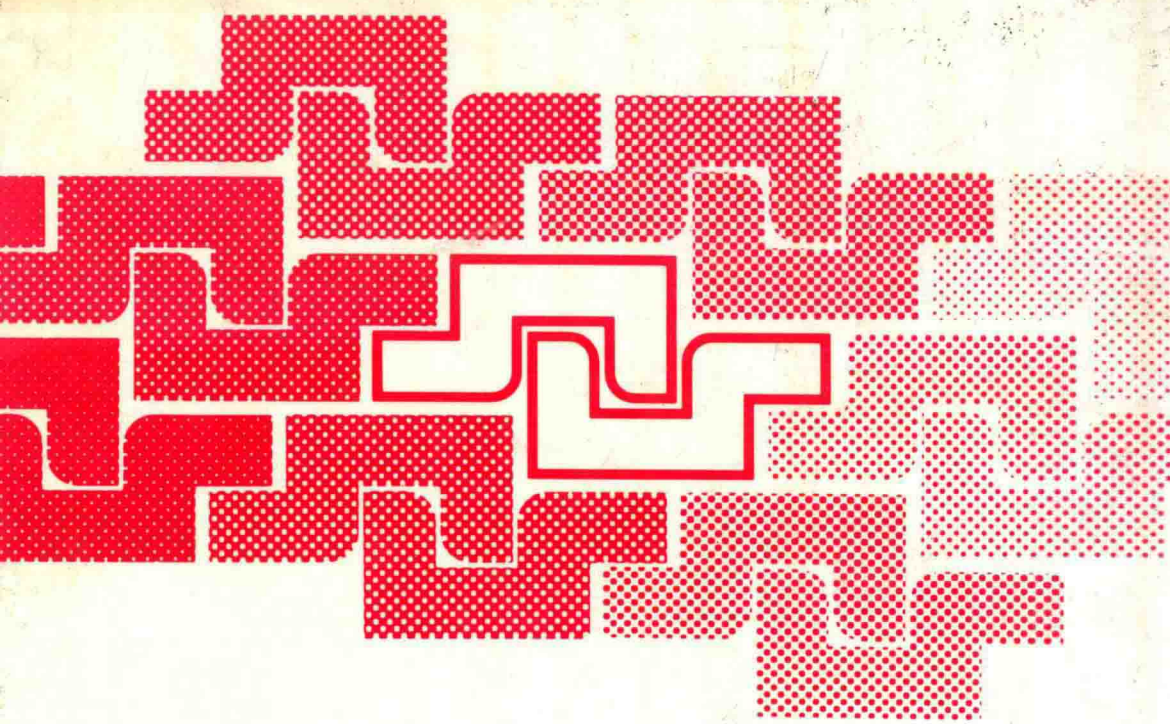

Advances in Paracrystalline Bacterial Surface Layers

Edited by
Terry J. Beveridge and
Susan F. Koval

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Advances in Bacterial Paracrystalline Surface Layers

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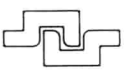

Series A: Life Sciences

PREFACE

This book is a compilation of the research which was presented during the NATO-Advanced Research Workshop (ARW) entitled "Advances in Bacterial Paracrystalline Surface Layers" held in London, Ontario, Canada during September 27 to 30, 1992. The organizing committee consisted of the two Workshop directors, S.F. Koval and T.J. Beveridge, and H. König, U.B. Sleytr and T.J. Trust; their summary statements about the significance and success of the NATO-ARW are in Chapter 37 of this book.

This was the third international workshop on bacterial S-layers and it demonstrated unequivocally how rapidly research is progressing. The Workshop was made possible by financial support from the North Atlantic Treaty Organization (NATO), the Medical Research Council of Canada (MRC), the Natural Sciences and Engineering Research Council of Canada (NSERC), and the Canadian Bacterial Diseases Network (CBDN) which is a Canadian National Centre of Excellence (NCE). We are very grateful for the support from all of these agencies since their financial aid made it possible to bring to London, Canada a truly international group of S-layer experts. We encouraged the attendance and participation of graduate students, postdoctoral fellows and research associates, and their presentations constitute the "Poster" section of this book. The NATO-ARW was an intense three day workshop held at a delightful secluded location (Spencer Hall) so that the delegates had both formal and informal occasions to interact and evolve new ideas.

Many individuals helped in the compilation of this book, In particular Elizabeth Copland, Beverley Sharpe and Susanne Schultze-Lam of the Department of Microbiology, University of Guelph, worked many hours word processing and formatting the book into camera-ready form. Ms Copland and Anita Evans (Department of Microbiology and Immunology, University of Western Ontario) were instrumental in helping us organize the Workshop.

- Terry J. Beveridge
Susan F. Koval, March, 1993

CONTENTS

I. INTRODUCTION

II. STRUCTURAL ANALYSIS OF S-LAYERS

III. S-LAYERS OF AGRICULTURAL AND ENVIRONMENTAL IMPORTANCE

IV. CHEMISTRY AND MOLECULAR BIOLOGY OF S-LAYERS

V. APPLICATIONS FOR S-LAYERS

VI. POSTER PRESENTATIONS

VII. SUMMARY STATEMENTS

I. INTRODUCTION

Chapter 1

A PERSPECTIVE ON S-LAYER RESEARCH

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It is almost forty years since Houwink (1953) demonstrated by electron microscopy a monolayer of macromolecules in a regular array, which we would now call an S-layer, on the external surface of the cell wall of an unidentified *Spirillum*. It was an elegant oddity for only a short time; within three years shadow-cast preparations displayed arrays on representatives of four more distinct genera. An ever increasing number of genera and species have been added to the list and continue to be recognized as having hexagonal (p6), tetragonal (p4), trimeric (p3), and oblique (p2) lattice forms; to these must be added some with layer upon layer, even with different symmetries expressed in each layer. Messner and Sleytr (1992) have tabulated from the literature more than 300 strains representing some 93 genera belonging to all the major phylogenetic groups of prokaryotes including *Archaea*. Forty years of research have generated a substantial body of knowledge which is available in several recent reviews on S-layers (Messner and Sleytr, 1992; Koval, 1988; Smit, 1986) as well as on the range of surface components of bacterial walls (Beveridge and Graham, 1991). More specialized areas of study include the self-assembly of units to form S-layers (Sleytr and Messner, 1989), participation of carbohydrates in the macromolecules (Messner and Sleytr, 1991), and the analysis of computer-enhanced micrographic images with generation of 3-D models (Hovmöller et al., 1988). The status of work in hand at the time of the previous workshop is published in a book (Sleytr et al., 1988) and this present volume is for current observations and concepts.

It is hard to describe today our feelings on first seeing an S-layer array and to explain how big a challenge it was to find the means to learn more about these compelling structures. Studies of cellular components were still primitive and micrographs were only a stimulus to speculation, while the analytical methods were cumbersome and peptide separation was at the level of paper chromatography. At the beginning, when the examples of S-layers were few, we derived pleasure and motivation because they seemed to be unique and beautiful. The excitements were yet to come after increasingly elegant images provided by electron microscopy impelled deeper research concerning the nature of the structures. Now, the variety

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of sophisticated approaches to analysis test our technological capability; they involve a shifting but selective marriage of microscopy, biochemistry, biophysical analysis, physiology, molecular genetics, crystallography, serology, pathology, and taxonomy, all of which developed at different rates. Interdisciplinary research has been applied to S-layers from the beginning and there are more questions than answers arising from our researches.

The form of research that will provide the next quantum-leap forward is not yet apparent, but it is certain that the qualities of creative curiosity, perceptiveness, patience, and readiness to cross disciplinary boundaries will still reward diligent research. This was true in past times when biological studies flowered in the decade 1945-1955; when electron microscopy was a prime stimulus; when bacteriologists were in process of realizing that bacteria needed to be thought of and described as cells (Stanier and van Niel, 1962), and when we learned that cells were susceptible to fractionation for the proper study of structure and function. This does not seem very revolutionary now, but, as we see by the later association with genetics and molecular techniques, this was a powerful if deceptively simple understanding that carried through to all the coming aspects of cell biology. We learned that diverse technologies must be applied if we were learn more about walls and their S-layers.

To us, at the time, the most crucial early development (allowing that there were many simultaneous discoveries) for what we were about to do was provided by the fractionation of bacterial cell walls assisted by the control of procedure provided by electron microscopy. The seminal studies of Salton and Horne (1951) and Salton (1953) showed that electron microscopy complemented biochemistry and that gram-positive and gram-negative cell walls were distinguishable in both biochemical and structural terms. These observations were soon elaborated by electron microscopy of sections showing varied complexity of layering (Kellenberger and Ryter, 1958) and by chemical and enzymatic fractional analysis of walls such as was accomplished by Weidel et al. (1960). Microbiologists were persuaded that significant developments needed the association of biochemistry and electron microscopy and we were gaining experience in our laboratory from a pioneer study on fractionation of bacterial endospores by P.C. Fitz-James (1953) combining biochemistry and structure. Finally, the development of the technique of negative staining (Brenner and Horne, 1959) and its application to electron microscopy of bacteria, viruses, and cell fractions was crucial to the display of macromolecules and their associations, both <u>in situ</u> and in fractions. The studies increased in number and depth in the 1960's; both the philosophy and the techniques were ready. We are still sailing along with the favourable winds they provided. However, we must be prepared to recognize now that the world of biological wonders of today is seductive and productive, but it is becoming excessively repetitive. We should be encouraged to look deeply into what surface science and physical chemistry can provide for new ways to think about how the microenvironments in cells operate and identify emerging problems in our sort of biology. A new intellectual and technical preparedness will be needed now, as it was 40 years ago, in the study of S-layers and bacterial cell walls, which still should form "model systems" for understanding aspects of structure and morphogenesis, regulation, macromolecular interactions in differentiation, and dynamic features of growth.

There are persistent and recurring deficiencies in our understanding of the biological significance of S-layers, some of which will be examined in this volume, and these should be kept in mind even while pursuing research that does not appear to be related to function. As Smit (1986) points out, structures that look alike do not have to function alike. It is tempting to think that location on a surface means that the structure has evolved in response to environmental and, by association, ecological

forces (Messner and Sleytr, 1992); this may be true in many cases. However, it is also clear that S-layers are a structural and even an integral component of the cell wall, which serves a complex of functions including being a chaperon to the somewhat sensitive requirements of the periplasm and the plasma membrane. Clearly, this is crucial to the S-layer-walled species of archaeobacteria. So functions may include both outward and inward concerns of the bacterial cell and of its dynamic cell wall. Some species of gram-negative S^+ eubacteria have provided S^- mutants which have not been shown to have other deficiencies.

The great number of species with S-layers probably conserve that structure by necessity in natural environments because S^- strains of S^+ species are seldom found in nature, despite the energy required to synthesize the protein. It seems likely that a lost S-layer has to be replaced if the clone is to avoid serious or fatal consequences. There should be alternative proteins with that potential, but not necessarily competent as an alternate, to fill the function. We should try to identify the sorts of basic properties that these molecules must have to be of service. There are some indications that this supposition might be worth considering. Strains of a single phenotypic and genetic species can show several distinctive lattice forms among their number, e.g. *Bacillus stearothermophilus* (Messner et al., 1984) and *Aquaspirillum serpens* (Boivin et al., 1985). A number of cellular enzymes form aggregates that display regular multimeric units in the electron microscope and at least one of them, when over-produced, forms tubular intracellular assemblies (Elmes et al., 1986) not unlike those formed by some S-layers when shed into the growth medium. Gas vacuoles (vesicles), flotation devices in many pelagic bacteria (Walsby, 1972), are probably the most extreme example of an intracellular assembly system inherent in a single species of protein and, like S-layers, having no interactive function. If species can control their density equilibrium by synthesizing or collapsing their gas vacuoles, it is not too much of a stretch of imagination to consider a response (to light, or pressure, or nutrients) that leads to synthesis or depolymerization of some other protein, even an S-layer. It seems unlikely that S-layers are derived from a single genetically conserved protein from archaean times and the variety of them suggests many independent adaptations. Therefore, the biological reasons for the existence of these structures need to be sought along with any related proteins from that lineage of bacteria. In addition, field studies should be extended to include the broadest range of ecosystems.

The relationships of the S-layer proteins in the families of bacterial proteins have yet to be worked out, and the techniques of molecular biology are likely to be of service. As pointed out long ago by Sleytr and Plohberger (1980), any protein capable of assembling a continuous sheet from anisotropic units (e.g. hydrophobic on one side) could form specific associations with other macromolecules to form complex membranes by cooperating with lipids or together with other macromolecular associations to form structurally and physiologically significant cell components. Certainly, there are S-layers integrated with the outer membrane of gram-negative bacteria to the degree that the membrane must be dissolved away from the protein layer in order to isolate it (Smith and Murray, 1990; Thompson et al., 1982). Of course, these examples in *Aquaspirillum sinuosum* and *Deinococcus radiodurans* may be recent evolutionary adaptations, but it is suggested (Sleytr and Plohberger, 1980) that a simple protein membrane capable of dynamic growth could have initiated a barrier membrane in the early stages of biological evolution. An idea that should not be forgotten.

Our laboratory has studied several bacteria that form multiple S-layers on their surfaces, and it is not clear whether or not this confers some functional significance. One might think that a single layer formed by a single protein species should be

sufficient for the needs of a bacterium; an understandable solution to some environmental problem. Even the formation of two overlaying layers, each formed by single but distinct proteins (Kist and Murray, 1984), might not be excessive because nature does not mind expending energy as long as the products work. However, it is hard to understand the formation of complex units requiring the assembly of several distinct proteins, some glycosylated and some not, for a functional layer a distance outside of the cell. This is the case for the punctate layer of *Lampropedia hyalina* (Austin and Murray, 1990). That such complexity evolves is the more mysterious because that layer combines with an underlying perforate layer to enclose the sheets of cells and plays a dynamic role in the enclosure and separation (division) of the square tablets of cells (Chapman et al., 1963) despite being some distance outside of the essential cell wall. So there are deeper mysteries.

S-layers provide for some of a number of bacterial strategies utilizing wall components to reduce the impact of dire environmental influences (Koval and Murray, 1986). Their diverse functions (see Messner and Sleytr, 1992 for an extensive analysis), varying among taxa of bacteria, must have some strong selective value if the S-layer is to persist. Some recognized functions may co-exist in many bacteria to form a barrier against predators, a sieve that retains useful and excludes damaging macromolecules, and a promoter of cell associations or adhesion to surfaces, but it is equally likely that the real basis for evolutionary selection is cryptic in each species and appropriate to their natural environment. It would have to be actively sought in their natural environments. Observations made on the efficacy of the barrier function against *Bdellovibrio bacteriovorus* as a bacterial predator, grazing protozoa, and some bacteriophages (S.F. Koval, Chapter 9) support this thesis as do the studies on roles in virulence and pathogenicity (M.J. Blaser and W.W. Kay, Chapters 15 and 17). The possible functions are many, varied, incompletely surveyed, and only partially understood. Clarification of the nature of functions is an important goal because of the remarkable ubiquity of these structured surfaces.

The study of the roles of S-layers as surface components having functions should be integrated with studies on a wider range of other functional structures associated with cell walls. Some of them are structured assemblies of proteins (such as pili, fimbriae, spinae, and flagella); others may be less ordered surface proteins of high molecular mass, or integral proteins for enzymatic or transport functions but with uncertain physical associations in wall structure. It may be too limiting to expect all the complex S-layers to be crystalline, and there may be some with a mosaic or fractal ordering. We have observed an arrangement that might be termed "regular irregularity" in *Leptotrichia buccalis* (Listgarten and Lai, 1975; R.G.E. Murray, unpublished observations). This particular surface is specifically reactive, e.g., recognizing and adhering to a specific sugar moiety, for the sole purpose of close physical association with other cells while the main barrier/filter function remains and is essentially non-reactive. Although S-layers may serve generally to cover-up and protect a functional macromolecular (and antigenic) mosaic that lies underneath, some components, e.g., side-chains of lipopolysaccharide, may protrude through the meshwork (Chart et al., 1984) to confuse observations but add to function in the disease state.

Our interest in diseases affecting our lives, directly or indirectly, ensures continued research on the importance of surface components in terms of pathogenicity and virulence. Whether or not some of the antigenic variations involved in human disease involve S-layers is not established. However, some of the pathogens have high molecular weight protein superficial components suspected of complicity even though an S-layer is not present. None so far give the clear example of a role for S-layers provided by *Aeromonas salmonicida* and relatives in causing

disease in salmonid fish (see T.J. Trust and W.W. Kay, Chapters 16 and 15), but S-layers have been identified on a number of pathogens (e.g., species of *Campylobacter, Cardiobacterium, Clostridium,* and *Bacillus*) and roles in disease may be defined. There are opportunities, perhaps, that need further exploration. An example is the recurrent fevers due to species of *Borrelia* which, like the S-layer equipped trypanosome parasites, must shift their surface antigens to evade the effects of antibody. There are other spirochaetes, e.g., *Leptospira interrogans*, which have highly host-specific serovars. In the plant pathogenic bacteria, there are highly host-specific pathovars of genetically defined species, whose surface components are likely to be involved. So there is still much to be done in this field that is not so very different from understanding what is involved in a defined species managing to produce variants with a different S-layer protein.

It is my prejudice that all research on bacteria and their surfaces must retain an awareness of the biological significance. The techniques of molecular biology and genetics will continue to be productive and may well provide indications of otherwise inaccessible relationships among genes and their products. However, productive molecular studies need a precise biological phenomenon, a special circumstance, or a product or process identified as significant to provide the essential basis for the research. It is probable that S-layers will be recognized on more and more species of bacteria and most of these will be unexciting variations on a theme. Further understanding needs concentration on exemplary bacteria and increased awareness and concern for the physiology and interactions of bacteria in their ecological niches. Such a breadth of interests and involvements (happily this exists despite the tendency to focus on S-layer structure) is essential to the recognition of stimulating novelties and fresh associations which will stimulate our researches.

Despite the incredible amount of work done on model systems concerning the regulation and behaviour, in sickness and in health, of the murein and some other components of bacterial walls, there is still much to learn about how the integrated and complex structure works. The S-layer forms a defined and productive part of this overall study and has some remarkable properties: elegance, multiple functions, influential associations, accessibility and variety. Diverse disciplines come into play, increase our fascination, and provide for an exemplary study in biology.

It is a particular pleasure to me and to my microbiological colleagues from the University of Western Ontario and University of Guelph, the organizers of this meeting, to hold the meeting here because of the years spent in our Departments of Microbiology studying S-layers together with a number of graduate students, post-doctoral fellows, and visiting scientists. Now we expand our contacts in the family of scientists by bringing together an international group experienced in the broadest range of research on S-layers. We are all the beneficiaries of this exchange of information and understanding derived from research and thought.

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