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UTILIZATION OF ARGININE AS AN ENERGY SOURCE FOR THE GROWTH OF *STREPTOCOCCUS FAECALIS*[1]

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ABSTRACT

DEIBEL, R. H. (American Meat Institute Foundation, Chicago, Ill.). Utilization of arginine as an energy source for the growth of *Streptococcus faecalis*. J. Bacteriol. **87**:988-992. 1964.—Although both *Streptococcus faecalis* and *S. faecium* (and its variety *durans*) hydrolyze arginine, the utilization of this amino acid as an energy source appears to have taxonomic utility, as only *S. faecalis* and its varieties can couple the resultant energy with growth processes. Utilization of arginine by *S. faecalis* in a semisynthetic, casein-hydrolysate medium requires small concentrations of a fermentable carbohydrate (0.05%), presumably for synthetic reactions. The arginine analogue, agmatine, is utilized as an energy source by *S. faecalis* but not by *S. faecium*, and only approximately 50% of the latter strains hydrolyzed this compound. Other ureido- and guanido-containing compounds tested were neither utilized as an energy source nor deaminated.

In recent years, a separation of the enterococcus division (as described by Sherman, 1937) into two distinct physiological types has been reported (Skadhauge, 1950; Shattock, 1955; Barnes, 1956; Deibel, Lake, and Niven, 1963). The *Streptococcus faecalis* type is characterized by its ability to grow in the presence of 0.04% tellurite; to reduce tetrazolium salts; to ferment sorbitol, glycerol (anaerobically), gluconate, and citrate; by the inability to ferment arabinose; and by the ability to grow in semisynthetic media without the addition of folic acid. The *S. faecium* type gives opposite reactions to these physiological tests.

The purpose of the present report is to relate another characteristic, the utilization of arginine as an energy source, that may be employed to differentiate these two physiological types of enterococci. Although all enterococcus types hydrolyze arginine, only *S. faecalis* can couple the resultant energy with growth processes.

MATERIALS AND METHODS

The source of strains and the methods used to maintain them were described in a previous report (Deibel et al., 1963). The complex basal medium employed to test for the utilization of arginine consisted of the following: Tryptone (Difco), 10 g; yeast extract (Difco), 5 g; K_2HPO_4, 5 g; sodium chloride, 5 g; distilled water, 1 liter; pH 7.0 to 7.2. Energy sources (glucose or L-arginine) were added at the 1.0% level prior to autoclaving. To matched culture tubes (18 mm) were dispensed 10 ml of the various media; the inoculum consisted of 1 drop of a 24-hr culture that was diluted 1:100. Growth was estimated in a Bausch and Lomb Spectronic-20 colorimeter at a wavelength of 600 mμ. All cultures were incubated at 37 C.

The semisynthetic, casein-hydrolysate medium previously described for the culture of group F streptococci (Deibel and Niven, 1955) was employed in these studies. The energy sources were added prior to autoclaving, and the test media were inoculated with 1 drop of a 24-hr culture that was washed twice in sterile distilled water and diluted tenfold.

For aerobic culture, 10 ml of medium were dispensed into 50-ml flasks, and the cultures were incubated on a reciprocating shaker (150 oscillations per min). Anaerobic conditions of incubation were achieved by placing the culture tubes in a 6-liter desiccator and flushing three times with a 95% nitrogen-5% carbon dioxide gas mixture. This mixture also constituted the final gas phase in the desiccator during incubation.

Ammonium nitrate was employed as a standard for the estimation of the ammonia produced from arginine. After centrifugation (10,000 × *g*

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for 10 min) to remove the cells, the culture media were diluted appropriately and 1 ml of sample was added to matched tubes, followed by 3 ml of water and 1 ml of Nessler's reagent (Harleco; Hartmen-Leddon Co., Philadelphia, Pa.). Optical density of the solution was determined at 480 mμ.

RESULTS

Growth in complex medium with arginine. The enterococcus collection was tested for the ability of the strains to utilize arginine as a source of energy. All 20 *S. faecalis* strains that were tested utilized arginine, in contrast to the inability of 25 *S. faecium* strains. The growth response of *S. faecalis* with arginine as the energy source was equivalent to that obtained with glucose, whereas the growth responses of *S. faecium* with arginine were either comparable to that obtained in the basal medium or stimulated to a limited extent (Table 1).

In subsequent experiments, an attempt was made to enhance arginine utilization among the *S. faecium* strains by providing a small concentration of glucose (0.05%) in the medium. A strain of *S. lactis* (HBN) was included, because previous results indicated that this organism, like *S. faecium*, could not utilize arginine, although it hydrolyzed the amino acid when tested by conventional procedures that employed glucose in the medium. The inclusion of glucose had no effect on the ability of *S. faecium* to utilize arginine, although it did facilitate ammonia production. However, this small concentration of fermentable carbohydrate afforded maximal growth of the *S. lactis* strain (Table 2).

TABLE 1. *Typical growth responses of enterococci with arginine as the energy source*

Strain	Addition to basal medium*		
	None	L-Arginine (1.0%)	Glucose (1.0%)
Streptococcus faecalis 10Cl	40†	95	110
S. faecalis K2A	40	100	100
S. faecium F24	15	20	105
R7	25	39	116
S. faecium var. *durans* 14Cl	26	30	110

* The complex medium was employed and the growth response was determined after 20 hr of incubation.

† Indicates optical density × 100.

TABLE 2. *Exemplary growth responses of enterococci and Streptococcus lactis to glucose and arginine*

Strain	Additions to basal medium*				
	None	Glucose (0.05%)	L-Arginine (1.0%)	Glucose (0.05%) + L-arginine (1.0%)	Glucose (1.0%)
Streptococcus faecalis 10Cl	38†	59	98	116	116
S. faecium F24	11	47	18	52	110
S. lactis HBN	23	58	42	110	110

* The complex medium was employed.

† Indicates optical density × 100. Growth response was determined after 24 hr of incubation. Further incubation did not afford added growth.

TABLE 3. *Effect of concentration on the utilization of arginine by representative enterococci and Streptococcus lactis*

Strain	Percentage of L-arginine in medium				
	0	0.5	0.75	1.0	3.0
Streptococcus faecalis K2A	38*	95	100	110	116
S. faecium F24	21	39	39	37	29
S. lactis HBN	46†	95	100	105	100
S. faecium F24	42†	50	52	48	46

* Indicates optical density × 100. Growth response determined after 24 hr of incubation in the complex medium.

† Glucose (0.05%) was added to the medium to culture the *S. lactis* strain. *S. faecium* was also tested in this medium, and also without the addition of glucose.

Optimal concentration of arginine. A range of arginine concentrations was tested to determine the optimal concentration for maximal growth, and, also, to determine the effect of higher concentrations on the growth response of *S. faecium*. A concentration of 3.0% did not enhance utilization with *S. faecium* F24, and approximately 1.0% appeared to be optimal for the growth of *S. faecalis* and *S. lactis* (Table 3).

Effect of oxygen tension on arginine utilization. Representative enterococcus strains and *S. lactis* HBN were cultured aerobically and anaerobically (see Materials and Methods) to determine the effect of oxygen tension on the

TABLE 4. *Effect of oxygen tension on the utilization of arginine by the enterococci and Streptococcus lactis*

Strain	Condition of incubation*	Additions to basal medium†				
		None	Glucose (0.05%)	Glucose (0.05%) + L-arginine (1.0%)	L-Arginine (1.0%)	Glucose (1.0%)
Streptococcus faecalis	Aerobic	47‡	78	80	58	140
K2A	Anaerobic	37	66	140	130	110
S. faecium	Aerobic	28	46	51	43	100
F24	Anaerobic	16	47	59	21	100
S. lactis	Aerobic	41	68	92	43	130
HBN	Anaerobic	13	53	113	29	116

* The procedures employed to obtain aerobic and anaerobic conditions are described in Materials and Methods.

† The complex basal medium was employed, and growth was estimated after 21 hr of incubation.

‡ Indicates optical density × 100.

TABLE 5. *Growth response of representative enterococci and Streptococcus lactis in semisynthetic media with arginine as the energy source*

Strain	Addition to basal medium*				
	None	Glucose (0.05%)	Glucose (0.05%) + L-arginine (1.0%)	L-Arginine (1.0%)	Glucose (1.0%)
Streptococcus faecalis K2A	0†	39	68	0	73
Streptococcus faecalis 10C1	0	43	65	0	72
S. faecium F24	0	23	36	0	86
Kia	0	31	34	0	91
S. lactis HBN	0	45	94	0	100

* The casein-hydrolysate medium described in Materials and Methods was employed, and the growth response was estimated after 24 hr of incubation.

† Indicates optical density × 100.

utilization of arginine. The growth response of both the *S. faecalis* strains and, to a lesser extent, the *S. lactis* strain was decreased when the arginine-containing cultures were incubated aerobically (Table 4). Aerobiosis did not enhance arginine utilization by *S. faecium*, as exemplified by strain F24.

Growth in semisynthetic media with arginine. The casein-hydrolysate medium described in Materials and Methods was employed in this phase of the study. Preliminary results with *S. faecalis* indicated that none of the strains evidenced growth in this medium with arginine as the energy source. However, a good growth response occurred with glucose as the energy source, indicating the nutritional adequacy of the medium. It was observed in subsequent experiments that the *S. faecalis* strains required a small concentration of glucose to enhance growth with arginine as the energy source; supplementation with 0.05% afforded maximal growth of both the *S. faecalis* strains and the *S. lactis* strain (Table 5). Thus, it would appear that the growth requirement of *S. faecalis* was similar to that of *S. lactis*; unlike the requirements for growth in complex media, both streptococci required a small "sparking" concentration of glucose in semisynthetic media.

The glucose requirement for arginine utilization in the semisynthetic medium was investigated further, and 22 carbohydrate substrates were tested (0.05% level) for their ability to replace glucose. It was observed that any of the carbohydrates that were fermented and supported growth of the respective strains in complex media were capable of replacing glucose. Consequently, it would appear either that a fermentable carbohydrate is required for "sparking" the utilization of arginine or, more conceivably, that the carbohydrate serves as a carbon source for synthetic reactions necessary for cell growth. Thus, a division of carbon and energy sources may be effected in these streptococci.

Relationship between growth and hydrolytic ability. Preliminary experiments demonstrated that both *S. faecalis* and *S. faecium* produced approximately the same concentration of ammonia when cultured with arginine. An experiment was performed to compare the relative rates of hydrolysis in growing cultures with

representative enterococcal types. The strains were cultured in the complex basal medium with 0.05% glucose and 1.0% L-arginine. The glucose was added to facilitate arginine hydrolysis and growth of the *S. faecium* strain. Arginine hydrolysis was followed by determining the ammonia produced, and growth was estimated turbidimetrically. The experiment demonstrated a decided lag in hydrolysis by the *S. faecium* strain (Fig. 1). From the growth rates, it would appear that the *S. faecium* strain fermented the glucose and achieved a maximal population determined solely by the glucose concentration employed. Arginine hydrolysis commenced considerably later (approximately 8 hr after reading maximal optical density), but ultimately the amount hydrolyzed approached that of *S. faecalis*. The concentration of ammonia produced from arginine by *S. faecalis* (and ultimately by *S. faecium*) approached the theoretical value of 2 μmoles of NH_3 per μmole of arginine. No explanation can be offered for the protracted lag in hydrolytic activity by the *S. faecium* strains; however, the experiment demonstrates that a basic difference(s) exists in either the overall mechanism of the hydrolysis or the energy-coupling reactions with growth processes by the two enterococcal types.

Utilization of other guanido- and ureido-containing compounds. Møller (1955) described a simplified method of detecting arginine dihydrolase activity in various Enterobacteriaceae. In this study, he described also an agmatine dihydrolase system and observed that a strain of *S. faecalis* produced ammonia from agmatine. In this present study it was observed that agmatine, like arginine, is utilized as a source of energy by *S. faecalis*, but not by *S. faecium*. In addition, only about 50% of the *S. faecium* strains were able to hydrolyze this compound.

None of 12 other guanido- and ureido-containing compounds tested as energy sources for the enterococci was capable of supporting growth. In addition, none was deaminated, as determined by a qualitative test for ammonia.

DISCUSSION

The hydrolysis of arginine by streptococci was first reported by Hills (1940), and was later employed as a taxonomic tool by Niven, Smiley, and Sherman (1942) to differentiate various streptococcal species. The latter investigators observed the characteristic hydrolysis of arginine

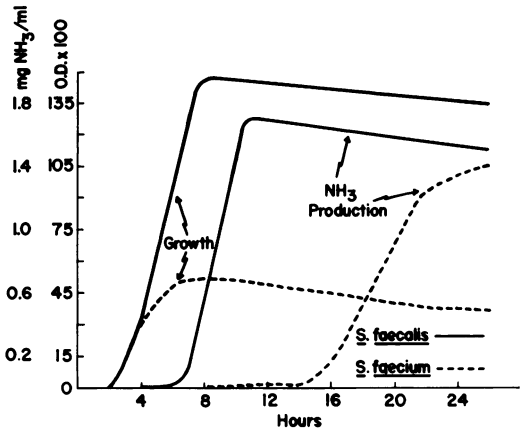

FIG. 1. *Comparison of the rates of growth and ammonia production in a complex medium by Streptococcus faecalis and S. faecium with arginine as the energy source. Medium contained 1.0% L-arginine and 0.05% glucose.*

by all enterococci, and noted the enhanced production of ammonia when glucose was added to arginine-containing media.

The arginine dihydrolase enzyme, as first named by Hills (1940), was studied intensively in several laboratories and was found to consist of a system of enzymes, the first of which hydrolyzes arginine to citrulline and ammonia (arginine desimidase). The hydrolysis of citrulline involves the formation of a high-energy phosphate bond (Slade and Slamp, 1952; Slade, 1953, 1954; Knivett, 1954; Korzenovsky and Werkman, 1952, 1953; Oginsky and Gehrig, 1952, 1953). Previously, Gale (1947) observed the liberation of energy in the hydrolysis of arginine by *S. faecalis* in studies concerned with the passage of amino acids across the cell wall. More recently, Jones, Spector, and Lipmann (1955), working with *S. faecalis* R (*S. faecium* in the current classification scheme), demonstrated the formation of carbamyl phosphate in citrulline hydrolysis. Bauchop and Elsden (1960) related the above observations with cell-free preparations back to growing cultures, and noted an increased growth response (above that accountable for the glucose added) when arginine supplemented the energy source. The increased increment of growth was related to the molar growth yield obtained with other energy sources, and could be equated in terms of adenosine triphosphate generated in the hydrolytic process. Although arginine was not employed as the sole energy source, it was demon-

strated conclusively that the total cell crop was increased proportionately to the arginine added. Thus, the organism was capable of coupling the energy liberated with growth processes, and the adenosine triphosphate generated could be estimated indirectly. These results agreed with the stoichiometry of the overall reaction in which a molar relationship was established for the amounts of arginine hydrolyzed and adenosine triphosphate formed.

From this current study, it would appear that Bauchop and Elsden (1960) employed a strain of *S. faecalis* and not *S. faecium*. It would appear that Knivett's (1954) strain was also a *S. faecalis*, whereas Slade's D10 strain was a *S. faecium* (Slade, *personal communication*). The results obtained in this study indicate that the growth of the *S. faecium* strains is slightly stimulated by arginine; however, the growth increments are small and inconsistent. These results may reflect a less efficient energy-coupling mechanism in *S. faecium* as compared to *S. faecalis*. Alternative and, perhaps, less likely explanations would be that these two enterococcus species possess different mechanisms of citrulline hydrolysis or that the *S. faecium* species may possess an uncoupling mechanism. Whatever the case, the energy differential between citrulline and ornithine is the same, and the inability of *S. faecium* to utilize the energy liberated reflects a deficiency in the overall economy of the cell.

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LITERATURE CITED

BARNES, E. M. 1956. Tetrazolium reduction as a means of differentiating *Streptococcus faecalis* from *Streptococcus faecium*. J. Gen. Microbiol. **14**:57–68.

BAUCHOP, T., AND S. R. ELSDEN. 1960. The growth of microorganisms in relation to their energy supply. J. Gen. Microbiol. **23**:457–469.

DEIBEL, R. H., D. E. LAKE, AND C. F. NIVEN, JR. 1963. Physiology of the enterococci as related to their taxonomy. J. Bacteriol. **86**:1275–1282.

DEIBEL, R. H., AND C. F. NIVEN, JR. 1955. The "minute" streptococci: further studies on their nutritional requirements and growth characteristics on blood agar. J. Bacteriol. **70**:141–146.

GALE, E. F. 1947. The assimilation of amino acids by bacteria. I. The passage of certain amino acids across the cell wall and their concentration in the internal environment of *Streptococcus faecalis*. J. Gen. Microbiol. **1**:53–76.

HILLS, G. M. 1940. Ammonia production by pathogenic bacteria. Biochem. J. **34**:1057–1069.

JONES, M. E., L. SPECTOR, AND F. LIPMANN. 1955. Carbamyl phosphate, the carbamyl donor in enzymatic citrulline synthesis. J. Am. Chem. Soc. **77**:819–820.

KNIVETT, V. A. 1954. Phosphorylation coupled with anaerobic breakdown of citrulline. Biochem. J. **56**:602–606.

KORZENOVSKY, M., AND C. H. WERKMAN. 1952. Bacterial metabolism of arginine. Arch. Biochem. Biophys. **41**:233.

KORZENOVSKY, M., AND C. H. WERKMAN. 1953. Conversion of citrulline to ornithine by cell-free extracts of *Streptococcus lactis*. Arch. Biochem. Biophys. **46**:174–185.

MØLLER, V. 1955. Simplified tests for some amino acid decarboxylases and for the arginine dihydrolase system. Acta Pathol. Microbiol. Scand. **36**:158–172.

NIVEN, C. F., JR., K. L. SMILEY, AND J. M. SHERMAN. 1942. The hydrolysis of arginine by streptococci. J. Bacteriol. **43**:651–660.

OGINSKY, E. L., AND R. F. GEHRIG. 1952. The arginine dihydrolase system of *Streptococcus faecalis*. I. Identification of citrulline as an intermediate. J. Biol. Chem. **198**:791–797.

OGINSKY, E. L., AND R. F. GEHRIG. 1953. The arginine dihydrolase system of *Streptococcus faecalis*. III. The decomposition of citrulline. J. Biol. Chem. **204**:721–729.

SHATTOCK, P. M. F. 1955. The identification and classification of *Streptococcus faecalis* and some associated streptococci. Ann. Inst. Pasteur Lille **7**:95–100.

SHERMAN, J. M. 1937. The streptococci. Bacteriol. Rev. **1**:1–97.

SKADHAUGE, K. 1950. Studies on the enterococci with special reference to the serological properties. Einar Munksgaards, Copenhagen.

SLADE, H. D., AND W. C. SLAMP. 1952. The formation of arginine dihydrolase by streptococci and some properties of the enzyme system. J. Bacteriol. **64**:455–466.

SLADE, H. D. 1953. Hydrolysis of arginine by soluble enzymes of *Streptococcus faecalis*. Arch. Biochem. Biophys. **42**:204–211.

SLADE, H. D. 1954. The metabolism of amino acids by streptococci, p. 65–86. *In* M. McCarty [ed.], Streptococcal infections. Columbia University Press, New York.