Improved Preparation Procedure for the Endogenous 115 kb Plasmid of *Rhodobacter capsulatus* AD 2 and Analysis of Restriction Pattern

Hans-Georg Koch and Jobst-Heinrich Klemme

Institut für Mikrobiologie und Biotechnologie der Universität Bonn, Meckenheimer Allee 168, D-53115 Bonn, Bundesrepublik Deutschland


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None of six published preparation procedures for circular bacterial plasmids was satisfactory to purify the endogenous plasmid of a nitrate-reducing strain (*AD 2*) of the phototrophic bacterium *Rhodobacter capsulatus*. By modifying the method of N. T. Hu and B. L. Marrs (1979), Arch. Microbiol. **121**, 61–69, the 115 kb plasmid of the latter strain was prepared to a highly pure state. Digestion of the plasmid with restriction endonuclease EcoRI yielded 21 fragments with sizes ranging from 0.3 to 18.4 kb. Except for the three largest ones (18.4 kb, 16.9 kb and 11.2 kb), all fragments were cloned into the vector plasmid pUC8, amplified in *E. coli* JM83 and characterized by HindIII restriction analysis.

Introduction

The majority of strains of purple non-sulfur bacteria investigated so far (*Rhodospirillum*, *Rhodobacter*, *Rhodobacter*, *Rhodobacter*; *Rhodopseudomonas*; *SDS*, sodium dodecyl sulfate). We have shown that nitrate and nitrite reductase genes are located and, probably, clustered on the endogenous 115 kb plasmid of *Rhodobacter capsulatus* AD 2 (Koch and Klemme, 1994). To facilitate analyses of the large cryptic plasmids of AD 2 and other *NO_3^-*-reducing *Rh. capsulatus* strains (Richardson et al., 1994), a reliable purification procedure was required. This paper describes a modification of the procedure of Hu and Marrs (1979) allowing the preparation of microgram quantities of the plasmid and gives data on the number and sizes of the DNA fragments obtained by restriction nucleases (EcoRI and HindIII) treatment.

Materials and Methods

*Rb. capsulatus* strains AD 2 and B10, *Rb. sphaeroides* 130, *R. rubrum* S1 and *E. coli* strain JM83 (as host for the vector plasmid pUC8) are kept in the culture collection of the institute. Phototrophic bacteria were grown and maintained under photosynthetic conditions (30 °C; illumination with tungsten lamps at about 2500 lux) in the RCV malate–ammonium sulfate medium described by Weaver et al. (1975). *E. coli* JM83 was grown at 37 °C in LB medium (Maniatis et al., 1982). Digestion of plasmid with EcoRI and HindIII restriction endonucleases, cloning of EcoRI-plasmid fragments in vector pUC8, transformation of *E. coli* JM83 with recombinant plasmids and agarose gel electrophoresis were performed according to standard techniques (Maniatis et al., 1982). Agarase and lysozyme were obtained from Boehringer, Mannheim; agarose from Serva, Heidelberg; Magic Miniprep from Promega, Madison, Wis. (U.S.A.); restriction enzymes from Gibco, Gaithersburg, Pa. (U.S.A.); and all other chemicals from E. Merck, Darmstadt.

Results and Discussion

As shown in previous publications, the nitrate-reducing *Rh. capsulatus* strain AD 2 contains a single 115 kb plasmid which is lacking in certain nitrate reductase-negative mutants (Willison, 1990; Witt and Klemme, 1991). To isolate the 115 kb plasmid from photosynthetically grown cells in microgram quantities, six preparation procedures published by other laboratories (including the methods of Bazaral and Helinski, 1968; Birnboim and Doly, 1979; and Hu and Marrs, 1979) were tested for their suitability.
the method of Bazaral and Helinski (1968) yielded satisfactory results with plasmids from photosynthetically grown cells of *Rb. sphaeroides* strain 130 (130 kb plasmid) and *R. rubrum* S1 (55 kb plasmid) it failed with *Rb. capsulatus* AD2.

By modifying the method of Hu and Marrs (1979), the AD2 plasmid was prepared in sufficient quantities and purity. 1. Cells from 100 ml of a photosynthetic culture were harvested by centrifugation and resuspended in 1.5 ml iced cold sucrose solution (25% [w/v] sucrose in 50 mM Tris-HCl, pH 8). 2. Cell lysis was initiated by addition of 25 mg lysozyme and gentle agitation for 10 min at low temperature (< 10 °C). 3. After addition of 1 ml of 0.25 M EDTA (pH 8), the tubes were inverted 4 times to mix the contents and stored on ice for 10 min. 4. Cellular lysis was completed by addition of 3.5 ml of a 10% (w/v) SDS solution (mixing and incubation as above). 5. Chromosomal DNA was precipitated from the lysate by addition of 8 ml of 5 M NaCl and incubation on ice for 2 h. The precipitate was removed by centrifugation (20,000×g; 4 °C; 30 min) and discarded. 6. After addition of 3 volumes of 96% ethanol and glycogen (5 μg/ml) the supernatant was incubated for 2 h at −20 °C. This step effectively removed the membraneous and proteinaceous contaminations of the plasmid preparation. 7. The precipitated plasmid DNA, collected by centrifugation (see above) was dissolved in TE buffer (10 mM Tris-HCl, pH 8, plus 1 mM EDTA) and then subjected to electrophoresis in a gel of low-melting agarose.

Residual DNA contaminations were removed by a another electrophoretic step. For this purpose, the plasmid-containing bands were cut out of the gel and subjected to a second agarose gel electrophoresis. Plasmid DNA was isolated from these gels by enzymatic digestion with agarase: 1. The agarose-slice was incubated for 2×20 min in three volumes of incubation buffer (10 mM EDTA; 100 mM NaCl; 50 mM Tris-HCl, pH 8) and subsequently heated at 68 °C for 10–15 min. 2. After temperature adaptation (37 °C, 15 min), agarase was added (20 U/ml) and the mixture incubated for 6–8 h at 37 °C with gentle agitation. 3. Plasmid DNA was precipitated by addition of three volumes of 96% ethanol (incubation at −20 °C for 2 h) and finally collected by centrifugation (see above).

Using the procedure outlined above, highly purified plasmid preparations with yields of 0.1–1 μg/100 ml culture were obtained. Treatment of the plasmid with *Eco*RI restriction endonuclease gave 21 fragments with sizes ranging from 0.3 to 18 kb. From these fragments, 18 were cloned in pUC8, amplified in *E. coli* JM83 and subjected to HindIII restriction analysis (Table I). Three of the *Eco*RI fragments (18.4; 16.9 and 11.2 kb, respectively) could not be cloned yet. Adding up the sizes of all single *Eco*RI fragments gave a total size of 118 kb. This value is in good agreement with that (115 kb) obtained for the native plasmid (Willison, 1990; Witt and Klemme, 1991).

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Table 1. Restriction fragments of 115 kb plasmid of *Rhodobacter capsulatus* AD2. Plasmid DNA was treated with *Eco*RI restriction nuclease and then subjected to agarose gel electrophoresis. The *Eco*RI fragments were cloned in pUC8, transformed to *E. coli* JM83 and amplified. The recombinant plasmids were purified from *E. coli* JM83 with the Magic Miniprep system (Promega, Madison) by following the instructions of the manufacturer and subjected to *Eco*RI/HindIII restriction analysis. Numbers represent sizes of primary *Eco*RI fragments and sizes of HindIII fragments of the latter (numbers in parentheses).


