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Biochemical characterization of *Xanthomonas campestris* pv. *arecae* causing leaf stripe in arecanut

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Abstract
Xanthomonas campestris pv. *arecae* is an important and destructive pathogen causing bacterial leaf stripe in arecanut. Attempt were made to ascertain the variability in *Xanthomonas campestris* pv. arecae**.** The tests conducted to know the biochemical properties of the pathogen. Hence, pathogen was subjected to various biochemical tests, the results indicated that, ammonia production, starch hydrolysis, fluorescent test and gram staining showed negative reaction whereas, positive reaction were observed in catalase test, liquefaction of gelatin, KOH test, methyl red test and hydrogen sulphide production.

Keywords: *Xanthomonas campestris* pv. *arecae*, Arecanut, Biochemical, Variability

Introduction
Arecanut (*Areca catechu* L) belongs to the family arecaceae is a palm is grown in most part of the tropical Asia and East Africa regions. Arecanut being affected by many biotic and abiotic factors, among that fungi, bacteria, virus, phytoplasma, causing heavy yield losses. The term Areca is derived from a Malayan language which means "cluster of nuts". Bacterial leaf stripe of arecanut is most devasticated disease because of its wide spread and distribution in Asian countries. [1] First time reported the occurrence of bacterial leaf stripe disease from Tumkur districts of Karnataka state in an endemic form. Young arecanut seedlings were severly affected by bacterial leaf stripe disease caused by *Xanthomonas campestris* pv. *arecae* leading up to 60% seedlings mortality. The initial symptoms include dark green water soaked stripes running alongside and parallel to the mid rib of the leaf lets. The lesions were covered with abundant quantity of bacterial exudates on the lower surface of the leaves. All the leaflets of a leaf may be affected resulting in complete or partial blighting of the leaf and in severe cases the entire crown may get killed. Well documented evidences are available to show that virulent strains of *Xanthomonas campestris* pv. *arecae* present in Asia are variable. The development of resistant varieties is the most economical and environmentally safe approach of disease management but for breeding long lasting resistant varieties and accurate knowledge of variation in the pathogen population is a prerequisite. Resistant varieties evolved against this disease; do not perform uniformly in all areas. It might be due to variability in the pathogen and pathogenic potential of the same pathogen. Hence, it becomes necessary to ascertain variability amongst the leaf stripe causing pathogen in particular region. An attempt has been made to identify the variation in *Xanthomonas campestris* pv. *arecae.*

Materials and methods
In the present study biochemical characters such as hydrolysis of starch, gelatin liquefaction, hydrogen sulphide production, catalase and KOH test, catalase, protein digestion and methyl red test by the pathogen were studied as per the methods described [2, 3].

Starch hydrolysis
The starch agar medium was used to carry out the starch hydrolysis. Potato starch (10 g) was added to 1000 ml of nutrient agar. 20 ml of sterilized cool medium was dispensed in to each of the Petri plates. After solidifying, starch agar plates were spot inoculated with loopful culture of the bacterium and incubated for five days at 27 ± 1°C. After incubation period is over, the plates were flooded with Lugol's iodine solution and observations were drawn for starch utilization by the bacterium.

Gelatin liquefaction
Fifteen ml of freshly prepared and autoclaved nutrient agar added with 0.4 per cent gelatin was poured into the sterilized Petri plates. After the medium gets solidified, spot inoculation using a tooth prick on the surface of the medium was done. Plates were incubated at 27 ± 1ºC for three days. After that plates were flooded with 10 ml of acid mercuric chloride solution. Observations were made for the formation of clear zone around the growth of the bacterium.

H_2S Production
The peptone broth was prepared and sterilized. A loopful culture of 48 hr. old test bacterium is inoculated in to the slants containing the peptone broth. Filter paper discs (What man No. 42) impregnated with 10 per cent solution of neutral lead acetate was taken and air dried and then inoculated. The sterilized stripes were placed in to the inoculated test tubes. The inoculated tubes were incubated at 27 ± 1ºC for 72 hrs. Observations were drawn for the H_2S production. Blackening of the stripes indicated the positive reaction.

Catalase test
A loopful of 48 hr sold slant growth of the bacterium was smeared on a slide and was covered with few drops of hydrogen peroxide (H_2O_2). The reaction will be positive if gas bubbles are produced.

Potassium hydroxide (KOH) solubility test
A loop full of bacteria was aseptically removed from culture plates with tooth pick, placed on glass slide in a drop of KOH (3 %) solution and stirred for ten second using a quick circular motion of hand, then the tooth pick was raised a few centimeter's above the slide and observed for the formation of viscid strand represent the bacterium as Gram-negative.

Protein digestion
For agar plate test, reconstituted powdered skim milk was sterilized and is mixed with sterile melted yeast extract nutrient agar (YENA) to obtain a10 per cent concentration and poured over the surface of nutrient agar in Petri plates. The plates were dried, spot inoculated and observed for a clear zone around the colonies after 3, 5 and 7 days.

Methyl red test
Methyl red indicator (0.1g methyl red dissolved in 300 ml of 95% ethanol and made up to 500 ml with distilled water) was added to test culture; change in color indicates the positive reaction.

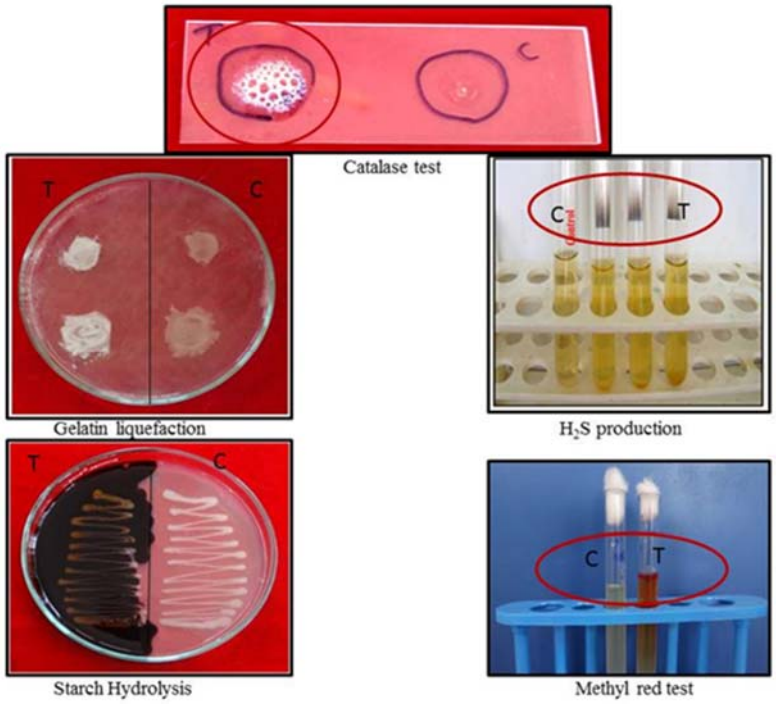
Fluorescent test
Test bacterium was streaked on petriplate containing Kings B medium (Protease peptone medium 20 g, Glycerol 10 ml, K_2HPO_4 1.5 g, $MgSO_4$ 1.5 g, Agar 15 g) and after two days incubation plates were observed under fluorescent microscope for further studies.

Gram reaction
Test culture was placed on clean slide then heat fix it and added one drop of crystal violet stain then allowed for two minutes then washed with tap water and air dried for two seconds. Then gently flood with Gram's iodine and allowed for 1 minute. Then again washed in tap water and air dried. Then added 95% ethyl alcohol decolorizing agent and allowed for two minutes. Then added secondary stain, safranin, allowed for one minute then wash and air dried. Then slide was observed under microscope for gram reaction.

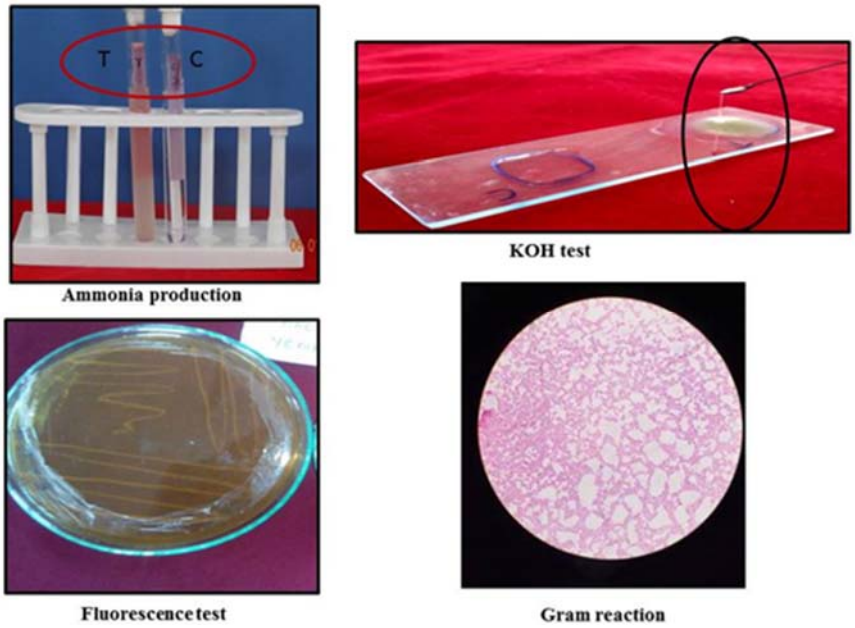
Results and discussion
Biochemical test revealed from table 2 and plate 2 that bacterium is a rod shaped strictly aerobic and gram negative organism. And also the bacterium was found positive for catalase reaction, liquefaction of gelatin, KOH test, protein digestion, methyl red test and produced hydrogen sulphide. Whereas, negative results were obtained for ammonia production, starch hydrolysis, fluorescent test and gram reaction.
The results of the present investigation were in accordance with the results obtained by [4, 5, 6] they reported that the *Xanthomonas* pathogen utilizes xylose, glucose, mannose, galactose, sucrose, lactose and positive tests for KOH string assay, H_2S, catalase, arginine dehydrolase test and raffinose but not maltose, glycerine and salicin. It readily hydrolyses the starch and liquefies the gelatin.

Table 1: Biochemical characterization of *Xanthomonas campestris* pv. *arecae*.

Sl. No.	BIOCHEMICAL TESTS	RESULTS
1.	Catalase test	+
2.	Gelatin liquefaction	+
3.	H_2S production	+
4.	Starch hydrolysis	+
5.	Methyl red test	+
6.	Ammonia production	-
7.	KOH test	+
8.	Fluorescent test	-
9.	Gram reaction	-



Plant 1: Biochemical characterization of *Xanthomonas campestris* pv. *arecae*.



Plant 2: Biochemical characterization of *Xanthomonas campestris* pv. *arecae.*

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