Isolation and Characterization of Prosthecae of *Asticcacaulis biprosthecum*

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Abstract. Prosthecae were sheared from cells of *Asticcacaulis biprosthecum* with a Sorvall omnimixer and purified by centrifugation. Removal of prosthecae resulted in no loss in viability. Purified prosthecae contain protein, carbohydrates and lipids. Certain enzymes that are present in the main body of the cell are also present in prosthecae: malic dehydrogenase (E.C. 1.1.1.37), alkaline phosphatase (E.C. 3.1.3.1), succinic dehydrogenase (E.C. 1.3.99.1), cytochrome c reductase (E.C. 1.6.2.a), and cytochromes. Other enzymes present in whole cells are not in prosthecae: isocitric dehydrogenase (E.C. 1.1.1.41), NADH oxidase (E.C. 1.6.99.3), glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49), and lactic dehydrogenase (E.C. 1.1.2.3). Swarmer cells (non-prosthecate) were purified by allowing prosthecate cells to attach to cheesecloth in the growth medium. Envelopes prepared from prosthecae were compared with envelope from swarmer cells. The two envelope fractions differed in the disc-gel electrophoretic patterns of proteins solubilized from them.

During the life cycle of the bacterium *Asticcacaulis biprosthecum*, each cell produces two prosthecae. Each prostheca is produced by a localized outgrowth of the cell envelope. The outer layers of the prostheca are continuous with the wall and plasma membrane of the cell, and the core of the prostheca opens directly to the cytoplasm of the cell (Pate *et al.*, 1973).

Studies on the fine structure of prosthecate bacteria of the caulobacter group (Cohen-Bazire *et al.*, 1966; Pate *et al.*, 1973) have shown that prosthecae of members of the genera *Caulobacter* and *Asticcacaulis* are homologous structures. They differ with respect to their position on the cell, having a polar location in the genus *Caulobacter* and an excentrical location in the genus *Asticcacaulis*. They also differ with respect to the presence of an adhesive holdfast material at their distal end, the holdfast being present at the ends of prosthecae of *Caulobacter* and absent from those of *Asticcacaulis*. The holdfast material in *A. biprosthecum* is located at one pole of the prosthecate cell.

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The function of prosthecae produced by these curious bacteria is still unknown. The purpose of the work reported here was to develop a method for removal and purification of intact prosthecae so that their composition and physiological properties could be studied. It was anticipated that information on the properties of prosthecae would provide clues to their function. We also wished to determine if the formation of prosthecae represents a biochemical as well as a morphological differentiation of the cell envelope. *A. biprosthecum* was chosen for this work due to the absence of holdfast material from the ends of prosthecae and the presence of two prosthecae on each cell.

**Materials and Methods**

**Reagents.** Acrylamide, N,N'-methylene bisacrylamide, N,N',N'-tetramethylenediamine, and riboflavin were provided by Eastman Kodak Co., Rochester, N.Y. Reduced nicotinamide adenine dinucleotide (NADH), sodium pyruvate, horse heart cytochrome c (type III), glucose-6-phosphate, isocitrate, phenazine methosulfate, Triton X-100, oricinol, tris (hydroxymethyl) amino methane (Tris), and deoxyribonuclease I (beef pancreas) were from the Sigma Chemical Co., St. Louis, Mo. Oxalacetate, nicotinamide adenine dinucleotide phosphate (NADP), p-nitrophenol phosphate and crystalline lysozyme (3x cryst) were from Nutritional Biochemicals Corp., Cleveland, Ohio. Sodium succinate and (2N) Folin phenol reagent were from the Fisher Scientific Co., Fair Lawn, N.J. Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), were from Schwartz/Mann, Orangeburg, N.Y. All other chemicals used were of reagent grade.

**Growth Conditions.** *A. biprosthecum* (ATCC 27554) was grown in the peptone-yeast extract (PYE) medium of Poindexter (1964) at 30°C. Growth was determined turbidometrically at 540 nm. When large numbers of cells were needed, a 20-liter carboy containing 15 liters of PYE was inoculated with a 2-liter culture of rapidly growing cells. The carboy was aerated with filter-sterilized air passed through two sintered glass spargers. Cells were harvested with a Sharples super centrifuge during late exponential growth (approximately 24 h after inoculation). Before harvesting, enough crushed ice was added to the culture so that some ice remained in the medium throughout centrifugation. The harvested cells were immediately suspended in 1200 ml of ice-cold 5 mM MgCl₂·6 H₂O in distilled water and stored on ice until used.

**Isolation of Swarmer Cells.** Advantage was taken of the adhesive property of prosthecate cells to separate them from swarmer cells. Cheesecloth was chopped into small pieces with a paper cutter, and 85 g of chopped cheesecloth were dispersed into two liters of PYE medium in a 4-liter flask equipped with a sintered glass sparger, a glass wool air filter, and an overflow tube. A late exponential phase (optical density = 0.7 to 0.8) 100-ml culture was used to inoculate the flask, and the culture was vigorously aerated at 30°C with filter-sterilized air. The cultures were harvested at an optical density of 0.25 to 0.30 after 20 to 24 h of growth. The suspension of swarmer cells was decanted from the shredded cheesecloth to which prosthecate cells were attached, and poured through eight layers of cheesecloth into a 2-liter beaker containing ice. From this point on, the temperature was maintained below 4°C. The chilled swarmer cells were harvested by centrifugation at 5000×g for 20 min, resuspended in 5 mM MgCl₂·6 H₂O, and washed once at 5000×g for 20 min. The washed swarmer cells were either frozen or used immediately.