A BIOCHEMICAL TEST FOR SEPARATING PARACOLON GROUPS

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The Arizona group of paracolon bacteria was first investigated by Peluffo *et al.* (1942). Studies on this group of organisms finally culminated in the report by Edwards *et al.* (1947) that established an antigenic scheme of classification independent of the Salmonella scheme. The Bethesda-Ballerup group of paracolons was definitively studied by West and Edwards (1954), although the original Ballerup strain was described by Kauffmann and Moeller (1940), while the Bethesda strains were first reported by Edwards *et al.* (1948). Kauffmann (1954) has combined the Bethesda-Ballerup group with *Escherichia freundii* as the only difference between them was rapid fermentation of lactose by *E. freundii*, while in the Bethesda group lactose fermentation may be delayed 3 to 10 days and occasionally may not occur.

Biochemically the Arizona and Bethesda-Ballerup groups are very closely related, the only constant difference being the liquefaction of gelatin by the Arizona group. This characteristic reaction may not occur with some strains of the Arizona group in less than 30 days. Moeller (1954*a*) reported on the value of the Braun KCN test for differentiating this group, although 2 of 35 strains in the Arizona group departed from the usual behavior. The KCN test as described by Moeller is difficult to control, as results may vary with the size of the inoculum. Moeller (1954*b*) also described methods for determining the distribution of the amino acid decarboxylases of the *Enterobacteriaceae* that were useful in classification. These tests required a special Danish peptone and close control for satisfactory evaluation of results. The need for a simple, reproducible method of determining decarboxylase activity useful in classification led to the present investigation.

MATERIALS AND METHODS

After several trials, a medium consisting of pancreatic digest of casein (U.S.P. XIV)[1], 15 g; dipotassium phosphate, 2 g; glucose, 1 g; and distilled water, 1000 ml, was found to be satisfactory for demonstration of lysine decarboxylase activity. Five ml of the medium is dispensed into screw-cap tubes (15 x 125 mm) and sterilized in the autoclave. The medium is inoculated with the organism under study and incubated at 37 C for 18 to 24 hours. After incubation 1 ml of 4 N NaOH is added and mixed. Two ml of chloroform is added and the tube shaken vigorously. The cadaverine is found in the chloroform phase after extraction. The tube is centrifuged to break the emulsion and separate sufficient clear chloroform extract for testing below the heavy layer of denatured protein present at the chloroform-medium interphase. One-half ml of the clear chloroform extract is removed with a pipette and placed in a 13 x 100 mm tube. To this is added 0.5 ml of 0.1 per cent ninhydrin (1,2,3,-triketohydrindene) in chloroform. The reaction mixture is observed at room temperature for 4 minutes. A positive reaction is evidenced by the occurrence of a deep purple color. Incubation of the reaction mixture at higher temperatures or for longer periods of time may result in the production of a false positive result because of the presence of minor ninhydrin-reacting compounds in the chloroform extract that were not identified.

The identity of the reacting substance was confirmed by growing positive strains in a synthetic medium in which glucose and lysine were present as the sole sources of carbon. The synthetic medium used was adequate to support the growth of many of the gram negative rods and contained dipotassium phosphate, 7.0 g; monopotassium phosphate, 3.0 g; ammonium sulfate, 1 g; glucose, 2.5 g; mineral salt solution, 10 ml dissolved in 1000 ml of water. The mineral salt solution contained magnesium sulfate, 10 g; calcium chloride, 1 g; and ferrous sulfate, 0.05 g dissolved in 1000 ml of water. This medium was dispensed in 5-ml quantities in screw-cap vials and one series of duplicate inoculations was supplemented with a 2 mg/ml of L-lysine monohydrochloride. After incubation the tubes were tested in the usual manner. No ninhydrin-react-

[1] Several different lots of Trypticase, BBL, and Casitone, Difco, were tested and all yielded uniform results.

ing compound was found in the synthetic medium without lysine.

Isolation of the major ninhydrin-reacting substance was accomplished by paper electrophoresis using a potential of 100 volts and 0.1 N-barbiturate buffer adjusted to pH 8.6. Under these conditions the substance migrated toward the negative electrode and could be located after drying by application of the 0.1 per cent ninhydrin in chloroform reagent.

RESULTS

This test has now been checked with more than 300 stock and routine cultures and results have been uniform, with a single exception. Fifty-two Providence group, 32 Bethesda group and 29 Arizona group type cultures were kindly supplied by Dr. P. R. Edwards. Stock cultures maintained in this laboratory included 30 Salmonellae of Edwards' typing series, 15 Shigellae, 15 Proteus species, 10 *Escherichia coli* and 10 Klebsiella strains. The balance were routine cultures submitted for identification. All Providence strains tested were negative. All Arizona strains were positive. All Bethesda-Ballerup strains were negative with the exception of the strain labeled Brazil 109. The correct classification of this strain as a member of the Bethesda-Ballerup group is in doubt as it does not utilize citrate or produce hydrogen sulfide and is sensitive to KCN. Proteus strains were irregularly positive. The salmonellae with the single exception of *S. paratyphi* (ETS #1) were positive, while all the Shigellae tested were negative. The *E. coli* and Klebsiella strains tested were also positive.

DISCUSSION

The ability of the gram negative rods to decarboxylate amino acids has long been recognized. Gale (1951) states that this activity is limited to those amino acids having at least one chemically active group other than the terminal carboxyl and α-amino groups. This process is a simple decarboxylation. Thus arginine is decarboxylated to agmatine, lysine to cadaverine, histidine to histamine, etc. These changes have been followed by shifts in hydrogen ion concentration as determined with a glass electrode or by changes in indicator solutions. The results secured with the presently described test indicate that the major component which reacts with the ninhydrin indicator is cadaverine, derived from the decarboxylation of the lysine present in the pancreatic digest of casein. This inference was checked by paper electrophoresis (figure 1). A small drop of chloroform extract derived from culture in the peptone medium was dried on filter paper, as was a small drop of the chloroform extract derived from a culture grown in the synthetic medium containing the single amino acid lysine. Electrophoresis was carried out as described and the ninhydrin-reacting spots from both extracts migrated toward the negative pole exactly the same distance from the starting point, indicating that they were identical. The chloroform extract derived from cultures in the synthetic medium containing lysine were concentrated by evaporation of the chloroform and subjected to electrophoresis. After drying, the spot was located with the ninhydrin reagent and a parallel portion cut from the paper, extracted with alcohol and tested for the presence of aliphatic amines with the dithiocarbamate reaction of Feigl (1947). A positive reaction was secured. The only amine derivable from lysine basic enough to migrate toward the negative pole under the conditions described is cadaverine.

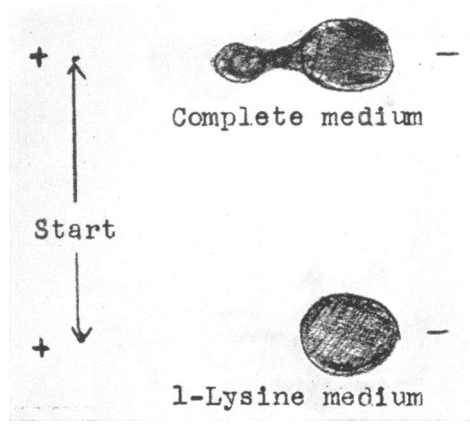

Figure 1. Electrophoretic pattern obtained with ninhydrin reagent. System: 0.1 N-Barbiturate buffer at pH 8.6, 100 volts, 90 minutes.

At least one more amino acid present in the peptone is decarboxylated by some of the organisms tested, as there was a minor ninhydrin-reacting component present in some of the chloroform extracts. This substance was not studied further as the primary purpose of this investigation was to devise a simple, reproducible biochemical test for separation of the Arizona and Ballerup-Bethesda groups. This minor compo-

nent may account for some of the positive reactions in other groups as both Gale (1951) and Moeller (1954*b*) have reported lysine decarboxylase absent in the Proteus group.

SUMMARY

A simple biochemical test has been presented that will separate strains of the Arizona and Bethesda-Ballerup groups. Results secured in testing other genera of the *Enterobacteriaceae* are recorded.

REFERENCES

EDWARDS, P. R., WEST, M. G., AND BRUNER, D. W. 1947 The Arizona group of paracolon bacteria. Bull. 499. Kentucky Agricultural Experiment Station, Lexington, Kentucky.

EDWARDS, P. R., WEST, M. G., AND BRUNER, D. W. 1948 Antigenic studies of a group of paracolon bacteria (Bethesda group). J. Bacteriol., **55**, 711–719.

FEIGL, F. 1947 *Qualitative analysis by spot tests*, 3rd ed. Elsevier Publishing Co., New York.

GALE, E. F. 1951 *The chemical activities of bacteria*, 3rd ed. University Tutorial Press, Ltd., London.

KAUFFMANN, F. 1954 *The Enterobacteriaceae*. Ejnar Munksgaard, Copenhagen.

KAUFFMANN, F. AND MOELLER, F. 1940 A new type of Salmonella (*S. ballerup*) with Vi-antigen. J. Hyg., **40**, 246–251.

MOELLER, V. 1954*a* Diagnostic use of the Braun KCN test within the *Enterobacteriaceae*. Acta Pathol. Microbiol. Scand., **34**, 115–126.

MOELLER, V. 1954*b* Simplified tests for some amino acid decarboxylases and for the arginine dihydrolase system. Acta Pathol. Microbiol. Scand., **36**, 158–172.

PELUFFO, C. A., EDWARDS, P. R., AND BRUNER, D. W. 1942 A group of coliform bacilli serologically related to the genus Salmonella. J. Infectious Diseases, **70**, 185–192.

WEST, M. G. AND EDWARDS, P. R. 1954 The Bethesda-Ballerup group of paracolon bacteria. Public Health Monograph No. 22. U. S. Govt. Printing Office, Washington, D. C.