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International Journal of Food Microbiology 36 (1997) 77–82

International Journal of Food Microbiology

Short communication

Amino acid requirements for the growth and enterotoxin production by *Staphylococcus aureus* in chemically defined media

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Received 3 July 1996; received in revised form 30 December 1996; accepted 30 December 1996

Abstract

The production of enterotoxins A, B, and C by five strains of *Staphylococcus aureus* was studied in a series of defined media, each differing from the complete defined medium in the lack of one amino acid. Valine was required for growth; arginine and cystine for growth and enterotoxin production. Omission of individual amino acid affected in different ways the yields of the toxins produced. © 1997 Elsevier Science B.V.

Keywords: Staphylococcus aureus; Enterotoxin; Amino acid requirement

1. Introduction

Staphylococcal food poisoning is a frequent foodborne disease that occurs in most countries of the world (Anunciacao et al., 1995) and caused by ingestion of food containing staphylococcal enterotoxin produced by certain strains of *Staphylococcus aureus* (Wieneke, 1991). Enterotoxins described to date have been designated enterotoxins A (SEA) through E (SEE). At least 75–87% of staphylococcal food poisoning outbreaks are due to SEA (Wieneke and Gilbert, 1987; Bergdoll, 1989) which may be produced under such conditions that may not support production of other enterotoxins.

Water activity (a_w) is an important factor that affects growth and enterotoxin production by staphylococci and there is currently great interest in the relationship between the accumulation of

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PII S0168-1605(97)01250-6

proline and the decline of enterotoxin production at lower a_w. *S. aureus*, which is able to grow at a reduced a_w, accumulates proline in their free intracellular pool (Anderson and Witter, 1982). We think that enterotoxin synthesis is strongly suppressed by proline accumulation in the amino acid pool at low a_w (Onoue et al., 1987). The aim of this work was to study the growth and enterotoxin formation by *S. aureus* strains, producing one or two of the toxins SEA, SEB and SEC in a series of defined media. Each medium differed from the complete defined medium in the lack of one amino acid.

2. Materials and methods

2.1. Strains used

Staphylococcus aureus FRI-100, S-6 and FRI-361 were obtained from M. S. Bergdoll (Food Research Institute, University of Wisconsin, Madison, WI). These organisms produce SEA, SEB and SEC, respectively. Strain K858, which produces SEA plus SEB, was associated with a food poisoning outbreak and isolated in this laboratory. Strain T7436, which produces SEA plus SEC, was obtained from H. Igarashi (Tokyo Metropolitan Research Laboratory of Public Health, Tokyo).

2.2. Chemically defined media

The composition of the chemically defined synthetic medium is shown in Table 1 (Pattee and Neveln, 1975). Amino acid requirements were determined in a series of defined media, each differing from the above medium in the lack of one amino acid (Emmett and Kloos, 1975).

2.3. Preparation of inoculum

A culture (1 ml) was diluted in 9 ml of the defined medium and it was further diluted tenfold serially. From an appropriate dilution 10 μl were inoculated to 50 ml of a defined medium (10^3 cfu/ml).

2.4. Culture conditions

All cultures were each grown in 50 ml of a defined medium in a 250-ml Erlenmeyer flask incubated at 35°C on a gyratory shaker at 100 rpm for the desired period of time. Samples of 5 ml were removed at different stages of incubation for measuring of growth and enterotoxin.

Table 1
Composition of chemically defined synthetic broth (Pattee and Neveln, 1975)

Ingredient	Amount
Amino acids	
L-glutamic acids	100 mg
L-serine	30 ng
L-methionine	3 mg
L-tyrosine	50 mg
L-alanine	60 mg
L-lysine	50 mg
L-threonine	30 mg
L-phenylalanine	40 mg
L-histidine	20 mg
Glycine	50 mg
L-tryptophan	10 mg
L-isoleucine	30 mg
L-valine	80 mg
L-leucine	90 mg
L-aspartic acid	90 mg
L-arginine	50 mg
L-proline	80 mg
L-cystine	20 mg
Glucose	5 g
Salts	
K_2HPO_4	7 g
KH_2PO_4	2 g
$Na_3citrate \cdot 2H_2O$	0.4 g
$MgSO_4$	0.05 g
$(NH_4)_2SO_4$	1 g
Vitamins	
Thiamine	1 mg
Niacin	1.2 mg
Biotin	0.005 mg
Ca pantothenate	0.25 mg
Purines and pyrimidines	
Adenine	5 mg
Guanine	5 mg
Cytosine	5 mg
Uracil	5 mg
Thymine	20 mg
Deionized water	1000 ml

Table 2
Growth and enterotoxin production by *Staphylococcus aureus* FRI-100, S-6 and FRI-361 in chemically defined synthetic media after 48 h of aerobic incubation

Medium	*S.aureus* FRI-100[b]		*S. aureus* S-6[b]		*S. aureus* FRI-361[b]	
	Viable count (cfu/ml)	SEA yield[c] (ng/ml) (%)	Viable count (cfu/ml)	SEB yield[c] (ng/ml) (%)	Viable count (cfu/ml)	SEC yield[c] (ng/ml) (%)
18AA[a]	1.6×10^9	1600 (100)	9.3×10^8	128 000 (100)	1.5×10^8	6400 (100)
18AA less Asp	6.8×10^8	640 (40)	3.3×10^8	16 000 (13)	1.3×10^8	3200 (50)
18AA less Ileu	2.5×10^9	1280 (80)	9.8×10^8	16 000 (13)	2.1×10^8	3200 (50)
18AA less Ala	6.7×10^8	640 (40)	8.0×10^8	16 000 (13)	6.2×10^7	1600 (25)
18AA less Lys	1.1×10^9	800 (50)	7.9×10^8	16 000 (13)	1.4×10^8	3200 (50)
18AA less Try	7.9×10^8	1280 (80)	6.4×10^8	32 000 (25)	2.3×10^7	3200 (50)
18AA less Glu	6.0×10^8	640 (40)	3.1×10^9	12 800 (10)	3.4×10^8	3200 (50)
18AA less Ser	1.4×10^8	640 (40)	1.3×10^9	32 000 (25)	8.0×10^7	800 (13)
18AA less Met	8.4×10^8	640 (40)	5.2×10^8	32 000 (25)	3.0×10^8	3200 (50)
18AA less His	1.6×10^9	1280 (80)	8.2×10^8	32 000 (25)	2.5×10^8	3200 (50)
18AA less Tyr	1.1×10^9	1280 (80)	4.3×10^8	32 000 (25)	3.6×10^8	800 (13)
18AA less Thr	1.4×10^9	640 (40)	5.1×10^8	16 000 (13)	3.5×10^8	3200 (50)
18AA less Leu	6.8×10^8	320 (20)	2.6×10^7	40 (0.03)	3.2×10^8	3200 (50)
18AA less Gly	1.1×10^9	640 (40)	1.4×10^9	32 000 (25)	2.4×10^5	<2
18AA less Phe	2.8×10^9	1280 (80)	1.1×10^9	16 000 (13)	5.4×10^8	800 (13)
18AA less Pro	7.0×10^8	<2	1.0×10^6	2 (<0.01)	1.7×10^7	160 (2.5)
18AA less Arg	7.0×10^5	<2	1.0×10^5	64 (0.05)	3.4×10^5	2 (0.03)
18AA less Val	3.6×10^2	<2	4.4×10^3	<2	7.4×10^2	<2
18AA less Cys	2.0×10^6	<2	4.0×10^5	4 (<0.01)	3.8×10^7	16 (0.25)

[a]The 18 amino acids are: aspartic acid (Asp), isoleucine (Ileu), alanine (Ala), lysine (Lys), tryptophan (Try), glutamic acid (Glu), serine (Ser), methionine (Met), histidine (His), tyrosine (Tyr), threonine (Thr), leucine (Leu), glycine (Gly), phenylalanine (Phe), proline (Pro), arginine (Arg), valine (Val), and cystine (Cys).
[b]Inoculum sizes were 10^3 cfu/ml.
[c]Percent of the yield in 18AA medium is presented in parentheses.

Table 3
Growth and enterotoxin production by *Staphylococcus aureus* K858 and T7436 in chemically defined synthetic media after 48 h of aerobic incubation

Medium	*S. aureus* K858[b]				*S. aureus* T7436[b]			
	Viable count (cfu/ml)	SEA yield[c] (ng/ml) (%)	SEB yield[c] (ng/ml) (%)	Ratio SEA/SEB (%)	Viable count (cfu/ml)	SEA yield[c] (ng/ml) (%)	SEC yield[c] (ng/ml) (%)	Ratio SEA/SEC (%)
18AA[a]	5.8×10^8	64 (100)	2560 (100)	1.0	6.2×10^7	256 (100)	64 000 (100)	1.0
18AA less Asp	4.5×10^8	16 (25)	320 (12.5)	2.0	4.4×10^7	256 (100)	64 000 (100)	1.0
18AA less Ileu	4.2×10^8	4 (6.25)	640 (25)	0.25	2.3×10^8	256 (100)	4000 (6.25)	16.0
18AA less Ala	4.4×10^8	16 (25)	1280 (50)	0.5	9.5×10^7	80 (31)	4000 (6.25)	5.0
18AA less Lys	3.2×10^8	8 (12.5)	2560 (100)	0.125	1.0×10^8	128 (50)	4000 (6.25)	8.0
18AA less Try	3.2×10^8	8 (12.5)	640 (25)	0.5	1.4×10^8	256 (100)	4000 (6.25)	16.0
18AA less Glu	5.1×10^8	64 (100)	64 (2.5)	40.0	9.5×10^7	16 (6.3)	200 (0.31)	20.0
18AA less Ser	3.7×10^8	64 (100)	320 (12.5)	8.0	4.9×10^7	32 (12.5)	2000 (3.13)	4.0
18AA less Met	5.6×10^6	8 (12.5)	8 (0.31)	40.0	1.1×10^8	128 (50)	32 000 (50)	1.0
18AA less His	3.6×10^8	8 (12.5)	2560 (100)	0.125	7.5×10^7	64 (25)	64 000 (100)	0.25
18AA less Tyr	2.0×10^6	8 (12.5)	16 (0.63)	20.0	6.8×10^7	64 (25)	3200 (5.0)	5.0
18AA less Thr	2.0×10^7	32 (50)	2 (0.08)	625.0	7.2×10^7	128 (50)	64 000 (100)	0.5
18AA less Leu	3.4×10^4	<2	<2		1.3×10^7	128 (50)	64 000 (100)	0.5
18AA less Gly	3.3×10^8	16 (25)	1280 (50)	0.5	6.8×10^7	64 (25)	4000 (6.25)	4.0
18AA less Phe	9.0×10^4	<2	2 (0.08)		2.0×10^8	128 (50)	64 000 (100)	0.5
18AA less Pro	2.2×10^6	<2	2 (0.08)		2.8×10^6	32 (12.5)	8 (0.01)	1250.0
18AA less Arg	8.0×10^5	<2	<2		3.3×10^4	<2	<2	
18AA less Val	6.2×10^3	<2	<2		1.0×10^2	<2	<2	
18AA less Cys	5.9×10^5	<2	<2		4.4×10^5	<2	16 (0.03)	

[a] Abbreviations are the same as in Table 2.
[b] Inoculum sizes were 10^3 cfu/ml.
[c] Percent of the yield in 18AA medium is presented in parentheses.

2.5. Viable count of staphylococci

Samples (1 ml) of the cultures were diluted in 9 ml of buffered peptone water and 0.1 ml of appropriate dilutions were spread onto salt egg yolk agar (Nissui Seiyaku, Tokyo). The plates were incubated for 48 h at 37°C.

2.6. Assay for staphylococcal enterotoxin

The cultures were centrifuged at 25 000 × g at 4°C for 15 min and enterotoxin assayed with an RPLA (reversed passive latex agglutination) test kit (SET-RPLA) for detection of enterotoxin supply by Denka Seiken, Tokyo (Igarashi et al., 1986).

3. Results and discussion

Amino acid requirements were determined for five *S. aureus* strains. Each amino acid was singly deprived of from the defined medium, and growth and enterotoxin production of *S. aureus* strains producing SEA, SEB and SEC one or two of the toxins were examined (Tables 2 and 3). Similar results were obtained in five experiments each with one strain.

S. aureus required several amino acids. Valine in all cases was required for growth and arginine and cystine for growth and enterotoxin production. The effects of proline on growth and enterotoxin production varied with the strain.

Enterotoxins are single polypeptide chains that contain relatively large amounts of lysine, aspartic and glutamic acids, leucine and tyrosine (Bergdoll, 1989). *S. aureus* did not require these amino acids for growth, but deprivation of any of them reduced enterotoxin production. Amino acid compositions of SEA, SED and SEE are similar and so are those of SEB and SEC, the number of proline residues in SEA and SED is four, whereas in SEB, SEC, and SEE, it is six or eight (Schantz et al., 1972; Huang and Bergdoll, 1970; Huang et al., 1967; Chang and Bergdoll, 1979; Borja et al., 1972).

In the absence of threonine, SEA production by strain K858 decreased by 50% of that in the complete defined medium, whereas SEB production decreased by only 0.08%. So, the ratio of percent production of SEA to that of SEB was 625. In the absence of glutamic acid or methionine, the corresponding ratio was 40.

In the absence of tyrosine, the ratio was 20 and the ratios in the absence of any other amino acid were smaller than this. In the absence of proline, the ratio of percent production of SEA to that of SEC by strain T7436 was 1250 (Table 3). In the absence of glutamic acid, the ratio was 20. The increase or decrease in the ratio varied depending on the amino acid. Overall, SEA production appeared to be much less responsive to deprivation of any amino acid than was SEB or SEC production.

In a previous paper dealing with the production of enterotoxins by *S. aureus* strain T7436 with NaCl as a humectant, we reported that SEA production was much less affected by a_w than was SEC (Onoue et al., 1987). Perhaps the same phenomenon is occurring in both proline deprivation in the proline-lacking medium and in the intracellular accumulation of proline at a lower a_w.

These results suggest that deprivation of individual amino acids in defined medium affects the yields of two different toxins produced by the same strain, in different ways.

Acknowledgements

The authors thank Dr T. Terayama of Tokyo Health Service Association for his helpful discussions. We thank Dr G. Sakaguchi of Japan Food Research Laboratories for his critical reading of the manuscript.

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