Nutritional Requirements for Growth of *Aerococcus viridans*

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SUMMARY

Vitamin requirements for growth in a casein hydrolysate medium were determined for 27 strains of *Aerococcus viridans* from diverse sources. Pantothenic acid, nicotinic acid and biotin were either absolutely required by, or markedly stimulatory to, all strains; none required thiamin, riboflavin, pyridoxine, folic acid or folinic acid. Tween 80 replaced the biotin requirement of most strains. Amino acid requirements were not sharply defined and varied from strain to strain. As the amino acid composition of the medium was simplified the amount of growth was decreased and most strains would not grow when biotin was replaced by Tween 80. A single purine base was required: either guanine or xanthine alone satisfied this requirement for each of the strains tested; adenine was a suitable alternative source for some strains. Exogenous pyrimidine was not required.

INTRODUCTION

There is increasing evidence that a relatively homogenous group of Gram-positive tetrad-forming cocci is widely distributed in our environment. These organisms may be referred to as *Aerococcus viridans* (Williams, Hirsch & Cowan, 1953) or *Pediococcus homari* (Deibel & Niven, 1960), and appear to be indistinguishable from the lobster pathogen *Gaffkya homari* (Aaronson, 1956; Deibel & Niven, 1960). These organisms are found in the air of occupied rooms (Williams *et al.* 1953), in meat curing brines (Deibel & Niven, 1960), in medicine bottles (Clausen, 1964), on raw and processed vegetables (Mundt, Graham & McCarty, 1967), in hospital environments (Kerbaugh & Evans, 1968) and in a variety of human infections (Colman, 1967). There have not been detailed reports of nutritional studies of these organisms, referred to here as *Aerococcus viridans*, although undocumented references to the requirements of one or two strains are included in papers by Aaronson (1956) and Sakaguchi & Mori (1969). The present study was undertaken to provide a survey of the nutritional requirements of a relatively large collection of cultures from diverse sources.

METHODS

Sources of strains. Included in this study were 14 strains isolated in our laboratory (MK-series) from the hospital environment (Kerbaugh & Evans, 1968), three strains

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received from Baird-Parker (strains 301, 302 and 303), four strains from Clausen (C2, C6, C7 and C9), five strains from Mundt (49-3, 49-7, 49-8, 61-8 and 62-4) and two strains from the American Type Culture Collection (10400 and 11563). Included as control cultures in some experiments were *Gaffkya tetragena* (ATCC 10875), *Pediococcus cerevisiae* (ATCC 8081) and enterococci from our departmental collection.

Media. Stock solutions of vitamins, amino acids, purines, pyrimidines and mineral salts were made in distilled water in acid-cleaned bottles. One ml. of a volatile preservative (Hutner, Cury & Baker, 1958) was added to 100 ml. volumes of these solutions, which were stored at 5°. Test media were adjusted to pH 7.0 to 7.2, dispensed in 10 ml. amounts in 18 mm. wide acid-cleaned borosilicate test-tubes, capped with Morton type stainless steel caps and sterilized at 121° for 13 min. The composition of the casein hydrolysate medium that served as the 'complete' medium for most of these studies is given in Table 1. Folinic acid (1 µg./l.) was added in some experiments. Amino acids (100 mg./l.) of the L-isomeric form replaced the hydrolysed casein in some experiments.

Table 1. *Casein hydrolysate medium for Aerococcus viridans*

Component	Per litre	Component	Per litre
Casein (acid-hydrolysed)	50 ml.	Biotin	100 µg.
L-Cystine	100 mg.	Folic acid	10 µg.
L-Tryptophan	100 mg.	Nicotinic acid	2 mg.
D-Glucose	10 g.	Calcium pantothenate	1 mg.
NH ₄ Cl	1 g.	Pyridoxine HCl	2 mg.
K ₂ HPO ₄	4 g.	Riboflavin	1 mg.
Adenine	5 mg.	Thiamin	1 mg.
Guanine	5 mg.	MgSO ₄ ·7H ₂ O	200 mg.
Uracil	5 mg.	FeSO ₄ ·7H ₂ O	10 mg.
Xanthine	5 mg.	MnSO ₄ ·4H ₂ O	10 mg.

Inoculation and serial transfer. Cultures were transferred three times at 24 hr intervals in BBL Trypticase Soy Broth. Test media were inoculated with one drop/tube and incubated at 30°. Growth was measured turbidimetrically at 24 hr intervals for 3 days, using a B & L Spectronic 20 colorimeter at 520 nm. From each tube of test medium that exhibited growth (optical density of at least 0.10) one drop was serially transferred to another tube of the same test medium. When growth occurred within 24 hr in this second transfer, a drop was transferred from it to a third tube of the medium. This procedure eliminated errors due to carry-over of nutrients in the inoculum. Results are generally presented as the optical density of the third transfer after 72 hr. When growth in the first or second transfer was insufficient to warrant serial transfer, the growth was recorded as 0.

RESULTS

Vitamin requirements. The vitamin requirements were determined for 27 of the 28 strains of *Aerococcus viridans*. One strain (C9) grew too poorly in the casein hydrolysate medium to determine its requirements and addition to the medium of folinic acid and Tween 80 did not elicit improved growth. Data for seven of the cultures are presented in Table 2; similar results were obtained with the other 20 strains. Control cultures that required thiamin, riboflavin, pyridoxine, folic acid and folinic acid were also included in the study to prove that the basal medium was free from contamination with these vitamins.

A three-vitamin casein hydrolysate medium was prepared (with only pantothenic acid, nicotinic acid and biotin) and 24 of the 27 cultures produced essentially the same growth as in the seven-vitamin medium (optical densities within ± 0.10). Of the other three cultures, two grew better in the three-vitamin medium and one grew better in the seven-vitamin medium. Addition of folinic acid to the three-vitamin medium did not stimulate growth of any of the cultures. Tween 80 (0.1 % (v/v)) completely eliminated the biotin requirement of most strains.

Table 2. *Vitamin requirements of representative strains of Aerococcus viridans*

All figures are optical density readings on the third transfer after 72 hr at 30°.

Strain	TSB*	Complete medium†	Vitamin omitted						
			Thi-amin	Ribo-flavin	Pyri-doxine	Panto-thenate	Nicotinic acid	Folate	Biotin
MK-32	0.79	0.53	0.45	0.64	0.52	0	0	0.54	0.24
MK-80	0.84	0.58	0.49	0.56	0.54	0	0	0.60	0
MK-95	0.98	0.70	0.56	0.80	0.75	0	0	0.68	0.37
MK-165	0.85	0.62	0.62	0.64	0.57	0	0	0.59	0
49-3	0.80	0.79	0.69	0.75	0.73	0	0	0.75	0.24
61-8	0.82	0.59	0.55	0.57	0.59	0	0	0.54	0
11563	0.62	0.31	0.21	0.29	0.35	0	0	0.34	0

* BBL Trypticase Soy Broth

† Medium described in Table 1.

Table 3. *Purine and pyrimidine requirements of representative strains of Aerococcus viridans*

All figures are optical density readings.

Medium	Strain number					
	MK-3	MK-53	MK-95	MK-132	MK-201	303
Complete*	0.54	0.53	0.63	0.55	0.55	0.69
Basal†	0.18	0	0.07	0	0.09	0.23
+adenine	0.44	0	0.62	0	0	0.42
+guanine	0.45	0.58	0.55	0.56	0.46	0.50
+xanthine	0.46	0.47	0.54	0.47	0.41	0.48
+uracil	0.16	0	0	0	0	0.29

* Medium described in Table 1 with 0.1 % Tween 80 added.

† Medium without purines or pyrimidine.

Amino acid requirements. It was determined that most cultures grew well in a medium with 18 L-amino acids as the nitrogen source, producing 80 to 100 % of the growth in the casein hydrolysate medium. One strain (MK-155) did not grow in the amino acid medium. An attempt was made to determine the specific amino acid requirements of three test strains by omitting individual amino acids from the medium. Cystine, methionine and tryptophan were absolute requirements of these three strains, and valine was required by two strains. Omission of several other amino acids markedly decreased the growth, but the three cultures differed in their response.

A medium containing the 10 amino acids required or apparently stimulatory to one or more of the three test organisms (arginine, glutamic acid, tyrosine, phenylalanine,

leucine, isoleucine, in addition to the four amino acids noted above) supported moderate growth (about 50 % of the growth in the casein hydrolysate medium) of 15 of 20 strains tested with both biotin and Tween 80 in the medium. Omission of biotin from this medium prevented growth of all strains.

Purine and pyrimidine requirements. Omission of the purines and pyrimidine singly from the casein hydrolysate medium did not show any requirement. However, as illustrated in Table 3, simultaneous omission of the purines and pyrimidine allowed little or no growth. Addition of either guanine or xanthine alone produced good growth of all strains tested; adenine alone sufficed for about half of the strains. Uracil was without apparent effect.

DISCUSSION

Despite individual differences among the various strains tested the nutritional requirements of this collection of aerococci seemed to be relatively homogeneous. Their vitamin requirements were particularly uniform, with pantothenic acid, nicotinic acid and biotin required by or markedly stimulatory to all strains and there was no evidence of other vitamin requirements. These vitamin requirements tended to differentiate these organisms from other species of Gram-positive cocci; they lacked the requirement for folinic acid that is characteristic of pediococci (Jensen & Seeley, 1954). Their lack of a requirement for thiamin or riboflavin differed from most streptococci and staphylococci.

Of interest were our results with *Gaffkya tetragena* ATCC 10875, which was found to have a low GC content (33 %) and to differ in other ways from the aerococci (Evans & Schultes, 1969); this culture required only nicotinic acid and thiamin. However, other strains received as *G. tetragena* had quite different vitamin requirements (unpublished observation).

In view of our results it is impossible to give credence to the report of Sakaguchi & Mori (1969) that two strains of aerococci required biotin, pantothenic acid, nicotinic acid, *p*-aminobenzoic acid, pyridoxine, riboflavin, thiamin, folic acid and folinic acid. They did not describe their methods, verify the identity of their cultures or present any data. We also question the report of Aaronson (1956), who implied, also without details of procedure or results, that the two strains he studied required pantothenic acid, nicotinic acid, biotin and thiamin. From our results one might infer a slight stimulatory effect of thiamin on some strains (e.g. MK-95); different basal media and techniques might enhance this effect.

Numerous efforts to select combinations of amino acid supplements that more specifically meet the nitrogen requirement of these organisms have not been successful. Hence, we have not devised what one could call a minimal defined medium for the aerococci. Further studies on the interrelationships between various nutrients such as amino acids and vitamin requirements may be warranted.

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