## THE USE OF STREPTOCOCCUS FAECALIS, A.T.C.C NO. 6057, AS A TEST ORGANISM IN A MICROBIOLOGICAL ASSAY

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Since Lunin discovered in 1881 the necessity of a group of "accessory factors," much time and energy has been spent with the B-complex vitamins. This increased interest in vitamins has necessitated the development of satisfactory analytical procedures. Since these vitamins occur naturally in such minute amounts, the conventional chemical and physical methods of analysis are in many cases not adequate. In 1939 Snell and Strong (9) reported a method of analysis using *Lactobacillus casei* as a test organism for riboflavin. Following this introduction of the use of lactic acid bacteria as test organisms for physiologically active substances, the technique of microbiological analysis has been utilized more extensively than any other method for amino acids and vitamins. In the last ten years, several lactic acid bacteria have been suggested and employed as test organisms. The more common ones are: *Lactobacillus arabinosus* 17-5 A.T.C.C. No. 8014, *Lactobacillus casei* A.T.C.C. No. 7469, *Lactobacillus delbrueckii* L.D.-5, *Lactobacillus pentosus*, *Leuconostoc mesenteroides* P-60, *Leuconostoc mesenteroides* A.T.C.C. No. 535, and *Strepto-*

*coccus faecalis R.* A.T.C.C. No. 8043. Recently this laboratory has developed a number of analytical procedures utilizing *Streptococcus faecalis* A.T.C.C. No. 6057 in microbiological assays. The present paper includes the details of analyses for riboflavin, calcium-pantothenate, vitamin $B_6$, niacin, and biotin using this organism.

## Experimental Procedure

*Streptococcus faecalis*, A. T. C. C. No. 6057, was isolated by Sherman (1) who reported that it has the ability to oxidize glycerol. The organism is maintained on a stock culture containing 1% glucose, 1% yeast extract, 1% peptone, and 1½% agar. The culture is transferred bi-weekly as a stab, incubated at 37° C. for twenty-four hours, and stored in the refrigerator.

The inoculum is prepared by transferring the organism to a broth containing 1% peptone, 1% glucose, and 1% yeast extract and incubating for eight hours. The cells are centrifuged, washed with sterile isotonic saline twice, and resuspended. Assay tubes are inoculated with one drop each of this last cell suspension. The tubes are chilled in an ice bath before and during inoculation.

After inoculation, the tubes are brought to 37° C. in a water bath and incubated for sixteen hours. At the end of the incubation period, the tubes are again chilled in an ice bath and the turbidity of the solution is measured in a Fisher Electrophotometer using a filter of 650 millimicrons. Just previous to measuring the turbidity of the tubes, the solutions are warmed to prevent the condensation of water vapor on the optical cells.

The basal medium is given in Table I. It should be pointed out that the buffer systems employed utilize sodium citrate and sodium acetate. The superiority of the citrate buffer in promoting growth has been adequately demonstrated for *S. faecalis R.* (7, 10), but its effect in preventing the precipitation of ferric phosphate has not been previously described. Since *S. faecalis* is also stimulated by the presence of citrate, the use of this ion in the buffer

TABLE I

*Composition of Basal Medium*
*Expressed in Amount/Tube Final Medium*

| | | | |
|---|---|---|---|
| Casein hydrolosate | 100 mg. | Na citrate | 100 mg. |
| Tryptophane | .5 mg. | Na Ac·$3H_2O$ | 50 mg. |
| Cystine | 1.5 mg. | $KH_2PO_4$ | 1 mg. |
| Glucose | 60. mg. | $K_2HPO_4$ | 1 mg. |
| Thiamine · HCL | 5. γ | Uracil | 0.1 mg. |
| Riboflavin | 2.5 γ | Adenine·$SO_4$ | 0.1 mg. |
| Ca-Pantothenate | 5. γ | Guanine·HCl·$2H_2O$ | 0.1 mg. |
| P A B A | 1. γ | $MgSO_4 \cdot 7H_2O$* | 8.0 mg. |
| Pyridoxamine | 10. γ | NaCl* | .4 mg. |
| Niacin | 5. γ | $FeSO_4 \cdot 7H_2O$* | .4 mg. |
| Folic Acid | .02 γ | $MnSO_4 \cdot 4H_2O$* | 1.2 mg. |
| Biotin | .04 γ | Na citrate* | .8 mg. |

*Modified Salt C

system serves several purposes. Further, the phosphate salts are reduced to such a concentration that, in the measurement of total growth, the desensitizing effect of the phosphate buffer system at the end point of the titration (pH 6.8) is reduced. The inorganic salts (other than phosphate salts) are similar to those employed by Roberts and Snell (8) in their Salt C solution except that sodium citrate is added to prevent the hydrolysis and subsequent precipitation of the ferric ion. Less glucose is used than in most other basal media but higher or lower concentrations will inhibit the growth rate of this organism. This low concentration of glucose had an added advantage in that

the carmelization during autoclaving is held to a minimum. The amounts of the other components in the medium are similar to those contained in other assay solutions.

## Results

The vitamin curves of riboflavin, biotin, calcium-pantothenate, niacin, and vitamin $B_6$ are shown in Fig. 1. The riboflavin curve is a fairly smooth curve and it is reproducible between 0 to 40 m$\gamma$ per tube to within 2 m$\gamma$. *Lactobacillus casei* will produce a curve between 0 and 500 m$\lambda$ per tube (9) which is less sensitive than the curve for this organism. Since the most sensitive part of the curve using *S. faecalis* is between 0 and 20 m$\gamma$ per tube (within this range the standard deviation of the tubes is .7), one can detect a significant difference of 1 m$\gamma$ per tube. The high sensitivity of this organism for riboflavin can be favorably compared to the sensitivity of *Leuconostoc mesenteroides* A.T.C.C. No. 10,100 (5).

The growth rate response curve of biotin shows that a maximum in the curve is reached when biotin is at a concentration of 2 m$\gamma$ per tube. The curve is the most sensitive between 0 and .5 m$\gamma$ per tube. This growth response curve appears to be more sensitive than that produced by *L. arabinosus* (11) which responds to biotin from 0 to 2.5 m$\gamma$ per tube.

Calcium-pantothenate reaches a maximum at 300 m$\gamma$ per tube. While this curve covers a longer range of concentration of the vitamins than that required by *L. arabinosus* 17-5 (3), *S. faecalis* gives reproducible results which will check favorably with assays using the previous organism.

The *L. arabinosus* 17-5 assay for niacin (6) covers a range of 0 to 500 m$\gamma$ per tube. However, there is a break in this standard curve between 300 and 350 m$\gamma$ per tube which limits the applicability of this organism in niacin determinations. *S. faecalis* produced satisfactory differences in growth response between 0 and 250 m$\gamma$ per tube of niacin; but since the slope of the curve is continually decreasing, a larger number of standard tubes are required for accurate determinations. The growth response curve of pyridoxamine is very sensitive between 0 and 8 m$\gamma$ per tube. However, before this assay can be run, it is necessary to irradiate the hydrolyzed casein with ultraviolet light. *S. faecalis* is sensitive to pyridoxine, pyridoxal, and pyridoxamine and complete details of a method of analysis for these compounds will be reported in a later paper.

## Discussion

To our knowledge, *Streptococcus faecalis* A.T.C.C. No. 6057 has not been previously utilized in any microbiological procedures. In recent years this organism has been used extensively by this laboratory in the analysis of some B vitamins occurring in plant material.

Kocher (4) describes the use of a strain of *S. faecalis* in the analysis of calcium-pantothenate and riboflavin. While the identity of the strain used in these assays is not specified, it is apparently not A.T.C.C. No. 6057 since it does not respond to niacin.

This organism can be used in assays of five of the B vitamins with sensitivity and reproducibility as good as or better than that obtained wth previously reported organisms. This organism produces vigorous growth at the end of sixteen hours of incubation and is not as fastidious in its general requirements as *L. casei* and some of the other test organisms. In considering the use of *S. faecalis* in the routine analysis of a large number of samples, this organism will produce vigorous growth even after being exposed to isotonic saline solution for a period of forty-five minutes. Tests run with *L. casei*, *L. arabinosus*, and *S. faecalis R.* show that these organisms will produce

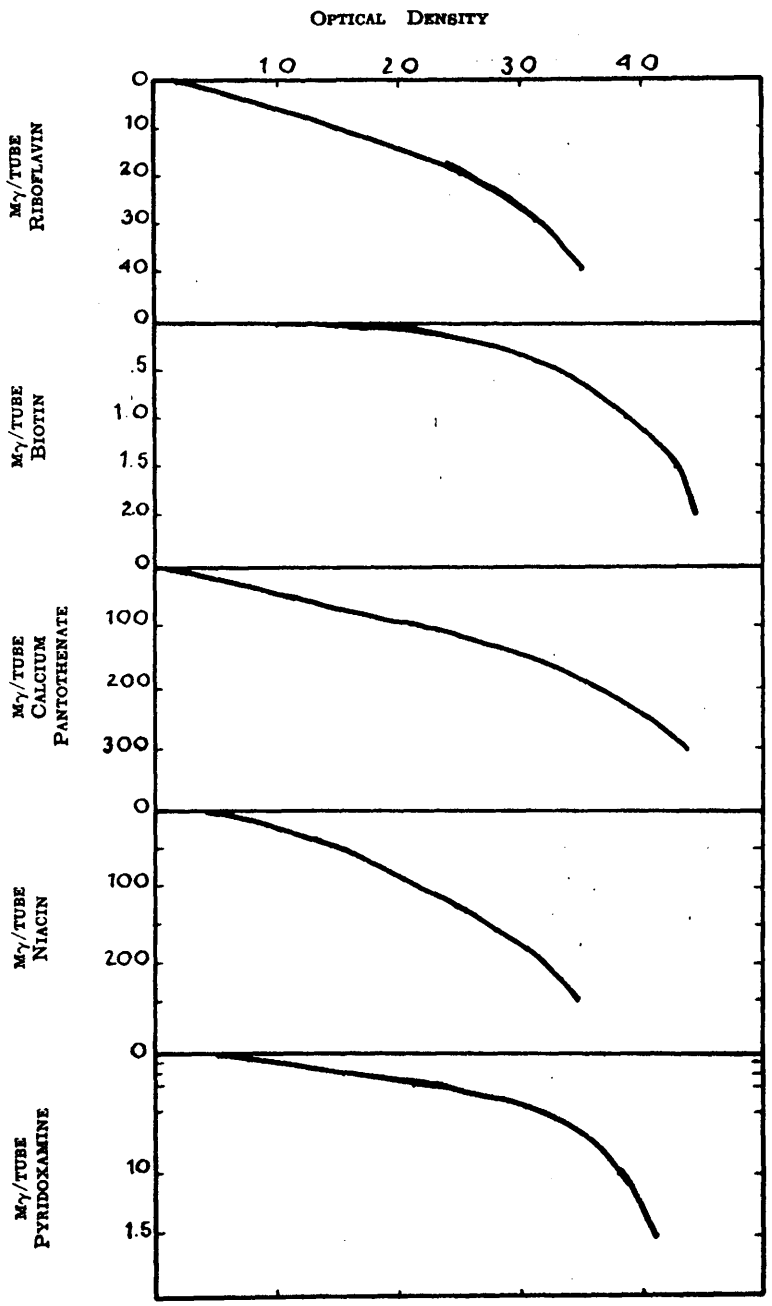


*FIGURE 1. Vitamin-turbidity Curves for S. faecalis A. A. T. C. No. 6057.*

erroneous results when exposed to saline for a similar period of time. Further, *S. faecalis* is not as fastidious with respect to optimum temperature requirements as the previously named organisms.

## Conclusions

*Streptococcus faecalis* A.T.C.C. No. 6057 appears to have a potentiality of being a valuable test organism in the analysis of some B vitamins and amino acids. The high sensitivity of this organism to relatively small amounts of some of the B vitamins is readily applicable to microbiological assays which compare very favorably with methods of analysis previously published. The lack of fastidious environmental requirements makes this organism appear much more favorable for routine analysis of a large number of samples. At the present time, the detailed nutritional requirements of this organism are being studied and these requirements along with methods of analysis of amino acids using *S. facalis* will be reported in the future.

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