CRYPTIC PLASMID IN BACILLUS PUMILUS ATCC 7065

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SUMMARY

Approximately 2% of the deoxyribonucleic acid (DNA) extracted from Bacillus pumilus ATCC 7065 can be isolated as covalently closed, circular duplex molecules. The 7065 plasmid-like DNA appears homogeneous with respect to size and has a molecular weight of approximately 6 million daltons. A biological function for this circular DNA element has not been determined.

INTRODUCTION

Biochemical evidence that would suggest the existance of plasmids (or plasmid-like extrachromosomal DNA) in sporeforming bacteria is limited to two reports. Carlton and Helinski (1) demonstrated that approximately 30% of the deoxyribonucleic acid (DNA) of a strain of B. megaterium can be isolated as covalently closed circular duplex molecules. The minicircles are heterogeneous in size and exhibit extensive base sequence homology with the chromosomal DNA of the host (2). No biological function has been attributed to these minicircles.

Evidence was recently presented demonstrating that a strain of B. pumilus (a species closely related to B. subtilis [3, 4, 5, 6]) harbors a plasmid (7). Approximately 3% of the DNA of B. pumilus NRS 576 can be isolated as covalently closed circular duplex molecules with a molecular weight of approximately 30 million daltons. Derivatives of NRS 576 which lack detectable plasmid DNA can be isolated by selecting variants that form spores at an elevated frequency relative to the oligosporogenic (plasmid$^+$) parent.

In the present report, we describe some of the biochemical properties of

a plasmid-like element isolated from another strain of B. pumilus, strain ATCC 7065. A biological function for this element has not been established.

MATERIALS AND METHODS

Bacillus pumilus ATCC 7065 was obtained from the culture collection of the Department of Microbiology, Scripps Clinic and Research Foundation, La Jolla, California. This strain is sensitive to the transducing bacteriophage PBP1 (8). For plasmid isolation, cells were inoculated from an exponentially growing penassay broth culture to a density of approximately 10^7 cells/ml in 40 ml of Spizizin's minimal glucose medium (9) containing 0.05% acid hydrolyzed casein, 250 µg/ml of deoxyadenosine and ^{3}H-thymidine (0.2 mCi, 0.002 mg, New England Nuclear). The culture was grown with vigorous shaking at 37°C to late log phase (approximately 5 x 10^8 cells/ml). Cells were washed, lysed and the lysate was mixed with cesium chloride and ethidium bromide (CsCl-EB) as previously described (7). The solution was centrifuged in the Ti50 rotor at 36,000 RPM for 40 hr. at 15°C. Dropwise fractions were collected and spotted to filter paper discs or precipitated with 5% TCA and counted as previously described (7). Sucrose gradient centrifugation, electron microscopy and buoyant density determinations were performed as reported previously (4, 7).

RESULTS AND DISCUSSION

The bulk DNA extracted from B. pumilus ATCC 7065 and purified by a phenol procedure (4) bands at a buoyant density of 1.702 g/cm^3 in CsCl gradients centrifuged to equilibrium in the analytical ultracentrifuge. No satellite peaks are detected at DNA concentrations of 2 or 20 µg/ml. Equilibrium centrifugation of isotopically labeled ATCC 7065 DNA in CsCl-EB gradients resolves the DNA into two components; a minor component (containing 2.3% [±0.3% in 3 determinations] of the total radioactivity recovered from the gradient) that bands at a buoyant density approximately 0.03 g/cm^3

greater than the major component (Figure 1A). In cleared lysates (7), the minor component generally represents 10 to 20% of the DNA recovered from the gradients (Figure 1B). The fractions containing the minor component

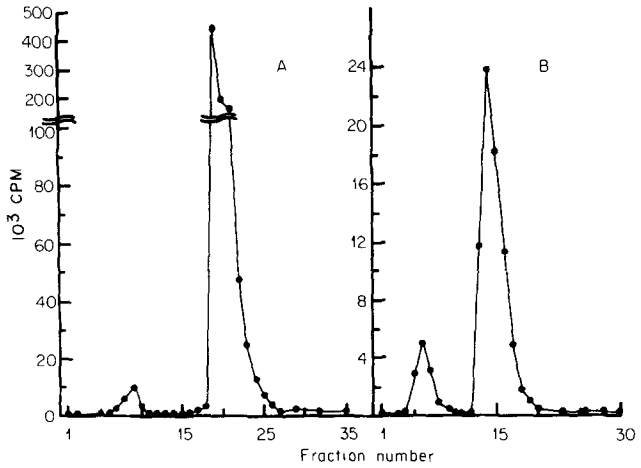

Figure 1. CsCl-EB gradient centrifugation of the DNA extracted from B. pumilus ATCC 7065. A, non-cleared lysate. B, cleared lysate. Buoyant densities of the major and minor peak fractions were 1.56 and 1.59 g/cm^3 (by refractometry [7]) respectively in both A and B. In both A and B, the 20 fractions corresponding to the top third of the gradients are not shown.

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were pooled, dialyzed exhaustively against TES buffer at 4°C and used in the following experiments. This material is referred to as 7065 plasmid DNA.

In neutral sucrose gradients, 7065 plasmid DNA is resolved into two components (Figure 2); a major component with a sedimentation velocity of 29S (±2%) and a minor component with a sedimentation velocity of 23S (±2%). The 29S and 23S peaks presumably represent the closed and open circular forms of the plasmid. In alkaline sucrose gradients, the plasmid is resolved into a fast sedimenting species (65S) representing the closed circles, and a slow sedimenting species (22S) consisting of the denatured open circles.

The molecular weight of the 7065 plasmid calculated from the sedimentation velocity of the covalently closed form in neutral sucrose gradients (29S) is 6.4 x 10^6 daltons, according to the equation described by Clowes (11).

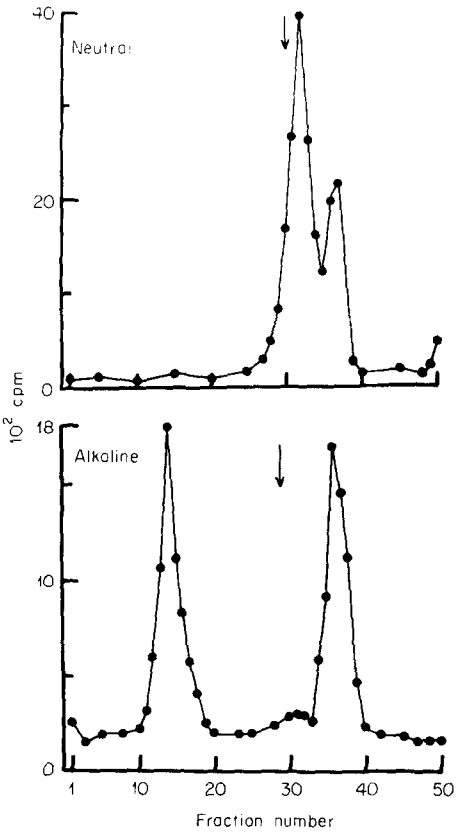

Figure 2. Centrifugation of 7065 plasmid DNA through neutral and alkaline 5 to 20% linear sucrose gradients (7). The sedimentation velocity of the T7 DNA was taken as 32S in neutral gradients and 37S in alkaline gradients (10). The arrow indicates the position of bacteriophage T7 DNA (labeled with ^{14}C-thymidine [7]) on such gradients. Centrifugation was in the SW 50.1 rotor at 34,000 RPM for 215 mins at 5°C. The top of the gradients is to the right.

Preliminary electron microscopic examination of the open circular form of the plasmid (not shown) is consistent with this molecular weight value.

The evidence presented in this and a previous study (7) indicates that two strains of B. pumilus harbor small, covalently closed circular duplex DNA molecules. In both strains, ATCC 7065 and NRS 576, the plasmids appear homogeneous in size with molecular weights of approximately 6 and 30 million daltons, respectively. Both plasmids can be isolated from host cells in amounts corresponding to 2 to 3% of the total DNA. Minimum estimates of the

number of copies of each plasmid per chromosome are 10 for the 7065 plasmid and 2 for the 576 plasmid. These calculations are based on the assumptions that the majority of the *in vivo* plasmid DNA can be isolated in the supercoiled configuration and that the molecular weight of the *B. pumilus* chromosome is similar to that of the *B. subtilis* chromosome (12, 13).

The presence of the 7065 plasmid in at least 10 copies per chromosome is a property similar to that of a class of *E. coli* plasmids whose replication is not tightly coordinated with the replication of the host chromosome; e.g., the Col E1 factor (11). The presence of the 576 plasmid in a more limited number of copies per chromosome may indicate that the replication of this element is more stringently coordinated with that of the host chromosome. Experiments to test these ideas are in progress.

In light of the limited number of reports of extrachromosomal DNA in sporeforming bacteria, it should be noted that the two strains of *B. pumilus* which have been shown to carry supercoiled DNA (strains NRS 576 and ATCC 7065) represent 20% of the strains examined in an initial screening. Recent studies suggest that other strains of *pumilus* may harbor similar elements. Efforts are under way to determine the biological function(s) of these elements.

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