

Morphological, Cultural, Physiological and Biochemical Characteristics of Bacterial Pustule of Soybean Caused by *Xanthomonas axonopodis* pv. *glycines*

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The identification of the pathogen *Xanthomonas axonopodis* pv. *glycines* involved in causation of the disease was done by conducting studies on its morphological, biochemical, cultural and physiological features of the pathogen as per the standard microbiological procedures. The bacterium was gram negative, rod shaped with round ends. The cells appeared as single, some in pairs and in clusters having single polar flagellum. The cells measured 0.4-0.25 x 1.25- 3.00 mm in size. The biochemical features of the pathogen revealed that the bacterium liquified the gelatin, produced the H₂S, positive for starch hydrolysis and acid produced by carbon sources (sucrose, glucose and dextrose) but it was negative for acid production by nitrogen (peptone) sources. Among the eight different solid media tested, NGA medium was significantly supported for the luxurious growth of the pathogen as evidenced by the maximum recovery of bacterial colonies (96.00). Significantly least number of colonies (39.40) was recorded on Xan-D medium. The XPS agar medium supported absolutely no growth of the bacterium. Circular, flattened, slightly raised, convex with yellow to bright yellow colour on SX and NA medium. They occurred singly or rarely in aggregates. Where as, on both YDCA and GYCA, colonies were circular, slightly raised, glistening with yellow colour. Highly irregular to irregular, slightly raised, light yellow coloured colonies were observed in NGA media. Optimum temperature requirement for the growth of pathogen was studied and found that the temperature level of 30°C was optimum in which significantly maximum numbers of bacterial colonies were recorded. The pathogen colonies were reduced below and beyond of 30°C. Similarly, the effect of hydrogen ion concentration on the growth of pathogen was studied and found that pH of 7.0 as optimum where maximum numbers of colonies were recorded. The colonies were found reduced beyond and below pH level of 7.0.

Keywords: *Xanthomonas axonopodis* pv. *glycines*, Morphology, Gram negative, biochemical, starch hydrolysis, cultural, NGA, physiological, temperature and pH.

Soybean (*Glycine max* (L.) Merrill) is a protein rich oilseed crop. Though, soybean is a legume crop but it is widely used as oilseed due to its poor cooking ability on account of inherent presence of trypsin inhibitor that limits its usage as pulse crop. It can supply the much needed

protein to human diets, because it contains more than 40 per cent protein of superior quality and all the essential amino acids particularly glycine, tryptophan and lysine, similar to cow's milk and animal proteins. Soybean also contains about 20 per cent oil with an important fatty acid, lecithin, Vitamin- A and D. In India, annual yield losses due to various diseases are estimated as 12 per cent of total production. Over hundred pathogens are known to affect soybean, of which 66 fungi, six bacteria and eight viruses have reported to be

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associated with soybean seeds (Sinclair, 1978).

In India, it occupies an area of 10.02 million ha with a production of 11.64 million tonnes and productivity of 1062 kg per ha (Anon., 2015). Madhya Pradesh is often called as 'Soybean state' or 'Fort of soybean' due to its contribution to the extent of 70 per cent of area and 64 per cent of production. In Karnataka, it is grown on an area of 0.29 million ha with production of 0.24 million tones and productivity of 828 kg per ha (Anon., 2015).

MATERIAL AND METHODS

Morphological variability

Shape

Smear of the isolates from the 24 hrs old cultures grown on nutrient glucose agar media was stained with methylene blue and examined under oil immersion for recording the shape of the bacterial cells (Salle, 1961).

For measuring the size of the bacterial cells, the method suggested by Salle (1961) was followed. Fifty cells of isolate was measured and the average size was worked out.

Cultural characters

To study the growth characteristics of the pathogen, various differential and selective/semi-selective media are used in this investigation. The experiment was carried out using Completely Randomized Design (CRD) and data were analysed statistically.

Biochemical characters

The biochemical characters such as starch hydrolysis, gelatin liquefaction, KOH production, H₂S production, carbon (sucrose, dextrose and glucose) and nitrogen (peptone) sources availability by the pathogen were studied as per the methods described by Salle (1961). The observation on utilization of all these sources by pathogen were recorded in terms of + or -ve reaction.

Physiological characters

Temperature and pH levels

The study was undertaken to assess the best supportive temperature and pH for the growth of *X. a. pv. glycines* using nutrient agar as basal medium. Serially diluted one ml of 10⁵ dilution was dispensed on to the surface of nutrient agar contained in sterilized petriplates. The suspension

was uniformly spread over the medium with the help of sterilized spreader to obtain well separated bacterial colonies. Then inoculated plates were incubated at different temperature and pH levels for 72 hr. Observations were made on the development of colonies on the inoculated plates kept at specific temperature and pH levels. Colonies were counted, recorded and data was analysed as per the statistical procedures

RESULTS AND DISCUSSION

Morphological characters

The cells measured 0.4 - 0.25 x 1.25 - 3.00 mm in size. The cells readily got stained with common stain crystal violet. Based on these characteristics the pathogen was identified as *Xanthomonas axonopodis* pv. *glycines* (Nakano) Dye.

Cultural characters

Efficacy of different media in supporting the growth of the pathogen

A total of two selective and five differential media were tested for the efficacy to support the growth of *Xanthomonas axonopodis* pv. *glycines*. Among them Nutrient Glucose Agar (NGA) medium was significantly supported for the luxurious growth of the pathogen as evidenced by the maximum recovery of bacterial colonies (96.00) Nutrient agar medium was the next best which gave 85.40. These two media were significantly differed with each other and also superior to other media under study. Yeast extract nutrient agar (82.80) Glucose yeast chalk agar (77.00) and SX media (67.00) were next best media in promoting the growth and development of bacterial colonies. Significantly least number of colonies (39.40) were recorded on Xan-D medium. The XPS agar medium supported absolutely no growth of the bacterium.

Colony morphology

On SX, YNA and NA media, colonies of the pathogen *Xanthomonas axonopodis* pv. *glycines* appeared as mostly circular to irregular, flattened, glistening, slightly raised, convex and yellow to bright yellow coloured, they occurred singly or rarely in aggregates. On Xan-D media, colony morphology with slightly raised character, circular to irregular and bright yellow colour was observed. Highly circular to irregular, slightly raised, light

Table 1a. Morphological and cultural characters of *Xanthomonas axonopodis* pv. *glycines* on different media

Media	No. of colonies of the bacterium	Size of the colony range (mm)	Average size of the colony (mm)	Colony characters		
				Colour	Shape	Appearance
Glucose yeast chalk agar (GYCA)	77.00 (8.83) *	1-5	2.5	Light yellow to yellow	Circular	Slightly raised
Nutrient agar (NA)	85.40 (9.30)	2-5	3.2	Bright yellow to Yellow	Circular	Flattened, glistening
Nutrient glucose agar (NGA)	96.00 (9.85)	1-4	3.2	Light yellow	Irregular	Slightly raised, slimy glistening
SX Agar	67.00 (8.25)	2-5	2.1	Bright yellow	Mostly circular	Slightly raised, glistening, convex
Xan-D	39.40 (6.36)	0.10-0.30	0.20	Bright yellow	Circular to irregular	Slightly raised
XPS Agar	0.00 (1.00)	0.0	0.0	-	-	-
Yeast Extract Dextrose Calcium Carbonate agar (YDCA)	42.00 (6.56)	1-5	2.4	Milky white	Circular	Flattened / slightly raised, glistening
Yeast Extract Nutrient Agar (YNA)	82.80 (9.15)	1-4	2.5	Light yellow	Mostly circular	Flattened
S.E.m±	0.52					
CD at 1%	1.95					

* $\sqrt{x+1}$ values

yellow coloured colonies were recorded on nutrient glucose agar and GYCA agar media. Purely milky white, circular, slightly/flattened, glistening colonies were observed on YDCA media.

Biochemical characters

Starch hydrolysis

The presence of starch hydrolysis was indicated by the presence of clear zones around the bacterial spot inoculum. The zone hydrolysed was measured in terms of cm *i.e.*, 3.5 cm.

Gelatin liquefaction

Observations were made for the formation of clear zone around the growth of the bacterium measured which was 0.5 cm in diameter.

KOH solubility

When the KOH alkali solution was placed on the inoculum on the slide that became sticky after five minutes when it lifted with the bacterial loop, a long thread was observed which indicated that the bacterium was gram –ve.

H₂S production

After four days the presence of H₂S was indicated by blackening of the filter paper. Where as the strips of filter paper with control (without inoculum) was pure white on which there was no dark band on the paper was observed.

Table 1b. Growth rate of *Xanthomonas axonopodis* pv. *glycines* on different differential and selective media

Medium	Hours after inoculation			No. of colonies after inoculation		
	24	48	72	24	48	72
Glucose yeast chalk agar (GYCA)	+	++	+++	54.2 (7.42) *	63.3 (8.01)	77.00 (8.83)
Nutrient agar (NA)	+	++	+++	70.0 (8.42)	80.22 (9.01)	85.40 (9.30)
Nutrient glucose agar (NGA)	++	+++	+++	88.50 (9.46)	96.00 (9.84)	96.00 (9.85)
SX Agar	++	++	+++	56.58 (7.58)	56.58 (8.14)	67.00 (8.25)
Xan-D	+	++	+++	25.25 (5.12)	35.48 (6.03)	39.40 (6.36)
XPS Agar	-	-	-	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Yeast Extract Dextrose Calcium Carbonate agar (YDCA)	+	++	+++	30.50 (5.61)	38.42 (6.27)	42.00 (6.56)
Yeast Extract Nutrient Agar (YNA)	++	+++	+++	70.00 (8.42)	80.86 (9.04)	82.80 (9.15)
S.Em±				0.30	0.12	0.52
CD at 1%				1.21	0.38	1.95

Note : Colony growth : ‘ - ’ no growth, ‘+’ less growth, ‘++’ moderate growth, ‘+++’ maximum growth

* $\sqrt{x+1}$ values

Table 2. Biochemical tests for *Xanthomonas axonopodis* pv. *glycines* causing bacterial pustule of soybean

Tests	Reaction
Starch hydrolysis	+
Gelatin liquefaction	+
KOH solubility	+
H ₂ S production	+
Acid production (carbon sources)	Sucrose +
	Dextrose +
	Glucose +
Acid production (nitrogen sources)	Peptone -

Note + : Positive - : Negative

Acid production from sucrose, dextrose, glucose and peptone

At 24, 48 and 72 hrs *X. axonopodis* pv. *glycines* showed very strong acid production for (carbon sources) sucrose, dextrose and glucose sources, which was observed by clear change in the colour from blue to straw yellow colour. Where as the pathogen failed to produce acid from nitrogen source (peptone) till 72 hr by no change in the colour (blue).

Physiological characters

Temperature requirement

The effect between the different temperature levels on the growth of *Xanthomonas axonopodis* pv. *glycines* was significant. The maximum number of colonies of the pathogen

Table 3. Effect of temperature on the growth of *Xanthomonas axonopodis* pv. *glycines*

Temp. levels (°C)	Number of colonies of the bacteria	Growth (O. D. Value) at 660 nm
10	0.00 (1.00) *	0.7460 (1.32) *
20	21.40 (4.73)	0.9240 (1.38)
25	77.40 (8.85)	1.1280 (1.45)
30	117.80 (10.89)	1.9380 (1.71)
35	48.20 (7.01)	1.0380 (1.45)
40	2.40 (1.84)	0.9840 (1.45)
45	0.00 (1.00)	0.0800 (1.03)
S.Em±CD at 1%	0.040.17	0.010.03

* $\sqrt{x+1}$ values**Table 4.** Effect of pH on the growth of *Xanthomonas axonopodis* pv. *glycines*

pH levels	Number of colonies of the bacteria	Turbidity index (Growth O.D. value at 660 nm)
4.0	1.80 (1.67) *	0.2520 (1.11)*
5.0	17.40 (4.28)	0.7540 (1.32)
6.0	64.40 (8.08)	0.7720 (1.33)
7.0	103.2 (10.20)	1.2560 (1.50)
8.0	72.60 (8.57)	0.8580 (1.36)
S.Em±CD at 1%	0.050.22	0.010.03

* $\sqrt{x+1}$ values

was recovered as 117.80 at the temperature level of 30°C followed by 77.40, 48.20, 21.40 at 25, 35 and 20°C respectively. The least number of colonies (2.40) at 40°C. The pathogen failed to grow at the extreme temperature levels of 10°C and 45°C.

The OD value was maximum (1.9380) at a temperature level of 30°C followed by 1.1280 and 1.0380 at 25, 35 and 35°C respectively. The least OD value was recorded (0.0800) in 45°C temperature indicating the turbidity of the pathogen.

Effect of hydrogen ion (pH) concentration

The data revealed that the effect of each pH level on the growth of the bacterium was found significant. The pathogen was found to grow between the pH levels of 4.0-8.0 with pH of 7.0 as optimum where significantly maximum numbers of colonies (103.20) were recorded. The next best pH level of the medium for the growth of the bacterium was 8.0 and 6.0 where in 72.60 and 64.40 respectively colonies were recorded which are on par with each other. The least growth of the bacterium was observed at pH of 4.0 (1.80) and 5.0 (17.40).

The OD value was maximum (1.2560) at a pH level of 7.0 followed by 0.8580 at 8.0. The OD value was on par with each other at pH 6.0 and 5.0 which were 0.7720 and 0.7540 respectively. The least OD value was recorded (0.2520) at 4.0 indicating the turbidity of the pathogen.

REFERENCES

1. Sinclair, J. B., The seed borne nature of some soybean pathogens. The effect of phomopests and *bacillus subtilis* on germination and their occurrence in soybean produced in Illinois. *Seed Sci. and Tech.*, 1978; **6**: 957-964.
2. Anonymous, *Directorate Reports and Summery Tables of Experiment*, AICRP on Soybean, Directorate of Soybean Research, 2015; Indore. p. 54.
3. Salle, A. J., Fermentation of carbohydrates and related compounds. In : *Laboratory Manual on Fundamental Principles of Bacteriology*, McGraw Hill Book Company, New York, 1961; p. 94-98.