

Penicillin-Binding Proteins of Bdellovibrios

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Received 29 February 1988/Accepted 10 May 1988

We examined the predacious gram-negative bacterium *Bdellovibrio bacteriovorus* 109J and free-living strains 109J-A1 and 109J-KA1 derived therefrom for penicillin-binding proteins (PBPs). We compared their PBPs with those of the host bacterium, *Escherichia coli*, and with those of a facultatively predacious bdellovibrio, *B. stolpii* UKi2, grown axenically. The multiple PBPs of the 109J strains and of UKi2 differed from each other and from those of *E. coli*, which suggests that screening for PBPs may be a convenient way to determine to what extent the bdellovibrios may represent a diverse group of organisms. A method for labeling furazlocillin and cefaperizone with iodine-125 is also described.

We have examined the predacious gram-negative bacterium *Bdellovibrio bacteriovorus* 109J and free-living strains 109J-A1 and 109J-KA1 derived therefrom for penicillin-binding proteins (PBPs). We have compared their PBPs with those of the host bacterium, *Escherichia coli*, and with those of a facultatively predacious bdellovibrio, *B. stolpii* UKi2, grown axenically (2).

B. bacteriovorus 109J was grown on stationary *E. coli* cells in dilute nutrient broth essentially as described by Shilo and Bruff (3). To 500 ml of *E. coli* RV cells grown to stationary phase in dilute nutrient broth were added 2×10^9 *B. bacteriovorus* 109J cells (multiplicity of infection, 1:200). The culture was aerated for 16 to 18 h, by which time lysis of the host bacteria was essentially complete. Five milliliters of 0.5% sodium glutamate was added to maintain the bdellovibrios. The bdellovibrios were isolated from the remaining host cells by differential centrifugation. Cells and cell debris were removed by centrifugation at $1,000 \times g$ for 5 min, and the bdellovibrios were recovered from the supernatant by centrifugation at $10,000 \times g$ for 20 min. The vibrios were washed and suspended in 0.5 ml of 10 mM phosphate buffer, pH 7.0. *B. bacteriovorus* 109J-A1 and 109J-KA1 and *B. stolpii* UKi2 were grown in 1% peptone-0.3% yeast extract at 30°C with aeration and concentrated and washed as described above. The cell pellets of the 109J strains were mustard, whereas the UKi2 cells were mauve. The cells were ruptured by prolonged sonication (5 min at 0°C) and the envelopes were collected by centrifugation at $100,000 \times g$ for 30 min.

The cell envelopes were labeled with either tritiated benzyl penicillin or furazlocillin iodinated with ^{125}I . Iodination of furazlocillin was carried out as follows. With the aid of syringes, 5 μl of 0.1 M KI, 20 μl of 0.025 M furazlocillin, and 20 μl of 0.1 M chloramine T were added to 2 mCi of Na^{125}I (carrier free, pH 10) contained in the rubber-stoppered vial received from New England Nuclear Corp. After being mixed well, the reaction mixture was incubated at room temperature for 30 s, and then 20 μl of 0.1 M sodium metabisulfite and 20 μl of 0.1 M KI were added to terminate the reaction. The iodination procedure was carried out in a charcoal filter safety cabinet in a hood. This method can also

be used to iodinate cefaperazone, but this product was less effective for binding to the PBPs of *E. coli*.

For partial purification by electrophoresis, the reaction mixture was transferred with the aid of a syringe and needle to a strip of Whatman 3 MM paper (1.5 by 22 in. [ca. 3.8 by 56 cm]) and air dried in the hood. The paper was wetted with 0.05 M ammonium bicarbonate. Electrophoresis on a flat plate apparatus was at 2,000 V for 1.5 h. After electrophoresis, the strip was air dried and exposed to X-ray film for 30 s to locate the radioactive materials. A broad band, located about 8 to 13 in. (ca. 20 by 33 cm) towards the positive electrode, was cut out and eluted with 0.002 M phosphate buffer, pH 7. About 1 ml of eluate was recovered and stored frozen in 50- μl aliquots. One microliter was sufficient to saturate the PBPs of the envelopes of *E. coli* cells harvested from 50 ml of mid-log-phase culture and suspended in 20 μl of buffer. Binding of radioactive β -lactam antibiotic to envelope PBPs and their separation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis was carried out as described previously (1).

Figure 1 compares the PBPs of *B. bacteriovorus* 109J (lane 2) with those of its host, *E. coli* RV (lane 1). This bdellovibrio has at least five PBPs. None correspond in size

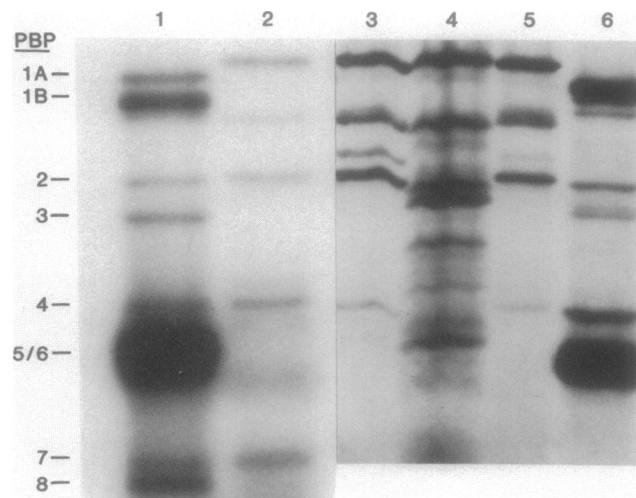


FIG. 1. PBPs of *Bdellovibrio* strains. Lanes: 1, *E. coli*; 2, strain 109J; 3, strain 109J-A1; 4, strain UKi2; 5, strain 109J-KA1; 6, *E. coli*.

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to the important high-molecular-weight PBPs of *E. coli*, though the smallest two are similar in size to PBP 4 and PBP 7 of *E. coli*. Lanes 3 through 6 of Fig. 1 compare the PBPs of the mutant strains of *B. bacteriovorus* 109J grown axenically with those of *B. stolpii* and *E. coli*. The PBPs of the 109J mutants are identical to those of the strain from which they were derived except that in addition they appear to have gained a PBP intermediate in size between PBPs 2 and 3. *B. stolpii* UKi2 is clearly different from the 109J strains. Although the three largest PBPs of the 109J strains are very similar but not identical to three of the main PBP bands of UKi2, UKi2 has at least five additional unique bands not present in 109J.

Thus, bdellovibrios, like other gram-negative prokaryotic organisms, possess multiple PBPs which presumably participate in the assembly of their murein sacculus. The tell-tale differences between 109J and UKi2 suggest that a study of

the PBP patterns of bdellovibrios might be a useful and convenient way to differentiate between members of this genus.

This work was supported by Public Health Service grant AI 05090 from the National Institutes of Health to J. T. Park.

We thank S. C. Rittenberg for cultures and advice.

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