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Nutritional and Metabolic Features of *Eubacterium suis*†

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We studied the nutritional and metabolic features of *Eubacterium suis*, an anaerobic animal pathogen that causes cystitis and pyelonephritis in pigs. Peptone-yeast extract-starch (PYS) medium, which contained Trypticase (BBL Microbiology Systems), yeast extract, starch, minerals, cysteine, and sodium carbonate, was shown to support excellent growth of this organism (absorbance at 600 nm = 1.8). Growth was considerably less (absorbance at 600 nm = 0.6) when the starch in the medium was replaced by maltose. Formate, acetate, and ethanol were the major products of fermentation of starch or maltose. The organism appears to require a fermentable carbohydrate for growth since the deletion of starch from PYS resulted in a negligible amount of growth. Growth decreased by ~20% when CO_2 was rigorously excluded from PYS minus Na_2CO_3. The deletion of only yeast extract from PYS resulted in a decrease in growth of about 75%, and the simultaneous deletion of both yeast extract and Trypticase resulted in negligible growth. When the yeast extract in PYS was replaced by a defined mixture of purine and pyrimidine bases, vitamins, and amino acids, growth was ≥80% that observed in PYS. The deletion of Trypticase from this medium resulted in no detectable growth, suggesting a possible peptide requirement for *E. suis* growth. Good growth (absorbance at 600 nm = 1.4) was obtained when adenine and uracil were substituted for the mixture of purine and pyrimidine bases in modified PYS; the substitution of pyridoxal, riboflavin, and nicotinic acid for the vitamin mixture gave comparable growth. The nutritional requirements of *E. suis* apparently reflect the fact that the organism adapts to its natural niche by doing away with certain biosynthetic capabilities which it does not seem to require.

Soltys and Spratling were the first to isolate an obligately anaerobic, gram-positive, nonspore-forming bacterium from pigs with infectious cystitis and pyelonephritis (27). They assigned this organism to the genus *Corynebacterium* primarily on the basis of its diphtheroid morphology, and proposed the name *Corynebacterium suis*. Cases of cystitis and pyelonephritis due to *C. suis* have since been described by several other investigators (6, 8, 15–18, 20, 26), and the disease syndrome is apparently well recognized in swine veterinary practice today (2). However, until recently, little has been published about the taxonomy, physiology, and nutritional characteristics of this organism.

Our recent studies showed that of the many substrates tested, *C. suis* ferments only maltose, starch, and glycogen and produces acetate, formate, and ethanol but not propionate as major end products of carbohydrate metabolism (31). Furthermore, rhamnose and lysine were shown to be the major cell wall components of this organism. These data suggested that *C. suis* does not belong in the genus *Corynebacterium* or *Propionibacterium*. On the basis of these and other data, we proposed that the organism be transferred to the genus *Eubacterium* as *Eubacterium suis* (31).

To date, there have been no nutritional or metabolic studies of *E. suis*. In this paper, we present the results of our recent studies of the nutrition and metabolism of *E. suis*.

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MATERIALS AND METHODS

Bacteria. *E. suis* Soltys 50052 (ATCC 33144) was obtained from M. A. Soltys, Ontario Veterinary College, Guelph, Ontario, Canada. The organism was maintained on slants of peptone-yeast extract-maltose medium as previously described (31).

Media. Basal peptone-yeast extract medium used in most of these studies was the modified, prereduced, anaerobically sterilized medium described by Holdeman et al. (12). The vitamin K-hemin solution was deleted, and 4 ml each of minerals 1 and 2 of Caldwell

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and Bryant (3) were added in place of the salts solution to give a final concentration of 0.48 mg of $(NH_4)_2SO_4$ per ml of medium. Peptone was replaced by Trypticase (10 mg/ml; BBL Microbiology Systems). In different experiments, either maltose or starch was added (10 mg/ml) as the primary energy source to the peptone-yeast extract medium; the resulting media will be subsequently referred to as PYM and PYS, respectively. The pH values of the media were adjusted to 6.7 with 10% NaOH. L-Cysteine-hydrochloride hydrate was added to the media immediately before the media were heated. All anaerobic procedures were carried out under 100% oxygen-free CO_2 gas phase except as mentioned otherwise. Media were prepared (31) and dispensed anaerobically (10 ml per tube [18 by 150 mm]), stoppered, and autoclaved in a press (15 lb/in^2 for 15 min at 121°C).

PYS medium or a modified PYS medium containing charcoal-treated Trypticase (see below) and, in place of the yeast extract, a defined mixture of purine and pyrimidine bases, amino acids, and vitamins (see below) was used for determining the nutritional requirements of this organism. Oxygen-free N_2 was the gas phase in all nutritional experiments in which modified PYS (see Fig. 2 and 3) was used. To determine vitamin requirements, we added single-vitamin solutions individually or in various combinations at the concentrations indicated to modified PYS without the vitamin mixture. Similarly, to determine purine and pyrimidine requirements, we added one or more individual stock solutions of these bases to modified PYS without the purine and pyrimidine bases.

Preparation of charcoal-treated Trypticase. Trypticase used in modified PYS medium was charcoal treated to remove vitamins and certain organic substances. Charcoal (10 g) was added to a solution of Trypticase (10 g/90 ml of double-distilled water) that was adjusted to pH 3.5 with glacial acetic acid. The solution was stirred for 1 h and then filtered through Whatman no. 1 filter paper, and its pH was readjusted to 6.5 with 10 M NaOH. The solution was again mixed with charcoal (5 g), stirred for 1 h, and refiltered, and its final volume was adjusted to 200 ml. Of this Trypticase solution, 20 ml was added to each 100 ml of medium.

Vitamins. Individual aqueous stock solutions of different vitamins in double-distilled water contained the following (milligrams per milliliter): thiamine-hydrochloride, 20; pyridoxal-hydrochloride, 5; calcium-D-pantothenate, 20; riboflavin, 20; nicotinic acid, 10; *p*-aminobenzoic acid, 1; biotin, 0.5; pyridoxine-hydrochloride, 10; pyridoxamine-hydrochloride, 5; and nicotinamide, 10. Vitamin solutions were filter sterilized and stored in the dark at 4°C in acid-cleaned, sterile test tubes. These stocks were added (1%, vol/vol) as needed aseptically and anaerobically to autoclaved, tubed media just before inoculation.

Modified PYS contained all of the above bases.

Amino acids. The stock solution of L-amino acids in water contained the following (milligrams per 100 ml): alanine, 220; arginine, 180; asparagine, 100; aspartate, 250; glutamate, 940; glycine, 20; histidine, 80; hydroxyproline, 20; isoleucine, 260; leucine, 380; lysine, 300; methionine, 140; phenylalanine, 200; proline, 320; serine, 300; threonine, 160; tryptophan, 40; tyrosine, 260; and valine, 260. The solution was stored frozen and added (10%, vol/vol) as needed to media before autoclaving was performed.

Purine and pyrimidine bases. Individual aqueous stock solutions (2 mg/ml) of adenine sulfate, guanine HCl·$2H_2O$, uracil, xanthine, and thymine were prepared. Guanine was solubilized by the addition of 10% HCl, and xanthine was solubilized with 10% NaOH. The stocks were autoclaved and stored at 4°C in the dark in acid-cleaned sterile tubes. These solutions were added (1%, vol/vol) as needed to autoclaved, tubed media just before inoculation.

All of the above bases were added to modified PYS.

Preparation of inocula for studies of nutritional and growth conditions. Cells from a mid-log culture of *E. suis* grown in 3.5 ml of PYS were aseptically harvested by centrifugation, washed twice with 3.5 ml of sterile sodium phosphate buffer (0.02 M; pH 7), and resuspended in the same buffer to give an absorbance at 600 nm of approximately 0.5. One drop of this washed culture (0.05 ml) in a sterile Pasteur pipette was used to inoculate 10 ml of the experimental medium. The organism was subcultured several times in each experimental medium to minimize the effect of nutrient carry-over from the original inoculum. All cultures were incubated at 37°C, and growth was monitored with a Spectronic 20 spectrophotometer (Bausch & Lomb, Inc.) by measuring the absorbance at 600 nm. Absorbance values given represent the means of the values for three tubes.

Fermentation balance: procedures and analyses. Cells were grown under N_2 gas phase in rubber-stoppered, round-bottomed flasks (250 ml) containing 100 ml of peptone-yeast extract, PYM, or PYS. Each flask was inoculated with 5 ml of a mid-log culture grown in the corresponding medium. Uninoculated flasks of the above media served as negative controls. All flasks were incubated at 37°C for 72 h. The total amount of gas produced was calculated as previously described (22). The proportions of H_2 and CO_2 in the gas phase were assayed as previously described (28) with a model 1400 gas chromatograph equipped with a thermal conductivity detector (Varian Associates). A stainless steel column (3.2 mm by 1.8 m) packed with Porapak R (80/100 mesh) was used for CO_2 determinations. Helium was the carrier gas (25 ml/min). A similar column packed with Molecular Sieve 5A (60/80 mesh) was used for H_2 determinations; nitrogen was the carrier gas. Dissolved CO_2 in the media was estimated as previously described (22).

At the end of incubation, each medium was acidified with 1 ml of 50% (vol/vol) H_2SO_4 and clarified by centrifugation. Portions of the clarified fermentation liquor were assayed directly for 2,3-butanediol (5), acetoin and diacetyl (32), and total carbohydrate content (13). A maltose standard curve was used for determining the total carbohydrate concentration in PYM and peptone-yeast extract medium, and a starch standard curve was used for determining the total carbohydrate concentration in PYS. Organic acids were extracted from the clarified fermentation liquor with ether and were identified and quantified (12) with a Varian model 1400 gas chromatograph equipped with a thermal conductivity detector and a column packed with 15% SP 1220–1% H_3PO_4 on Chromosorb W AW (100/120 mesh). Helium was used as the carrier gas (25 ml/min). The column was maintained at 135°C, the injector was maintained at 175°C, and the detector was maintained at 195°C. Volatile distillates (19) of the

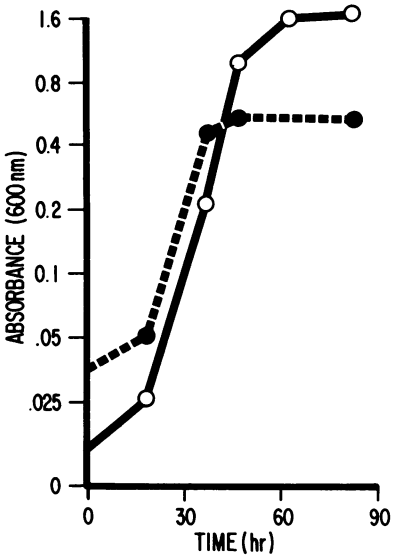

FIG. 1. Growth curves for *E. suis* in PYS (○) and PYM (●) media. Growth is expressed as in Table 2.

clarified fermentation liquor were assayed for alcohol end products with a Varian model 2400 gas chromatograph equipped with an H_2 flame ionization detector. The column was packed with Porapak Q and maintained at 170°C; the H_2 flow was 30 ml/min, and the nitrogen (carrier gas) flow was 30 ml/min.

Fermentation balance calculations were made by the method of Wood (33).

Materials. Purine and pyrimidine bases, hemins, maltose, amino acids, and vitamins were obtained from Sigma Chemical Co., and soluble starch and yeast extract were obtained from Difco Laboratories. Porapak Q and R, SP-1220, Chromosorb W AW, and Molecular Sieve 5A were obtained from Supelco Inc., Bellefonte, Pa.

RESULTS

Fermentation balances for growth on maltose and starch. Maltose was the most common energy source used in the past for growing *E. suis* (16, 26, 27, 31). The results of this study show that growth in PYM was less than half that in PYS (Fig. 1). Hence, in all subsequently described experiments, PYS medium was used for cultivating *E. suis*. Formate, acetate, and ethanol were the only detectable metabolic products produced from starch or maltose fermentation (Table 1). Detectable levels of diacetyl, acetoin, 2,3-butanediol, H_2, CO_2, propionate, lactate, succinate, propanol, and butanol were not produced in either PYS or PYM medium.

Effects of different components of PYS on growth. Growth in PYS minus Trypticase was comparable to that in PYS (Table 2); however, it took considerably longer (81 h) for maximal absorbance to be reached in PYS minus Trypticase, which indicates that Trypticase was stimulatory but not required for growth in this medium. Growth was comparable in PYS or in PYS containing only 0.1% (wt/vol) yeast extract (data not shown). However, the complete deletion of yeast extract from PYS resulted in only about one-third the growth observed in complete PYS (Table 2). These results show that neither Trypticase nor yeast extract alone served as an optimal nitrogen source for growth in PYS medium. Thus, both yeast extract and Trypticase appear to contain nutrients required for optimal growth of *E. suis*. When both yeast extract and Trypticase were deleted from PYS, negligible growth resulted (Table 2). This suggests that *E. suis* is unable to use the ammonium sulfate present in the medium as a sole source of nitrogen.

The deletion of soluble starch from PYS resulted in a decrease in growth of about 95% (Table 2). This low level of growth was consistently seen even after several serial transfers in this medium. The small amount of growth seen in this medium may have been due to trace amounts of carbohydrate known to be present in yeast extract (*BBL Manual*, p. 163, *BBL Microbiology Systems*, 1973).

To determine whether CO_2 was a nutritional requirement for growth, we rigorously excluded CO_2 from PYS as previously described by Dehority (4) and used N_2 gas phase. Total growth in this medium decreased by about 20% (Table 2), but the rate of growth was not affected, as compared with that observed in PYS. This indicated that CO_2 is stimulatory but not required for growth.

In modified PYS medium (see Materials and

TABLE 1. Fermentation balance for *E. suis* grown with maltose or starch as the energy source[a]

Medium	Metabolic product produced (mmol/100 mmol [hexose equivalent])			Carbon recovery (%)	OR balance	C_1:C_2 ratio
	Formate	Acetate	Ethanol			
PYM	156.9	98.8	83.0	86	0.95	0.86
PYS	155.2	146.8	87.1	104	0.89	0.66

[a] Results are expressed as the means of the values obtained from at least two experiments. All values were corrected for products present in the uninoculated medium and in inoculated PY medium.

TABLE 2. Effects of single and double deletions from PYS on *E. suis* growth

Deletion(s)	Growth[a]
None	1.87 (41)
Salts	1.80 (57)
Cysteine	1.80 (41)
Trypticase	1.70 (81)
Na_2CO_3 and CO_2[b]	1.30 (41)
Yeast extract	0.70 (58)
Starch	0.07 (61)
Trypticase + cysteine	1.70 (58)
Yeast extract + cysteine	0.49 (63)
Trypticase + yeast extract	0.03 (41)

[a] Growth is expressed as the mean of maximal absorbance at 600 nm in three tubes (18 by 150 mm). Numbers in parentheses refer to hours of incubation required for maximal growth.

[b] CO_2 and Na_2CO_3 were deleted, and N_2 was used as the gas phase.

Methods) containing a defined mixture of 20 amino acids, growth was 80% that observed in PYS. When Trypticase was then deleted from this medium, there was negligible growth (absorbance at 600 nm = 0.02 [mean result of three tubes at 41 h of incubation, when maximal growth was attained]) in comparison with growth in PYS. In contrast, the deletion of amino acids from modified PYS medium resulted in no decrease in growth; in fact, slightly better growth (absorbancy at 600 nm = 1.82 [mean result of three tubes at 87 h, when maximal growth was attained]) was observed in this medium than in the medium without any deletions (absorbancy at 600 nm = 1.43 [mean result of three tubes at 87 h of incubation, when maximal growth was attained]). This dramatic stimulation of growth by Trypticase in a medium containing a full complement of amino acids and ammonium sulfate was rather surprising and strongly suggests a possible peptide requirement for the growth of this organism.

Vitamin requirements. The growth response of *E. suis* to various additions of vitamins was tested in modified PYS minus vitamins as described in Materials and Methods. Growth was negligible when no vitamins were added (Fig. 2). Single additions of pyridoxal, riboflavin, or nicotinic acid resulted in only marginal growth. Slight stimulation of growth was observed when pyridoxal plus riboflavin or pyridoxal plus nicotinic acid but not riboflavin plus nicotinic acid was added (data not shown). A combination of pyridoxal, riboflavin, and nicotinic acid supported growth comparable to that obtained in the medium with the full complement of all 10 vitamins.

Purine and pyrimidine requirements. In modified PYS without the defined mixture of purines and pyrimidines or with only uracil present, growth was negligible (Fig. 3). The single addition of adenine resulted in a slight increase in growth. The addition of both adenine and uracil gave growth comparable to that obtained in the medium with the full complement of purine and pyrimidine bases. The combined addition of adenine, guanine, thymine, and xanthine gave growth that was no better than that obtained when only adenine was added (data not shown).

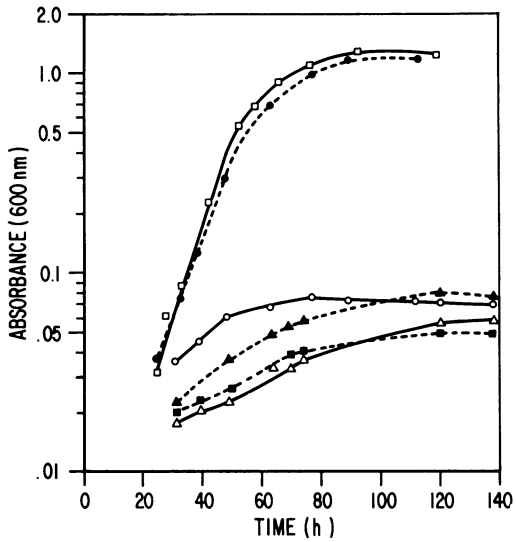

FIG. 2. Effects of vitamins on *E. suis* growth. PYS medium was modified by the addition of charcoal-treated Trypticase and the replacement of yeast extract with a defined mixture of purine and pyrimidine bases. Single-vitamin solutions were added individually or in various combinations to modified PYS as described in the text. Symbols: □, full complement of vitamins; ●, pyridoxal, riboflavin, and nicotinic acid; ▲, nicotinic acid; ○, pyridoxal; △, riboflavin; ■, no vitamins.

DISCUSSION

We previously reported that *E. suis* fermented only maltose, starch, and glycogen but not glucose or many other carbohydrate and noncarbohydrate substrates (31). The results of this study show that a fermentable carbohydrate appeared to be required as an energy source for growth (Table 2) and that growth was more than twofold greater in PYS than in PYM. These results could be explained by the fact that a number of bacteria are known to metabolize disaccharides and oligosaccharides by phosphorolytic cleavage, whereby the energy of the glycosidic link is conserved (1, 9, 25). Consequently, ATP, normally required for the activation of sugar to sugar-1-PO_4, is conserved for each glycosidic link cleaved by phosphorylic cleavage. Therefore, metabolism of starch-derived oligosaccha-

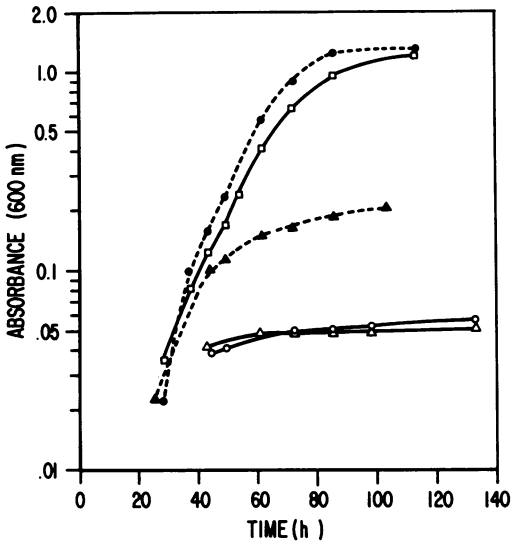

FIG. 3. Effects of purine and pyrimidine bases on *E. suis* growth. PYS medium was modified by the addition of charcoal-treated Trypticase and the replacement of yeast extract with a defined mixture of vitamins. Stock solutions of individual purine and pyrimidine bases were added singly or in various combinations to the modified basal medium as described in the text. Symbols: □, full complement of purines and pyrimidines; ●, adenine and uracil; ▲, adenine; ○, uracil; △, no purines or pyrimidines.

rides by *E. suis* would result in a considerably greater conservation of ATP, as compared with metabolism of an equivalent quantity of maltose. This, perhaps, explains why we observed greater growth in PYS medium than in PYM medium. Further research is clearly needed to define the carbohydrate metabolism in this organism in greater detail.

The results show that formate, acetate, and ethanol are the main products produced from fermentation of starch or maltose by *E. suis*. C_1 recovery ratios and oxidation reduction (OR) balance values for both PYM and PYS suggest that an oxidized C_1 compound was missing (Table 1). Almost identical results were obtained when this fermentation balance experiment was repeated three times. In spite of numerous attempts, we failed to demonstrate that CO_2 was a product of metabolism of *E. suis*. Even so, we can not completely rule out the possibility that small amounts of CO_2 may indeed be produced during the metabolism of starch or maltose by *E. suis* and that much of this CO_2 is fixed by the organism for biosynthetic purposes. However, the more likely possibility is that the missing C_1 compound is formate, which may have been underestimated because the sensitivity of the chromatographic method used for the determination of formate is not very high, especially at higher levels of formate. If the missing C_1 product is assumed to be formate and the fermentation balance for growth in PYM, for example, is recalculated, the C_1:C_2 ratio would be 1.0, the OR balance would be 1.08, and the carbon recovery value would be 1.01. Further studies in which ^{14}C-labeled substrates are used and key metabolic enzymes are assayed should be useful in defining the possible pathways involved in the metabolism of starch and maltose by *E. suis*.

Approximately equimolar amounts of ethanol and acetate were produced in PYM, whereas almost twice as much acetate as ethanol was produced in PYS. Since the conversion of acetyl coenzyme A to acetate via acetyl phosphate results in the synthesis of an additional molecule of ATP (9), it appears that more ATP might be generated from fermentation of starch than fermentation of maltose. This may at least partially account for the higher level of growth attained with starch as the energy source.

Although it would seem reasonable to expect that NH_4^+, presumably readily available to the organism in vivo owing to its urease activity, satisfies the nitrogen requirements of this strain, our studies indicated that an organic nitrogen source was required, at least in vitro. Furthermore, when Trypticase was absent, NH_4^+, a full complement of free amino acids, or both could not satisfy the nitrogen requirements of the organism; the addition of Trypticase to the medium, however, dramatically restored growth. These data suggest that Trypticase serves as a source of peptides (and perhaps other nutrients) required for the growth of *E. suis*. The requirement for peptides even in the presence of a full complement of free amino acids has been observed for a few other organisms (14, 21).

We have previously shown that *E. suis* differs from other members of the genus *Corynebacterium* on the basis of cell wall composition, fermentation end products, and biochemical characteristics (31). The data presented here show that *E. suis* differs from other animal pathogenic corynebacteria in its nutritional requirements as well. For example, almost all strains of the type species *Corynebacterium diphtheriae* require pimelic acid, nicotinic acid, and β-alanine (24). Van Eseltine et al. (30) have reported that *Corynebacterium renale*, the etiological agent of infectious bovine pyelonephritis, can use ammonium ion as the sole source of nitrogen, although other workers have indicated that amino acids, in addition to NH_4^+, are required (10, 11). *Corynebacterium bovis* has also been shown to utilize ammonium ion as its sole nitrogen source and to require unsaturated long-chain fatty acids for growth. Some strains of *C. bovis* have been shown to require nicotinic acid (24). Little is

known about the nutritional requirements of other species of animal pathogenic corynebacteria, including *Corynebacterium pseudotuberculosis* and *Corynebacterium kutscheri* (24). More recent studies showed that *Corynebacterium pyogenes* requires adenine, uracil, riboflavin, and thiamine and that a defined mixture of amino acids can serve as a source of nitrogen only in the presence of inositol (C. A. Reddy and A. McClellan, unpublished data). In this investigation, *E. suis* was not tested for growth in medium containing free amino acids plus inositol.

The nutritional requirements of *E. suis* also appear to differ from those of the anaerobic coryneforms (propionibacteria). Ferguson and Cummins (7) reported that these organisms grow well when a basal salts medium is supplemented with glucose, pantothenate, biotin, thiamine, and many amino acids. Nicotinamide is required for optimal growth only of *Propionibacterium acnes* strains. The vitamin requirements of these species reflect the need for those cofactors which are essential to the propionic acid fermentation pathway. Since *E. suis* does not utilize this pathway, it is not surprising that its vitamin requirements differ. In addition, Ushijima (29) and Ferguson and Cummins (7) have shown that guanine, adenine, and a combination of both are stimulatory but not required for the growth of the anaerobic coryneforms. *E. suis* required both adenine and uracil for maximal growth.

E. suis produces pyelonephritis almost exclusively in female animals, often in association with pregnancy and parturition (26). Perhaps in its natural milieu the organism utilizes cellular stores of glycogen, the chief reserve carbohydrate of animals, as the main energy source. It is unlikely that the organism has access to maltose or starch in situ. Filterable peptides, vitamins, and nucleic acid bases are also available to the organism in its natural niche. The nutritional requirements of *E. suis* apparently reflect its adaptation to its natural niche: the organism does away with biosynthetic abilities that it does not seem to require.

LITERATURE CITED

1. **Alexander, J. K.** 1961. Characteristics of cellobiose phosphorylase. J. Bacteriol. **81**:903–910.
2. **Blood, D. C., and J. A. Henderson.** 1974. Diseases caused by *Corynebacterium* sp., p. 300–307. *In* Veterinary medicine, 4th ed. The Williams & Wilkins Co., Baltimore.
3. **Caldwell, D. R., and M. P. Bryant.** 1966. Medium without rumen fluid for nonselective enumeration and isolation of rumen bacteria. Appl. Microbiol. **14**:794–801.
4. **Dehority, B. A.** 1971. Carbon dioxide requirement of various species of rumen bacteria. J. Bacteriol. **105**:70–76.
5. **Desnuelle, P., and M. Naudet.** 1945. Microdosage colorimetrique du formol: son application au dosage du glycerol, de l'ethylene et du propyleneglycol. Bull. Soc. Chim. Fr. **12**:871–873.
6. **Dijkstra, R. G.** 1969. Cysto-pyelonephritis bij varkens veroorzaakt door *Corynebacterium suis*. Tijdschr. Diergeneeskd. **94**:393–394.
7. **Ferguson, D. A., Jr., and C. S. Cummins.** 1978. Nutritional requirements of anaerobic coryneforms. J. Bacteriol. **135**:858–867.
8. **Frijlink, F. P. A., J. E. van Dijk, and J. Goudswaard.** 1969. Een hemorrhagischenecrotiserende cysto-pyelonefritis bij een drachtige zeug, veroorzaakt door *Corynebacterium suis*. Tijdschr. Diergeneeskd. **94**:389–393.
9. **Gottschalk, G.** 1979. Catabolic activities of aerobic heterotrophs, p. 114–115. *In* Bacterial metabolism. Springer-Verlag, New York.
10. **Hirai, K., S. Shimahura, and R. Yanagawa.** 1969. Minimum medium for *Corynebacterium renale* and filamentous growth due to deficiency or excess of inorganic ions. Jpn. J. Vet. Res. **31**:145–149.
11. **Hirai, K., and R. Yanagawa.** 1967. Nutritional requirements of *Corynebacterium renale*. Jpn. J. Vet. Res. **15**:121–134.
12. **Holdeman, L. V., E. P. Cato, and W. E. C. Moore (ed.).** 1977. Anaerobe laboratory manual, 4th ed. Virginia Polytechnic Institute and State University, Blacksburg, Va.
13. **Johnson, R. R., T. L. Balwani, L. J. Johnson, K. E. McClure, and B. A. Dehority.** 1966. Corn plant naturity. II. Effect on *in vitro* cellulose digestibility and soluble carbohydrate content. J. Anim. Sci. **25**:617–623.
14. **Kihara, H., O. A. Klatt, and E. E. Snell.** 1952. Peptides and bacterial growth. III. Utilization of tyrosine and tyrosine peptides by *Streptococcus faecalis*. J. Biol. Chem. **197**:801–807.
15. **Larsen, J. L.** 1970. *Corynebacterium suis* infectioner hos svin. Nord. Vet. Med. **22**:422–431.
16. **Munro, R., and F. Wond.** 1972. First isolation of *Corynebacterium suis* in Hong Kong. Br. Vet. J. **128**:29–32.
17. **Narucka, U., and J. F. Westendorp.** 1971. *Corynebacterium suis* bij het varken. Tidjschr. Diergeneeskd. **96**:399–404.
18. **Narucka, U., and J. F. Westendorp.** 1972. *Corynebacterium suis* bij het varken. II. Tidjschr. Diergeneeskd. **97**:647–652.
19. **Neish, A. C.** 1952. Analytical methods for bacterial fermentations. Report no. 46-8-3. National Research Council of Canada, Ontario.
20. **Percy, D. H., H. L. Ruhnke, and M. A. Soltys.** 1966. A case of infectious cystitis and pyelonephritis of swine caused by *Corynebacterium suis*. Can. Vet. J. **7**:291–292.
21. **Pittman, R. A., and M. P. Bryant.** 1964. Peptides and other nitrogen sources for the growth of *Bacteroides ruminocola*. J. Bacteriol. **88**:401–410.
22. **Reddy, C. A., M. P. Bryant, and M. J. Wolin.** 1972. Characteristics of S organism isolated from *Methanobacillus omelianskii*. J. Bacteriol. **109**:539–545.
23. **Reddy, C. A., C. P. Cornell, and A. M. Fraga.** 1980. Chemically defined growth medium for *Corynebacterium pyogenes*. Am. J. Vet. Res. **40**:843–845.
24. **Rogosa, M., C. S. Cummins, R. A. Lelliott, and R. M. Keddie.** 1974. Coryneform group of bacteria, p. 599–632. *In* R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
25. **Sheth, K., and J. K. Alexander.** 1969. Purification and properties of β-1,4-oligoglucan: orthophosphate glucosyltransferase from *Clostridium thermocellum*. J. Biol. Chem. **244**:457–464.
26. **Soltys, M. A.** 1961. *Corynebacterium suis* associated with a specific cystitis and pyelonephritis in pigs. J. Pathol. Bacteriol. **81**:441–446.
27. **Soltys, M. A., and F. R. Spratling.** 1957. Infectious cystitis and pyelonephritis of pigs: a preliminary communication. Vet. Rec. **69**:500–504.
28. **Uffen, R. L.** 1976. Anaerobic growth of a *Rhodopseudomonas* species in the dark with carbon monoxide as sole carbon and energy substrate. Proc. Nat. Acad. Sci. U.S.A. **73**:3298–3302.

29. **Ushijima, T.** 1966. Nutritional requirements of some anaerobic species in the genus *Corynebacterium*. Med. Biol. **72**:214–216.
30. **Van Eseltine, W. P., W. M. Cox, and S. Kadis.** 1977. Minimal nitrogen requirements of *Corynebacterium renale* strains. Am. J. Vet. Res. **39**:123–127.
31. **Wegienek, J., and C. A. Reddy.** 1981. Taxonomic study of "*Corynebacterium suis*" Soltys and Spratling: proposal of *Eubacterium suis* (nom. rev.) comb. nov. Int. J. Syst. Bacteriol. **32**:218–228.
32. **Westerfeld, W. W.** 1945. A colorimetric determination of blood acetoin. J. Biol. Chem. **161**:495–502.
33. **Wood, W. A.** 1961. Fermentation of carbohydrates and related compounds, p. 63–65. *In* I. C. Gunsalus and R. Y. Stanier (ed.), The bacteria, vol. 2. Academic Press, Inc., New York.