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# Outbreak of grapevine bacterial canker disease in India[1)]

by

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**Ausbreitung eines Reben-Bakterienbrandes in Indien**

Zusammenfassung: In Indien wurde bei Reben die starke Zunahme einer durch Bakterien verursachten Brandkrankheit festgestellt. Die an Blattspreite, Blattstiel und Sproßachse ausgelösten Symptome waren eckig begrenzt, erhaben und brandig verfärbt. Starker Befall führte zum Absterben der Blätter und zu Störungen des Triebwachstums. Als neuer Wirt neben der Rebe wurde Mango (*Mangifera indica* L.) nachgewiesen. Als Erreger der Krankheit wurde das Bacterium *Xanthomonas campestris* pv. *viticola* identifiziert. Dieses bildet in Verbindung mit Salicin und Aesculin Säuren; für *Xanthomonas*-Arten ist eine solche Reaktion ungewöhnlich.

Key words: bacteria, disease, grapevine bacterial canker disease, GVBCD, Xanthomonas campestris pv. viticola, leaf, shoot, symptomatology, physiology, biochemistry, variety of vine, mango, India.

## Introduction

Grapevine (*Vitis vinifera* L.) is an important fruit crop and grown in many parts of the world. Due to development of wine and raisin production in India, this crop has attained industrial significance. During the last few years, the crop has suffered severely from a bacterial canker disease caused by *Xanthomonas campestris* pv. *viticola* (NAYUDU) DYE (CHAND and KISHUN 1988; KISHUN and CHAND 1988). The disease causes about 60—80 % loss in yield in severely infected vineyards. The disease was first reported from India in 1972 (NAYUDU 1972). No further information is available since then, despite its sudden spread and menace in major grape growing areas of the country. Hence, it was felt necessary to investigate the disease in more detail.

## Materials and methods

### Isolation and pathogenicity

Infected samples were collected in Maharashtra, Andra Pradesh and Karnataka (India) from seedless grapes (Thompson Seedless, Tas-e-Ganesh, Sonaka and Manik Chaman). Isolations were made from infected leaves, petioles and canes on nutrient agar by streak plate method (HAYWARD 1983) and the culture was purified by single colony isolation.

Pathogenicity of each isolate was confirmed on 1-year-old grapevine cv. Thompson Seedless raised under glasshouse conditions. The inoculum was prepared by suspending the 72 h old growth of bacterium in sterile water ($2 \times 10^6$ cfu/ml) and inoculated by pinprick method (KOIZUMI 1971). Inoculated plants were incubated at 90 % relative humidity for 48 h and then transferred to glass house (RH 70 —80 %).

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### Cultural, morphological and biochemical characters

All the isolates were grown on nutrient and SX (SCHAAD and WHITE 1974) agar media for colony characters. The gram stain, KOH solubility, nitrate reductase, gelatin liquification, arginine dehydrolase and catalase were determined as described by FAHY and HAYWARD (1983). The methyl red test, production of $H_2S$, indole, urease, fermentative metabolism, growth at 4 and 35 °C and in 3 % NaCl were tested by method of DYE (1968). The basal medium of PALLERONI and DOUDOROFF (1972) was used for utilization of different carbon compounds as sole source of carbon. Production of acid with different carbon sources was studied in medium 'C' (DYE 1968).

### Host range

Neem (*Azadirachta indica* A. JUSS.), *Achyranthus aspera* L., *Centella asiatica* (L.) ORBAN, bell pepper (*Capsicum annuum* L.), tomato (*Lycopersicon esculentum* MILL.), mango (*Mangifera indica* L.), lantana (*Lantana camara* L.), *Lagasca mollis* CAV., guava (*Psidium guajava* L.). promegranate (*Punica granatum* L). castor (*Ricinus communis* L.), sapota (*Achrus sapota* L.), lemon (*Citrus limon* BURMF.), parthenium (*Parthenium hysterophorus* L.), fig (*Ficus carica* L.) and papaya (*Carica papaya* L.) which were present in the vicinity of vineyards were inoculated with the bacterium. Observations on disease development were recorded regularly up to 45 d.

The grapevine cvs Thompson Seedless, Sonaka, Manik Shaman, Anab-e-Shahi, Ugni blanc and Bangalore blue were also inoculated.

## Results and discussion

### Symptoms

Initial symptoms of disease appear as small water-soaked lesion surrounded by a yellow halo on the lower surface of the leaf. These lesions enlarge (2—5 mm) in due course and become angular and cankerous (Figs. 1 and 2). Sometimes the lesions coalesce and form large patches. Severely infected leaves give a blighted appearance and remain firmly attached to the cane even after complete drying. Lesions are brown to black, elongated (0.5—8 cm) and cankerous on petioles and canes (Figs. 3 and 4). In advanced stages of infection, stunting, cracking and irregular growth of canes were also observed. On the berries lesions are brown to black; cankerous and severely infected berries are small and shrivelled.

### Isolation and pathogenicity

The pathogen was isolated from leaf, petiole and cane on nutrient agar after 3 d of incubation. Maximum populations (46 colonies/plate) were recovered from canes and petioles. Very low (2 colonies/plate) recovery came from leaf isolates. *Erwinia herbicola* was recovered in every isolation. Its presence was maximum in leaves and minimum in canes. This may be the major factor in lower recovery of pathogenic bacteria from leaves.

On inoculation of leaves (cv. Thompson Seedless), all isolates were found to produce typical disease symptoms within 10 d, whereas it was 20 d in petioles and canes. Initially water-soaked symptoms appeared around the margin of a pinprick which gradually increased in size and transformed into angular lesions. The same bacterium was recovered from the lesions on reisolation.

## Cultural and morphological characters

Bacterial colonies on nutrient agar were white, round, smooth, glistening with entire margin and on SX agar it produced a clear starch digestion zone (3—4 mm). Bacterium was a gram negative rod with rounded ends, motile by single polar flagellum and 0.4—1.2 × 2—3 μm in size.

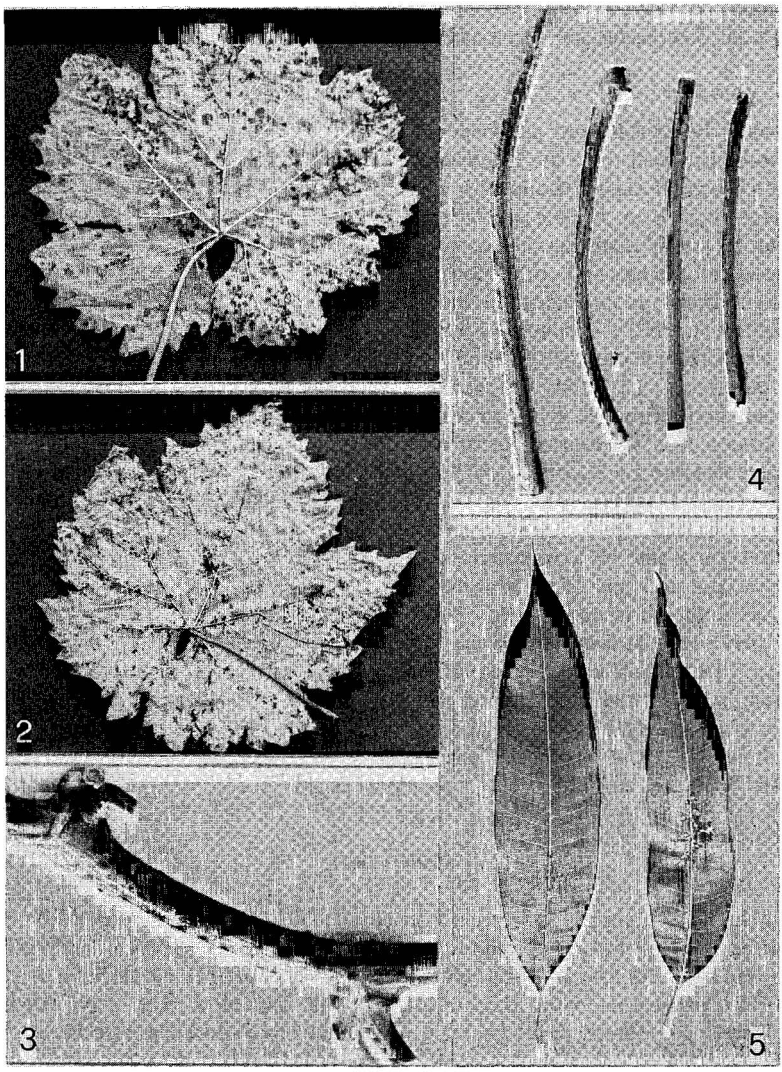


Fig. 1: Grapevine bacterial canker on leaf lamina.
Fig. 2: Symptoms along the veins.
Figs. 3 and 4: Cankerous symptoms on cane and petioles.
Fig. 5: Infection on mango leaves.

Abb. 1: Bakterienkrebs der Rebe auf der Blattspreite.
Abb. 2: Symptome längs der Blattadern.
Abb. 3 und 4: Krebssymptome an Sproß und Blattstielen.
Abb. 5: Infektion von Mangoblättern.

### Physiological and biochemical characters

The results presented in the table clearly indicate that the disease causing bacterium was negative in potato soft rot, arginine dehydrolase, tobacco hypersensitivity, nitrate reduction and production of fluorescent pigment on King medium B. Starch hydrolysis, growth at 4 and 35 °C, oxidative mode of nutrition and other characters were typical to *Xanthomonas* spp. On the basis of these characters, the bacterium was identified as *Xanthomonas campestris* pv. *viticola* (NAYUDU) DYE (NCPPB 3611).

### Host range

Among the 16 different plant species tested (besides grapevine), only mango was found to be infected with the bacterium. On mango leaves, *X. campestris* pv. *viticola* produces brown to black lesions within 10 d of inoculation. These lesions were found to grow continuously up to 30 d and afterwards become restricted (Fig. 5). These symptoms can be easily distinguished from mango bacterial canker by the slower growth, non-cankerous lesions and under-developed yellowish halo.

Among the grapevine cultivars inoculated, Anab-e-Shahi and Bangalore blue were found less susceptible. Grapevine is reported to be infected by many bacterial diseases (BHIDE *et al.* 1972; NAYUDU 1972; BRADBURY 1973; DE CLEANE and DE LEY 1976; HOPKINS 1977; MARTELLI *et al.* 1986). The bacterial disease of grapevine reported by NAYUDU (1972) can be distinguished by its cankerous lesions on leaves, petioles, canes and fruits. Hence, it is appropriate to name the disease 'grapevine bacterial canker'. The bacterium isolated by NAYUDU (1972) was earlier identified as *Pseudomonas viticola*. DYE (1978) redefined its taxonomic position and named it *X. campestris* pv. *viticola* based on few characters. Additional biochemical and physiological characters of the bacterium are reported here which support the view of DYE (1978) and confirm the identity of the pathogen. The present isolates produce acid with salicin and esculin, which is rare for *Xanthomonas* spp. (MOFFETT and CROFT 1983).

Occurrence of *X. campestris* pv. *viticola* on mango is not observed in nature but its ability to infect this plant under artificial inoculation is an important discovery. Thus the mango which is grown abundantly in grape growing areas of India may serve as an additional source of inoculum for the pathogen. The neem which is reported a collateral host for *X. campestris* pv. *viticola* (NAYUDU 1972) was not infected by our isolates collected from different grape growing areas of India.

## Conclusion

This study confirmed that a recent outbreak of grapevine bacterial canker disease is due to more extensive cultivation of susceptible seedless cultivars. *X. campestris* pv. *viticola* was found to be associated with this disease. Mango may serve as a collateral host for the pathogen during the off-season. The disease is mainly spread through infected cuttings. Hence, strict quarantine measures should be adopted to check the further spread of the disease. Propagating material should be restricted to vineyards free of the disease.

## Summary

A severe outbreak of grapevine bacterial canker disease has been noticed in India. Disease symptoms produced on leaf blade, petiole and cane were angular, raised, and cankerous. Severe infection leads to the death of leaves and affects the growth of

Physiological and biochemical characters of *Xanthomonas campestris* pv. *viticola* isolates

Physiologische und biochemische Charakterisierung der Isolate von *Xanthomonas campestris* pv. *viticola*

| Characters | Reaction | | |
|---|---|---|---|
| | Leaf isolate | Petiole isolate | Cane isolate |
| Production of $H_2S$ | + | + | + |
| Production of indole | – | – | – |
| Nitrate reduction | – | – | – |
| Hydrolysis of gelatin | + | + | + |
| Hydrolysis of starch | + | + | + |
| MR and VP test | – | – | – |
| Proteolysis of milk | – | – | – |
| Mode of glucose utilization | Oxidative | Oxidative | Oxidative |
| Catalase | + | + | + |
| Arginine dehydrolase | – | – | – |
| Urease | – | – | – |
| Salt tolerance (3 %) | + | + | + |
| Hydrolysis of: Tween 80 | + | + | + |
| Tween 60 | + | + | + |
| Tween 40 | + | + | + |
| Tween 20 | + | + | + |
| Levan production | + | + | + |
| Potato soft rot | – | – | – |
| Hypersensitivity in tobacco leaves | – | – | – |
| Growth at: 4 °C | + | + | + |
| 35 °C | + | + | + |
| Pigment on King's medium B | – | – | – |
| D,L-phenyl deaminase | – | – | – |
| Utilization of carbon sources: Malonate, mannitol, L-sorbose, lactose, glucose, sucrose, starch, mannose, trehalose, galactose, salicin, raffinose | + | + | + |
| Acid production from glucose, sucrose, fructose, starch, esculin, salicin, trehalose, lactose, mannose, arabinose, cellobiose, ribose, melibiose, galactose, L-sorbose, D-xylose, mannitol | + | + | + |
| Dulcitol, adonitol, rhamnose, inulin, sorbitol, tartaric acid | – | – | – |

+ = positive, – = negative.

canes. Mango (*Mangifera indica* L.) was found as a new collateral host. The causal bacterium was identified as *Xanthomonas campestris* pv. *viticola*. Production of acid with salicin and esculin was found, which is a rare character for *Xanthomonas* spp.

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