PHENYL ALANINE DEAMINASE TEST

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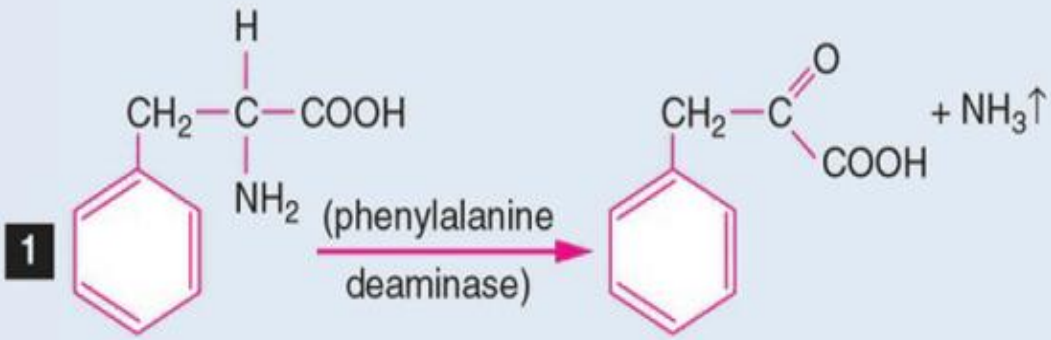
AIM:

To determine the ability of an organism to produce enzyme Deaminase

PRINCIPLE:

- Phenylalanine agar , also known as phenylalanine deaminase medium contains nutrients and DL – phenylalanine.
- The phenylalanine serves as the substrate for enzymes which are able to de-aminate it to form phenyl pyruvic acid.
- Microorganisms that produce phenylalanine deaminase remove the amine (NH2) from phenylalanine.
- The reaction results in the production of ammonia (NH3) and phenyl pyruvic acid.
- The phenyl pyruvic acid is detected by adding a few drops of 10% ferric chloride which acts as a chelating agent.
- A green colored complex is formed between these two compounds indicating a positive test.

MECHANISM OF THE PHENYLALANINE DEAMINASE REACTION



L–phenylalanine —(enzyme)→ phenylpyruvic acid + ammonia

2 phenylpyruvic acid + ferric ion (Fe^{+++}) —acid→ green complex

MEDIA COMPOSITION:

- DL- Phenylalanine : 2 gm
- Yeast extract : 3 gm
- Sodium chloride : 5 gm
- Disodium phosphate : 1 gm
- Agar : 12 gm
- Distilled water : 1 L
- ph : 7.3

REQUIREMENTS :

Type	Amount	Quantity
1) Phenylalanine slant		1
2) 24 hr old culture suspension		2ml
3) 10% Aqueous Ferric chloride		2ml
4) Nichrome wire loop		1
5) Incubator		1

Protocol

- Inoculate Phenylalanine slant with given culture suspension
- Incubate the slant at 37 degrees for 24 hours
- After incubation add 2-3 drops of 10% aqueous ferric chloride solution to the slant

Result:

- The test is positive if the medium turns dark green. The green color is a result of degradation product produced from phenylalanine. It will eventually combine with iron compound in the acidic environment leading to dark green color.

- The result is negative if the color of the medium didn't change. It remains straw to yellow in color.

Positive result
(Formation of dark green color)

Negative result
(No color change)

EXAMPLES:

- Examples of organisms that test positive for phenylalanine deaminase test are:

1) Proteus sp

2) Morganella sp

3) Providenica sp

4) Proteus vulgaris

5) Proteus mirabilis

- Examples of organisms that test negative for phenylalanine deaminase test are:

1) Escherichia coli

2) Enterobacter aerogenes

Uses:

- Phenylalaine deaminase test is used to differentiate members of the genera *Proteus*, *Morganella*, and *Providencia* (+ve) from other members of **Enterobacteriaceae** which give negative results.

THANKYOU