Volume 211, number 1, 53–58 FEB 04362 January 1987

Structure of the porin from a bacterial stalk

J.P. Chalcroft, H. Engelhardt and W. Baumeister

Max-Planck-Institut für Biochemie, D-8033 Martinsried, FRG

Received 5 November 1986

The stalks (hyphae) of a prosthecate bacterium, directly sampled from the water surface of a hot pond, show extended regular patterns on their envelope in the electron microscope. Image processing revealed a structure of the crystalline complexes which is very similar to the gross morphology of the *Escherichia coli* porins OmpC and OmpF. The natural two-dimensional crystal of the outer membrane protein has p3 symmetry and a lattice constant of 7.95 nm. The three-dimensional structure of the stalk porin has been determined to an almost isotropic resolution of 1.7 nm. The reconstruction revealed a complex network of channels within the membrane matrix with a triplet of pores merging into a common outlet, similar to the structure of the *E. coli* porin OmpF in reconstituted membranes. In addition, a blindly ending pore exists which appears to be connected to the continuous pores via small channels. The significance of the regularly arrayed porin cylinders with respect to the shape and function of the stalks is discussed.

Pore-forming protein; Outer membrane; Shape determination; Two-dimensional crystal; Three-dimensional electron microscopy; (*Hyphomicrobium*)

1. INTRODUCTION

The outer membranes of gram-negative bacteria contain few protein species, above all pore-forming proteins for the passive transport of ions and small molecules across the membrane. The structure of outer membranes of phylogenetically distant bacteria may differ remarkably. At present there are at least four known types of outer membranes, differing in protein composition and supramolecular organization, namely the regular outer membrane proteins of the Chlamydiae [1,2] and of *Pseudomonas acidovorans* [2,3], the outer membranes of *Deinococcus radiodurans* (Karrenberg, F., Wildhaber, I. and Baumeister, W., submitted), and of the Enterobacteria [4,5]. Among them, only the porins of *E. coli* have been studied extensively and are, therefore, often (erroneously) regarded as representative of most gram-negatives. The matrix porins OmpC, OmpF and PhoE were reconstituted in 2-D crystals in artificial lipid membranes, and their structures have been studied by electron microscopy [6–8]. Recently, the 3-D structure of the OmpF porin has been described by Engel et al. [9]. To study structure-function relationships of the porins, e.g. pore size and mechanism of pore opening and closing, it is advantageous to have available natural 2-D crystals (which are a prerequisite for high-resolution electron microscopy) providing the functional supramolecular arrangement of proteins in the outer membrane.

Here, we report on the 3-D structure of an Enterobacteria-type porin which forms 2-D crystals in vivo. The reconstruction has been obtained from a bacterium possessing long stalks with a remarkably regular surface lattice, i.e. the outer membrane protein in a crystalline array. A similar (halophilic) organism also possessing stalks with regularly arranged complexes reminiscent of the *E. coli* porins in projection has been recently described by Kessel et al. [10].

Correspondence address: H. Engelhardt, Max-Planck-Institut für Biochemie, D-8033 Martinsried, FRG

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

2. MATERIALS AND METHODS

The bacteria were obtained by direct sampling of the surface layer of the largest hot water pond in Kuirau park, an active geothermal region situated in urban Rotorua, New Zealand. The average temperature of the region sampled was 65°C, the pH being 6.5.

Small pieces of mica, about 4–6 mm square, bearing carbon films were gently lowered into the hot water at an angle, so that the carbon film floated off. In order to prevent loss or damage to the delicate carbon films during incubation, they were floated within vertical pyrex glass tubes about 25 mm in diameter and 120 mm high, which could be closed by glass stoppers above, but were open below. After incubation for a few minutes up to several hours the carbon films were carefully retrieved by immersing a new freshly cleaved piece of mica beneath the liquid surface and bringing it up out of the liquid below the film. The samples were immediately stained with 2% (w/v) uranyl acetate, the film floated off, and retrieved onto a freshly flamed 400 mesh copper grid. As a variant the films were floated on a 50% (v/v) glycerol solution and remounted on a mica piece prior to staining.

Micrographs were obtained using the extreme tilt holder of Chalcroft and Davey [11] in a Philips 420 electron microscope, operated at 100 kV with a magnification of 36000×. Tilting at non-constant increments followed the protocol of Saxton et al. [12] and ranged from −2.8 to 77.7°. The total dose suffered by the specimen during a whole tilt series was about 35000 electrons/nm^2; the comparison of the first and last (untilted) images of the series provided no indication of significant radiation damage out to a resolution of at least 1.7 nm. Suitable areas (512 pixels square) of the micrographs were densitometered with a step size of 20 μm, i.e. 0.57 nm at the specimen level. Correlation averaging [13] was performed by applying references obtained from quasi-optical filtration of the Fourier transforms in order to separate the information of the superimposed lattices in the flattened stalk, in principle following the procedure of [10,14]. For 3-D reconstruction the hybrid real space/Fourier space approach [12] was applied, starting with unit cells extracted from correlation averages of one continuous tilt series.

3. RESULTS AND DISCUSSION

The bacterium displayed in fig.1 is representative of most cells collected from the water surface. This thermophilic organism shows a remarkable morphological differentiation into a cell body, about 1 μm in length and 0.5 μm wide, one or two stalks (hyphae) of variable lengths emanating from the cell poles, and sheaths covering a portion of 0.2–0.5 μm of the stalks (resembling a collar). Stalks with a maximum length of about 100 μm were observed. The basic morphology of the bacterium is reminiscent of the pleomorphic Hyphomicrobiaceae [15,16], suggesting that our organism may be related to this group of prosthecate bacteria.

The envelopes of the cell body as well as of the stalks, and the sheaths are regularly arrayed (fig.1); at least two different lattices could be identified. The sheaths appear to have a spiral structure with subunits in a low symmetry arrangement (p1 or p2); the cell body and the stalks show lattices with p3 symmetry and almost identical lattice constants. The structure of the stalk has been studied in detail by electron microscopy and image processing. Since the lattices of the top and bottom part of the envelopes were superimposed on each other in the flattened stalks, it was necessary to separate them by quasi-optical filtration to obtain appropriate references for cross-correlation and averaging. The unit cell positions in the top and bottom membrane are illustrated in fig.2.

The average (fig.3) shows a 3-fold symmetric protein/lipid complex with large stain-filled pores (lattice constant 7.95 nm, i.e. a center-to-center spacing of 9.2 nm). This structure is remarkably similar to the gross morphology of the complexes of the outer membrane proteins OmpF [7] and OmpC [6] from *E. coli* in projection, strongly suggesting that the walls of the stalks made up of 2-D crystalline porin complexes are an extension of the outer membrane. Although the lipid composition of the membrane remains to be analyzed and we do not yet have functional evidence for a membrane pore, the striking structural similarity seems to justify the identification as a porin. Because of the crystallinity the arrangement of the unit cells on the surface of the stalk cylinder can be described in terms of a helix. In flattened stalks (which are usually observed in our negatively stained and air-

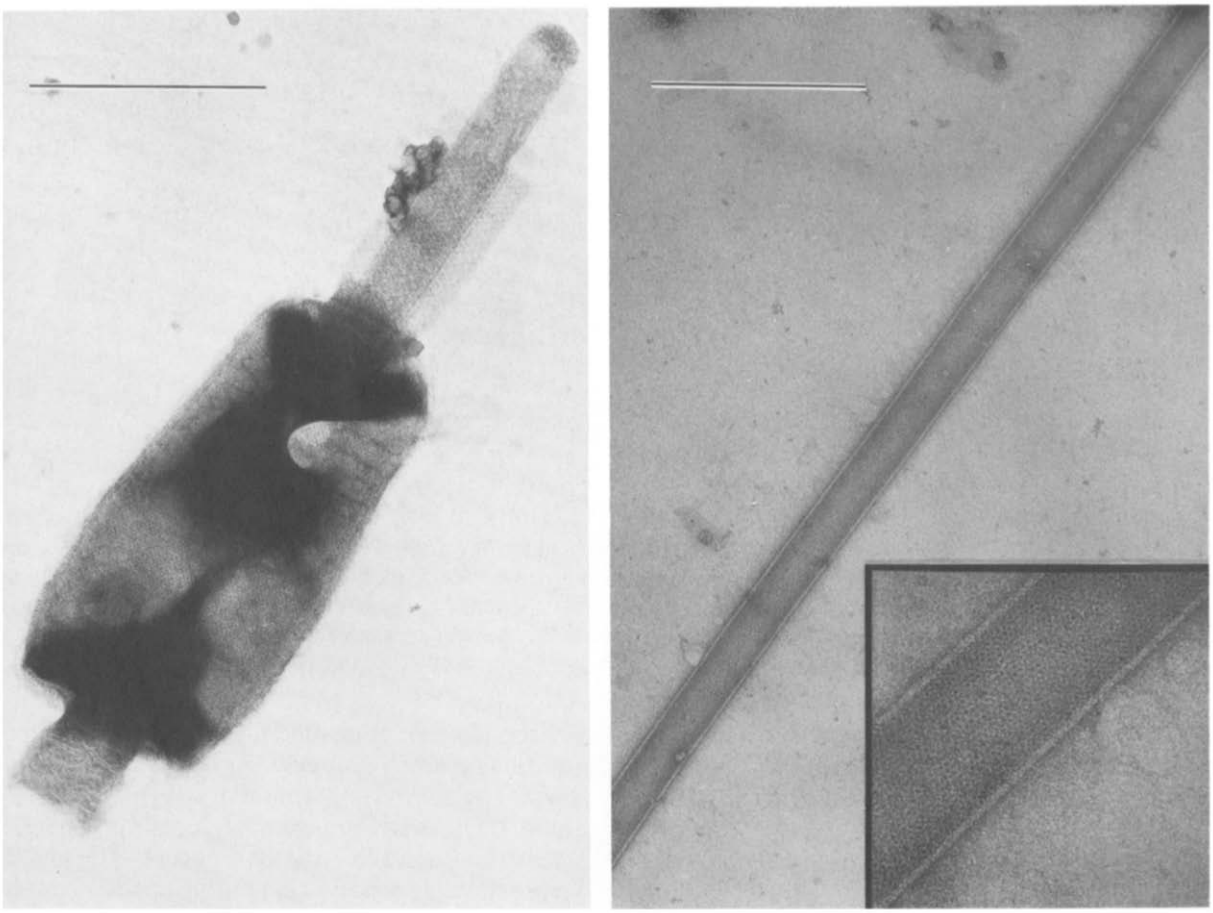

Fig.1. (A, left) Bacterial cell collected from the surface of a hot pond. The cell body and the stalk have regular surface structures with p3 symmetry, the sheaths, one covering part of the stalk, show an oblique lattice. (B, right) Part of a long stalk. The inset (image size 270 nm) shows the area selected for averaging and 3-D reconstruction. Bars, 0.5 μm.

dried preparations) the helices are transformed into a zig-zag pattern. This arrangement of the unit cells can be emphasized visually in the Moiré pattern of the superimposed peak lists in fig.2C, if an oblique viewing angle is chosen by tilting the peak lists around the longitudinal axis of the stalk (fig.2D). 24 unit cells appear to fit into one complete helical turn of the parallel four-stranded helix. The crystalline arrangement of the complexes implies that the porin protein has a shape-determining effect. The diameter of each individual stalk along its longitudinal axis remains, in fact, extremely constant (fig.1). Moreover, the diameters of different samples agree closely (112 ± 7 nm, 13 samples), although they do not appear to be absolutely invariable. The constant diameter of the stalks extending over many micrometers (a stalk of 1 μm length contains about 2950 unit cells or 8850 porin polypeptides) suggests that this crystalline array is well ordered; thus, the stalks are particularly suitable for structural analysis. A 3-D reconstruction of the porin complexes out to an almost isotropic resolution of 1:7 nm has been obtained.

At this resolution the stain-filled pores show a remarkably complex network within the membrane matrix (fig.4). On one of the surfaces the orifices of all pores (a triplet of pores belongs to one unit cell) have equivalent shapes and sizes (fig.4A,B). About halfway through the membrane

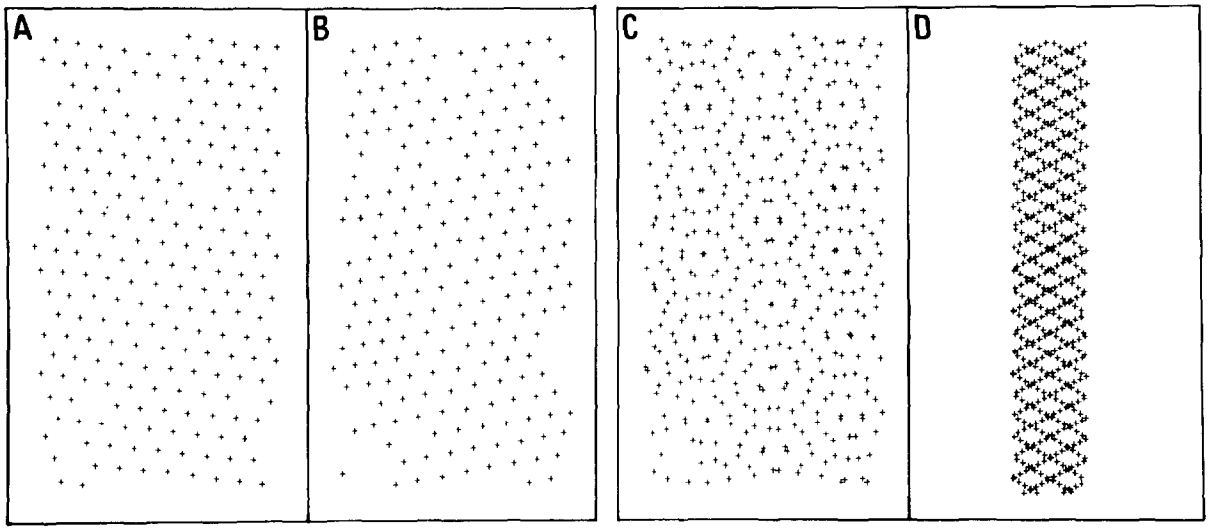

Fig.2. Unit cell positions ('peak lists') in the stalk shown in the inset of fig.1B. The positions were obtained by cross-correlating the original images with references created by quasi-optical filtration of the Fourier transform, selecting the two lattices A and B separately. In panel C the two peak lists were combined to illustrate the Moiré pattern in the original image. Panel D shows the ideal lattices of panel C (derived from the lattice base vectors) but now tilted around the *y*-axis by 75° to render the mutual arrangement of the unit cells in the top and bottom membrane visible.

the pores become narrower and appear to be connected to an additional, blindly ending pore via small channels (at the 'central' 3-fold axis, fig.4C).

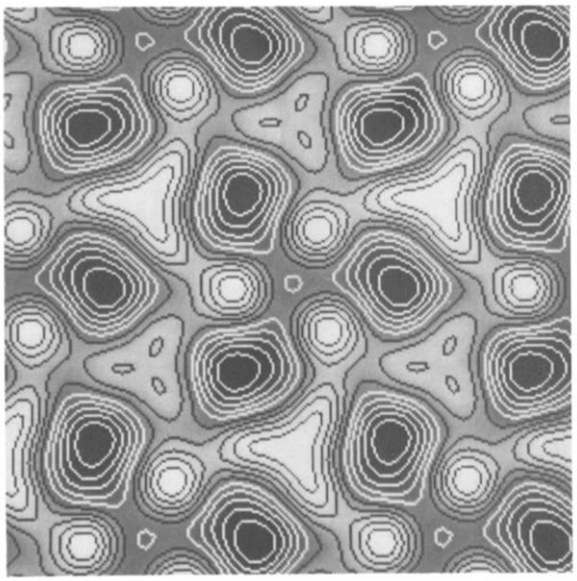

Fig.3. Projection through the 3-D reconstruction of the regular outer membrane protein from the bacterial stalk. Dark regions indicate stain-filled pores, bright areas protein and lipid. Center-to-center spacing, 9.2 nm.

Three adjacent pores (marked in fig.4) merge into a common larger pore near the opposite membrane face at one of the two 'subsidiary' 3-fold symmetry axes. This situation is similar to that of the 3-D structure of the *E. coli* porin OmpF in reconstituted membranes recently described by Engel et al. [9]. Several artificial 2-D crystals of *E. coli* porins exist [6–8], differing, however, in the space groups, lattice constants and lipid-to-protein ratios. Besides the apparently good crystallinity of the porin described here, the stalks have the advantage of representing the porin complexes in their natural state and environment. Thus, the arrangement of proteins and pores in the membrane should provide valuable information as to structure-function relationships (Engelhardt, H., Chalcroft, J.P. and Baumeister, W., in preparation). An interesting aspect of the stalk structure, and the identification of a porin as its main constituent, is that it may contribute to understanding of the function of this appendage. The length of the stalks of *Hyphomicrobia* was observed to be dependent on the nutrient concentration [17]; cells in phosphate-depleted media, for example, developed extremely long stalks [17,18]. Since phosphate starvation stimulates the production and assembly of porin in outer membranes of

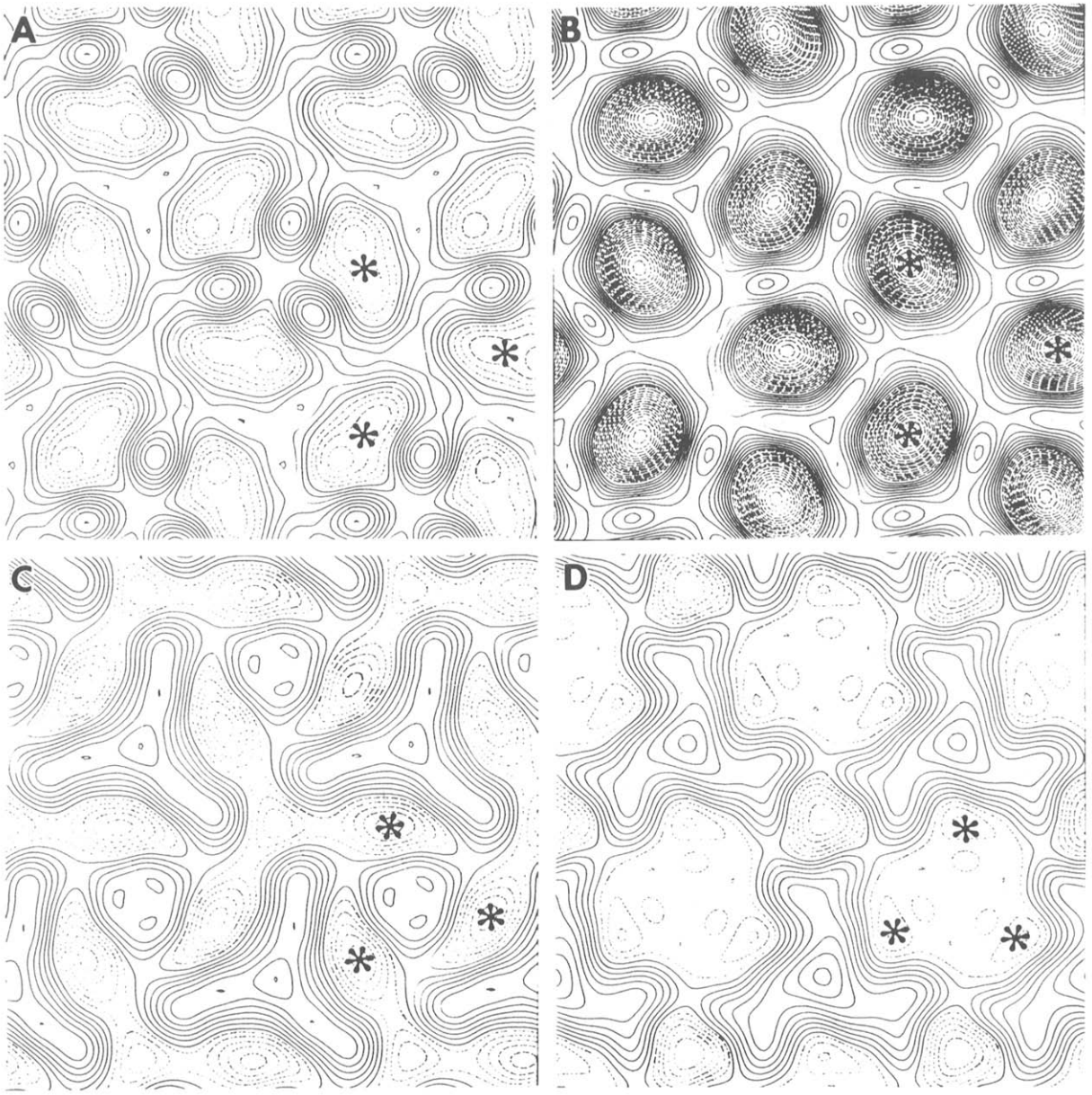

Fig.4. Horizontal sections through the 3-D reconstruction of the regular outer membrane protein. The sections were collected at 0.15 nm spacings in the z-direction and have a distance (nm) from the central plane of (A) −1.05, (B) −0.15, (C) 0.75 and (D) 1.65. Stain-filled pores are indicated by dotted lines; pores merging into a common outlet are marked by asterisks. Lattice spacing, 9.2 nm.

Enterobacteria and Pseudomonads [19,20], the development of tightly packed porin in long stalks possibly is an analogous response to nutrient starvation, in order to enlarge the surface area and, hence, the capacity of molecule transport. (A stalk of 4–5 μm length doubles the cell surface.) Isolated prosthecae of *Asticcacaulis biprosthecum* have indeed been found to be involved in nutrient uptake [21,22].

REFERENCES

[1] Chang, J.-J., Leonard, K., Arad, T., Pitt, T., Zhang, Y.-X. and Zhang, L.-H. (1982) J. Mol. Biol. 161, 579–590.

[2] Baumeister, W. and Engelhardt, H. (1987) in: Electron Microscopy of Proteins (Harris, J.R. and Horne, R.W. eds) vol.6, Academic Press, New York, in press.

[3] Chalcroft, J.P., Engelhardt, H. and Baumeister, W. (1986) Arch. Microbiol. 144, 196–200.

[4] Lugtenberg, B. and Van Alphen, L. (1983) Biochim. Biophys. Acta 737, 51–115.

[5] Benz, R. (1985) Crit. Rev. Biochem. 19, 145–190.

[6] Chang, C.-F., Mizushima, S. and Glaeser, R.M. (1985) Biophys. J. 47, 629–639.

[7] Dorset, D.L., Engel, A., Häner, M., Massalski, A. and Rosenbusch, J.P. (1983) J. Mol. Biol. 165, 701–710.

[8] Jap, B. (1986) in: Proc. 44th Annu. Meet. Electron Microsc. Soc. America (Bailey, G.W. ed.) pp.164–165, San Francisco Press, San Francisco.

[9] Engel, A., Massalski, A., Schindler, H., Dorset, D.L. and Rosenbusch, J.P. (1985) Nature 317, 643–645.

[10] Kessel, M., Radermacher, M. and Frank, J. (1985) J. Microsc. 139, 63–74.

[11] Chalcroft, J.P. and Davey, C.L. (1984) J. Microsc. 134, 41–48.

[12] Saxton, W.O., Baumeister, W. and Hahn, M. (1984) Ultramicroscopy 13, 57–70.

[13] Saxton, W.O. and Baumeister, W. (1982) J. Microsc. 127, 127–138.

[14] Hegerl, R., Cejka, Z. and Baumeister, W. (1986) in: Proc. 44th Annu. Meet. Electron Microsc. Soc. America (Bailey, G.W. ed.) pp.148–149, San Francisco Press, San Francisco.

[15] Hirsch, P. (1974) Annu. Rev. Microbiol. 28, 391–444.

[16] Dow, C.S. and Whittenbury, R. (1979) Developmental Biology of Prokaryotes (Parish, J.H. ed.) Studies in Microbiol., vol.1, pp.139–165, University of California Press, Berkeley.

[17] Harder, W. and Attwood, M.M. (1978) Adv. Microb. Physiol. 17, 303–359.

[18] Whittenbury, R. and Dow, C.S. (1977) Bacteriol. Rev. 41, 754–808.

[19] Poole, K. and Hancock, E.W. (1986) J. Bacteriol. 165, 987–993.

[20] Bauer, K., Benz, R., Brass, J. and Boos, W. (1985) J. Bacteriol. 161, 813–816.

[21] Porter, J.S. and Pate, J.L. (1975) J. Bacteriol. 122, 976–986.

[22] Larson, R.J. and Pate, J.L. (1976) J. Bacteriol. 126, 282–293.