APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1991, p. 618–621
0099-2240/91/020618-04$02.00/0

Vol. 57, No. 2

Staphylococcus aureus Growth and Type 5 Capsular Polysaccharide Production in Synthetic Media

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Received 20 July 1990/Accepted 26 November 1990

The production of type 5 capsular polysaccharide by *Staphylococcus aureus* in synthetic media was investigated. The influence of medium components on capsular polysaccharide synthesis appeared to relate to the presence or absence of the component rather than to concentration gradient. The production of type 5 capsular polysaccharide was linked to energy availability and energy source, but not to carbohydrate concentration or carbon/nitrogen ratio. Regulation of capsular polysaccharide production by *S. aureus* in response to medium changes would appear to differ from that typically displayed in other organisms that produce polysaccharides.

Staphylococcus aureus produces capsular polysaccharides (CP) that have been defined antigenically into at least eight serotypes (16, 17). Two of these, types 5 and 8, have been found to account for between 70 and 90% of all clinical isolates (1, 5, 8, 9, 13). The development of a synthetic medium is desirable for the study of product formation and physiology and simplifies isolation of polysaccharides for chemical characterization and clinical investigation.

The objective of this study was to develop a synthetic medium for the production of type 5 CP by *S. aureus* and then simplify this medium, identify required components, and examine the influence of individual components on type 5 CP production. The approach suggested by Monod (22) was taken to identify limiting factors and energy sources in the synthetic medium and their influence on CP production.

S. aureus Reynolds, the prototype strain for type 5 CP, was used (10, 17). The strain was taken from lyophilized stock culture and maintained on Columbia medium (no. 0944; Difco Laboratories, Detroit, Mich.) agar slants at room temperature. Columbia broth (no. 0944; Difco) was used as a preculture medium for all experiments presented here in an effort to minimize variation from acclimation (6, 14, 18). All cultures and precultures were incubated at 37°C on a rotary shaker. Precultures were washed and resuspended in physiological buffered saline, and 0.1 ml of inoculum was used per 20 ml of synthetic medium. Total type 5 CP concentration (cell-bound CP plus cell-free CP) was determined by a two-step inhibition enzyme-linked immunosorbent assay (ELISA), using monoclonal antibodies as described previously (5).

Bacterial growth yield (Y_g) is defined as the final optical density (OD) minus the initial OD measured immediately after inoculation. One OD unit, measured at 620 nm, was equal to 0.40 g of bacterial dry cell mass per liter. Growth rate (μ) was calculated by using at least five OD measurements in the log growth phase. Calculations made with less than five measurements are presented as "estimated μ." Specific type 5 CP yield (Y_{CP5}) is defined as the total type 5 CP (nanograms per milliliter) divided by the corresponding OD value. The Y_{CP5} measured in the log growth phase is designated log-Y_{CP5}, and the Y_{CP5} measured in the stationary phase is designated final-Y_{CP5}.

Demonstration of CP production was first attempted by using commercially available mammalian cell tissue culture medium (Eagle medium; GIBCO, France) supplemented with vitamins and other components (Table 1). Supplemented Eagle medium supported good growth and type 5 CP production (final OD = 1.37; μ = 0.534/h; final-Y_{CP5} = 3,784 ng/ml · OD).

We wanted to make a simpler medium that would still give high type 5 CP production and to investigate the necessity of individual medium components for CP production. Frantz medium, which was adapted for CP production by *Neisseria meningitidis* (11), was modified for the cultivation of *S. aureus* and the production of type 5 CP. A synthetic Frantz medium (SFM) was developed based on the amino acid composition of yeast extract (3) supplemented with lactose, additional glutamic acid, and salts (Table 1). SFM gave superior growth (4.08 OD) to supplemented Eagle medium, but specific CP production was similar (final-Y_{CP5} = 3,107 ng/ml · OD) and the growth rate was less (μ = 0.118).

The concentrations of all amino acids in SFM, except cystine, threonine, and glutamic acid, were reduced to 1, 10, and 50% of the concentrations found in full-strength SFM (respectively, 1% SFM, 10% SFM, and 50% SFM) to determine the influence of amino acid concentration on growth and CP production in this medium. Reducing the concentration of amino acids resulted in less growth, demonstrating that this medium is limited by one or more of the 10 amino acids which were diluted. The lowest concentration (1% SFM) supported a significantly lower final-Y_{CP5} than 50% SFM and full-strength SFM (α = 0.05). The 10% SFM yielded approximately the same amount of cell mass as supplemented Eagle medium. The formula for this medium is given in Table 1.

Components were eliminated from SFM individually to determine their effect on type 5 CP production and growth (Table 2). *S. aureus* Reynolds did not grow to any significant extent (final OD, <0.1) when arginine, histidine, isoleucine, leucine, valine, or magnesium chloride was eliminated from SFM.

Ten amino acids were required for growth by this strain: arginine, cystine, histidine, isoleucine, leucine, lysine, me-

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TABLE 1. Synthetic media for *S. aureus* Reynolds growth and CP production

Compound	g/liter in given medium					
	SEM[a]	SFM	10% SFM	Base-10[b]	Base-16[c]	Base-21[d]
Arginine	0.126	0.8	0.08	0.6	0.6	0.6
Histidine	0.042	0.4	0.04	0.6	0.6	0.6
Isoleucine	0.052	1.0	0.10	0.6	0.6	0.6
Leucine	0.052	1.4	0.14	0.6	0.6	0.6
Lysine	0.073	1.4	0.14	0.6	0.6	0.6
Methionine	0.015	0.4	0.04	0.6	0.6	0.6
Valine	0.046	1.0	0.10	0.6	0.6	0.6
Cystine	0.036	0.4	0.40	0.6	0.6	0.6
Threonine	0.048	0.8	0.80	0.6	0.6	0.6
Phenylalanine	0.032	0.8	0.08	0.6	0.6	0.6
Proline	0.115				0.6	0.6
Aspartic acid	0.133				0.6	0.6
Alanine	0.089				0.6	0.6
Serine	0.105				0.6	0.6
Glycine	0.075				0.6	0.6
Ornithine					0.6	0.6
Tyrosine	0.036	0.8	0.08			0.6
Tryptophan	0.010	0.2	0.02			0.6
Glutamine						0.6
Hydroxyproline						0.6
Asparagine	0.150					
Glutamic acid	1.470	1.30	1.30			0.6
Lactose	2.000	2.00	2.00			
$MgCl_2 \cdot 6H_2O$	0.400	0.40	0.40	0.20	0.20	0.20
NaH_2PO_4	5.520	5.50	5.52	0.28	0.28	0.28
K_2HPO_4	9.200	9.20	9.20	2.25	2.25	2.25
NH_4Cl	6.000	6.00	6.00			
KCl	0.540	0.54	0.54			
NaCl	6.000	6.00	6.00	6.00	6.00	6.00
$FeCl_3 \cdot 6H_2O$	0.120	0.12	0.12	0.006	0.006	0.006
Biotin	0.0006	0.0006	0.0006	0.0001	0.0001	0.0001
Nicotinamide	0.048	0.048	0.048	0.008	0.008	0.008
Thiamine	0.012	0.012	0.012	0.002	0.002	0.002

[a] SEM, Supplemented Eagle medium was made by using modified Eagle medium.
[b] Medium with 10 amino acids.
[c] Medium with 16 amino acids.
[d] Medium with 21 amino acids.

thionine, phenylalanine, threonine, and valine. The standard utilized in this study to define required amino acids was growth in excess of 0.5 OD unit, as our objective emphasized the development of a medium useful for production of type 5 CP. This standard is generally more rigorous than that utilized in other nutritional studies (6, 21). By using a standard of detectable growth, only arginine, histidine, leucine, and valine would be identified as absolute requirements.

In SFM, final-Y_{CP5} was found to be independent of total bacterial growth, carbon/nitrogen ratio (0.71 to 5.16), iron concentration (0.0012 to 0.165 g/liter), calcium (0 and 0.054 g/liter), magnesium (0.0239 to 0.479 g/liter), or sodium (1.60 to 3.51 g/liter). This is in contrast to studies conducted with most other organisms (2, 7, 12, 19, 20, 23, 25–28).

Based on previous results, a new medium was formulated for testing. In this medium, all amino acids were added at the same concentration to simplify formulation, and the medium was designated base-10 for the 10 amino acids it contained. Base-10 medium contained all components present in SFM except lactose, glutamic acid, tyrosine, tryptophan, ammonia, and KCl (Table 1).

This formulation did not support adequate growth, but approximately one inoculum of five would grow in this medium and reach an OD as high as 0.3 unit (maximum) after 72 h. When the deleted components were added back individually, it was found that the addition of either lactose or glutamic acid resulted in luxuriant growth. It was therefore concluded that base-10 medium supplied the essential nutrients required by *S. aureus* Reynolds, but required the

TABLE 2. Effect of deleting components of SFM on total *S. aureus* Reynolds growth and type 5 CP yield

Component deleted	Mean final OD	SD	Mean final-Y_{CP5} (ng/ml · OD)	SD	No. of expt
None	4.08	0.61	3,107	2,496	10
Lactose	2.97	0.23	152[a]	148	3
Glutamic acid	3.77	0.23	3,069	1,864	3
Cystine	0.15[a]	0.11	700[a]	509	3
Tyrosine	3.57	0.38	7,674[a]	4,379	3
NH_4Cl	4.96	0.21	518[a]	198	3
Tryptophan	2.10[a]	1.47	5,941	2,168	3
Threonine	0.29[a]	0.05	2,203	1,480	3
Phenylalanine	0.23[a]	0.02	2,115	919	3
Arginine[b]	0.006	0.001	1,314	148	3
Histidine[b]	0.008	0.003	933	404	3
Isoleucine[b]	0.028	0.028	733	252	3
Leucine[b]	0.007	0.03	1,166	404	3
Lysine[c]	0.13[a]	0.08	173[a]	110	3
Methionine	0.145[a]	0.065	1,900	584	3
Valine[b]	0.007	0.03	1,167	404	3
$FeCl_3 \cdot 6H_2O$	0.225	0.041	5,676	538	4
$MgCl_2 \cdot 6H_2O$[b]	0.085	0.037	5,807	1,994	4

[a] Significantly different from complete medium at $\alpha = 0.05$, analysis of variance on natural log conversion of the data; OD > 0.1 only.
[b] Not included in statistical analysis; OD < 0.1 for all data.
[c] Lysine monohydrochloride.

TABLE 3. Energy sources utilized by *S. aureus* Reynolds and associated CP production

Medium	Substrate concn (g/liter)	Final OD	Final-Y_{CP5}	Estimated μ[a]	Final pH
Base-10[b]		0.02	ND[c]	ND	ND
Base-10 + lactose	1.0	1.05	1,809	0.148	6.20
Base-10 + glutamic acid	1.2	2.22	999	0.156	7.78
Base-10 + glutamate[d]	1.2	1.74	1,034	0.194	7.67
Base-10 + ornithine	0.6	0.13	1,154	0.078	6.44
Base-10 + proline	0.6	1.80	815	0.141	7.02
Base-10 + aspartic acid	0.6	1.00	1,535	0.073	6.65
Base-10 + alanine	0.6	0.02	ND	ND	ND
Base-10 + serine	0.6	0.42	1,352	0.081	6.57
Base-10 + glycine	0.6	0.02	ND	ND	ND
Base-10 + glutamine	0.6	1.26	887	0.101	6.96
Base-10 + hydroxyproline	0.6	0.14	857	0.081	6.45
Base-10 + tryptophan	0.6	0.09	ND	ND	ND
Base-10 + tyrosine	0.6	0.02	ND	ND	ND
Base-10 + pyruvate	1.0	0.28	2,667	0.081	ND
Base-10 + acetate	1.0	0.02	ND	ND	ND
Base-16[b]		3.12	2,699	0.168	7.75
Base-21[b]		6.75	3,753	0.265	8.15
Base-16 + lactose	1.0	1.35	2,666	0.136	ND
Base-16 + glutamic acid	0.6	3.00	6,000	0.142	ND

[a] Estimated growth rate calculated from less than five datum points.
[b] See Table 1 for formulation.
[c] ND, Not determined.
[d] Monosodium glutamate.

TABLE 4. Comparison of lactose- and glutamate-supplemented base-10 media for production of type 5 CP by *S. aureus* Reynolds

Medium[a]	Y_g (OD units)	μ (h^{-1})	Log-Y_{CP5} (ng/ml · OD)	Final-Y_{CP5} (ng/ml · OD)	Ratio, final-Y_{CP5}/ log-Y_{CP5}
Base-10 + lactose	1.05	0.117	7,149	18,969	2.65
Base-10 + glutamate	1.71	0.192	2,061	939	0.46

[a] Lactose at 1 g/liter; monosodium glutamate at 1.2 g/liter.

addition of an easily utilizable energy source to support significant growth.

Table 3 presents results with base-10 medium supplemented with various additional compounds. All compounds that supported growth also supported CP production. Media containing multiple amino acid additions tended to have greater final-Y_{CP5} values. Significant final-Y_{CP5} values were obtained even in the absence of lactose. Further study was carried out with lactose and glutamate as supplements to base-10 medium.

Comparison of final-Y_{CP5} for the two substrates shows that lactose was the superior substrate for CP production even though greater total growth was achieved with glutamate (Table 4). Growth yield was superior per mole with lactose, but more growth was achieved per weight with monosodium glutamate. Lactose supported better CP production on both a molar and a weight basis than monosodium glutamate. The log-Y_{CP5} was also greater with lactose despite the greater growth rate with glutamate.

The influence of amino acid concentration on growth and CP production was investigated in base-10 medium supplemented with 1.0 g of lactose per liter as an energy source. As was found for SFM, the growth yield curve for amino acid concentration did not exhibit a simple relationship (Fig. 1). In contrast to SFM, base-10 medium was not amino acid limited. The concentration of amino acids and the ratio of amino acids to lactose did not influence final-Y_{CP5}.

Amino acid concentration was maintained at 0.6 g/liter and lactose concentration was varied between 0.1 and 10 g/liter. Growth increased linearly with increased lactose concentration to a maximum OD of approximately 2.5 (Fig. 2). At concentrations of ≥2 g of lactose per liter, acid production exceeded buffer capacity, resulting in a final pH of <6.0, and the maximum achievable final growth yield was pH limited. Final-Y_{CP5} was not influenced by lactose concentration in this range.

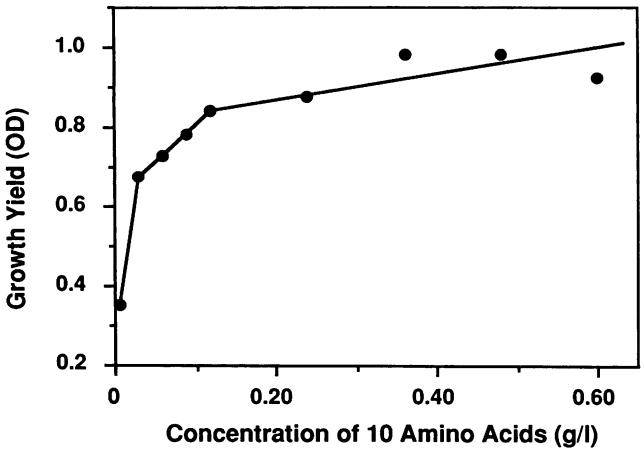

FIG. 1. Growth yield of *S. aureus* Reynolds in response to changing amino acid concentration in base-10 medium supplemented with lactose at 1.0 g/liter.

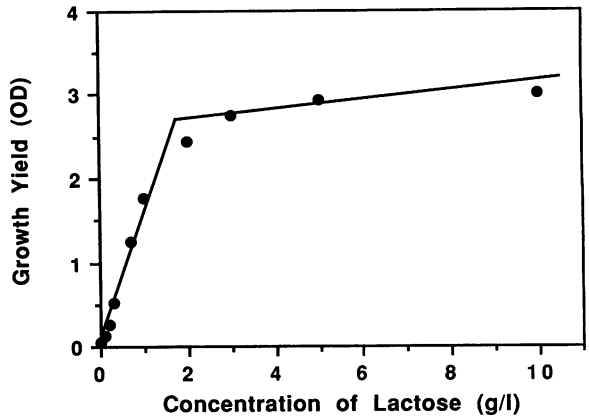

FIG. 2. Growth yield of *S. aureus* Reynolds in response to changing lactose concentration in base-10 medium.

The relation between growth and glutamate as an energy source was also examined in base-10 medium. The relationship appeared to be biphasic (Fig. 3). The first slope occurred at glutamate concentrations of <1 g/liter. The second relation was observed at between 1 and 5 g of glutamate per liter. At glutamate concentrations of >5 g/liter, final pH rose above 8.0 and the maximum growth yield was limited by the buffer capacity of the medium. Maximum growth was reached at an OD of 4.5. Final-Y_{CP5} was not correlated with glutamate concentration.

In conclusion, it was found that the production of type 5 CP was linked to energy availability and energy source, but not to carbohydrate concentration or carbon/nitrogen ratio. The influence of medium components on CP synthesis appeared to relate more to the presence or absence of the component than to concentration gradient. However, in contrast to results obtained with other products of *S. aureus*, no individual medium component was identified as being an absolute controlling factor for type 5 CP production (4, 15,

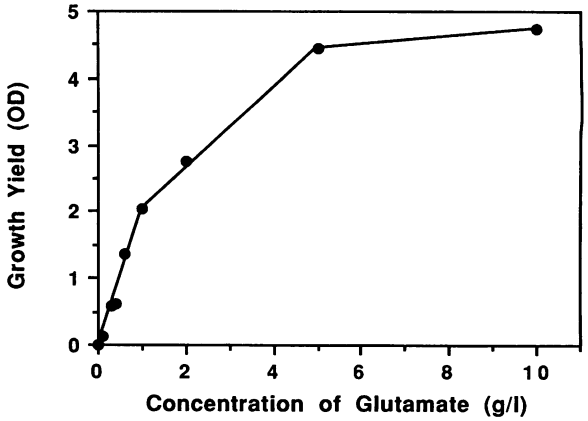

FIG. 3. Growth yield of *S. aureus* Reynolds in response to changing monosodium glutamate concentration in base-10 medium.

21, 24). Regulation of CP production by *S. aureus* in response to medium changes would appear to differ from that typically displayed in other organisms that produce polysaccharides (2, 7, 12, 19, 20, 23, 25–28). The results we present may be useful as a basis for further research to delineate the biosynthetic pathways of *S. aureus* CP production.

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

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