JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Nov. 2013. Vol. **7**(Spl. Edn.), p. 149-158

Identification of Newly Isolated Xanthomonades by Using Pathological, Chemical and Physiological Tests

Khalid A. Ali AbdelRahim[1,2]*, M.M. Ibrahim[1,3] and Gehan A. El-Gaaly[4]

[1]Department of Botany and Microbiology, College of Science, King Saud University P.O. Box 2455, Riyadh 11451, Saudi Arabia.
[2]Department of Botany, Faculty of Science, Sohag University, 82524 Sohag, Egypt,
[3]Department of Botany and Microbiology, Faculty of Science, Alexandria University, P.O. Box 21511, Alexandria, Egypt.
[4]Department of Botany and Microbiology, Female Section, College of Science, King Saud University, P.O. Box 22452, Riyadh, 11495, Saudi Arabia.

(Received: 06 August 2013; accepted: 19 October 2013)

Thirty newly isolated *Xanthomonas*-strains from different host were characterized by pathological, chemical and physiological tests.All strains were Gram-negative with short rods. Scanning electron microscopy showed that the newly isolated strains are polar-monoflagellated short rods.The bacteria were oxidase-negative, utilized glucose oxidatively and not fermentatively. All strains were able to hydrolyse starch and esculin. All of the strains tested grew on 0.02% TTC and were unable to grow on 0.1% TTC. Some of newly tested strains induced a typical hypersensitive reaction (HR) in tobacco leaves, but all the strains tested induced a typical HR in tomato leaves. Therefore, it is suggested to use tomato leaves instead of tobacco leaves for testing the HR inducing capability of *Xanthomonas* strains. The Rfvalues of TLC test and UV spectrophotometric analysis of newly tested strains pigments (xanthomonadin) were similar to those used as reference strains.

Key words: Xanthomonads, Xanthomonadin, Chemotaxonomic, HR test.

During the last years several new *Xanthomonas* isolates were obtained by the Göttingen Collection of Phytopathogenic Bacteria (GSPB). These strains originated from new host plants (*Catharanthus* spp., *Lobelia* spp., *Isotomaaxillaris*) for which diseases caused by xanthomonads had not been reported earlier. Only abstracts or short reports on these strains have been published (Poschenrieder *et al.*, 1988; Mavridis *et al*., 2000; Mavridis and Rudolph, 2002; Poschenrieder *et al.*, 2002). The aim of these studies was for further characterization of these new *Xanthomonas* strains in order to denote correct or new species or pathovar names.

Secondly, the so–called highly virulent strains (HVS) causing disease on cotton were obtained. These strains caused different symptoms on cotton leaves than the known races of *X. c*. pv. *malvacearum*. As shown in Fig. 1 the HV strains cause marked necrotic areas on cotton leaves which enlarge more and more until leaf drop. In contrast, the known races of *X. c*.pv. *malvacearum* cause water-soaked leaf spots on cotton leaves (Fig. 2) which may transform into necrotic spots but rarely cover the whole leaf (Ahmed *et al*., 1995; Kucera and Rudolph, 1998; Huang *et al.*, 2000).

The first steps concerning primary characteristics of the new*Xanthomonas*

* To whom all correspondence should be addressed.
Tel.: +966146476940; Fax: +96614675833;
E-mail: kabderaheem@ksu.edu.sa

strainsincluded microscopic investigation, Gram staining, electron microscopic investigation to detect the motility organs (flagella), some physiological and pathological tests and finally examination of the unique pigment for xanthomonads, xanthomonadin.

Isolations from plant tissue and soil debris often yield many yellow-pigmented bacteria. It may be difficult to distinguish colonies of *Xanthomonas* spp. visually from colonies of saprophytic bacteria by color, especially with *Xanthomonas* species that do not produce copious amounts of extracellular polysaccharide (EPS). Because the yellow xanthomonadin pigments of *Xanthomonas*are unique to the genus, pigment extraction and chromatographic analysis can be an important tool for bacterial genus identification (Irey and Stall, 1982; Suryawanshi, *et al*., 2011; Minhaj *et al*., 2002).

Starr (1944) and Starr and Stephens (1964) showed in early comparative studies that (i) all Xanthomonas strains examined synthesized similar pigments (as detected by chromatographic and spectral absorption properties), (ii) these pigments seemed to be unique to the genus Xanthomonas not similar to those formed by other yellow-pigmented bacteria and (iii) these pigments were membrane bound (Stephens and Starr, 1963). Further studies (Anderwes*et al.*, 1973; 1976; Starr *et al.*, 1977) revealed that the Xanthomonas pigments consist of mixtures of brominated, aryl-polyene esters, collectively called xanthomonadin (Fig. 1). It became clear by 1977 that xanthomonadins from different xanthomonads differed in bromination and methylation (Starr *et al.*, 1977). Some exceptional *Xanthomonas campestris* pathovarsgrow withcolonies of white color, such as the pathovars*manihotis, phyllanthi, ricini, vitiswoodrowii, vesicatoria, mangiferaeindicae, azadirachtae and pedalii* and the earlier species Xanthomonasmaltophilia(now named Stenostrophomonasmaltophilia) (van den Mooter, 1984).

Studies with *X. albilineans*, *X. arboricola*pv. *pruni*, *X. campestris*, *X. fragariae*, *X. axonopodis*, *X. arboricola*pv. *juglandis*, *X. hyacinthi*, *X. axonopodis*pv. *phaseoli*, *X. translucens*, *X. vesicatoria* and *X. ampelina* (currently *Xylophilusampelinus*) indicated that the bromination and methylation patterens of xanthomonadins were useful for identification of members within the genus (Chun, 2000). Starr *et al.* (1977) showed distinctive electronic absorption and chemical properties of the pigments from *Xylophilusampelinus*, suggesting that this species does not belong in the genus *Xanthomonas*. Xanthomonadins have not been reported from any other bacteria, including those yellow pigmented *Pseudomonas spp*., which might be thought to be related to *Xanthomonas*. Palleroni and Doudoroff (1972) reported that the pigments of *Pseudomonas vesicularis*, *P. mendocina*, *P. flafa*, and *P. palleroni* are carotenoids but not xanthomonadins. Also the major pigments of *Pseudomonas paucimobilis* are carotenoids (nostoxathin).

Therefore, unique pigments (xanthomonadin) produced by *Xanthomonas* strains are considered an adequate chemotaxonomic marker for the genus *Xanthomonas*, so thatxanthomonads can easily be distinguished from the many other genera of yellow-pigmented bacteria which are isolated from the plants also (Goodfellow*et al.*, 1976).

Xanthomonadin production is controlled by a gene cluster consisting of seven transcriptional units (Poplawsky*et al.*, 1993, Fig. 2) and is regulated by an extracellular bacterial pheromone (Poplawsky and Chun 1995; Chun and Poplawsky 1997). The gene cluster appears to be present in all xanthomonads including the white variant *X. manihotis* (Poplawsky and Chun 1995). One of these transcriptional units,*pigB*, encodes a diffusible factor (DF) that is involved in both pigment and extracellular polysaccharides production (Poplawsky and Chun 1997). Hence, single site *pigB* mutants are typically white and produce less EPS. Goel*et al.* (2001) isolated an ethyl methane sulfonate (EMS) which induced a pigment-deficient mutant of *X. oryzae*pv. oryzae which is also virulence deficient and aromatic amino acids autotrophic. A genomic clone that restores pigmentation, prototrophy, and virulence of this mutant was isolated by functional complementation (Goel*et al*., 2001). Characterization of this clone indicated that shikimate dehydrogenase, an enzyme participating in the amino acid biosynthetic pathway, is required for production of pigments and virulence at normal level in *X. oryzae*pv. *oryzae*(Goel*et al*., 2001).

Pigments deficient mutants of

Xanthomonasjuglandis and *X. oryzae*pv. *oryzae*were reported to be more sensitive to photobiological damage than the wild type strains suggesting that the pigments may provide protection against photo damage in the presence of oxygen (photodynamic damage) (Jenkins and Starr, 1982; Poplawsky and Chun, 1998). The majority of pigment-deficient mutants that have been isolated from several xanthomonads are prototrophs and virulence proficient upon wound inoculation (Poplawsky*et al.*, 1993; Tsuchiya *et al.*, 1982; Durgapal, 1996). Pheromone, xanthomonadin and extracellular polysaccharide production may be required for epiphytic survival by *X. c*. pv. *campestris* on crucifer leaves (Poplawsky and Chun 1995). It is well known that epiphytic survival is essential for xanthomonads to establish a successful infection (Rudolph, 1993). When both xanthomonadin and EPS production are deficient, populations of *X. c*. pv. *campestris* are as much as 1000-fold lower in planta. This resulted in significantly fewer lesions (from 8.7 to 1.7 lesions per leaf) in spray-inoculated crucifer leaves (Poplawsky and Chun, 1995).

MATERIALS AND METHODS

Cultures of 48 strains of *Xanthomonas* were included in these studies, as listed in table 1. Sixteen strains from well identified xanthomonads belonging to the pathovars*malvacearum*, *campestris*and *juglandis* were used asreference strains; 32 strains were newly isolated from different host plants. Ten strains were isolated from *Lobelia sp*. (Campanulaceae), 9 strains from *Isotomaaxillaris* (Campanulaceae), 10 strains from *Gossypium sp*. (Malvaceae) and 3 strains from *Catharanthuspusillus* (Apocynaceae). The bacteria were cultivated on nutrient agar plates to reduce the slime production.

Physiological tests.

Gram staining.

The Gram stain was applied to air dried films of bacteria by standard techniques (Schaad, 1980).

Oxidase test

The oxidase test was performed according to Kovacs, 1956.

Oxidation-fermentation test

Oxygen requirements were determined with the glucose semi-solid medium of Hugh and Leifson (1953).

Growth on TTC

Bacteria were streaked onto nutrient agar (8 g Difco nutrient broth consisting 3 g beef extract and 5 g peptone plus 15 g agar per liter) containing 0.02% or 0.1% triphenyl-tetrazolium chloride (TTC). Enough of sterile, autoclaved solution of TTC was added to autoclaved NA to make the final concentrations. Plates were checked for bacterial growth after 4 days incubation at 30 °C.

Starch hydrolysis

Starch hydrolysis was performed as described by Lelliott and Stead, 1987. Starch hydroly-sis was indicated by the presence of clear zones in the black-stained medium around or under the colonies. Reddish zones indicated that starch had been partially hydrolyzed to dextrins.

Esculin hydrolysis

Esculin, a glucoside, is cleaved by all *Xanthomonascampestris*pathovars yielding glucose and dihydrocoumarin (dark brown compound). Esculin utilization is indicated by the development within 3—4 days of a progressive blackening of the medium or disappearance of fluorescence when observed under ultra-violet light(Sands, 1990).

Electron microscopic test

The newly isolated strains were investigated using scanning electron microscopy to find out whether the bacterial cells showed the same characteristics as the well-known xanthomonads concerning the number and position of flagella.

Pathogenicity tests

Bacteria from 48 h-old slant agar (NGA) cultures were suspended in 1 to 5 ml of sterile tap water. These suspensions were injected into the intercellular spaces of intact leaves of tobacco (cultivar Xanthi) and tomato (cultivar Lyconorma) with a hypodermic needle (Klement,, 1990).

Analytical methods for pigments determination.

Thin layer chromatography (TLC) of pigments

1. A colony was streaked onto nutrient agar (8 g Difco nutrient broth consisting of 3 g beef extract and 5 g peptone plus 15 g agar per liter). The nutrient agar should not contain additional carbohydrates because the resulting copious slime will interfere with chromatography of the pigments.

2. After 48 h of growth, the bacteria were scraped from the surface and added to 3 ml of spectrophotometry grade methanol in a test tube with a screw cap. Enough bacteria should be added to the methanol to give a turbidity equivalent to nearly 10^{10} CFU/ml.
3. The capped tube was placed in a boiling water bath until the pigment had been extracted from the bacteria (solution becomes yellow).
4. The solution was centrifuged at 1,000 g for 15 min to remove cell debris.
5. The supernatant was decanted and the methanol was evaporated in a water bath at 50-60 C ° until the optical density of the pigment extract reached 0.4 at 443 nm.
6. 5 µl were spotted on a precoated, thin-layer chromatography sheet of silica gel 60 of 0.2 mm thickness (E. Merck, Darmstadt, Germany). A total of 25 µl per spot were applied, allowing each 5 µl amount to dry before the next portion was applied.
7. The plate was placed in a developing apparatus with anhydrous spectrophotometry grade methanol as solvent. The solvent front was allowed to move approximately 10 cm.
8. The yellow spots were outlined with a pencil when the silica gel was still wet. A *Xanthomonas* reference strain was included as a control.

Absorption spectrum of pigments

Pigments of selected strains were extracted by scraping the growth from 4-day-old NA plates and suspending the cells in 4 ml of methanol in screw-cap tubes. The tubes were immersed in a water bath at 90 °C for 5 min and then centrifuged at 1400 x g for 15 min. The absorption spectrum of the pigment extracts was determined with a Perkin-Elmer Lambda 15 UV/VIS double-beam scanning spectrophotometer by cooperating with a working team (Prof. Laatsch) from the Department of Organic Chemistry of the University of Göttingen.

RESULTS

Physiological tests

All of the newly isolated strains were Gram-negative bacteria with short rods. The bacteria were oxidase-negative, utilized glucose oxidatively and not fermentatively, produced acid

Table 1. Strains used in this study

Number of Strains	Origin and date of isolation	Host plant	Pathovar suggested name (Newly isolated)	Pathovar Name (Reference)
8	(6) Nicaragua, 1986; (1) USA; (1) Sudan, 1991	Cotton	-	*X. c*Pv. *malvacearum,* Race 18
6	(5) Turkey, 1987; (1) USA, 1986.	Cotton	-	*X. c*Pv. *malvacearum,* Race 6
1	(1) Germany, 1994.	Brassicaceae	-	*Xanthomonas campestrispv. campestris*
1	(1) ATCC 29078	Juglans	-	*Xanthomonas campestrispv. juglandis*
10	(7) Burkina Faso, 1984; (3) Sudan, 1994	Cotton	HV Strains	-
10	(9) Germany, 2000; (1) Netherland, 2000.	*Lobelia sp.*	*Xanthomonas campestrispv. Lobelia*	-
9	(9) Germany, 2001.	*Isotomaaxillaris*	*Xanthomonas campestrispv. isotoma*	-
3	(3) India, 1997	*Catharanthus pusillus.*	*Xanthomonas campestrispv.catharanti*	-

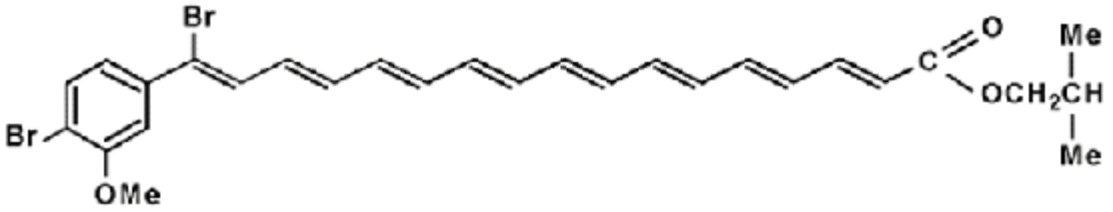


Fig.1. Structure of xanthomonadin I produced by *Xanthomonasjuglandis*, reproduced from Andrewes *et al.,* 1973

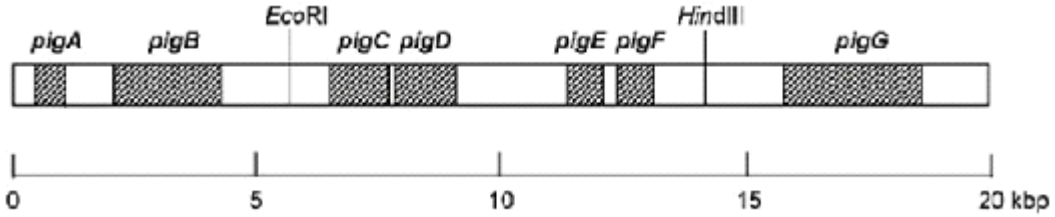
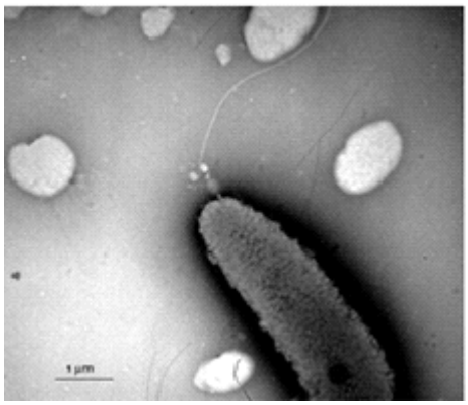
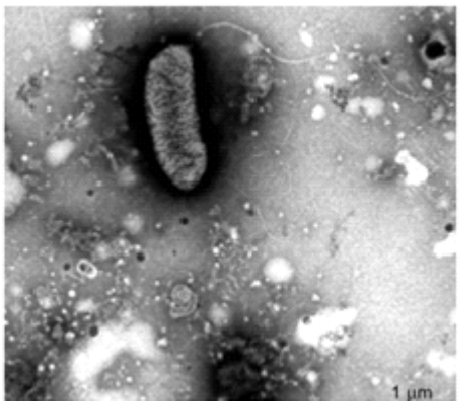


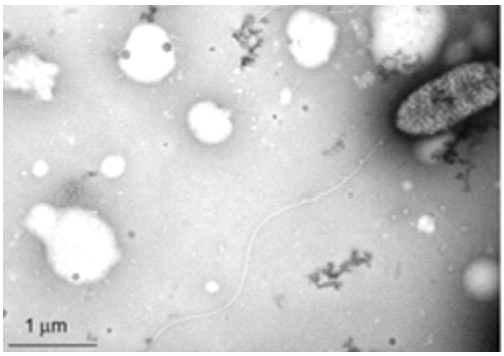
Fig.2. The xanthomonadin gene cluster from*Xanthomonascampestris*pv. *campestris*. Reproduced from Poplawsky*et al.,* 1993



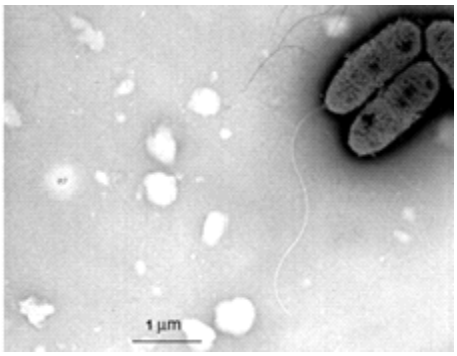
a) Scanning electron micrograph of *Xanthomonas* strain isolated from *Isotoma* (GSPB 3088)



b) Scanning electron micrograph of *Xcm*, HV strain isolated from cotton (GSPB 3006)

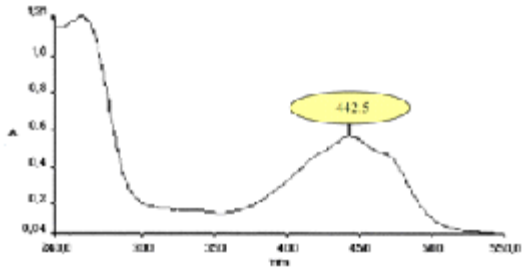


c) Scanning electron micrograph of *Xanthomonas* strain isolated from *Lobelia* (GSPB 3030)

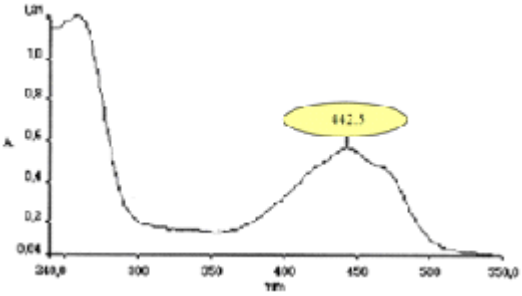


d) Scanning electron micrograph of *Xanthomonas*, isolated from *Catharanthu* (GSPB 2801)

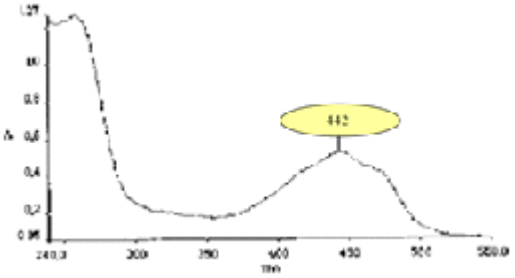
Fig. 3. Scanning electron micrographs of representative strains from newly isolated *Xanthomonas*pathovarsshowing the mono-polar flagellum



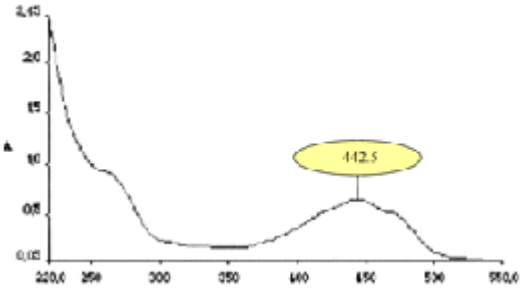
a) *Xcm* race 18 ®



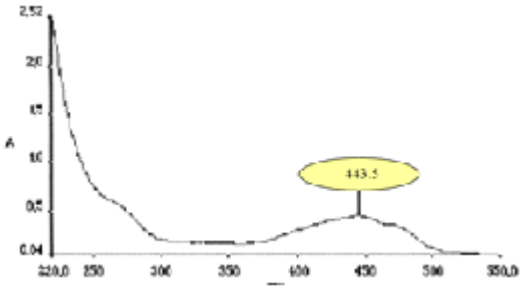
e) strains isolated from *Lobelia*



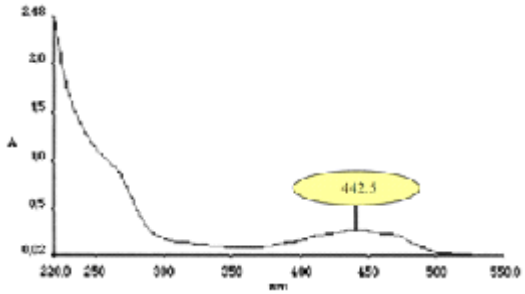
b) Strains isolated from C*atharanthus*



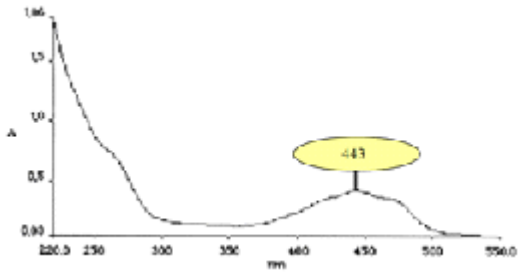
f) *Xcm* race 6®



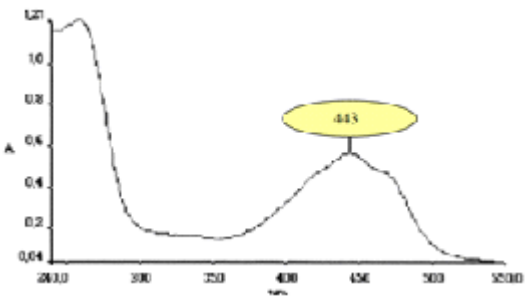
c) *X. campestrispv. Juglandis*®



g) Strains isolated from *Isotoma*



d) *Xcm*(HVS)



h) *Xcpv.campestris*®

Fig. 4(a-h): UV spectroscopy of pigments ofrepresentative strains belongs to newly and References® used in this study

from glucose. All strains tested grew on 0.02% TTC and were unable to grow on 0.1% TTC. All strains were positive in the starch hydrolysis test as well as in esculin hydrolysis.

Electron microscopic investigation

The results of scanning electron microscope investigation showed that all of these newly isolated strains are polar-monoflagelatted short rods (Figs.3, a-d) which are typical for the known xanthomonads.

Pathogenicity tests

The hypersensitive reaction (necrosis) of tobacco leaves indicated phytopathogenicity. All strains induced a typical hypersensitive reaction (HR) intomato, but not all tested strains induced (HR) intobacco leaves.

Analytical methods for Pigments determination

Thin layer chromatography (TLC)

Most of the strains tested showed an Rf value of exactly 0.48 whereas the values of the other strains ranged from 0.45 to 0.49. These values are similar to those used as reference strains.

Absorption spectra of pigments

According to The UV scanning spectrophotometer experiments all of the strains tested including reference strains showed approximately the same absorption maxima at 440 to 445 nm which are characteristical for the xanthomonadin pigments Figs. 4 (A - H).

DISCUSSION

The physiological tests used in this study were the ones listed in Bergey‘s Manual (Buchanan and Gibbons, 1974) for characterizing *Xanthomonas*. All xanthomonadsare Gram-negative rods, sometimes slightly curved with rounded ends. The cell length is variable, even within the same strain (Swings *et al.,* 1993).The cultures used in this study were uniform in all the tests used; they were Gram negative short rods occurring mostly alone or in pairs. Chains and filaments also occurred as discussed by Swings *et al.*(1993). The oxidase test was also uniform to the xanthomonds (negative reaction). The results of starch hydrolysis tests were positive which corresponds with the reactions known for most pathovars of *Xanthomonas* (Sands, 1990). Also all of the strains tested were able to cleave esculin yielding glucose and dihydrocoumarin (positive reaction). This result also is typical for all *Xanthomonas* pathovars except *X. fragariae* (Sands, 1990).

Most bacterial plant pathogens are oxidative (respiratory) but erwinias are fermentative (Lelliott and Stead 1987). Xanthomonads are obligate aerobic bacteria. In the oxidation-fermentation test of glucose, acid is formed in open tubes by all strains of the *campestris* complex, *X. oryza*, *X. populi* and *X. fragariae*. No acid is produced, either in opened or in sealed tubes, by *X. axonopodis*, *X. albilineans* and *X. maltophilia* (Swings *et al*., 1993). Most yellow pigmented xanthomonads are inhibited by 0.02% TTC, all of them are inhibited by 0.1 % TTC (Lelliott and Stead, 1987). Our results showed that the all of strains tested were able to grow on 0.02% of TTC and were not able to grow on 0.1% TTC. Thus, all the physiological tests proofed that the new strains belong to the genus *Xanthomonas*.

As the genus *Xanthomonas*is currently defined, yellow color and pathogenicity tests are the most reliable characteristics that can be used for identification. The use of pathogenicity to distinguish xanthomonads is probably due to the absence of saprophytic members in this genus. Necrosis of inoculated test plants (tobacco and tomato) was evidence for phytopathogenicity of the new strains. Tomato was the superior test plant for detection of pathogenicity by *Xanthomonas,* butTobacco was the less reliable for proving phytopathogenicity. It has also been noted by other authors that many *Xanthomonas*pathovars do not induce a HR in tobacco leaves (Klement, 1990). This method is extremely suitable for all phytopathogenicpseudomonads. There are some difficulties with xanthomonads the concentrations of which should be higher than 10^8 cells ml^{-1}. Plants also should be incubated at low temperature (18 ^oC) for two days before inoculation and at 30 – 32 ^oC after inoculation with xanthomonads(Klement, 1990). Since tomato leaves (cultivar Lyconorma) developed a typical HR after inoculation with all the newly isolated strains as well as with the *Xanthomonas* reference strains it is suggested to use tomato leaves instead of tobacco leaves for testing the phytopathogenicity of supposed xanthomonads.

The new strains tested were motile rodes

with monopolar flagella (monotrichous). According to Swings *et al.*(1993), *Xanthomonas* cells are motile, sometimes sluggishly. *X. maltophilia* (now named *Xenostrophomonas*) and *X. albilineans* both have cells that move rapidly. *Xanthomonascampestris*pv. *Graminis*is non-motile. *X. populi*contains motile and non-motile strains. *X. maltophilia* is multiflagellated.

The $R_f$values of bacterial pigments which were studied by Irey and Stall (1982) averaged at 0.45 (range of 0.42 to 0.49), whereas the pigments extracted from other yellow bacteria gave spots with R_f values considerably different from those of *Xanthomonas*. The R_f values of the other yellow pigmented bacteria ranged from 0.07 to 0.85, with some extracts having up to four distinct spots. The authors found that the pigments of the yellow bacteria were more distinct on thin layer plates, and did not fade as quickly after development as did the pigments of xanthomonads. The workers also demonstrated that the absorption maxima of crude pigment extracts of 11 selected strains belonging to *X. albilineans*, *X. axonopodis*, and other pathovars of *X. campestris* had an absorption maximum at 443 nm which differed from the absorption of pigments extracted from non *Xanthomonas* yellow pigmented bacteria. According to Starr *et al*. (1977) the absorption spectra of different *Xanthomonas* pigments ranged from 421.2 to 451.2. These authors classified the *Xanthomonas* strains studied according to the absorption spectrum of pigments into 15 groups. The most frequently encountered xanthomonadin pigments were those in pigment group 7 (dibrominated, M^+ 560, λ_{max}443-445 nm) and pigment group 10 (monobrominated, M^+ 481, λ_{max} 440-441 nm).

In our experiments the R_f values after TLC ranged from 0.45 to 0.49, and many strains showed R_f values of exactly 0.48. The absorption maxima of the pigments varied from 439.5 to 444 nm, and most often a $\lambda_{max}$of 442.5 was determined.

Thus, the yellow pigments of all the new *Xanthomonas* strains were identified as xanthomonadins. However, we could not find specific Rf-values or absorption maxima for the different pathovarsand strains tested. It appears as if mono– and dibrominated pigments occurred in every pathovar tested.

In conclusion, the phytopathological, physiological and biochemical tests confirmed that all the new strains tested belonged to the genus *Xanthomaons.* Specific features of the different pathovars tested could only be established by infection experiments on their respective host plants.

ACKNOWLEDGMENTS

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding this work through research group no RGP-VPP 297

REFERENCES

1. Ahmed, N.E., Rudolph, K.,Mavridis, A. Occurrence and distribution of *Xanthomona scampestris*pv. *malvacearum*races in Sudan. Phytomedizin, Mitteil. d. Deutschen Phytomedizin. Gesellschaft.,1995; **25**(2): 13 - 14.
2. Goel, K.G., Lakshmi, R.,Sonti, R.V. Pigment and virulence deficiencies associated with mutations in the *aroE* gene of *Xanthomonasoryzae*pv. oryzae. *Appl. Environ. Microbiol.*,2001; **67**: 245-250.
3. Andrewes, A.G., Hertzberg, S., Liaaen-Jensen, S., Starr, M.P. The *Xanthomonas* "carotenoids" - non-carotenoid, brominated, aryl-polyene esters. *Acta Chemica Scandinavica*,1973; **27**: 2383-2395.
4. Andrewes, A.G., Jenkins, C.L., Starr, M.P., Shepherd, J., Hope, H. Structure of xanthomonadin I, a novel dibrominated aryl-polyene pigment produced by the bacterium *Xanthomonasjuglandis*. Tetrahedron Letters, 1976; **45**: 4023-4024.
5. Buchanan, R.E., Gibbons, N.E. Bergey's Manual of Determinative Bacteriology, 8th ed. Williams and Wilkins, Baltimore, MD, USA,1974.
6. Chun, W.C. Xanthomonadins, unique yellow pigments of the genus *Xanthomonas*. The Plant Health Instructor.APS Education Center Advanced Topics,2000.
7. Chun, W., Cui, J.,Poplawsky, A.R. Purification, characterization and biological role of a pheromone produced by *Xanthomona scampestris* pv. *campestris. Physiol. Mol. Plant* Pathol., 1999; **51**: 1-14.
8. Durgapal, J.C..Albino mutation in rice bacterial blight pathogen.*Curr. Sci.*,1996;**70**: 15.
9. Goodfellow, M., Austin, B., Dickinson, C.H. Numerical taxonomy of some yellow-pigmented

bacteria isolated from plants. *J. Gen. Microbiol.*,1976; **97**: 219-233.

10. Huang, X., Mavridis, A., Rudolph, K. Charakterisierung der Eigenschaften der neuen, "hochvirulenten" Stämme von *Xanthomonas campestris* pv. *malvacearum*, dem Erreger der eckigen Blattfleckenkrankheit der Baumwolle. *Mitt. Biol. Bundesanst. Land- Forstwirtsch*, 2000; **376**: 531.
11. Hugh, R.,Leifson, E. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various Gram negative bacteria. *J. Bacteriol.*,1953; **66**: 24-25.
12. Irey, M.S., Stall, R.E.Value of xanthomonadins for identification of pigmented *Xanthomonascampestris*pathovars. Pages 85-95 in: Proc. Fifth Int. Conf. Plant Path. Bacteria. Published by C.I.A.T., Cali, Columbia,1982.
13. Jenkins, C.L., Starr, M.P. The brominated aryl polyene (xanthomonadin) pigments of *Xanthomonasjuglandis* protect against photobiological damage. Curr.Microbiol.,1982; **7**: 537-326.
14. Klement, Z. Tobacco (HR) test for the quick demonstration of pathogenicity. In: Klement, Z., Rudolph, K., and Sands, D. C., edts., Methods in Phytobacteriology. Akadémiai Kiadó, Budapest, 1990; 101-102.
15. Kovacs, N. Identification of *Pseudomonas pyocyanea* by the oxidase reaction. *Nature*, London,1956; **178**: 703.
16. Kucera, F., Rudolph, K. Charakterisierung von hochvirulenten Stämmen von *Xanthomonas campestris* pv. *malvacearum* (Smith) Dye (*Xcmal)* aus dem Sudan. Phytomedizin, Mitteilungen Deutsch. Phytomedizin. Gesellsch., 1998; **28** (1): 69-70.
17. Lelliott, R.A., Stead, D.E. Methods for Diagnosis of Bacterial Diseases of Plants. In: Preece, T. F., V2 ed. Methods in Plant Pathology, 1987.
18. Mavridis, A., Chand, R., Chaurasia, S., Rudolph, K. Eine neue Bakteriose an*Catharanthus*. Phytomedizin, Mitteilg. Deutsch. Phytomedizin. Gesellsch., 2000; **30**(1): 7.
19. Mavridis, A., Rudolph, K. Ist die Lobelienkultur durch eine neue Bakteriose gefährdet? Phytomedizin, Mitteilg. Deutsch. Phytomedizin. Gesellsch., 2002; **32**(2): 49-50.
20. Minhaj A.,. Suryawanshi, A.R, More, D.R. and Baig, M.M.V. Genetic and Pathological Diversity among Xanthomonas axonopodis pv citri Strains causing Citrus Canker in Maharashtra, India. *J Pure & Appl Microbiol*, 2012; **6**(3): 1219-1226.
21. Palleroni, N.J., Doudoroff, M. Some properties and taxonomic subdivisions of the genus *Pseudomonas*. *Ann. Rev. Phytopathol.*,1972; **10**: 73-100.
22. Poplawsky, A.R., Chun, W. A *Xanthomona scampestris* pv. *campestris* mutant negative for production of a diffusible signal is also impaired in epiphytic development. *Phytopathology*, 1995; **85**: 1184.
23. Poplawsky, A.R., Chun, W. *pigB* determines a diffusible factor needed for extracellular polysaccharides slime and xanthomonadin production in *Xanthomonascampestris*pv. *campestris*. *J. Bacteriol.*,1997; **179**: 439-444.
24. Poplawsky, A.R., Chun, W. *Xanthomona scampestris* pv. *campestris* requires a functional *pigB*for epiphytic survival and host infection. *Mol. Plant-Microbe Interact*.,1998; **11**: 466-475.
25. Poplawsky, A.R., Kawalek, M.D.,Schaad, N.W. A xanthomonadin-encoding gene cluster for the identification of pathovars of *Xanthomona scampestris*. Mol. Plant- Microbe Interact., 1993; **6**: 545-552.
26. Poschenrieder, G., Felgentreu, D., Schäfer, K. Eine neue Bakteriose an *Isotoma axillaris* (Syn. *Laurentia axillaris*). Phytomedizin, Mitteilg. Deutsch.Phytomedizin. Gesellsch.,2002; **32** (1): 44-45.
27. Poschenrieder, G., Lohweg, E.,Gerlach, W.P. Eine neue Bakteriose an *Lobelia erinus* "Richardii", hervorgerufen durch *Xanthomonas campestris*. Gärtnerbörse u. Gartenwelt,1988; **50**: 2204-2205.
28. Rudolph, K. Infection of the plant by *Xanthomonas*.In: Swings, J. G. and Civerolo, E. L. (edts). Xanthomonas, pp. 193-264, Chapman and Hall, London, 1993.
29. Sands, D.C. Physiological criteria - determinative tests. In: Klement, Z., Rudolph, K., and Sands, D. C., edts., Methods in Phytobacteriology. AkadémiaiKiadó, Budapest, 1990; 134-143.
30. Schaad, N.W. Initial identification of common genera.Pages 2-3 in: N. W. Schaad, ed., Laboratory Guide for Identification of Plant Pathogenic Bacteria. *Amer. Phytopathol. Soc.*, St. Paul, MN.,1980;72 pp.
31. Starr, M.P. Studies of phytopathogenic bacteria. CornellUniversity Abstracts of Theses for 1944, pp. 349-350.
32. Starr, M.P., Jenkins, C.L., Bussey, L.B.,Andrewes, A.G. Chemotaxonomic significance of the xanthomonadins, novel brominated, aryl-polyene pigments produced by bacteria of the genus *Xanthomonas*. *Arch. Microbiol.*, 1977; **113**: 1-9.
33. Starr, M.P., Stephens, W.L. Pigmentation and taxonomy of the genus *Xanthomonas*.J. *Bacteriol.*,1964; **87**: 293-302.

34. Stephens, W.L., Starr, M.P. Localization of carotenoid pigment in the cytoplasmic membrane of *Xanthomonas juglandis. J. Bacteriol.*, 1963; **86**: 1070-1074.
35. Suryawanshi, A.R., More, S.M. and Baig, M.M.V. Molecular Characterization of Xanthomonas citrii Pathovars from Marathwada Region. *J Pure & Appl Microbiol*, 2011; **5**(2): 807-812.
36. Swings, J., Vauterin L.,Kersters, K.The bacterium *Xanthomonas*. In: Swings, J. G., and Civerolo, E. L. (edts). *Xanthomona* spp. 121-146, Chapman and Hall, 1993.
37. Tsuchiya, K., Mew. T.W.,Wakimoto, S. Bacteriological and pathological characteristics of wild type and induced mutants of *Xanthomona scampestris*pv. *oryzae*. Phytopathology, 1982; **72**: 43-46.
38. Van den Mooter, M. De taxonomie van het plant pathogene bakterien geslacht *Xanthomonas* Dowson 1939. *Ph.D. thesis*, Rijksuniversiteit Gent, Belgium,1984.