Production of type 5 capsular polysaccharide by *Staphylococcus aureus* grown in a semi-synthetic medium

B. DASSY,^{1,2} W. T. STRINGFELLOW,^{2†} M. LIEB² and J. M. FOURNIER^{2*}

¹Division de Biochimie, Université Pierre et Marie Curie, Paris, France

²Département d'Ecologie, Institut Pasteur, F-75724 Paris Cedex 15, France

(Received 22 October 1990; accepted 18 December 1990)

The concentration of the type 5 capsular polysaccharide (CP) antigen of *Staphylococcus aureus* can be measured directly in cultures or cell suspensions by a two-step inhibition enzyme-linked immunosorbent assay (ELISA), using monoclonal antibodies. CP was synthesized during growth on a variety of carbon substrates and its production was not affected by the nature of the carbon source. High levels of yeast extract inhibited CP formation. CP was synthesized in batch culture at the same rate during exponential growth as in the post-exponential phase. Post-exponential CP production contributed at least half the final amount of CP measured. This phenomenon was observed in different culture media, although the specific yield of polysaccharide varied from one medium to another. Post-exponential CP production was observed in the pH range 6–7, but not at pH 8. Post-exponential production was strictly dependent on oxygen availability and did not occur under anaerobic conditions.

Introduction

Exopolysaccharides produced by a wide range of micro-organisms are receiving increasing attention due to their participation in pathogenic and symbiotic processes in plants and animals and their involvement in general interactions between micro-organisms and their environment (Sutherland, 1988; Whitfield, 1988; Wilkinson, 1958). Moreover, some microbial polysaccharides have an industrial value due to their gel-forming or emulsifying properties, their use in the preparation of rare sugars (Luong *et al.*, 1988; Paul *et al.*, 1986) and their potential use as bacterial vaccines (Jennings, 1990; Robbins & Schneerson, 1990).

The involvement of capsular polysaccharides (CP) in the invasiveness of many bacteria is well established. The enhanced virulence of encapsulated bacteria is generally attributed to their resistance to phagocytosis (Roberts *et al.*, 1989; Whitfield, 1988; Wilkinson, 1958).

CP may also be involved in the adhesive capacity of some bacteria (Sutherland, 1988; Whitfield, 1988).

Only a few well-encapsulated mucoid isolates of *Staphylococcus aureus* were identified before the 1980s. Although *S. aureus* causes bacteremia (Sheagren, 1984), it was not conclusively proved until recently that CP formation was common in clinical isolates of this organism (Karakawa & Vann, 1982; Robbins & Schneerson, 1990). The existence of CP has been demonstrated by morphological, immunological, biochemical and epidemiological studies. Transmission electron microscopy, using antibodies reacting with CP, showed that these antigens are exposed on the surface of *S. aureus* (Chomarat *et al.*, 1989; Hochkeppel *et al.*, 1987; Lee *et al.*, 1987). A classification scheme, based on direct bacterial agglutination and immunoprecipitation of cell extracts with polyclonal antisera identified eight capsular types (Karakawa *et al.*, 1985). Several epidemiological studies of encapsulated *S. aureus* from human clinical isolates have shown that two capsular serotypes, 5 and 8, account for about 70 to 80% of all isolates, with no strain possessing more than one serotype (Arbeit *et al.*, 1984; Boutonnier *et al.*, 1989; Hochkeppel *et al.*, 1987; Sompolinsky *et al.*, 1985).

In contrast to these areas of investigation, little is presently understood concerning the physiology and biochemistry of CP biosynthesis by *S. aureus*. Capsule

† Present address: Department of Environmental Sciences and Engineering, School of Public Health, The University of North Carolina, Chapel Hill, NC 27599-7400, USA.

Abbreviations: CP, capsular polysaccharide; mAb, monoclonal antibody; MFM-YE, modified Frantz medium plus yeast extract diffusate; MFM-CA, modified Frantz medium plus Casamino acids diffusate.

formation is usually demonstrated with fresh isolates of human or animal origin, and repeated subcultivation on an artificial medium results in decreased capsule synthesis (Johne *et al.*, 1989; Opdebeeck *et al.*, 1985, 1988; Rather *et al.*, 1986; Witte, 1975). Expression is also influenced by the medium in which capsule production is tested. Growth in Staphylococcus Medium 110, which contains high concentrations of carbohydrate and NaCl, encourages the production of mucoid or capsular material (Caputy & Costerton, 1984; Johnne *et al.*, 1989; Opdebeeck *et al.*, 1988; Rather *et al.*, 1986; Yoshida & Ekstedt, 1968). CP production occurs in both liquid (Lee *et al.*, 1987) and solid media (Chomarat *et al.*, 1989; Hochkeppel *et al.*, 1987; Johnne *et al.*, 1989). The production of CP may also be affected by the incubation period, but the few results concerning this point are incomplete and contradictory (Falcieri *et al.*, 1987; Kitzrow *et al.*, 1975; Wiley, 1968).

Apart from these partial findings, there is no other information available on the formation of CP by *S. aureus* grown under a range of controlled environmental conditions. An impediment to such a study has been the lack of a quantitative and specific assay for CP that can be applied to unpurified extracts. Recently, a staining method for the measurement of *S. aureus* CP was described (Mattila, 1989). However, the use of a staining technique is not highly specific. Monoclonal antibodies (mAbs) reactive with type 5 and type 8 CP of *S. aureus* have now been developed and allow the measurement of CP concentration directly in cultures and cell suspensions by a two-step inhibition, enzyme-linked immunosorbent assay (ELISA) (Albus *et al.*, 1988; Boutonnier *et al.*, 1989; Poutrel *et al.*, 1988).

The work reported here is part of an investigation undertaken to assess the effects of medium composition and environmental factors on the production of type 5 CP by *S. aureus* when grown in batch culture. For the present study, we compared several classic media for type 5 CP production and selected the most efficient, which was a semi-synthetic medium. The time course of growth and CP production was followed in this medium, and the influence of medium composition, pH and dissolved oxygen was examined.

Methods

Bacterium. *S. aureus* strain Reynolds is the prototype strain for type 5 CP (Fournier *et al.*, 1987; Karakawa *et al.*, 1985). It was stored lyophilized or in deep agar slants.

Culture media. The following classic media were used: Brain Heart Infusion (Difco), Nutrient Broth (Diagnostics Pasteur, France), Mueller-Hinton, Columbia and Staphylococcus Medium 110 (all Difco).

Frantz medium, a semi-synthetic medium for the production of

capsular polysaccharide by *Neisseria meningitidis* (Gottschlich *et al.*, 1969), was modified for the cultivation of *S. aureus*. The basal medium contained (l⁻¹) 1.3 g glutamic acid, 12 mg cystine, 0.4 g MgCl₂ · 6 H₂O, 5.7 g Na₂HPO₄ · 12 H₂O, 6 g NH₄Cl, 90 mg KCl, 6 g NaCl and 2 g lactose. The basal medium was adjusted to pH 6.5 with NaOH and supplemented with 2% (v/v) yeast extract diffusate (MFM-YE) or with 2% (v/v) Casamino acids diffusate, 1 mg thiamin and 1 mg nicotinamide (MFM-CA). The diffusates were obtained as follows: 100 g yeast extract (Difco) or Casamino acids (Difco) were placed in a dialysis bag and submerged in 500 ml 9 g NaCl l⁻¹ for 2 d at 4 °C with a magnetic stirrer. About 250 ml of diffusing material was obtained, and sterilized by filtration.

Nine ml of the above media, supplemented with 1.5% (w/v) agar (Difco), was placed in 17 mm screw cap tubes, to make agar slants.

Growth conditions. Bacteria were grown either on agar slants, in flasks, or in a 6 l fermenter, at 37 °C.

Unless otherwise specified, agar slants were incubated with loose caps permitting good air exchange. After growth, bacterial cells were washed from the agar slant and suspended in 10 ml phosphate-buffered saline, pH 7, for analysis and flask inoculation.

Flasks were inoculated with an overnight culture from the same medium, and placed in a rotary water-bath shaker (200 r.p.m.).

The fermenter had a working volume of 4.5 l and was equipped with a draft-tube agitation system (Biolafite, France). An autoclavable, galvanic-type electrode was used to measure dissolved oxygen tension, expressed as percentage saturation. The pH was automatically controlled by the addition of 1 M-NaOH or 1 M-acetic acid. The culture was agitated at a constant speed of 500 r.p.m. and aerated at a rate of 3 l of air min⁻¹. For anaerobic culture, nitrogen gas was used instead of air at a rate of 1 l min⁻¹. The fermenter was inoculated with 100 ml of an overnight culture from the same medium in a shake flask, and samples containing cells suspended in the growth medium were taken for analysis.

Bacterial growth was followed by measuring the optical density of an appropriate dilution of the cell suspension at 620 nm (OD₆₂₀). For 1 OD₆₂₀ unit, plate counts of viable organisms gave a mean value of 5×10^9 bacteria ml⁻¹ and dry weight determinations a mean value of 0.4 mg ml⁻¹. This relationship was observed with all the media used.

Type 5 CP assay. Bacterial cell suspensions were autoclaved at 121 °C for 30 min, to release type 5 CP from the cell (Fournier *et al.*, 1987). After centrifugation, the supernatant was retained and stored at -20 °C prior to type 5 CP assay. For samples taken from broth cultures, this supernatant contained total CP (cell-bound CP plus culture fluid CP).

For comparison, type 5 CP was also extracted by addition of lysostaphin (Sigma) at 0.1 mg ml⁻¹ to cell suspensions, incubation at 37 °C overnight, and centrifugation. These conditions have been shown to give optimal lysis (Huber & Huber, 1989). The CP contents determined in the supernatants were not significantly different for autoclaving and lysostaphin lysis.

Type 5 CP content was determined by a two-step inhibition ELISA using mAbs as previously described (Boutonnier *et al.*, 1989). In step one, mAbs were bound to CP present in the samples. A plate was blocked with 0.5% (w/v) gelatin in PBS at 37 °C for 1 h. After washing with PBS/Tween, the wells received 100 µl volumes of test samples and 100 µl of mAbs diluted in PBS/Tween, supplemented with 0.5% gelatin, to a concentration giving a final A₄₉₂ of 0.2–0.5 as determined by preliminary titration. In step two, CP was quantified by measurement of non-bound antibodies remaining after step one using CP-coated microtitre plates. After incubation at 37 °C for 1 h and then overnight at 4 °C, 100 µl samples from each well of the original plate one were transferred to a second plate which had been previously coated with purified CP and blocked with gelatin. This plate was incubated at 37 °C for 1 h and, after washing with PBS/Tween,

Table 1. Type 5 CP production by *S. aureus* strain Reynolds grown for 24 h on agar slants

Data shown are one representative set of data from four separate experiments.

Agar slant	Loose cap			Airtight cap		
	OD ₆₂₀	Type 5 CP (µg ml ⁻¹)	Final Y _{CP5} *	OD ₆₂₀	Type 5 CP (µg ml ⁻¹)	Final Y _{CP5} *
Brain Heart Infusion	5.43	2.840	0.523	1.70	0.014	0.008
Nutrient Broth	1.59	0.910	0.572	1.02	0.195	0.191
Mueller-Hinton	1.06	0.638	0.602	0.79	0.095	0.120
Columbia	2.38	0.939	0.395	1.50	0.057	0.038
Medium 110	1.89	4.773	2.525	1.02	0.167	0.163
MFM-YE	1.72	16.150	9.390	1.10	0.615	0.559

* The final Y_{CP5} is the concentration of type 5 CP divided by the OD₆₂₀ value for the bacterial suspension [µg CP ml⁻¹ (OD₆₂₀ unit)⁻¹].

conjugate was added to the wells and the plate was incubated at 37 °C for 45 min. After washing with PBS/Tween, enzyme substrate was added and, after 10 min at room temperature, the reaction was stopped and the A₄₉₂ was read.

For each ELISA run, negative controls (wells not receiving test samples but PBS/Tween supplemented with 0.5% gelatin) and titration of purified CP were done to determine the sensitivity of the assay. A standard titration curve was plotted for the absorbance of serially diluted samples (0.06–1000 ng ml⁻¹) of purified CP. The amounts of CP in the supernatants of autoclaved bacterial cell suspensions assayed at different dilutions were determined from the standard titration curve and expressed in µg ml⁻¹.

Chemicals. All chemicals were analytical or reagent grade and purchased from Prolabo or Merck.

Analysis of results. The type 5 CP final specific yield (final Y_{CP5}) is defined as the total type 5 CP (µg ml⁻¹) divided by the corresponding bacterial growth (OD₆₂₀) value at the end of growth.

Results

Type 5 CP production in different classic media

When grown on different solid media, *S. aureus* strain Reynolds exhibited significant variations in type 5 CP production that were not correlated with total cell growth (Table 1). MFM-YE gave the highest specific yield of type 5 CP, and good results were also obtained with Medium 110. Type 5 CP production by strain Reynolds also varied in different liquid media (Table 2). CP production did not correlate with total cell growth or with the final pH of the culture. MFM-YE and MFM-CA gave the highest yields, followed by Medium 110.

In all media used, type 5 CP was closely associated with the bacterial cell. The CP concentration in the supernatant never exceeded 5% of the total CP concentration, and washing in PBS did not result in removal of CP from the cells.

Table 2. Type 5 CP production by *S. aureus* strain Reynolds grown for 24 h in liquid media in shake flasks

Data shown are one representative set of data from five separate experiments. Y_{CP5} is defined in the footnote to Table 1.

Liquid medium	OD ₆₂₀	Type 5 CP (µg ml ⁻¹)	Final Y _{CP5}	Final pH
Brain Heart Infusion	7.98	2.070	0.259	7.83
Nutrient Broth	2.50	0.620	0.248	8.15
Mueller-Hinton	3.41	2.750	0.806	8.16
Columbia	8.80	9.000	1.020	7.88
Medium 110	3.15	11.300	3.587	4.80
MFM-YE	2.18	9.250	4.243	6.11
MFM-CA	1.79	8.140	4.547	6.00

Fig. 1. Effect of yeast extract diffusate concentration (in MFM-YE) on growth (○) and type 5 CP production (□) by *S. aureus* strain Reynolds grown for 24 h in shake flasks. Overnight cultures from MFM-YE agar slants were used as inocula. Bars represent means ± SEM of four independent experiments.

Type 5 CP production in modified Frantz media

The effects of the concentration of yeast extract diffusate in MFM-YE on growth and type 5 CP production by strain Reynolds were examined in a series of shake flask experiments (Fig. 1). The final pH was,

Fig. 2

Fig. 2. Growth (○) and type 5 CP production (□) by *S. aureus* strain Reynolds in MFM-YE in a fermenter without pH control. ▲, Dissolved oxygen; △, pH.

Fig. 3. Relationship between type 5 CP production and growth of *S. aureus* strain Reynolds. The data are from the experiment depicted in Fig. 2.

Fig. 3

respectively, 6.2, 6.5 and 6.9 in media containing 0.5, 3 and 8% (v/v) yeast extract diffusate. Numbers of cells increased with the yeast extract concentration. Type 5 CP production rose with increasing yeast extract concentration up to 2% (v/v), but fell at higher concentration. The same experiment done with increasing concentrations of Casamino acids diffusate (in MFM-CA) gave a similar peak of CP production at 2% (data not shown).

Type 5 CP production in MFM-CA containing various carbon substrates was also examined in shake flasks. For this experiment, basal MFM-CA was prepared without lactose and glutamic acid, compounds that can be used as carbon sources by *S. aureus* (Keller *et al.*, 1978). The carbon substrates, added at 2 g l⁻¹ to this basal medium, were: glucose, lactose, galactose, sucrose, maltose, mannose, mannitol, glycerol, sodium citrate, sodium pyruvate and glutamic acid. The addition of each of these carbon substrates increased the cell yield, compared to the basal medium, while the final Y_{CP5} was not significantly different from one substrate to another (data not shown). MFM-YE base was not used for this experiment because yeast extract contains sugars (Atkinson & Mavituna, 1983).

Growth and type 5 CP production during batch culture

Growth and type 5 CP production by strain Reynolds were followed in MFM-YE in a fermenter, without pH

Fig. 4. Relationship between type 5 CP production and growth of *S. aureus* strain Reynolds in MFM-YE in a fermenter with the pH controlled at 6.0 (○) or 8.0 (●).

control, for 24 h at 37 °C (Fig. 2). Exponential growth continued for 4 h, with a growth rate $\mu = 0.75 \text{ h}^{-1}$, followed by a slow growth phase for 5 h with a growth rate of about 0.12 h^{-1} . After 9 h, no further growth was detectable. Type 5 CP production lasted for 9 h, during both exponential growth and slow growth phases, with a mean production rate $\mu_{CP} = 0.65 \text{ h}^{-1}$. At the end of the slow growth phase, production stopped. The pH decreased during the exponential growth and slow growth phases, and then increased during the stationary phase. From 24 to 48 h, growth and type 5 CP concentration did not change, but the pH continued to increase up to 7.0

Fig. 5

Fig. 5. Growth (○, ●) and type 5 CP production (□, ■) by *S. aureus* strain Reynolds in MFM-YE medium under aerobic (open symbols) or anaerobic (filled symbols) conditions, in a fermenter with the pH controlled at 7.0.

Fig. 6

Fig. 6. Relationship between type 5 CP production and growth of *S. aureus* strain Reynolds under aerobic (○) and anaerobic (●) conditions. The data are from the experiment depicted in Fig. 5.

(data not shown). Similar results were obtained with MFM-YE prepared with yeast extract at 2 g l⁻¹. The dialysis of yeast extract had no influence on CP production.

The relationship between type 5 CP production and growth was further examined by plotting the CP concentration versus OD₆₂₀ (Fig. 3). This gave two slopes, corresponding to the exponential growth phase and the slow growth phase. For cultures in MFM-YE in both shake flasks and fermenters (three independent cultures in each case) without pH control, the second slope was always eight- to ninefold higher than the first one, although the final Y_{CP5} varied from one culture to another. This phenomenon was also observed in shake flask cultures with Brain Heart Infusion or Columbia media (data not shown).

Influence of pH control on type 5 CP production

When the pH was controlled at 6.0 (Fig. 4) or 7.0 (Figs 5 and 6) in a fermenter, CP was produced in both exponential and slow growth phases and there was the same correlation between the two slopes of a plot of CP production versus OD₆₂₀. By contrast, when the pH was controlled at 8.0, the exponential growth rate decreased to 0.52 h⁻¹, and CP production was proportional to cell mass for the whole growth period, giving only one slope on the CP versus OD₆₂₀ plot (Fig. 4).

Effect of anaerobic growth on type 5 CP production

A comparison between growth on solid media with either loose or airtight caps (Table 1) showed that oxygen

supply increased not only the total cell growth, but also the final Y_{CP5} . Different final specific yield ratios were observed depending on the medium (Table 1).

Growth and type 5 CP production by strain Reynolds were followed under aerobic and anaerobic conditions in MFM-YE for 30 h in a fermenter maintained at pH 7.0 and 37 °C (Fig. 5). Results obtained under aerobic conditions at pH 7.0 were the same as those obtained without pH control (Fig. 2). Under anaerobic conditions, the bacteria grew exponentially with a growth rate of 0.30 h⁻¹, and the maximum cell mass was 55% of that obtained under aerobic conditions. Type 5 CP production was proportional to the cell mass, with a mean production rate of 0.27 h⁻¹ during the exponential growth phase. The final type 5 CP concentration was tenfold lower than that observed under aerobic conditions. Plotting type 5 CP production against growth gave two slopes under aerobic conditions and one slope under anaerobic conditions (Fig. 6).

Discussion

The amount of type 5 CP formed by *S. aureus* strain Reynolds was influenced by the culture conditions. Comparison of the final specific yields presented in Tables 1 and 2 shows that variations in CP production followed similar patterns in both solid and liquid media. It seems that the physical state of the culture medium has little influence on CP production. The same observation was made with *Haemophilus influenzae* (Anderson *et al.*, 1976). In contrast, important variations in the expression of cell wall proteins have been observed for staphylococci

grown in liquid as opposed to solid media (Cheung & Fischetti, 1988).

Our work confirms earlier findings of enhanced CP production by *S. aureus* grown in Medium 110 (Caputy & Costerton, 1984; Yoshida & Ekstedt, 1968). We describe a medium that is simpler than Medium 110, but which is as efficient for CP production, despite its low concentrations of salt and carbohydrate, two factors which were assumed to be important for capsule production (Yoshida & Ekstedt, 1968). Media closely related to our semi-synthetic medium, containing Casamino acids and glycerol, have been used previously for the study of capsule formation by *S. aureus* (Wiley, 1968; Witte, 1975).

Exopolysaccharide production is dependent on the available carbon source for bacteria such as *Streptococcus pneumoniae*, *Escherichia coli*, *Lactobacillus*, *Pseudomonas*, *Agrobacterium* and *Erwinia*, and both the amount and composition of the capsular material can be affected (Linton, 1990; Sutherland, 1988; Wilkinson, 1958). Our results show that there is no such dependence for type 5 CP production by *S. aureus* strain Reynolds. The multiplicity of catabolic pathways and the ability of *S. aureus* to utilize various carbon sources under both aerobic and anaerobic conditions might explain this lack of influence of specific carbon source on CP production (Wilkinson, 1958).

High concentrations of dialysed yeast extract in MFM-YE (above 2% v/v) inhibited CP formation. High levels of yeast extract diffusate have been shown to inhibit delta-haemolysin production by *S. aureus* (Turner, 1978). One or several inhibitory factors might be present in yeast extract. For the production of CP, such factors may be UMP and UDP, which strongly inhibit the synthesis of lipid-linked sugar intermediates (Chiu, 1988).

Production of capsular material during growth of *S. aureus* has been previously observed (Kitzrow *et al.*, 1975; Wiley, 1968), but the relationship of CP production to growth had not been investigated. In the present study, type 5 CP synthesis by strain Reynolds was shown to continue when cell growth slows down, and CP production in the post-exponential growth phase contributed at least half the final amount of CP measured. Significant post-exponential polysaccharide production has been observed with other bacteria such as *Lactobacillus* (Dunican & Seeley, 1965), *H. influenzae* (Anderson *et al.*, 1976), *Aerobacter* (Wilkinson, 1958) and *Azotobacter* (Sutherland, 1988). As the pH continues to decrease after the end of the exponential phase, it can be surmised that lactose was still available for polysaccharide synthesis during the slow growth phase. Substances not required for polysaccharide synthesis but which act as growth-limiting nutrients should give an increased amount of

polysaccharide per cell. When these substances are depleted, the synthesis of protein and most growth factors is inhibited, but the production of polysaccharides and lipids may continue (Wilkinson, 1958). Furthermore, the study of specific activities of precursor-forming enzymes involved in the formation of exopolysaccharide by *Pseudomonas* showed a constitutive biosynthetic system (Williams & Wimpenny 1980).

Post-exponential production of exoproteins by several *S. aureus* strains has also been observed (Coleman *et al.*, 1978; Schlievert & Blomster, 1983). The ratio of exoproteins to bacterial density increased after the end of the exponential growth phase, and was interpreted as a competition between intracellular protein and exoprotein synthesis.

pH influences the production of exopolysaccharides by several bacterial species (Masson & Holbein, 1985; Wilkinson, 1958). Using the serum/soft agar technique, Chomarat *et al.* (1989) found that certain *S. aureus* strains that possessed capsular antigen and formed diffuse-type colonies at pH 7.2 exhibited compact growth at pH 8.4, corresponding to a non-capsulated phenotype. Our results also indicate that pH influences CP synthesis by strain Reynolds. CP production was sustained after the exponential growth phase in the pH range 6.0–7.0, but at pH 8.0 there was no continuation of CP synthesis after the end of this phase, and the final specific yield of CP was significantly lower.

The comparison between cells grown on agar slants with loose and airtight caps had previously been made but with Columbia medium only (Hochkeppel *et al.*, 1987). Results from fermenter cultures confirmed that oxygen supply increased CP production and allowed the continuation of CP synthesis after the end of the exponential growth phase. Higher oxygen supply enhances exopolysaccharide production by *Pseudomonas* (Bayer *et al.*, 1990), *Alcaligenes* (Lawford & Rousseau, 1989) and *Xanthomonas* (Suh *et al.*, 1990). It is assumed that cells actively synthesizing exopolysaccharide have an oxygen demand associated with the energy requirements for biosynthesis (Linton, 1990; Wilkinson, 1958). The intracellular energy level could also explain the higher exoprotein production by *S. aureus* in aerobic as opposed to anaerobic culture (Coleman, 1985; Jarvis *et al.*, 1973; Schlievert & Blomster, 1983).

The culture system developed here could be a useful tool in future investigations on the physiology of CP production by *S. aureus*. Furthermore, the use of a simple medium containing only low molecular mass components will facilitate isolation of the polysaccharide for clinical investigation and vaccine development.

We are grateful to F. Nato and J. C. Mazie (Hybridolab, Institut Pasteur, Paris) for providing monoclonal antibodies to *S. aureus* capsular polysaccharide type 5.

References

- ALBUS, A., FOURNIER, J. M., WOLZ, C., BOUTONNIER, A., RANKE, M., HØIBY, N., HOCHKEPPEL, H. & DÖRING, G. (1988). *Staphylococcus aureus* capsular types and antibody response to lung infection in patients with cystic fibrosis. *Journal of Clinical Microbiology* **26**, 2505–2509.
- ANDERSON, P., PITT, J. & SMITH, D. H. (1976). Synthesis and release of polyribophosphate by *Haemophilus influenzae* type b *in vitro*. *Infection and Immunity* **13**, 581–589.
- ARBEIT, R. D., KARAKAWA, W. W., VANN, W. F. & ROBBINS, J. B. (1984). Predominance of two newly described capsular polysaccharide types among clinical isolates of *Staphylococcus aureus*. *Diagnostic Microbiology and Infectious Diseases* **2**, 85–91.
- ATKINSON, B. & MAVITUNA, F. (1983). *Biochemical Engineering and Biotechnology Handbook*, p. 61. New York: The Nature Press/Macmillan.
- BAYER, A. S., EFTEKHAR, F., TU, J., NAST, C. C. & SPEERT, D. P. (1990). Oxygen-dependent up-regulation of mucoid exopolysaccharide (alginate) production in *Pseudomonas aeruginosa*. *Infection and Immunity* **58**, 1344–1349.
- BOUTONNIER, A., NATO, F., BOUVET, A., LEBRUN, L., AUDURIER, A., MAZIE, J. C. & FOURNIER, J. M. (1989). Direct testing of blood cultures for detection of the serotype 5 and 8 capsular polysaccharides of *Staphylococcus aureus*. *Journal of Clinical Microbiology* **27**, 989–993.
- CAPUTY, G. G. & COSTERTON, J. W. (1984). Immunological examination of the glycocalyxes of *Staphylococcus aureus* strains Wiley and Smith. *Current Microbiology* **11**, 297–302.
- CHEUNG, A. L. & FISCHETTI, V. A. (1988). Variation in the expression of cell wall proteins of *Staphylococcus aureus* grown on solid and liquid media. *Infection and Immunity* **56**, 1061–1065.
- CHIU, T. (1988). Biosyntheses of galactosyl lipids and polysaccharide in *Streptococcus mutans*. *Biochimica et Biophysica Acta* **963**, 359–366.
- CHOMARAT, M., ICHIMAN, Y. & YOSHIDA, K. (1989). Protection of mice by a pseudodiffuse strain of *Staphylococcus aureus* possessing polyvalent capsular type antigen. *Journal of Medical Microbiology* **28**, 129–136.
- COLEMAN, G. (1985). A comparison of the patterns of extracellular proteins produced by the high α -toxin-secreting organism *Staphylococcus aureus* (Wood 46) during aerobic and anaerobic growth. *Journal of General Microbiology* **131**, 405–408.
- COLEMAN, G., JAKEMAN, C. M. & MARTIN, N. (1978). Patterns of total extracellular protein secretion by a number of clinically isolated strains of *Staphylococcus aureus*. *Journal of General Microbiology* **107**, 189–192.
- DUNICAN, L. K. & SEELEY, H. W. (1965). Extracellular polysaccharide synthesis by members of the genus *Lactobacillus*: conditions for formation and accumulation. *Journal of General Microbiology* **40**, 297–308.
- FALCIERI, E., VAUDAUX, P., HUGGLER, E., LEW, D. & WALDVOGEL, F. (1987). Role of bacterial exopolymers and host factor on adherence and phagocytosis of *Staphylococcus aureus* in foreign body infection. *Journal of Infectious Diseases* **155**, 524–531.
- FOURNIER, J. M., HANNON, K., MOREAU, M., KARAKAWA, W. W. & VANN, W. F. (1987). Isolation of type 5 capsular polysaccharide from *Staphylococcus aureus*. *Annales de l'Institut Pasteur/Microbiologie* **138**, 561–567.
- GOTTSCHLICH, E. C., YUNG LIU, T. & ARTENSTEIN, M. S. (1969). Human immunity to the meningococcus. III. Preparation and immunochemical properties of the group A, group B, and group C meningococcal polysaccharides. *Journal of Experimental Medicine* **129**, 1349–1365.
- HOCHKEPPEL, H. K., BRAUN, D. G., VISCHER, W., IMM, A., SUTTER, J., STAEBLI, U., GUGGENHEIM, R., KAPLAN, E. L., BOUTONNIER, A. & FOURNIER, J. M. (1987). Serotyping and electron microscopy studies of *Staphylococcus aureus* clinical isolates with monoclonal antibodies to capsular polysaccharide type 5 and 8. *Journal of Clinical Microbiology* **25**, 526–530.
- HUBER, M. M. & HUBER, T. W. (1989). Susceptibility of methicillin-resistant *Staphylococcus aureus* to lysostaphin. *Journal of Clinical Microbiology* **27**, 1122–1124.
- JARVIS, A. W., LAWRENCE, R. C. & PRITCHARD, G. G. (1973). Production of staphylococcal enterotoxins A, B and C under conditions of controlled pH and aeration. *Infection and Immunity* **7**, 847–854.
- JENNINGS, H. J. (1990). Capsular polysaccharides as vaccine candidates. *Current Topics in Microbiology and Immunology* **150**, 97–127.
- JOHNE, B., JARP, J. & HAAHEIM, L. R. (1989). *Staphylococcus aureus* exopolysaccharide *in vivo* demonstrated by immunomagnetic separation and electron microscopy. *Journal of Clinical Microbiology* **27**, 1631–1635.
- KARAKAWA, W. W., FOURNIER, J. M., VANN, W. F., ARBEIT, R., SCHNEERSON, R. S. & ROBBINS, J. B. (1985). Method for the serological typing of the capsular polysaccharides of *Staphylococcus aureus*. *Journal of Clinical Microbiology* **22**, 445–447.
- KARAKAWA, W. W. & VANN, W. F. (1982). Capsular polysaccharides of *Staphylococcus aureus*. *Seminars in Infectious Diseases* **4**, 285–293.
- KELLER, G. M., HANSON, R. S. & BERGDOLL, M. S. (1978). Molar growth yields and enterotoxin B production of *Staphylococcus aureus* S-6 with amino acids as energy sources. *Infection and Immunity* **20**, 151–157.
- KITZROW, D., BLOBEL, H. & SCHAEF, W. (1975). Isolation of capsular substances from *Staphylococcus aureus*. *Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene (Series A)* **233**, 305–313.
- LAWFORD, H. & ROUSSEAU, J. (1989). Effect of oxygen on the rate of β -1,3-glucan microbial exopolysaccharide production. *Biotechnology Letters* **11**, 125–130.
- LEE, J. C., BETLEY, M. J., HOPKINS, C. A., PEREZ, N. E. & PIER, G. B. (1987). Virulence studies, in mice, of transposon-induced mutants of *Staphylococcus aureus* differing in capsule size. *Journal of Infectious Diseases* **156**, 741–750.
- LINTON, J. D. (1990). The relationship between metabolite production and the growth efficiency of the producing organism. *FEMS Microbiology Reviews* **75**, 1–18.
- LUONG, J. H. T., MULCHANDANI, A. & LEDUY, A. (1988). Kinetics of biopolymer synthesis: a revisit. *Enzyme and Microbial Technology* **10**, 326–332.
- MASSON, L. & HOLBEIN, B. E. (1985). Influence of nutrient limitation and low pH on serogroup B *Neisseria meningitidis* capsular polysaccharide level: correlation with virulence for mice. *Infection and Immunity* **47**, 465–471.
- MATTILA, T. (1989). A staining technique to measure capsular polysaccharides of *Staphylococcus aureus* on filter membranes. *Journal of Microbiological Methods* **9**, 323–331.
- OPDEBEECK, J. P., O'BOYLE, D. & FROST, A. J. (1985). The expression of capsule in serum-soft agar by *Staphylococcus aureus* isolated from human clinical sources. *Journal of Medical Microbiology* **19**, 275–278.
- OPDEBEECK, J. P., WATSON, D. L. & FROST, A. J. (1988). Colony morphology of *Staphylococcus aureus* in serum-soft agar following *in vivo* and *in vitro* growth. *Veterinary Microbiology* **16**, 87–91.
- PAUL, F., MORIN, A. & MORSAN, P. (1986). Microbial polysaccharides with actual potential industrial applications. *Biotechnological Advances* **4**, 245–259.
- POUTREL, B., BOUTONNIER, A., SUTRA, L. & FOURNIER, J. M. (1988). Prevalence of capsular polysaccharide types 5 and 8 among *Staphylococcus aureus* isolates from cow, goat, and ewe milk. *Journal of Clinical Microbiology* **26**, 38–40.
- RATHER, P. N., DAVIS, A. P. & WILKINSON, B. J. (1986). Slime production by bovine milk *Staphylococcus aureus* and identification of coagulase-negative staphylococcal isolates. *Journal of Clinical Microbiology* **23**, 858–862.
- ROBBINS, J. B. & SCHNEERSON, R. S. (1990). Polysaccharide–protein conjugates: a new generation of vaccines. *Journal of Infectious Diseases* **161**, 821–832.
- ROBERTS, I. S., SAUNDERS, F. K. & BOULNOIS, G. J. (1989). Bacterial capsules and interactions with complement and phagocytes. *Biochemical Society Transactions* **17**, 462–464.
- SCHLIEVERT, P. M. & BLOMSTER, D. A. (1983). Production of staphylococcal pyrogenic exotoxin type C: influence of physical and chemical factors. *Journal of Infectious Diseases* **147**, 236–242.
- SHEAGREN, J. N. (1984). *Staphylococcus aureus*: the persistent pathogen. *New England Journal of Medicine* **310**, 1368–1373, 1437–1442.

- SOMPOLINSKY, D., SAMRA, Z., KARAKAWA, W. W., VANN, W. F., SCHNEERSON, R. & MALIK, Z. (1985). Encapsulation and capsular types in isolates of *Staphylococcus aureus* from different sources and relationship to phage types. *Journal of Clinical Microbiology* **22**, 828–834.
- SUH, I. S., HERBEST, H., SCHUMPE, A. & DECKWER, W. D. (1990). The molecular weight of xanthan polysaccharide produced under oxygen limitation. *Biotechnology Letters* **12**, 201–206.
- SUTHERLAND, I. W. (1988). Bacterial surface polysaccharides: structure and function. *International Review of Cytology* **113**, 187–231.
- TURNER, W. H. (1978). The effect of medium volume and yeast extract diffusate on delta-hemolysin production by five strains of *Staphylococcus aureus*. *Journal of Applied Bacteriology* **45**, 291–296.
- WHITFIELD, C. (1988). Bacterial extracellular polysaccharides. *Canadian Journal of Microbiology* **34**, 415–420.
- WILEY, B. B. (1968). Capsule size, coagulase production, and egg virulence among certain strains of *Staphylococcus aureus*. *Canadian Journal of Microbiology* **14**, 685–689.
- WILKINSON, J. F. (1958). The extracellular polysaccharides of bacteria. *Bacteriological Reviews* **22**, 46–73.
- WILLIAMS, A. G. & WIMPENNY W. T. (1980). Extracellular polysaccharide biosynthesis by *Pseudomonas* NCIB 11264. Studies on precursor-forming enzymes and factors affecting exopolysaccharide production by washed suspensions. *Journal of General Microbiology* **116**, 133–141.
- WITTE, W. (1975). Capsule formation in *Staphylococcus aureus* as a reason for nontypability by phages. *Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene (Series A)* **233**, 447–451.
- YOSHIDA, K. & EKSTEDT, R. D. (1968). Relation of mucoid growth of *Staphylococcus aureus* to clumping factor reaction, morphology in serum-soft agar, and virulence. *Journal of Bacteriology* **96**, 902–908.