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Effects of Culture Conditions on Production of Type 5 Capsular Polysaccharide by Human and Bovine *Staphylococcus aureus* Strains

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Two *Staphylococcus aureus* strains, the prototype human Reynolds strain and a bovine isolate, were grown in different complex media and in a synthetic medium (D. Taylor and K. T. Holland, J. Appl. Bacteriol. 66:319–329, 1989) and compared for their ability to produce type 5 capsular polysaccharide. Cell-bound and cell-free type 5 capsular polysaccharide were measured by a new one-step competition enzyme-linked immunosorbent assay. The total production and the proportion of cell-bound type 5 capsular polysaccharide were dependent on the nature of the medium, the duration of the culture, and the strain. Both strains produced more type 5 capsular polysaccharide when cultivated in the synthetic medium than when cultivated in complex media. The best yield of type 5 capsular polysaccharide, about 300 μg/ml of medium, was obtained with strain Reynolds grown for 48 h with shaking in the synthetic broth containing glucose as a carbon source.

Capsular polysaccharides (CP) have been identified in clinical isolates of *Staphylococcus aureus*, and serological distinction of 11 CP types has been proposed (19, 30). By using specific polyclonal or monoclonal antibodies, it has been shown that two CP types, 5 and 8 (CP5 and CP8), account for 70 to 80% of *S. aureus* strains isolated from different human sites (4, 14, 30). Similar results have been obtained for isolates from cow, goat, and ewe milk (25) and for isolates from some other farm animals (27).

Some evidence indicates that the production of capsular material is preferentially induced when *S. aureus* is grown in certain media. By use of the serum soft agar test, a conversion from compact to diffuse morphology has been observed when human and bovine isolates, grown on brain heart infusion (BHI) broth, were cultured on modified *Staphylococcus* medium 110 (mod 110) (24, 28, 32, 38, 39). The CP material was also detected in vivo, in the sera of animals during experimental endocarditis and focal infection (3, 5), by use of the prototype CP8 Becker strain. The CP material was also detected in vivo on *S. aureus* involved in bovine mastitis and in the milk from an infected mammary gland (16, 33). Since mod 110 and milk are rich in lactose, these results suggest that lactose might increase expression of CP. However, Watson and Watson (36) claimed that surface components expressed by mastitis isolates cultivated in the presence of either whey or mod 110 or supplemented media with lactose are of a different nature. This conclusion was based on morphological examination of *S. aureus* by electron microscopy.

Depending on culture conditions, the capsular material of *S. aureus* has been shown to play a part in virulence and in resistance to phagocytosis. Strains grown on mod 110 agar (37) or in milk (22) were more virulent to mice than the same strains grown in BHI agar or Trypticase soy broth. Also, staphylococci grown in raw milk showed enhanced virulence for bovine skin (7). However, Albus et al. (2), using a mouse model, reported that the type 5 human strain Reynolds cultivated in Columbia broth was not more virulent than a capsule-negative or capsule-deficient mutant. They suggested that microencapsulated strains do not share the biologic properties associated with highly encapsulated *S. aureus* strains. The outermost surface components of staphylococci, in particular CP, are known to have a major role in phagocytosis (18–20). Mastitis isolates in medium supplemented with milk exhibited more hydrophilic properties (23) and increased resistance to phagocytosis by polymorphonuclear leukocytes (34) than those cultivated in a conventional medium. Moreover, it was demonstrated that antibodies elicited in mice by injection of a CP5 conjugated to exotoxin A of *Pseudomonas aeruginosa* promoted type-specific opsonization of *S. aureus* by human polymorphonuclear leukocytes (10). Taken together, these findings strongly suggest that differences in the environment as well as the metabolism of the organisms might directly affect CP production and subsequent virulence. Thus, quantitative determination of CP production in relation to culture conditions is desirable, particularly when isolation and purification of large amounts of CP and clinical investigations of virulence are required.

There are few data available on the quantitative production of CP by *S. aureus* relative to the composition of the medium and environmental factors. A final yield of 1 mg of purified CP5 and CP8 per g of wet cell pellet was obtained when Columbia agar was used for growth (11, 12). Similar values were found with typeable coagulase-negative staphylococci (26). It was reported that the production of CP5 in synthetic media (SM) was linked to energy availability and energy source but not to carbohydrate concentration or carbon/nitrogen ratio (31). Recently, several classical media were compared for CP5 production by strain Reynolds, and a semisynthetic medium was developed (9). However, these studies were limited to the human prototype strain, and it cannot be excluded that the influence of conditions of growth on CP production might be different for other isolates. Moreover, the semisynthetic medium proposed (9), although superior to classical media, supported a low biomass and CP production.

In the work reported here, the prototype human strain Reynolds and a bovine isolate, cultivated in different complex media, were compared for their ability to facilitate CP5 pro-

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TABLE 1. Summary of the different growth conditions used for the *S. aureus* strains

Medium	Conditions used for[a]:	
	Human strain (Reynolds)	Bovine strain (159.14)
BHI	a	a
BHI-whey	a	a
Columbia	a, b	a, b
Mod 110	a	a
SM-lactose	a	a
SM-glucose	a, c	a

[a] Symbols: a, liquid medium without shaking; b, solid medium; c, liquid medium with shaking.

duction. The time course production of cell-bound and cell-free CP was monitored in the different media. In addition, the influences of shaking and of the nature and concentration of carbohydrates in a defined SM on CP production were investigated. For measurement of CP5 concentration, a new quantitative assay by a one-step competition enzyme-linked immunosorbent assay (ELISA) was developed.

MATERIALS AND METHODS

Bacterial strains. Two isolates of *S. aureus* were used: strain Reynolds, the prototype strain for CP5 (17), and strain 159.14, isolated from a subclinical case of bovine mastitis belonging to the CP5 serotype as demonstrated previously (25). The strains were kept freeze-dried in the collection of our laboratory.

Culture media and procedures. The different media and conditions of growth used for each strain are shown (Table 1).

Columbia, BHI broth (Difco Laboratories, Detroit, Mich.), and mod 110 (38) were chosen because they are currently used for cultivation of *S. aureus* and also because only few data (9) exist on the ability of these different media to induce production of CP5. To determine if some milk constituents induce expression of capsule as suggested by Watson and Watson (36), bacteria were also grown in BHI broth supplemented with whey (10% [vol/vol]), prepared by rennet precipitation from defatted bovine milk.

Since an SM which would give a high yield of CP5 and simplify its purification is desirable, we tested the 18-amino-acid medium described by Taylor and Holland (35) which supports high biomass regardless of the strain of *S. aureus* used. To determine the influence of the carbohydrate source, either lactose or glucose, at a final concentration of 10 g/liter, was incorporated in the SM. Further studies were carried out with different glucose concentrations, namely, 1, 10; and 50 g/liter.

All amino acids, nicotinic acid, thiamine hydrochloride, glucose, lactose, and ammonium sulfate were obtained from Sigma Chemical Co. (St. Louis, Mo.), and all other chemicals were from Serva (Heidelberg, Germany).

For all experiments, to minimize variation from acclimation, strains from lyophilized stock cultures were precultured in Columbia broth at 37°C for 18 h and then inoculated on Columbia agar. Cells were resuspended in physiological buffered saline (PBS), and the suspensions used to inoculate the test media in flasks were standardized by measuring the optical density at 600 nm (OD_{600}). When desired, cultures were incubated at 37°C with orbital shaking at 150 rpm (G10 gyrotory shaker; New Brunswick Co., Inc., New Brunswick, N.J.).

Growth was assayed by spectrophotometry at 600 nm and by bacterial wet cell mass.

Preparation of test samples. Bacteria cells collected on Columbia agar or after centrifugation at 2,500 × *g* for 30 min on the different broth media tested were suspended in 2 ml of PBS and autoclaved at 120°C for 1 h to extract cell-bound CP5 (11).

Supernatants of every broth medium containing cell-free CP were autoclaved under similar conditions. All autoclaved samples were stored at −20°C until used. The volumes of supernatants and wet weights of the bacterial pellets were determined.

Determination of CP5 concentration by ELISA. Both cell-bound CP and cell-free CP were quantified by a competition ELISA. Purified CP5 was prepared as described by Fournier et al. (11). Polyclonal anti-CP5 serum was raised in rabbits immunized three times per week for 3 weeks with heat-killed (70°C for 1 h) strain Reynolds as described previously (17).

A flat-bottom microplate (Nunc, Roskilde, Denmark) was coated with a solution of CP5 (1 μg/ml of PBS) conjugated with cyanogen chloride (Sigma) to poly-L-lysine (Sigma) by previously described methods (6, 13). After incubation at 37°C for 2 h, the plate was washed with PBS supplemented with 0.1% (vol/vol) Tween 20 (Sigma), blocked with gelatin (0.5% gelatin in PBS; Prolabo, Paris, France) by incubation at 37°C for 1 h, and washed with PBS-Tween. Fifty microliters of each dilution of each test sample or dilution of purified CP5 and 50 μl of an appropriate dilution (OD of 0.3 to 0.5 by indirect ELISA using purified CP5) of rabbit polyclonal antiserum were added to the wells. After incubation at 37°C for 1 h and washing with PBS-Tween, an anti-rabbit peroxidase-conjugated immunoglobulin G (heavy and light chain specific; Jackson Immuno-research, Inc., West Grove, Pa.) was added, and the plate was incubated at 37°C for 1 h. Washing with PBS-Tween was followed by addition of the peroxidase substrate. After 1 h at room temperature with agitation, the OD_{414} was read with an automated microplate reader (Titertek Multiscan MCC 340; Flow Laboratories SA, Puteaux, France). The CP content of samples assayed at various dilutions was determined by interpolation from a standard titration curve of purified CP5. It was established that a linear relationship between the percentage of competition and a range of CP concentrations existed from 2 to 125 ng/ml. The coefficient of variation, a measure of repeatability of the measures, was 12%. For each ELISA, the titration of CP for the standard curve and diluted test samples was performed in duplicate. Autoclaved sterile medium corresponding to the samples tested was incorporated as a control.

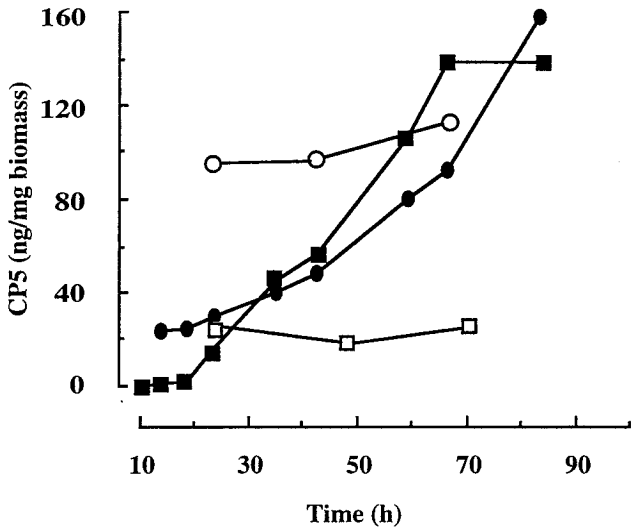

FIG. 1. Total production of CP5 by *S. aureus* human strain Reynolds (○ and ●) and by *S. aureus* bovine strain 159.14 (□ and ■) cultivated on Columbia agar (open symbols) and in Columbia broth (filled symbols) without shaking.

RESULTS

CP5 production in Columbia broth and Columbia agar (Fig. 1). On Columbia agar plates, regardless of the strain used, CP5 production remained stable after 24 h of culture. In contrast, production increased in Columbia broth in relation to the duration of incubation of the culture, and production was greater in Columbia broth than on Columbia agar when the culture was prolonged after 68 h.

In the liquid medium, production yields were similar for the two strains after 80 h of cultivation, i.e., about 160 ng/mg of biomass. However, when organisms were harvested from Columbia agar plates, strain Reynolds produced 100 ng of CP5 per mg of biomass, an amount fivefold greater than that produced by bovine strain.

Growth and CP5 production in different complex and synthetic liquid media without shaking. Growth curves recorded for human Reynolds and bovine strains are shown in Fig. 2 and 3, respectively.

Initially, growth was delayed in the defined SM when compared with that in the complex media. All of the various complex media supported reasonable growth, with the highest and lowest growth yields obtained in Columbia and mod 110 broths, respectively, for both strains (Table 2). The cell yields recorded for the bovine strain were greater than those of the Reynolds strain for each medium tested.

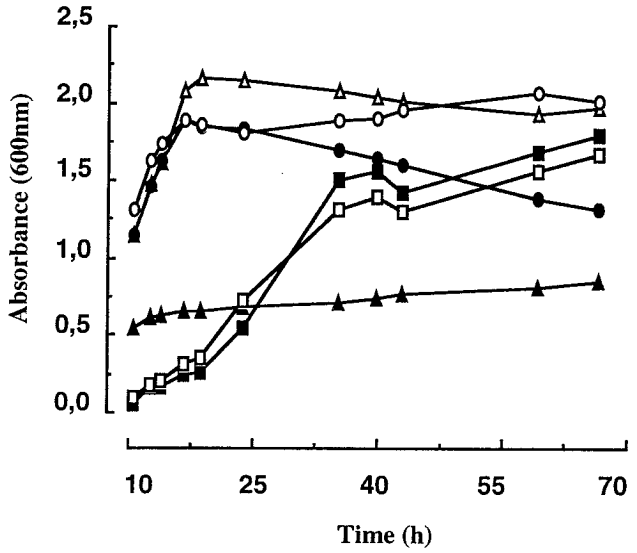

FIG. 2. Growth of *S. aureus* human strain Reynolds cultivated without shaking in the following liquid media: BHI broth (●), BHI-whey broth (○), Columbia broth (△), mod 110 (▲), SM containing glucose at 10 g/liter (□), and SM containing lactose at 10 g/liter (■). Data shown are a representative set of data from two separate experiments.

When grown on different media, the two strains exhibited significant variations in CP5 production that were independent of bacterial growth. More CP5 was produced per milliliter of culture in the two SM than in the different complex media tested. The SM with lactose supported the highest CP production for the bovine strain, whereas the highest production for the human strain was recorded with the SM supplemented with glucose.

The amounts of CP5 produced per milligram of biomass (Fig. 4 and 5) and also per milliliter of culture (data not shown) increased markedly during the stationary phase of growth in SM. Very little CP5 was produced by *S. aureus* bovine cells grown in BHI and in BHI-whey broths. Because staphylococci may release soluble CP5 during growth in broth cultures, we measured the CP5 concentrations in culture supernatants of different media and compared them with the concentrations of cell-associated CP. Our results indicated that the proportion of CP5 bound to the bacteria was dependent on three parameters, i.e., the nature of the medium used, the duration of the culture, and the strain (Table 3).

TABLE 2. Cell yield and CP5 production by *S. aureus* strains cultivated for 67 h in different liquid media without shaking[a]

Medium	Human strain (Reynolds)			Bovine strain (159.14)		
	OD_{600}	CP5 produced		OD_{600}	CP5 produced	
		μg/ml	μg/mg[b]		μg/ml	μg/mg
SM-lactose 10[c]	1.80	2.60	1.20	2.90	1.60	0.41
SM-glucose 10[c]	1.70	3.70	1.70	2.90	1.20	0.28
Mod 110	0.90	0.60	0.30	2.20	0.82	0.28
Columbia broth	2.00	0.27	0.09	3.90	0.85	0.13
BHI-whey broth	2.00	0.41	0.19	3.00	ND[d]	ND
BHI broth	1.30	0.30	0.13	3.00	0.04	ND

[a] Data shown are a representative set from two separate experiments.
[b] Wet biomass.
[c] 10, 10 g/liter (final concentration).
[d] ND, not detected.

Whatever the medium or the strain used, the proportion of cell-associated CP5 either remained constant or decreased along with the duration of growth, but it never increased. After 60 h of growth, relative to the total amount of CP5, the highest concentrations in the supernatants were recorded in Columbia and mod 110 broths for bovine and Reynolds strains, respectively. Supplementation of SM with lactose or glucose had no effect on the proportion of cell-associated CP5. However, the proportion of cell-associated CP5 for the human strain was twofold greater when compared with that of the bovine strain (67% versus 32%).

Growth and CP5 production in liquid SM-glucose with shaking. Our objective was to define a simple medium and conditions of growth that would give high CP5 production.

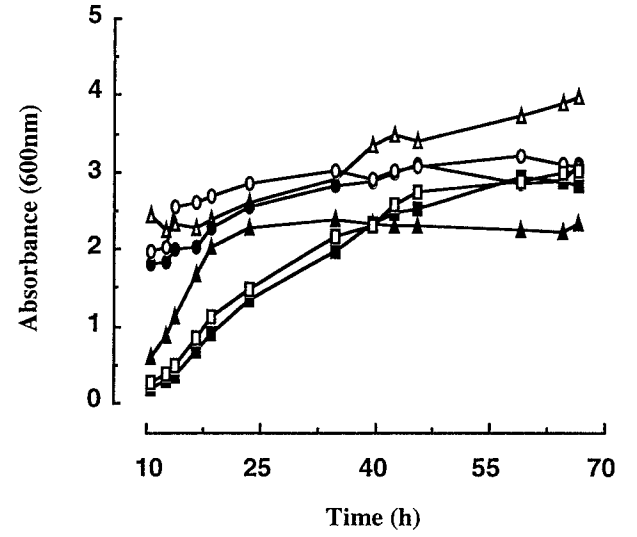

FIG. 3. Growth of *S. aureus* bovine strain 159.14 cultivated without shaking in the following liquid media: BHI broth (●), BHI-whey broth (○), Columbia broth (△), mod 110 (▲), SM containing glucose at 10 g/liter (□), and SM containing lactose at 10 g/liter (■). Data shown are a representative set of data from two separate experiments.

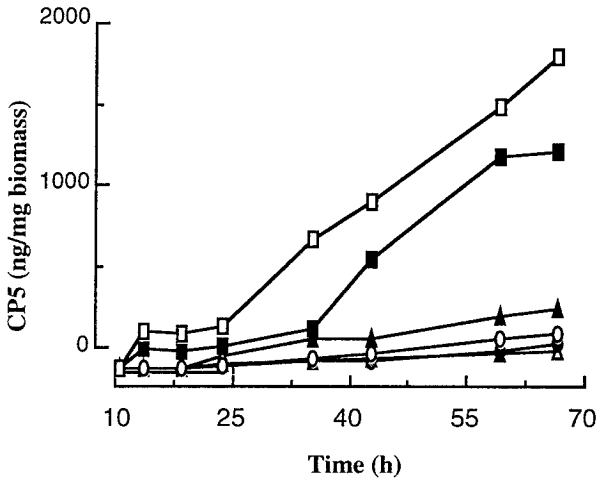

FIG. 4. Total production of CP5 by *S. aureus* human strain Reynolds cultivated without shaking in the following liquid media: BHI broth (●), BHI-whey broth (○), Columbia broth (△), mod 110 (▲), SM containing glucose at 10 g/liter (□), and SM containing lactose at 10 g/liter (■). Data shown are a representative set of data from two separate experiments.

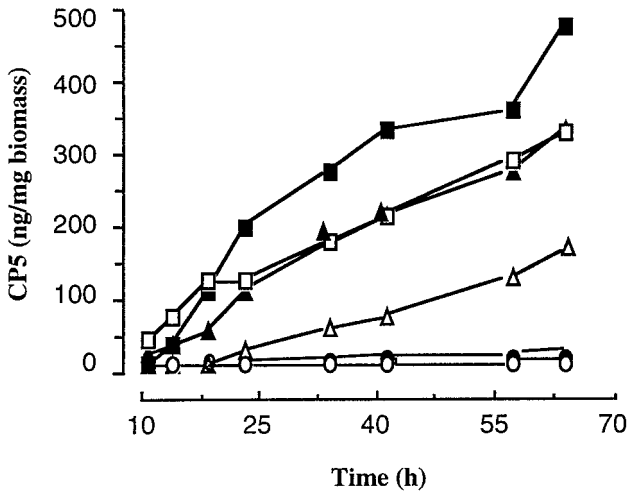

FIG. 5. Total production of CP5 by *S. aureus* bovine strain 159.14 cultivated without shaking in the following liquid media: BHI broth (●), BHI-whey broth (○), Columbia broth (△), mod 110 (▲), SM containing glucose at 10 g/liter (□), and SM containing lactose at 10 g/liter (■). Data shown are a representative set of data from two separate experiments.

Indeed, we investigated the influence of shaking, which increases dissolved oxygen, and of glucose concentration on CP production by the human strain because the Reynolds strain supported the best CP production and the glucose was the best substrate in SM for this strain. As shown in Table 4, growth yield and CP5 production after 48 h of culture were not significantly influenced by glucose concentration. With shaking, maximum growth reached, with a concentration of 25 g of glucose per liter, an OD of about 22. The OD value recorded with a concentration of 10 g/liter was 12-fold greater than that for cells cultivated under the same conditions but without shaking (Table 2). The total amount of CP produced by strain Reynolds in these conditions was about 300 μg/ml of broth and did not change after 48 h of growth, whereas the CP5 concentration in the supernatant did not exceed 20% of the total CP concentration.

DISCUSSION

There are a few reports (9, 21, 31) on the influence of culture conditions that would augment the production of the prevalent CP5 and CP8 of *S. aureus* isolates. All of the previous studies were conducted only with prototype strains, Reynolds and Becker, for CP5 and CP8, respectively. In this study, we examined and compared different parameters for their potential effect on production of CP5 by human and bovine *S. aureus* strains. Recently, Dassy et al. (9) described a semisynthetic medium simpler than mod 110 and as efficient for CP5 production by *S. aureus* strain Reynolds. Although Stringfellow et al. (31) reported a higher CP5 production by strain Reynolds grown in a liquid SM than by the same strain grown in complex media, the production recorded by these authors remained relatively low. In the present study, we selected a more efficient SM and investigated the influence of the carbohydrate concentration and of shaking.

TABLE 3. Time course of cell-associated CP5 production by *S. aureus* strains grown in different liquid media without shaking[a]

Medium	CP5 production (% relative to total) by:					
	Human strain (Reynolds)			Bovine strain (159.14)		
	24 h	35 h	60 h	24 h	35 h	60 h
SM-lactose 10[b]	NT[c]	NT	65.8	55.8	45.8	28.4
SM-glucose 10[b]	51.4	69.8	67.0	51.0	39.7	32.3
Mod 110	26.4	24.4	33.3	79.9	70.7	72.7
Columbia broth	40.0	42.9	46.5	88.8	71.8	23.1

[a] Data shown are a representative set from two separate experiments.
[b] 10, 10 g/liter (final concentration).
[c] NT, not tested.

TABLE 4. Time course of CP5 production by *S. aureus* Reynolds strain grown with shaking in liquid synthetic medium containing different concentrations of glucose[a]

Glucose concn (g/liter)	OD_{600} maximum	CP5 Concn μg/ml)			
		24 h	48 h	72 h	96 h
1	20.3	44.0 (89.3)[b]	357.0 (93.0)	348.0 (87.0)	365.0 (79.2)
10	21.2	135.0 (91.0)	250.0 (79.0)	323.0 (75.3)	341.0 (68.6)
25	22.1	180.0 (92.4)	321.0 (84.0)	336.0 (80.4)	328.0 (73.8)

[a] Data shown are a representative set from two separate experiments. For each experiment, CP5 production was determined by an ELISA on three different days.
[b] Values in parentheses are the percentages of cell-associated CP5.

Our results indicate that the amount of CP5 formed by *S. aureus* was influenced by culture conditions and also that variations in CP production did not follow similar patterns for the two strains tested. Figure 1 shows that cells grown on solid Columbia medium yielded higher quantities of CP5 than those yielded by cells grown in Columbia broth after 24 h of culture, which is the duration of culture used in previous studies. This datum explains and justifies the choice of solid Columbia medium by most of the authors (11, 21). However, the amount of CP5 increased greatly in Columbia broth, without shaking, after the end of the exponential growth phase (Fig. 1).

Our work confirms earlier findings of reduced and enhanced CP5 production by *S. aureus* grown in BHI broth and mod 110, respectively (9, 38).

Watson and Watson (36) reported that *S. aureus* grown inside the udder, or in a standard nutrient broth supplemented with milk whey, produced a large, well-defined pseudocapsule outside the cell wall, on the basis of its appearance by electron microscopy. However, this pseudocapsule has never been defined biochemically. Likewise, resistance to phagocytosis and expression of CP5 are enhanced when *S. aureus* mastitis isolates are cultivated on milk agar (34). In our experiment, BHI broth supplemented with whey failed to facilitate CP5 production by *S. aureus*. This result may be explained by the presence of inhibitory factors in the BHI broth, but further studies must be undertaken to specify the role of milk components in promoting CP5 production by *S. aureus*.

S. aureus can utilize various carbon sources for growth and CP production. Dassy et al. (9) examined CP5 production by *S. aureus* Reynolds in a semisynthetic medium containing various carbon substrates. They observed that the final yield of CP5 was not significantly different from one substrate to another. In the present study, the synthetic medium defined by Taylor and Holland (35) appeared to be the most suitable for CP5 production. The two carbohydrate sources incorporated in the SM, lactose and glucose, supported the same cell yields. However, CP5 production was better in the SM containing glucose for the human strain Reynolds, whereas the bovine strain elaborated a little more CP5 in the SM supplemented with lactose.

Bearing in mind that the bovine strain was isolated from milk which is rich in lactose, this difference may be related to the different origins of these two strains.

Works recently reported by the team of Dassy and Stringfellow (9, 31), who used for the first time a quantitative and specific assay for CP5, gave the following precious information on the formation of CP5 by *S. aureus* Reynolds grown under a range of controlled environmental conditions. (i) CP5 was synthesized during exponential and postexponential growth phases. (ii) CP5 production was linked to energy availability and energy source but not to carbohydrate concentration or carbon/nitrogen ratio. (iii) CP5 production was increased by oxygen supply. Our results confirm all these findings. However, the SM containing glucose (35) that we used was more efficient for growth and CP production by *S. aureus* Reynolds than the SM defined by Stringfellow et al. (31). We obtained 300 μg of CP5 per ml of medium compared with their 20 μg/ml, and the yield of CP5 elaborated per milligram of cells was 1.5-fold lower in their medium than in ours. That could result from the fact that the carbohydrate substrate chosen by these authors was lactose, which seemed less efficient than glucose in promoting CP5 synthesis by *S. aureus* Reynolds. Above all, the growth of strain Reynolds was 10-fold greater in our medium than in that described by Stringfellow et al. (31). The medium defined by these authors contained 10 amino acids compared with 18 in the medium we used. We suggest that the eight supplementary amino acids are necessary for optimum growth. A synthetic medium with the same 18 amino acids was described for slime production by coagulase-negative staphylococci (15). All of these amino acids were required to obtain growth, whatever the staphylococcal strains used.

The expression of extracellular and cell-bound proteins in *S. aureus* is controlled by a regulatory locus called accessory gene regulator (*agr*). Most of these exoproteins, many of which play a role in the pathogenesis of *S. aureus*, are positively regulated by *agr* and are produced mainly after the cessation of active exponential growth (1, 29). Recently, Dassy et al. (8) investigated the involvement of *agr* in expression of CP5, and their results indicated that CP5 synthesis was controlled by this locus. The medium we used, which was defined for the production of *S. aureus* extracellular proteins (35), allowed good CP5 synthesis as well. That is in keeping with the hypothesis proposed by Dassy et al. (8). This medium could be useful for further investigations on the regulation of CP5 expression by *agr*.

Our study also indicated that the production of CP5 and the proportion of CP5 bound to the bacteria were dependent on three parameters: the nature of the medium, the duration of the culture, and the strain used. Capsule formation is usually demonstrated with fresh isolates of human or animal origin, and repeated subcultivation on an artificial medium results in decreased capsule synthesis (16). These data could explain the contradictory results obtained concerning encapsulated *S. aureus* virulence and phagocytosis resistance (2, 18, 22, 37). A drawback of these studies was the lack of a quantitative and specific assay for CP that can be applied to unpurified extracts. Thus, the simple one-step competition ELISA we developed for measurement of CP5 concentration, like the two-step inhibition ELISA proposed by Stringfellow et al. (31), may constitute a useful tool for future clinical investigations.

In conclusion, the use of the simple medium described here, containing only low-molecular-mass components and supporting good growth and CP5 production by *S. aureus*, will facilitate purification of CP5, clinical investigation, and vaccine development.

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REFERENCES

1. **Abdelnour, A., S. Arvidson, T. Bremell, C. Ryden, and A. Tarkowski.** 1993. The accessory gene regulator (*agr*) controls *Staphylococcus aureus* virulence in a murine arthritis model. Infect. Immun. **61:**3879–3885.
2. **Albus, A., R. D. Arbeit, and J. C. Lee.** 1991. Virulence of *Staphylococcus aureus* mutants altered in type 5 capsule production. Infect. Immun. **59:** 1008–1014.
3. **Arbeit, R. D., and R. M. Dunn.** 1987. Expression of capsular polysaccharide during experimental focal infection with *Staphylococcus aureus*. J. Infect. Dis. **156:**947–952.
4. **Arbeit, R. D., W. W. Karakawa, W. F. Vann, and J. B. Robbins.** 1984. Predominance of two newly described capsular polysaccharide types among clinical isolates of *Staphylococcus aureus*. Diagn. Microbiol. Infect. Dis. **2:**85–91.
5. **Arbeit, R. D., and M. J. Nelles.** 1987. Capsular polysaccharide antigenemia in rats with experimental endocarditis due to *Staphylococcus aureus*. J. Infect. Dis. **155:**242–246.
6. **Barrett, D. J., M. Triggiani, and E. M. Ayoub.** 1983. Assay of antibody to group A streptococcal carbohydrate by enzyme-linked immunosorbent assay. J. Clin. Microbiol. **18:**622–627.
7. **Brock, J. H., A. Turvey, and B. Reiter.** 1973. Virulence of two mastitis strains of *Staphylococcus aureus* in bovine skin: enhancement by growth in high carbohydrate-high salt medium or in raw milk. Infect. Immun. **7:**865–872.
8. **Dassy, B., T. Hogan, T. J. Foster, and J. M. Fournier.** 1993. Involvement of the accessory gene regulator (*agr*) in expression of type 5 capsular polysaccharide by *Staphylococcus aureus*. Microbiology **139:**1301–1306.
9. **Dassy, B., W. T. Stringfellow, M. Lieb, and J. M. Fournier.** 1991. Production of type 5 polysaccharide by *Staphylococcus aureus* grown in a semi-synthetic medium. J. Gen. Microbiol. **137:**1155–1162.
10. **Fattom, A., R. Schneerson, S. C. Szu, W. F. Vann, J. Shiloach, W. W. Karakawa, and J. B. Robbins.** 1990. Synthesis and immunological properties in mice of vaccines composed of *Staphylococcus aureus* type 5 and type 8 capsular polysaccharides conjugated to *Pseudomonas aeruginosa* exotoxin A. Infect. Immun. **58:**2367–2374.
11. **Fournier, J. M., K. Hannon, M. Moreau, W. W. Karakawa, and W. F. Vann.** 1987. Isolation of type 5 capsular polysaccharide from *Staphylococcus aureus*. Ann. Inst. Pasteur/Microbiol. **138:**561–567.
12. **Fournier, J. M., W. F. Vann, and W. W. Karakawa.** 1984. Purification and characterization of *Staphylococcus aureus* type 8 capsular polysaccharide. Infect. Immun. **45:**87–93.
13. **Gray, B. M.** 1979. ELISA methodology for polysaccharide antigens: protein coupling of polysaccharides for adsorption to plastic tubes. J. Immunol. Methods **28:**187–192.
14. **Hochkeppel, H. K., D. G. Braun, W. Vischer, A. Imm, S. Sutter, U. Staeubli, R. Guggenheim, E. L. Kaplan, A. Boutonnier, and J. M. Fournier.** 1987. Serotyping and electron microscopy studies of *Staphylococcus aureus* clinical isolates with monoclonal antibodies to capsular polysaccharide types 5 and 8. J. Clin. Microbiol. **25:**526–530.
15. **Hussain, M., J. G. M. Hastings, and P. J. White.** 1991. A chemically defined medium for slime production by coagulase-negative staphylococci. J. Med. Microbiol. **34:**143–147.
16. **Johne, B., J. Jarp, and L. R. Haaheim.** 1989. *Staphylococcus aureus* exopolysaccharide in vivo demonstrated by immunomagnetic separation and electron microscopy. J. Clin. Microbiol. **27:**1631–1635.
17. **Karakawa, W. W., J. M. Fournier, W. F. Vann, R. Arbeit, R. S. Schneerson, and J. B. Robbins.** 1985. Method for the serologic typing of the capsular polysaccharides of *Staphylococcus aureus*. J. Clin. Microbiol. **22:**445–447.
18. **Karakawa, W. W., A. Sutton, R. Schneerson, A. Karpas, and W. F. Vann.** 1988. Capsular antibodies induce type-specific phagocytosis of capsulated *Staphylococcus aureus* by human polymorphonuclear leukocytes. Infect. Immun. **56:**1090–1095.
19. **Karakawa, W. W., and W. F. Vann.** 1982. Capsular polysaccharides of *Staphylococcus aureus*. Semin. Infect. Dis. **4:**285–293.
20. **Karakawa, W. W., and D. A. Young.** 1979. Immunological specificity of heat-stable opsonins in immune and nonimmune sera and their interaction with non-encapsulated strains of *Staphylococcus aureus*. Infect. Immun. **25:** 175–186.
21. **Lee, J. C., S. Takeda, P. J. Livolsi, and L. C. Paoletti.** 1993. Effects of in vitro and in vivo growth conditions on expression of type 8 capsular polysaccharide by *Staphylococcus aureus*. Infect. Immun. **61:**1853–1858.
22. **Mamo, W., M. Lindahl, and P. Jonsson.** 1991. Enhanced virulence of *Staphylococcus aureus* from bovine mastitis induced by growth in milk whey. Vet. Microbiol. **27:**371–384.
23. **Mamo, W., F. Rozgonyi, S. Hjertéen, T. Wadström.** 1987. Effect of milk on surface properties of *Staphylococcus aureus* from bovine mastitis. FEMS Microbiol. Lett. **48:**195–200.
24. **Opdebeeck, J. P., A. J. Frost, D. O'Boyle, and N. L. Norcross.** 1987. The

expression of capsule in serum-soft agar by *Staphylococcus aureus* isolated from bovine mastitis. Vet. Microbiol. **13:**225–234.
25. **Poutrel, B., A. Boutonnier, L. Sutra, and J. M. Fournier.** 1988. Prevalence of capsular polysaccharide types 5 and 8 among *Staphylococcus aureus* isolates from cow, goat and ewe milk. J. Clin. Microbiol. **26:**38–40.
26. **Poutrel, B., C. Mendolia, L. Sutra, and J. M. Fournier.** 1990. Reactivity of coagulase-negative staphylococci isolated from cow and goat milk with monoclonal antibodies to *Staphylococcus aureus* capsular polysaccharide types 5 and 8. J. Clin. Microbiol. **28:**358–360.
27. **Poutrel, B., and L. Sutra.** 1993. Type 5 and 8 capsular polysaccharides are expressed by *Staphylococcus aureus* isolates from rabbits, poultry, pigs, and horses. J. Clin. Microbiol. **31:**467–469.
28. **Rather, P. N., A. P. Davis, B. J. Wilkinson.** 1986. Slime production by bovine milk *Staphylococcus aureus* and identification of coagulase-negative staphylococcal isolates. J. Clin. Microbiol. **23:**858–862.
29. **Recsei, P., B. Kreiswirth, M. O'Reilly, P. Schlievert, A. Gruss, and R. P. Novick.** 1986. Regulation of exoprotein gene expression in *Staphylococcus aureus* by *agr.* Mol. Gen. Genet. **202:**58–61.
30. **Sompolinsky, D., Z. Samra, W. W. Karakawa, W. F. Vann, R. Schneerson, and Z. Malik.** 1985. Encapsulation and capsular types in isolates of *Staphylococcus aureus* from different sources and relationship to phage types. J. Clin. Microbiol. **22:**828–834.
31. **Stringfellow, W. T., B. Dassy, M. Lieb, and J. M. Fournier.** 1991. *Staphylococcus aureus* growth and type 5 capsular polysaccharide production in synthetic media. Appl. Environ. Microbiol. **57:**618–621.
32. **Sutra, L., C. Mendolia, P. Rainard, and B. Poutrel.** 1990. Encapsulation of *Staphylococcus aureus* isolates from mastitic milk. Relationship between capsular polysaccharide types 5 and 8 and colony morphology in serum-soft agar, clumping factor, teichoic acid and protein A. J. Clin. Microbiol. **28:** 447–451.
33. **Sutra, L., and B. Poutrel.** 1990. Detection of capsular polysaccharide in milk of cows with natural intramammary infection caused by *Staphylococcus aureus*. Am. J. Vet. Res. **51:**1857–1859.
34. **Sutra, L., P. Rainard, and B. Poutrel.** 1990. Phagocytosis of mastitis isolates of *Staphylococcus aureus* and expression of type 5 capsular polysaccharide are influenced by growth in the presence of milk. J. Clin. Microbiol. **28:**2253–2258.
35. **Taylor, D., and K. T. Holland.** 1989. Amino acid requirements for the growth and production of some extracellular products of *Staphylococcus aureus*. J. Appl. Bacteriol. **66:**319–329.
36. **Watson, D. L., and N. A. Watson.** 1989. Expression of a pseudocapsule by *Staphylococcus aureus*: influence of cultural conditions and relevance to mastitis. Res. Vet. Sci. **47:**152–157.
37. **Xu, S., R. D. Arbeit, and J. C. Lee.** 1992. Phagocytic killing of encapsulated and microencapsulated *Staphylococcus aureus* by human polymorphonuclear leukocytes. Infect. Immun. **60:**1358–1362.
38. **Yoshida, K., and R. D. Ekstedt.** 1968. Relation of mucoid growth of *Staphylococcus aureus* to clumping factor reaction, morphology in serum-soft agar, and virulence. J. Bacteriol. **96:**902–908.
39. **Yoshida, K., M. Takahashi, and Y. Takeuchi.** 1969. Pseudocompact-type growth and conversion of growth types of strains of *Staphylococcus aureus* in vitro and in vivo. J. Bacteriol. **100:**162–166.