AgriCos e-Newsletter
ISSN: 2582-7049
AgriCos e-Newsletter
Open Access Multidisciplinary Monthly Online Magazine
Volume: 02 Issue: 11 November 2021 Article No: 05

Identification of Bacteria Based on Their Ability to Utilise Amino Acids through Biochemical Detection Technique

Aarti S. Gauns[1], Archana Anokhe[2] and Vinay Kalia[3]
[1]Research Scholar, Pathology, ICAR-Indian Agricultural Research Institute, New Delhi
[2]Scientist, Entomology, ICAR-Indian Agricultural Research Institute, New Delhi
[3]Principal Scientist, Entomology, ICAR-Indian Agricultural Research Institute, New Delhi

SUMMARY

Decarboxylases are a group of enzymes which act by hydrolysing an amino acid to form an amine. Decarboxylase test is mainly performed to differentiate bacteria in Enterobacteriaceae family from other gram-negative bacteria. The decarboxylation of the amino acid yields an alkaline pH and a change in colour of pH indicators bromocresol and cresol red from orange to purple.

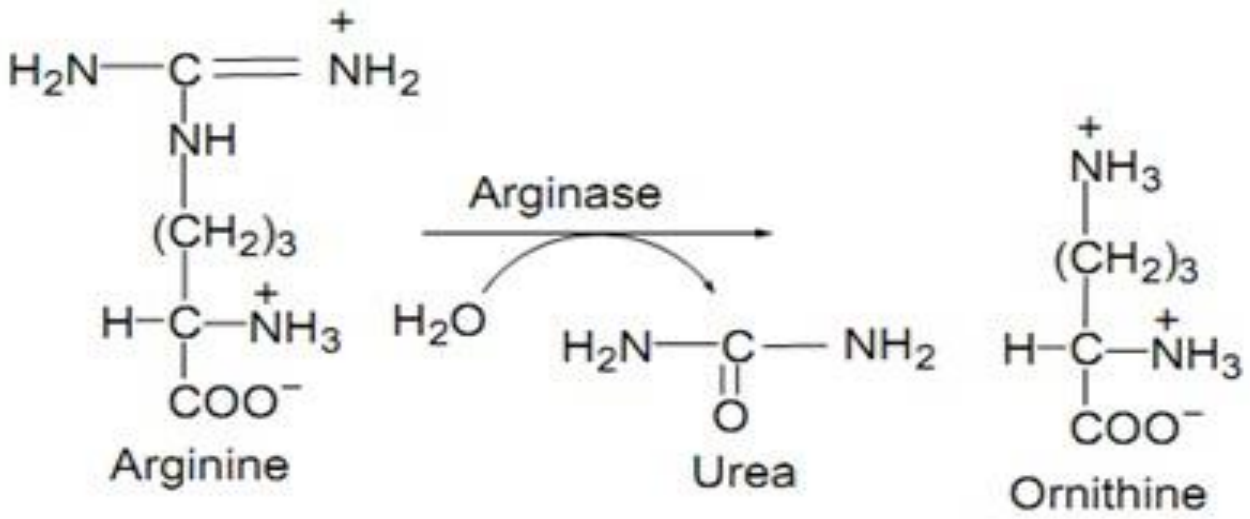
INTRODUCTION

Determining the ability of bacteria to produce enzymes that either deaminate, hydrolyse, or decarboxylate certain amino acids is often used in identification. Three different decarboxylase enzymes are produced by organisms that catalyse the metabolism of amino acid; ornithine decarboxylase, arginine decarboxylase, and lysine decarboxylase. The production of these enzymes is taken as an important parameter for the differentiation of bacteria present in the Enterobacteriaceae family on the basis of their ability to produce the enzyme decarboxylase. Different Amino acids are metabolized differently by gram negative aerobic bacteria, facultative anaerobic bacteria and gram positive cocci.

Principle and procedure of detecting decarboxylase producing bacteria:

Decarboxylases are a group of substrate specific enzymes which reacts with the carboxyl (COOH) portion of amino acids, forming alkaline-reacting amines and by-product carbon dioxide. Some microorganisms possess such enzymes which allow their detection. The test thus measures the enzymatic ability (decarboxylase) of an organism to decarboxylate (or hydrolyse) an amino acid to amine in turns increases pH. The increased pH of the medium is detected by colour change of the pH indicators, bromocresol purple and cresol red from yellow to purple. The decarboxylation of specific amino acid results in formation of Lysine to Cadaverine, Ornithine- to Putrescine and Arginine to Citrulline

In another reaction, Arginine is hydrolysed to ornithine i.e. arginine is first converted to citrulline via Di hydrolase reaction, in which NH2 group is released from arginine further Citrulline is converted to ornithine. Ornithine decarboxylation results in putrescine formation.



(Source:https://microbeonline.com/decarboxylation-test-types-uses-principles)

Fig: Hydrolysis of Arginine to Ornithine by Arginase

For detection of Glucose-Nonfermenting Organisms, prepare a suspension (McFarland No. 5 turbidity standard) in brain-heart infusion broth from an 18 to 24 hr old culture growing on 5% sheep blood agar. Inoculate each of the three decarboxylase broths (arginine, lysine, and ornithine) and the control broth (no amino acid) with four drops of bacterial suspension and then add a 4-mm layer of sterile mineral oil to each tube. Incubate these tubes at 35°C to 37°C in ambient temperature. Examine the tubes at regular intervals of 24, 48, 72, and 96 hours. Inoculate each of the three tubes with one drop of an 18- to 24-hour brain-heart infusion broth culture. Add a 4-mm layer of sterile mineral oil above it and Incubate the cultures for 4 days at 35°C to 37°C in ambient temperature and then examine tubes at 24, 48, 72, and 96 hours' intervals. The Positive test will be turbid purple to faded-out yellow-purple colour (alkaline) whereas the negative test will be having bright clear yellow colour (acid) or no change (non-fermenting rods). Control tube: remain its original colour or turn yellow.

Examples:

- Enteric Gram-negative bacteria and *Vibrio*, *Plesiomonas*, and *Aeromonas* can be identified to a species level.
- *Stenotrophomonas* and *Burkholderia* (lysine and arginine) Fluorescent *Pseudomonas* (arginine)
- Coagulase-negative staphylococci (ornithine) and viridans group streptococci (arginine)
- Non-glucose-fermenting, Gram-negative bacteria (arginine)
- Spreading indole-negative *Proteus* (ornithine)

Media and composition:

Decarboxylase Test Medium Base (Moeller's is used for testing amino acid decarboxylase activity. Other media like Motility-indole-ornithine medium (MIO) and Lysine iron agar can also be used. The Final pH of the experiment is set as 6.7 ±0.2 at 25°C. The composition of the decarboxylase mediums are, Peptic digest of animal tissue (5.0), Yeast extract (0.3), Dextrose (1.0), Bromo cresol purple (0.1), Cresol red and Pyridoxal (0.005 each)

Fig: Positive test (Left) with turbid purple/faded yellow-purple colour (alkaline), Negative test (Center) with bright clear yellow colour (acid) or no change (non-fermenting rods), Control tube (Right) with original colour intact.

Applications:

- Decarboxylase test is used to differentiate the members of the Enterobacteriaceae family with closely related physiological characteristics.
- Arginine decarboxylase is useful in the identification of *Enterococcus* to the species level;like *Enterococcus faecalis* and *Enterococcus faecium* are arginine positive but Enterococcus *avium* is arginine negative.
- Lysine decarboxylase is used to differentiate between *Salmonella* (+) and *Shigella* (-).

Limitation:

- The test does not measure the amount of intracellular enzyme initially and detect it only when it has reached sufficient quantity to cause a pH change in the medium.
- The fermentation of dextrose in the medium causes the acid colour change. However, it would not mask the alkaline colour change brought about by a positive decarboxylation reaction.

CONCLUSION

This test measures the enzymatic ability (decarboxylase) of bacteria to decarboxylate or hydrolyze an amino acid to form an amine. Besides identification of Enterobacteriaceae, the ornithine decarboxylase test is important, especially for separating members of *Klebsiella-Enterobacter-Serratia* group and for identifying species of *Proteus*.

REFERENCES

Anupama. S, (2020) Decarboxylase Test- Principle, Procedure, Results, Uses (microbenotes.com)

Sagar A, (2019). Amino Acid Decarboxylase Test - Procedure, Uses and Interpretation (microbiologyinfo.com)

Decarboxylase test: Principle, Procedure, Result Interpretation, uses and (universe84a.com)

Fay, G. D., & Barry, A. L. (1972). Rapid ornithine decarboxylase test for the identification of enterobacteriaceae. Applied microbiology, 23(4), 710–713.

Pilsucki, R. W., N. W. Clayton, V. J. Cabelli, and P. S. Cohen. 1979. Limitations of the Moeller lysine and ornithine decarboxylase tests. Appl. Environ. Microbiol. 37:254-260.