	0/ 81(0 %)	-(ca. 2.0×10 ⁻²)
no cell	28 C	0/ 45(0 %)	-(ca. 2.0×10 ⁻⁴)
17 days cell	37 C	0/7 (0 %)	-(ca. 1.5×10 ⁻⁵)
2 days cell	37 C	0/6 (0 %)	-(ca. 5.0×10 ⁻⁵)
no cell	37 C	Not tested	-(less than 5.0×10 ⁻⁶)

a) Days after protoplast isolation.

b) Number of pCOR1⁻ isolates/Number of isolates tested.

c) Number of survived bacteria/Number of inoculated bacteria.

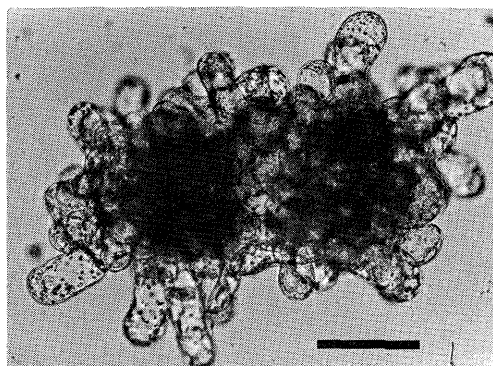


Fig. 1. Tobacco plant cells (40days) used in co-cultivation experiment. Bar : 100μm

Table 2. Loss of pCOR1 plasmid in *P. syringae* pv. *atropurpurea* NIAES 1309 by co-cultivation with tobacco cells

Tobacco cell (growth stage ^{a)})	Inoculated bacterial conc. (cell/ml)	Incubation period (day)	Frequency of loss ^{b)}
Xanthi nc (40days)	1×10^7	12	5/50(10%)
X. nc (40days)	1×10^8	6	5/75(7%)
X. nc (40days)	1×10^8	12	17/48(35%)
X. nc (60days)	1×10^8	8	6/64(9%)
X. nc (60days)	1×10^8	14	8/66(12%)
Xanthi (60days)	1×10^8	8	2/46(4%)
X. (60days)	1×10^8	14	5/66(8%)

a) Days after protoplast isolation.

b) Number of pCOR1⁻ isolates/Number of isolates tested.

faciens is lost at high temperature⁹⁾.

Next, we tried to culture pv. *atropurpurea* NIAES 1309 together with plant cells of older growth stage (40 and 60 days) at 28 C. As shown in Table 2, pCOR1 plasmid was lost at high frequencies ranging from 4% to 35% in these co-cultivation experiments. The highest frequency (35%) was obtained in the co-cultivation with 40 days old plant cells (Fig. 1). These values fell within the range of the frequencies in the inoculated plants previously reported⁴⁾.

These results suggest that tobacco plant cells, especially around 40 days old cells, may produce some substance effective to lose pCOR1 plasmid from the bacterium. The substance may inhibit the gene expression of replication or partition of pCOR1 plasmid, or it may affect favourably on the survival of plasmid-free bacteria. To our knowledge, the fact that virulence related plasmid of phytopathogenic bacteria is lost by co-cultivation with plant cells has not been reported in any other bacteria.

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和 文 摘 要

沼田 司・佐藤 守・酒井富久美：タバコ培養細胞との共存培養による *Pseudomonas syringae* pv. *atropurpurea* の pCOR1 プラスミドの高頻度離脱

P. syringae pv. *atropurpurea* NIAES 1309 株をタバコ培養細胞と共存培養させると条件によって本菌のコロナチン産生支配プラスミド pCOR1 が高頻度に除去された。その離脱率はタバコ細胞の生育日数に影響され17日間培養のタバコ細胞との共存培養で3%, 40日間培養細胞で7~35%, 60日間培養細胞で4~12%の率でpCOR1 の離脱が起こった。一方2日間培養細胞(プロトプラスト)との共存培養,あるいは培地のみでは全く起こらなかった。これらの結果より,本菌のプラスミドの離脱は分裂増殖したタバコ細胞から産生される何らかの物質により誘導されるものと推察した。

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