INDOLE TEST

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AIM :

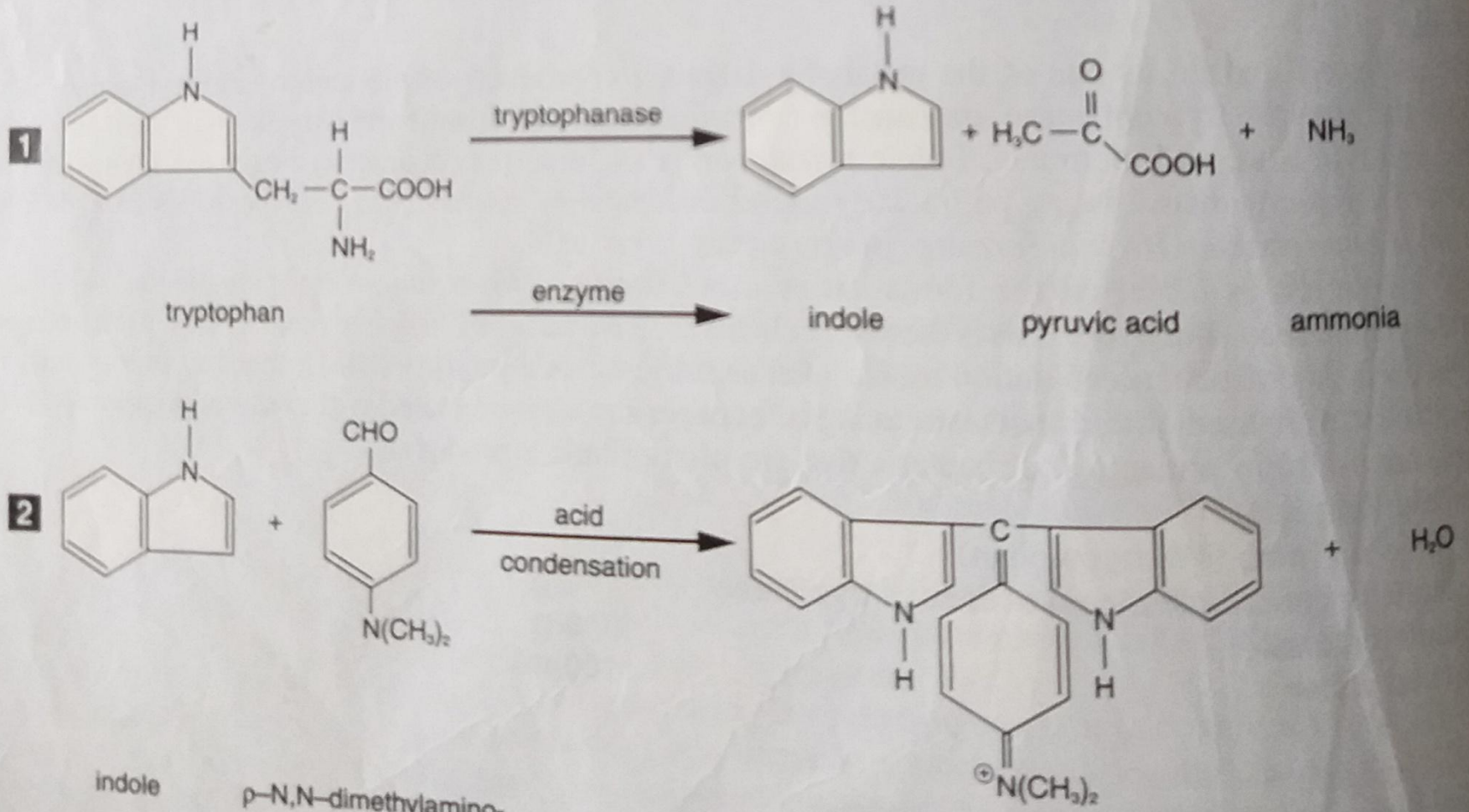
- To detect the Indole production by the bacteria .

PRINCIPLE :

The tryptophan undergoes deamination and hydrolysing by bacteria that produce the enzyme tryptophanase .The product of the reaction is indole which is generated by the intermediate biomolecule indole pyruvic acid .

The indole test is a biochemical test performed to determine the ability of the microorganisms to convert tryptophan into indole. The indole reacts with the p-dimethylaminobenzaldehyde give red coloured complex. This is the active chemical in Kovac's reagent. The use of the Kovac's reagent has also been used described for combined test media such as sulfide indole motility (SIM), motility indole ornithine (MIO).

MECHANISM OF THE INDOLE REACTION



REQUIREMENTS TABLE

Sr.No	Name of the Apparatus	Volume	Quantity
	Media and Reagents		
1.	Sterile 1% Tryptophan Broth	5ml	_
2.	Kovac's reagent	1ml	_
3.	Appropriate Suspension	1ml	_
	Miscellaneous		
1.	Nichrome wire loop	_	_
2.	Incubator at 35 C For 18 to 24 hrs	_	_

COMPOSITION :

1 %Tryptophan Broth Composition :

Sr. No	Ingredients	gm/L
1.	Peptone or pancreatic digest of caesin (trypticase)	2
2.	Sodium chloride	0.5
3.	Distilled water	100ml
4.	pH 7.5 (0.2)	_

Kovac's Reagent composition :

Sr.No	Ingredients	gm/L
1.	p-dimethylaminobenzaldehyde	10
2.	Isoamyl alcohol or pure amyl	150ml
3.	Concentrated HCl	50ml

PROTOCOL :

Inoculate the tryptophan broth with appropriate sample

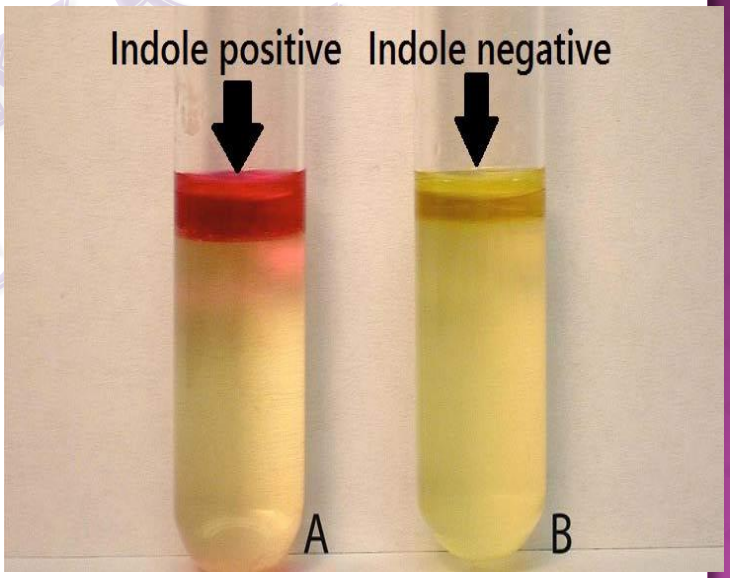
Incubate at 35 C for 18 to 24 hrs

At the end, add 15 drops of Kovac's reagent.

NOTE: Kovac's reagent can be replaced by Ehrlich's reagent. If Ehrlich's reagent is used. This step sholud be preceded by the addition of 1 ml xylene.

OBSERVATION :

1. After addition of Kovac's reagent red colour complex observe on the surface of broth, it indicates that the test is Indole positive.
2. After addition of Kovac's reagent if red colour complex not observe on the surface of the broth,it indicates that the test is Indole negative.



RESULTS

- After addition of Kovac's reagent red colour complex form within a seconds on the surface of the tryptophan broth. Hence , the organism produce tryptophanase enzyme and able to hydrolyze tryptophan.

After addition of Kovac's reagent red colour complex not form on the surface of the tryptophan broth. Hence , the organism does not produce tryptophanase enzyme and unable to hydrolyze tryptophan.

EXAMPLES :

I. Indole test positive organisms- Escherichia coli , Enterococcus faecalis ,Vibrio spp , Proteus spp, etc

II. Indole test negative organisms- Klebsiella pneumoniae, Lactobacillus spp ,Pseudomonas spp , Enterobacter spp, etc.