Bacteriocin Production in *Agrobacterium tumefaciens*

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Received May 17, 1984

**ABSTRACT.** *Agrobacterium tumefaciens* C58 forms “plaques” during layer cultivation. The “plaques” were shown not to be caused by the presence of a temperate bacteriophage or by random contamination. The “plaques” and their central microcolonies were used to repeatedly isolate cultures producing an antibiotic substance against the original strain *A. tumefaciens* C58, other nopaline strains, some octopine strains of *A. tumefaciens* and some strains of the related genus *Rhizobium*. The substance is thus a bacteriocin; in analogy to agrocin 84 and D286 it was named agrocin C58. The agrocin is not inactivated by trypsin. Its production by strain C58 was found only on cultivation on solid but not liquid media. The producing isolate of *A. tumefaciens* C58 (strain C58i2) contains neither plasmid pTiC58 nor the plasmid analogous to pAgK84 which controls the production of agrocin 84 in *A. radiobacter* K84.

Tumorigenic strains of *Agrobacterium tumefaciens* are intensively studied as hosts of the Ti plasmid; the T-region of the plasmid may act as a vector for the incorporation of exogenous DNA into plant cells (Kahl and Schell 1982). Our study of the properties of *A. tumefaciens* C58 and some other nopaline and octopine strains of the species revealed the formation of plaques and confluent lyses in a bacterial lawn on agar plates.

*A. tumefaciens* was described to harbour a large number of virulent and temperate bacteriophages (Manasse et al. 1972; Vervliet et al. 1974). We excluded the possibility that the plaques and confluent lyses are caused by the phages and showed that their formation is caused by a minor fraction of the cell population which produces an antibiotic substance inhibiting the growth and division of other cells of the same species. The formation of an antibacterial substance, agrobacteriocin I, by *A. tumefaciens* was described by Stonier (1960). Some of its further properties were studied by Beardsley et al. (1962). Henderson et al. (1983) discovered the formation of bacteriocin (agrocin D286) in a set of *A. tumefaciens* strains newly isolated from plant tumours. The production of agrocin 84 which has important practical applications, by *A. radiobacter* K84, is being intensively studied (Moore and Warren 1979; Ellis and Kerr 1979).

The strain *A. tumefaciens* C58, frequently used in plant genetic engineering, has not yet been known to produce an agrocin-like substance. We describe the conditions for plaque formation, compare the efficiency spectrum
of the active substance with that of agrocin 84, and provide evidence that
the production of the substance is not controlled by a plasmid. Preliminary
results have been published (Nečásek et al. 1983).

MATERIALS AND METHODS

Strains. The strain \textit{A. tumefaciens} C58M was a kind gift of Dr. P. Márton
(Biological Centre, Hungarian Academy of Sciences, Szeged); most of the other
Agrobacterium strains were from Dr. M. Bezděk (Institute of Biophysics, Cze-
choslovak Academy of Sciences, Brno). Strain C58ZII was from the collection
of the Department of Plant Breeding Theory, Institute of Experimental Botany,
Czechooslovak Academy of Sciences, České Budějovice. Strains of the genus
Rhizobium were obtained from Dr. H. Marečková (Research Institute of
Plant Production, Prague).

Media. Minimal (MM) and complex (CM) media were prepared according
to Langley and Kado (1972) and solidified with 2 \% agar. Stock cultures
were maintained on nutrient agar No. 1 (Lachema, Czechoslovakia) with 0.5 \%
sucrose (NA1) which was also used in the production of the active substance.

Determination of 3-ketolactose. The formation of 3-ketolactose was tested
by a plate method according to Bernaerts and De Ley (1963).

Elution of antibiotic substance. The substance was eluted from the cultures
on NA1 according to Cooksey and Moore (1982).

Detection of plasmid DNA. Isolation of plasmid DNA and its detection by
electrophoresis on agarose gel was carried out according to Casse et al. (1979).

Cultivation. The cultures were incubated at 28 °C. Cultures for determina-
tion of sensitivity against the tested substance were incubated for 2 d on
MM while those used for production of the substance were incubated for 2 d
on CM. These cultures were used to inoculate NA1 (20 mL in a 90 mm diameter
Petri dish), 3--5 drops of the inoculum being placed on individual plates.
After a 2-d incubation the cells of the grown colonies were killed by chloro-
form fumes and left for further 16 h in the absence of chloroform. The cell
mass was then removed and in the places of the colonies the agar was cut
into cylinders 6 mm in diameter which were used for activity testing.

Testing of antibiotic activity. Isolates of \textit{A. tumefaciens} C58 and the strain
\textit{A. radiobacter} K84 were tested for the formation of antibiotic substances
according to Spiers (1980) on MM with 2 \% agar. In this plate method
a positive result is represented by formation of inhibition zones 10 mm in
diameter or larger, the diameter being usually 15—26 mm.

RESULTS

The biochemical mutants of \textit{A. tumefaciens} C58 were tested by a layer
technique used conventionally in the study of bacteriophages (Adams 1959).
We repeatedly found the formation of “plaques” and sometimes also “con-
fluent lyses” (Plate 1). Most “plaques” were 0.5—3 mm in diameter,
turbid, and contained a central microcolony; these features simulate the
presence of a temperaSe, spontaneously induced bacteriophage. The same
finding was made in strains \textit{A. tumefaciens} C58ZII, C58C9 and B6S. On the
other hand, strains \textit{A. tumefaciens} T37, B6, B6-806 and 37 400 formed no
plaques even in extensively repeated experiments. To determine whether
the formation of “plaques” is not caused by a random phage or bacterial