Short Communication

Plasmid Detection and Isolation in Strains of *Clostridium acetobutylicum* and Related Species

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Summary. Twenty-one strains of *Clostridium acetobutylicum*, *C. butylicum* and *Clostridium saccharoperbutylacetonicum* were examined. Seven of them contained extrachromosomal DNA molecules, with a size ranging from 2.6 to more than 50 megadaltons. Strain M1 carries a small plasmid of 2.6 megadaltons, AB10 at least one plasmid of 2.6 megadaltons, AB12 one plasmid of 5.2 megadaltons, AB14 and AB16 a plasmid of about 7 megadaltons and a large one of more than 50 megadaltons, AB17 carries at least one plasmid of 6.7 megadaltons and AB18 two plasmids (4–6 megadaltons and 10–12 megadaltons). All of them are cryptic as at present no function can be correlated with their presence in a bacterial strain.

Several species of the genus *Clostridium* have been shown to contain plasmids. The plasmids best characterized up to now are from *Clostridium perfringens*. The strain CPN 50 (Ionesco et al. 1976; Brefort et al. 1977) harbors the transferable or cotransferable plasmid pIP404 of 5.5 megadaltons (Md) which encodes the production of a bacteriocin. The strain CP 590 (Sebald and Brefort 1975; Brefort et al. 1977) harbors the plasmids pIP401 (transferable coreistance to chloramphenicol and tetracycline) (37 Md), pIP402 (coreistance to erythromycin and clindamycin) (41 Md) and pIP403 (bacteriocin production) (6.5 Md). Other plasmids have been revealed in *C. perfringens*, associated with the production of a bacteriocin (Mihelec et al. 1978), antibiotic resistance (Rood et al. 1978), toxin production (Duncan et al. 1978), caseinase (Blaschek and Solberg 1981). Their presence was also observed in following species of pathogenic and nonpathogenic *Clostridia*: *C. botulinum* (Scott and Duncan 1978), *C. tetani* (Laird et al. 1980), *C. cochlearium* (Pan-Hou et al. 1980) and in four strains of *C. butyricum* (Minton and Morris 1981) where three cryptic plasmids of 3.9 Md, 5.2 Md and 4.3 Md were isolated.

The aim of our work was to investigate the presence of plasmids among a large range of acetone-butanol producing strains of *Clostridium acetobutylicum* and related species.

The following strains of *C. acetobutylicum* were used: AB4 (→NCIB 619); AB7 (→NCIB 8653); AB11 (→NRRL 594); AB12 (→NRRL 598); AB14 (→NCIB 2951); AB15 (→NCIB 6441); AB16 (→NCIB 6442); AB17 (→NCIB 6443); AB18 (→NCIB 6444); AB19 (→NCIB 6445); AB Kn2; AB Kn8; AB Kn9B; AB Kn9J; AB Kn13; AB Kn15; AB Kn17. (→recent isolates from K. Nahapetian and M. Sebald, Laboratory Collection).

The following strains of related species were also studied: *C. butylicum* AB9 (→NRRL 592); AB10 (→NRRL 593); *C. saccharoperbutylacetonicum*: AB27 (→ATCC 13564), M1 (→Dr. S. Hongo, Fukuoka, Japan).

Routinely, bacterial cells were cultured overnight in TGY medium. Their DNA was extracted after an alkaline denaturation-renaturation technique, slightly modified from Casse et al. (1979). Larger production of plasmid DNA was performed according to Hansen and Olsen (1978), followed by purification in cesium chloride-ethidium bromide density gradient centrifugation. Electrophoresis was carried out on a vertical slab gel apparatus in 0.7% agarose, 6 mm thick, at 50 V (60 mA) for 3 h (Meyers et al. 1976).

The gels were stained in 1.5 μg/ml ethidium bromide, for 1 h. The DNA bands were visualized by long wave UV transillumination before photography.

The molecular weights of the plasmids were calculated from their relative mobility in 0.7% agarose gels compared to the CCC DNAs from plasmids RP4::Tn7 (44.5 Md), PM2 (6.3 Md), pBR322 (2.6 Md), as standards.

Plasmid DNA was detected in seven strains AB10, AB12, AB14, AB16, AB17, AB18, (Fig. 1A) and M1 (Fig. 2B). Each plasmid appears on the photographs as one or several bands. Besides, one band corresponds to contaminating chromosomal DNA, which is more or less visible from sample to sample.

Whether one or more plasmids coexist in the same strain is subject to tentative interpretation since the same plasmid, under different conformations, covalently closed circular CCC, open circular OC, linear L or multimeric forms, migrates as different bands on the same gel.

In 14 strains, no plasmid DNA could be detected as bands on the agarose gels.

**Strain AB10**

DNA prepared from AB10 displays up to six different bands (Fig. 1A – lane 2, Fig. 1B – lanes 1 and 4), more or less visible depending on the sample. The most intense band in front of the migration (a) is accompanied by two others (b) (c), of fainter intensity. These bands were still
Fig. 1. A Agarose gel electrophoresis of DNA extracted from strains of Clostridium acetobutylicum (Casse et al. 1979). Lane 1: Standard DNA markers. Bands are indicated by arrows, from bottom to top: pBR322 CCC DNA, (2.6 Md); pBR322 L DNA; PM2 CCC DNA (6.3 Md); chromosomal DNA; PM2 OC DNA; RP4::Tn7 CCC DNA (44.5 Md). Other lanes: different bands are indicated by black points on the left. Lane 2 AB10, Lane 3 AB12, Lane 4 AB14, Lane 5 AB16, Lane 6 AB17, Lane 7 AB18. B Purified DNA from AB10 (lane 1) and AB12 (lane 2), and the same DNA after freezing-and-thawing treatment (lanes 4 and 3).

visible, after purification of CCC DNA from AB10 by CsCl density gradient centrifugation. They were more visible when the DNA sample had been submitted to several freeze-and-thawing treatments (Fig. 1 B – lane 4) (Fig. 2A – lane 1). Compared to the small plasmid pBR322 (Fig. 2A – lane 2) under three different conformations, the bands (a) (b) (c) respectively, may represent the CCC, L and OC forms of a small plasmid of 2.6 Md, equivalent to that of pBR322 DNA.

Three other bands are only faintly visible (Fig. 1 B lane 4) (d) (e) (f). Possibly one more, or several larger plasmids are present in this strain, in addition to the smaller one.

Strain AB12

DNA prepared from AB12 migrates as one main band (a) (Fig. 1A – lane 3 and Fig. 1B – lanes 2 and 3) which is sometimes associated, depending on the experiments, with two other bands of slower migration (b) (c). After purification of AB12 CCC DNA, electrophoresis on agarose gel revealed only one band (Fig. 1 B – lane 2). Bands (b) and (c) appeared after several successive freezing-and-thawing treatments of the DNA extract (Fig. 1 B – lane 3). Band (a) probably corresponds to the CCC form of a plasmid of 5.2 Md, the other bands probably represent other configurations of the same plasmid.

Strains AB14 and AB16

DNAs prepared from AB14 and AB16 display comparable patterns (Fig. 1A – lanes 4 and 5). Three bands can be observed on the gels, beside one band corresponding to chromosomal DNA. The faster one probably corresponds to the CCC form of a plasmid comparable in size to the plasmid of AB17 of 6–8 Md. The third band may be the

Fig. 2. A Agarose gel electrophoresis of purified AB10 DNA submitted to freeze-and-thawing treatment. Lane 1: AB10 DNA, bands a, b, c. Lane 2: pBR322 from bottom to top: CCC DNA (2.6 Md, L DNA, OC DNA. B Agarose gel electrophoresis of M1 DNA. Lane 1: M1 DNA. Lane 2: pBR322 from bottom to top: CCC DNA, L DNA, OC DNA

OC form of the same plasmid. The slower band on top of the gradient may correspond to the CCC form of a larger plasmid of more than 45 Md. If so, only one discrete band of CCC DNA is expected, since the OC form of such a large plasmid probably cannot enter the gel, and the L fragments migrate with linear chromosomal DNA fragments. This interpretation needs to be confirmed after purification of the extracted DNAs.

Strain AB17

DNA prepared from strain AB17 migrates as two bands (Fig. 1A – lane 6). The main band which is the fastest one, represents the CCC configuration of plasmid DNA. The second band is absent from the gel when purified DNA is electrophoresed. It reappears when purified DNA is submitted to several successive freezing- and-thawing treat-