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Uracil and Pyruvate Requirements for Anaerobic Growth of Staphylococci[1]

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Sixty-six strains of staphylococci recently isolated from human skin and identified as members of the three recognized species of staphylococci, and 21 stock strains representing most of the Baird-Parker subgroups of staphylococci and micrococci were studied. All 16 skin strains of *Staphylococcus aureus* demonstrated weak to moderate anaerobic growth in the basal medium, growth stimulation by either uracil or pyruvate, and best growth when both were added. The 20 skin strains of *S. epidermidis* responded similarly to *S. aureus* but with a tendency toward heavier growth. The 28 isolates of *S. saprophyticus* generally gave little or no growth in the basal medium, no increase due to pyruvate alone, a weak response to uracil alone, and, with three exceptions, gave moderate to good growth when both supplements were present. The Baird-Parker strains from subgroups S-II and S-III responded like *S. epidermidis;* those from subgroups S-V, S-VI, M-1, M-2, M-3, and M-6 generally responded like *S. saprophyticus;* and those from subgroups M-4 and M-5 failed to grow anaerobically in all media.

One of the first studies published on the nutritional requirements of bacteria reported that for anaerobic growth *Staphylococcus aureus* required uracil and pyruvate in addition to the nutrients required for aerobic growth (8). Much later, Jones et al. (4) reported that *S. epidermidis* could grow anaerobically in a complex medium with either glucose or pyruvate as the energy source and that uracil was essential. The biochemical basis for these added nutritional requirements for anaerobic growth of *S. aureus* has been investigated only recently and found to result from the inability of its dihydroorotate dehydrogenase to function under anaerobic conditions and a deficiency in recycling electron transport cofactors (6).

The ability of staphylococci to grow anaerobically has been accepted as a major criterion for differentiating them from the aerobic micrococci (2). However, one of the three recognized species of staphylococci (*S. saprophyticus*) often gives equivocal results on this test, and in some strains only a small number of the individual cells in a population appear to initiate growth under anaerobic conditions (3). The present study was designed to reexamine the uracil and pyruvate requirements for anaerobic growth of *S. aureus* and *S. epidermidis* and to determine the relationship of these nutrients to the anaerobic growth of *S. saprophyticus*.

MATERIALS AND METHODS

Cultures. Two groups of cultures were included in this study: 66 strains of staphylococci that had been isolated recently from human skin and characterized as *S. aureus*, *S. epidermidis*, or *S. saprophyticus* (5, 9): and 21 older stock strains that were classified by Baird-Parker (1) into a series of subgroups that are not specifically correlated with currently accepted species.

Media. Most experiments were conducted using as the basal medium a casein hydrolysate medium slightly modified from that used in previous studies of *Aerococcus viridans* (7). The biotin concentration was reduced to 1 μg/liter, sodium citrate (2 g/liter) was added, and uracil was omitted. Uracil (5 mg/liter) and pyruvate (2.5 g/liter) were added to appropriate portions of experimental media.

Procedure. The experimental media were prepared and tubed in 7.5-ml volumes in 18.0-mm borosilicate tubes, autoclaved at 15 lb/in^2 for 10 min, chilled in an ice bath, inoculated with one loopful of 24-h cultures grown in Trypticase soy broth (BBL), placed in GasPak (BBL) anaerobic jars, and incubated at 35 C for 3 days. Growth was measured as optical density at 600 nm in a Bausch and Lomb Spectronic 20 spectrophotometer.

RESULTS

Skin staphylococci. Table 1 presents data obtained on four representative strains of each

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TABLE 1. *Effect of uracil and pyruvate on anaerobic growth of representative strains of staphylococci from human skin*[a]

Strain	Additions to basal medium			
	None	Uracil	Pyruvate	Uracil and pyruvate
Staphylococcus aureus				
WK-1	0.16	0.45	0.50	0.88
WK-11	0.28	0.70	0.47	0.86
WK-12	0.40	0.83	0.67	1.00
WK-57	0.38	0.83	0.58	1.10
S. epidermidis				
WK-18	0.23	0.64	0.92	1.20
WK-22	0.04	0.54	0.04	0.82
WK-51	0.56	0.93	0.72	1.40
WK-61	0.82	0.84	1.00	1.30
S. saprophyticus				
WK-4	0.06	0.30	0.10	0.90
WK-15	0.03	0.10	0.01	0.35
WK-32	0.00	0.04	0.00	0.67
WK-56	0.00	0.12	0.00	0.78

[a] Growth is expressed as optical density at 600 nm after anaerobic incubation at 35 C for 3 days.

of the three species studied. All 16 strains of *S. aureus* gave weak to moderate growth in the basal medium. Either uracil or pyruvate gave a marked increase in growth, generally in the range of doubling the optical density achieved in the basal medium. The simultaneous addition of uracil and pyruvate resulted in somewhat better growth than the addition of either alone.

The 20 strains of *S. epidermidis* were somewhat less uniform in their response than were the *S. aureus*. Growth in the basal medium ranged from very slight (six strains achieving an optical density of less than 0.10) to heavy (four strains having an optical density of 0.80 or more). Except for the strains with heavy growth in the basal medium (e.g., WK-61) growth was increased by uracil. All but three strains (e.g., WK-22) grew better when pyruvate was added, and best growth was obtained when both pyruvate and uracil were added.

The 28 strains of *S. saprophyticus* showed the most striking requirement for uracil and pyruvate. All of these strains gave little or no growth in the basal medium and in the medium with pyruvate added. The majority also gave little or no growth when uracil was added, but eight strains (e.g., WK-4) gave weak to moderate growth (optical density 0.20 to 0.37). In the medium with both pyruvate and uracil, 21 of the 28 strains gave moderately heavy growth but generally somewhat less than the growth of strains of the other two species, particularly *S. epidermidis*. Three strains of *S. saprophyticus* achieved little or no anaerobic growth even when uracil and pyruvate were present, and four strains (e.g., WK-15) gave weak to moderate growth.

Baird-Parker strains. The results obtained with the 21 stock strains of Baird-Parker are presented in Table 2. The subgroup classification listed was that obtained on these strains when originally studied by Baird-Parker (1). The four strains representing subgroups S-II and S-III gave a response to uracil and pyruvate that is typical of *S. epidermidis* (Table 2). No strains from subgroup S-IV were available for this study. The three strains from S-V and S-VI grew rather poorly, but the one strain that grew best (BP-9) behaved similar to *S. saprophyticus*. The eight strains from subgroups M-1, M-2, and M-3 also gave results that were characteristic of *S. saprophyticus*, except for strain BP-5 that appeared like a weak-growing strain of *S. epidermidis*. The three strains of M-4 and M-5 failed to grow anaerobically and the three strains of M-6 responded like *S. saprophyticus*. Strains of M-7 and M-8 were not included since they are synonymous with the species *Micrococcus luteus* and *M. roseus* that are incapable of anaerobic growth. The deoxyribonucleic acid base composition of 12 of these 21 strains has been determined in other studies (3) and is also listed in Table 2. It can be seen that all of them were in the range that is typical of the genus *Staphylococcus*.

TABLE 2. *Effect of uracil and pyruvate on anaerobic growth of stock strains of biotypes of staphylococci*

Strain	Baird-Parker subgroup	DNA[a] base composition (mol% G+C)	Additions to basal medium			
			None	Uracil	Pyruvate	Uracil and pyruvate
BP-7	S-II		0.49[b]	1.08[b]	0.82[b]	1.10[b]
BP-12	S-II		0.13	0.78	0.97	1.10
BP-2	S-III	32.6	0.07	0.14	0.73	0.73
BP-34	S-III		0.05	0.08	0.40	0.58
BP-49	S-V		0.00	0.08	0.23	0.26
BP-9	S-VI		0.02	0.28	0.06	0.74
BP-21	S-VI	32.4	0.00	0.01	0.00	0.10
BP-19	M-1		0.00	0.00	0.09	0.18
1557	M-1	32.7	0.14	0.20	0.13	0.97
BP-5	M-2	33.2	0.02	0.41	0.46	0.74
BP-15	M-2	32.0	0.00	0.01	0.07	0.72
BP-16	M-2	35.2	0.00	0.27	0.10	0.44
BP-3	M-3	34.7	0.09	0.14	0.14	0.97
BP-4	M-3	33.1	0.02	0.03	0.09	0.55
BP-10	M-3	31.5	0.00	0.06	0.03	0.78
BP-43	M-4		0.00	0.01	0.00	0.00
BP-45	M-5		0.00	0.01	0.00	0.00
BP-47	M-5	33.9	0.00	0.00	0.00	0.00
BP-8	M-6		0.00	0.10	0.03	0.35
BP-23	M-6	32.3	0.00	0.08	0.01	0.24
1463	M-6	33.4	0.08	0.57	0.09	0.88

[a] DNA, Deoxyribonucleic acid.
[b] Optical density at 600 nm after anaerobic incubation at 35 C for 3 days.

Basal medium modifications. Individual deletions from the basal medium demonstrated that the purines (adenine, guanine, and xanthine), citrate, and NH_4Cl were not required for anaerobic growth. A simplified basal medium omitting all of these ingredients was used to determine the requirement for uracil and pyruvate for anaerobic growth of 19 of the skin staphylococci and five of the Baird-Parker strains. The results were essentially the same as were obtained with the more complex basal medium.

DISCUSSION

This study has verified the previous reports that uracil and pyruvate are required for optimum anaerobic growth of *S. aureus* and *S. epidermidis*. However, under the conditions of this study, most strains of these two species achieved moderate to good anaerobic growth when either of these nutrients were omitted. In contrast, virtually all strains of *S. saprophyticus* showed an absolute requirement for added uracil for anaerobic growth and a strong requirement for pyruvate.

Correlation of nutritional requirements with the classification scheme of Baird-Parker on the basis of these studies is questionable because of the limited number of strains of the different subgroups and because of the poor growth of many of these strains. These same strains grew poorly in the Trypticase soy broth under aerobic conditions. This reduced growth response of older stock cultures as compared to more recent isolates seems to be a fairly common occurrence that casts considerable doubt on the significance attached to type and neotype strains.

Four cultures that in agar shake cultures produced only a few scattered colonies in the anaerobic zone (3) were included in the present study (BP-19, BP-21, BP-23, and BP-47). Three of these four strains gave only slight anaerobic growth in the liquid medium with both uracil and pyruvate present and the other strain failed to grow. If the scattered colonies in the agar shake cultures were mutant cells with the capability of anaerobic growth, one would expect in broth cultures that such mutant cells would grow out and produce heavy growth within the 3-day incubation period. Limited experiments extending the incubation period up to 6 days have failed to demostrate a continued increase in growth response. Rather, it seems that the poor aerobic growth as shown in the Trypticase soy broth cultures is limited even further by anaerobic conditions. Perhaps these might be likened to metabolically injured cells

that are unable to repair their injury or to leaky mutants with regard to anaerobic growth.

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