JOURNAL OF BACTERIOLOGY
Vol. 87, No. 2, p. 293-302 February, 1964
Copyright © 1964 by the American Society for Microbiology
Printed in U.S.A.

PIGMENTATION AND TAXONOMY OF THE GENUS *XANTHOMONAS*

MORTIMER P. STARR AND WILLIAM L. STEPHENS[1]

Department of Bacteriology, University of California, Davis, California

Received for publication 26 August 1963

ABSTRACT

STARR, MORTIMER P. (University of California, Davis), AND WILLIAM L. STEPHENS. Pigmentation and taxonomy of the genus *Xanthomonas*. J. Bacteriol. **87**:293-302. 1964.—The colonies formed by phytopathogenic bacteria of the genus *Xanthomonas* are typically yellow in color. This chromogenesis stems from the presence in all yellow xanthomonads of a particular carotenoid pigment. This unique "*Xanthomonas*-carotenoid," which has not been found in any yellow nonxanthomonad, is characterized herein to the extent required for its recognition by relatively simple screening procedures. In general, the occurrence of a carotenoid "alcohol" with absorption maxima at 418, 437, and 463 mμ (petroleum ether)—in a gram-negative, polarly-flagellated, oxidative, rod-shaped bacterium—would suggest placement of that microorganism in the genus *Xanthomonas*.

Many of the phytopathogenic bacteria are pigmented, and this chromogenesis is an important determinative trait used practically in the isolation and identification of these microorganisms. Nevertheless, with a few exceptions (Starr, 1944, 1958; Starr and Saperstein, 1953), only incidental attention has been devoted to the nature of their pigments.

Most of the chromogenic phytopathogenic bacteria are referred to the genera *Pseudomonas* and *Xanthomonas* of the order Pseudomonadales. In naming the genus *Xanthomonas*, Dowson (1939) emphasized that the yellow color is one of the more significant characteristics of its species. This emphasis upon production of a yellow growth, and its use as a primary determinative feature for these bacteria, prompted a general study of the yellow coloring matter of *Xanthomonas*, which had been shown earlier (Starr, 1944) to be carotenoid in nature. Our work on the localization of this pigment in the cytoplasmic membrane of *X. juglandis* appeared recently (Stephens and Starr, 1963).

The present contribution substantiates our belief that a unique carotenoid occurs universally in the yellow members of the genus *Xanthomonas*, and that it is not found in a representative collection of yellow nonxanthomonad bacteria, including several which are erroneously referred to *Xanthomonas*. The possible taxonomic and determinative implications of these observations are discussed. The precise chemical nature of the unique *Xanthomonas* pigment is considered here only to the extent required for its recognition by relatively simple screening procedures.

MATERIALS AND METHODS

Cultures. Cultures were obtained from the International Collection of Phytopathogenic Bacteria (ICPB) maintained at the Department of Bacteriology, University of California at Davis, from the general stock culture collection of the same department, and from the American Type Culture Collection (ATCC). The following species (names, as received) and strains were used: *Cellulomonas biazotea* (2074); *C. fimi* (2544); *Erwinia ananas* (ICPB-EA133); *E. lathyri* (ICPB-EL102); *Flavobacterium acidificum* (ATCC-8366); *F. ferrugineum* (ATCC-13524); *F. heparinum* (ATCC-13125); *F. meningisepticum* (ATCC-13253); *F. resinovorum* (ATCC-12524); *P. rimaefaciens* (ICPB-PR107); *P. syringae* (ICPB-PS169); *Pseudomonas* sp. (safflower-662-11-1-yellow); *X. badrii* (ICPB-XB103); *X. begoniae* (ICPB-XB8); *X. beticola* (ICPB-XB109Sm^r); *X. carotae* (ICPB-XC139); *X. cassiae* (ICPB-XC141); *X. corylina* (ICPB-XC12); *X. desmodii-gangeticii* (ICPB-XD106); *X. geranii* (ICPB-XG10); *X. holcicola* (ICPB-XH3); *X. hyacinthi* (ICPB-XH110); *X. juglandis* (ICPB-XJ103); *X. lespedezae* (ICPB-XL2); *X. manihotis* (ICPB-XM12); *X. nakatae-olitorii* (ICPB-XN101); *X. oryzae* (ICPB-XO101); *X. pelargonii* (ICPB-XP8); *X. phaseoli* (ICPB-

[1] Present address: Department of Biology, Chico State College, Chico, Calif.

XP104); "X." *stewartii* (ICPB-SS1, SS11, SS12, SS18); *X. taraxaci* (ICPB-XT11); "X." *trifolii* (ICPB-XT109, "*Bacterium herbicola*" 2554); "X." *uredovorus* (ICPB-XU104); *X. vasculorum* (ICPB-XV24); *X. vesicatoria* (ICPB-XV3). The quotation marks indicate our belief that these species ("X." *stewartii*, "X." *trifolii*, and "X." *uredovorus*) are not correctly assigned to the genus *Xanthomonas*.

Cultivation of the bacteria. Before use in this study, each culture was checked for purity, Gram stain reaction, cell morphology, motility, and flagellation. The xanthomonad stock cultures were grown on "YDC" agar slants consisting of 1.0% Difco yeast extract, 1.0% glucose, 2.0% finely divided $CaCO_3$, and 1.5% agar. These were transferred to Difco nutrient broth (100 ml per 250-ml Erlenmeyer flask); the flasks were incubated at 28 C on a rotary shaking machine operating with an amplitude of 1.5 in. at a rate of 160 rev/min. After 24 hr, the 100-ml cultures were checked microscopically for purity, and then transferred *in toto* aseptically to 500 ml of nutrient broth contained in 2-liter Erlenmeyer flasks, which were incubated for 24 hr under the same conditions. After a second purity check, the cells were harvested in a Servall centrifuge (type SP/X) at 3,440 $\times$ *g*; a typical xanthomonad yielded about 4 g of moist packed cells per liter of culture.

Of the 20 nonxanthomonad cultures, 18 were cultivated in a similar manner; however, because most of these cultures appeared to form less pigment than the true *Xanthomonas* spp., additional flasks of culture medium were used, and the cells were harvested in a Sharples Super-Centrifuge at 50,000 rev/min. *F. ferrugineum* and *Pseudomonas* sp. "662-11-1-yellow" did not produce pigmented cells when cultivated in the above manner; they were grown on YDC agar plates at 30 C and, after sufficient growth, the pigmented cells were scraped off.

Solvents. The following solvents were all reagent grade of Allied Chemical Corp., New York, N.Y.: petroleum ether (b.r. 30 to 60 C), methyl alcohol (absolute), diethyl ether (anhydrous), and benzene. The petroleum ether was further purified by passage through a silicic acid column, and the diethyl ether was redistilled and stored over reduced iron.

Extraction of the pigments. Approximately 50 ml of absolute methanol were added per g of moist bacterial cells, and the container was immersed in hot water to bring the methanol quickly to the boiling point and thus extract the pigments in a very few minutes. The solution was then cooled, and centrifuged to remove the cells. Direct controls revealed that this brief exposure to an elevated temperature produced no observable injurious effects on the pigments.

Separation of types of pigments. The methanol extract of each organism was partitioned between immiscible solvents to separate the carotenoids into four empirical groups: "hydrocarbons," "alcohols," "esters," and "acids." It must be emphasized that these terms, as applied to crude pigment preparations, do not necessarily relate to actual chemical structure—rather, they mean that the crude pigment partitions in the manner expected of pure carotenoid hydrocarbons, alcohols, esters, or acids. There is a substantial effect of impurities upon this partition behavior, and much confusion has resulted from any more rigorous interpretation of such partition tests. This partition procedure (Fig. 1) is a modification of the scheme of Sobin and Stahly (1942) which in turn derives from the method of Kuhn and Brockmann (1932). We recommend close adherence to the following procedural details as essential for reproducibility.

Petroleum ether was added to the methanolic cell extract to make a monophasic system, after which enough water was added to give a 90% alcohol concentration (neglecting, however, the $<2\%$ water in the cell extract). The mixture was shaken in a separatory funnel, but very gently to avoid formation of an emulsion. To insure complete separation, the petroleum ether layer was extracted repeatedly with fresh samples of 90% methanol, and the methanol phase was similarly partitioned several times against fresh petroleum ether. This partition test should first be performed on a small sample; if all pigments remain in the methanol (i.e., are hypophasic), the bulk of the methanolic extract may then be saponified and partitioned against diethyl ether.

The petroleum ether layer was then washed with distilled water by means of a continuous-flow device, dehydrated with sodium sulfate, and taken to dryness in vacuo with a Rinco evaporator. The dried material may contain carotenoid hydrocarbons and esters. This residue was then dissolved in 3% KOH-methanol, and saponified at 40 C for 3 hr. An equal volume of petroleum ether was added, followed by enough water to give a 90% alcohol concentration; then

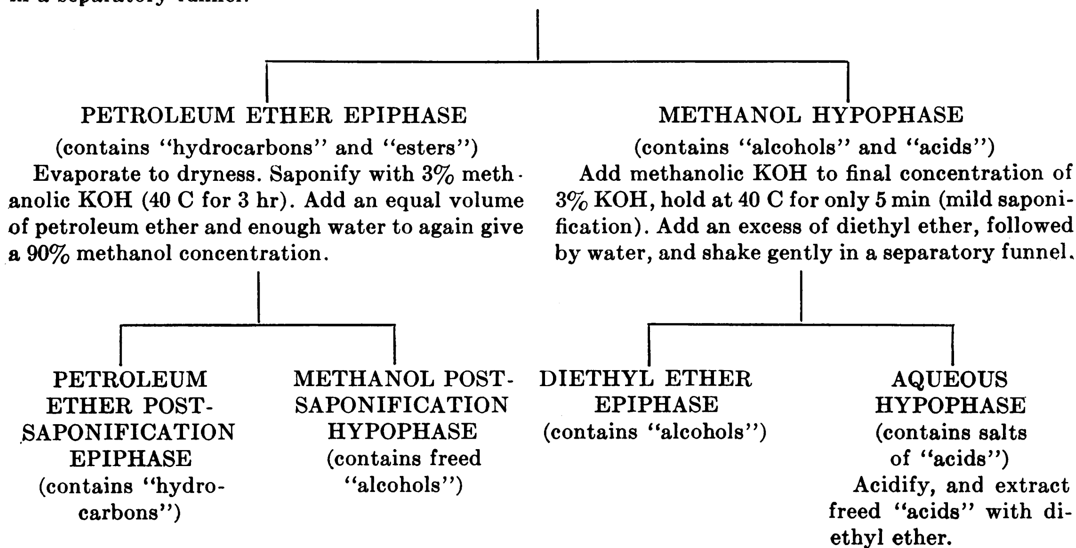

FIG. 1. *Scheme for empirical separation of bacterial carotenoid pigments.*

the mixture was shaken very gently in a separatory funnel. The petroleum ether epiphase was removed, washed, dehydrated with sodium sulfate, and dried in vacuo. The individual carotenoid hydrocarbons (i.e., carotenes) in the residue were then separated by column chromatography.

The de-esterified carotenoid alcohols in the KOH-methanol hypophase were transferred into diethyl ether by the addition first of that solvent and then of distilled water. The ether layer was washed free of the methanol-KOH with water, freed of water by treatment with a saturated solution of NaCl followed by the addition of anhydrous sodium sulfate, and then dried in vacuo. These de-esterified carotenoid alcohols were then separated by adsorption chromatography.

Returning now to the original methanolic hypophase (or the original methanolic cell extract), we deviated slightly from the Sobin-Stahly procedure, which calls for addition of just enough KOH to make the solution alkaline to litmus. Instead, KOH-methanol was added to the methanolic solution of the pigments to a final KOH concentration of 3% and the solution was heated gently (40 C for 5 min); then 2 volumes of diethyl ether were added to make a monophasic system, followed by enough water to separate two layers. This mixture was shaken very gently in a separatory funnel. The hypophase was drained off and, if still colored, was partitioned repeatedly against fresh samples of diethyl ether. The ether layer, which contained the carotenoid alcohols, was withdrawn, washed, dehydrated, and evaporated to dryness by the methods previously mentioned. It must be emphasized that the best possible extraction of the carotenoid alcohols (i.e., xanthophylls) requires that the water be added to the homogeneous solution of diethyl ether and methanolic pigment extract. Strain (1938) showed that alcohol or acetone solutions of xanthophylls form colloidal solutions when diluted with water and, moreover, the pigments cannot be extracted from these solutions with solvents that are immiscible with water. The formation of these colloidal solutions occurs most easily in the presence of the saponified materials and the complex substances which are in the alkaline lipid extracts.

Any carotenoid acids would remain in the aqueous phase in the form of their salts, and might be recovered by the addition of dilute hydrochloric acid, followed by extraction with diethyl ether.

The method described above was used on the crude methanol extracts primarily to isolate carotenoids of similar solubility properties, and

thereby to effect a certain amount of purification prior to chromatography. It should again be stressed that partition tests performed upon such crude lipid extracts can give quite different results from those performed on pure carotenoids, and that the designations "hydrocarbon," "alcohol," "ester," and "acid" are merely a convention. The presence of other lipids interferes with the partitioning of the different carotenoid types. Partition tests used in the chemical characterization of a carotenoid should be performed on a pure sample.

Chromatographic adsorption. After separating the carotenoids into the aforementioned groups, the individual carotenoids of each group were isolated by column chromatography. The extracts were adsorbed from dry benzene solutions onto columns of magnesia-Celite, 1:1 by weight (MgO, Baker's chemically pure analyzed; Celite, L-665-A of Johns-Manville, New York, N.Y.). We used Pyrex chromatography tubes (16 by 130 mm) with coarse-porosity fritted discs sealed in the inner member of the $^{14}/_{35}$ ground joint. Columns were packed dry with gentle suction, then washed with benzene forced through the adsorbent with nitrogen. Loading of the samples onto the columns and development of the chromatograms were also accomplished with nitrogen under positive pressure. Development was generally achieved with benzene. After separation of the pigments, the columns were allowed to run dry, and the zones were separated mechanically and eluted with methanol.

Spectrometric analysis. All spectrometric analyses were performed with a Beckman recording spectrophotometer (model DK-2). The absorption maxima of the individual carotenoids were determined in petroleum ether, benzene, and absolute methanol. The spectrophotometer was calibrated with a Hanovia mercury lamp and checked periodically with the wavelength calibration standards listed in Beckman instruction manual 305-A.

RESULTS

Behavior upon partition tests. The procedure described for separating the four major types of carotenoids can be abridged somewhat for the pigments of *Xanthomonas* species. Since these particular pigments were always hypophasic when a 90% methanolic crude extract was partitioned against petroleum ether, this partition need be tried only on a small sample to verify the absence of epiphasic pigments. No epiphasic pigments were found in any xanthomonads, and the bulk of the crude methanol extract was further processed as though it were the "original methanolic hypophase."

Chromatograms and absorption maxima. Table 1 contains the results of the chromatographic and spectrophotometric observations of the pigments found in the xanthomonads. The designations given to the pigments in this table are arbitrary and serve merely to identify the specific carotenoids. Zones are numbered in the order in which they appear on the columns from top to bottom.

Of the 20 *Xanthomonas* cultures examined, all 19 yellow-pigmented species contained carotenoids; only the colorless *X. manihotis* was devoid of these pigments. All carotenoids found in each of the yellow species had partition behaviors, in crude extracts, like those of carotenoid alcohols. In every case, the pigment in at least one zone of the chromatogram, when eluted and examined photometrically, showed an absorption spectrum with maxima of (418), 437, and 463 mμ in petroleum ether; (435), 454, and 481 mμ in benzene; and (420), 441, and (468) mμ in methanol. (Parentheses around an absorption maximum indicate a shoulder or broad central peak centered around that approximate wavelength.) More often, the pigments in two separate zones of the column possessed this particular set of maxima. In such cases, the pigments in the upper zone (designated type 2) appear to be oxidized or oxidatively deteriorated forms of the pigment in the lower zone, the "universal pigment" of the yellow xanthomonads.

Pigments 1 and 2 are further distinguishable from one another on the basis of spectral configuration (Fig. 2). It should not be inferred that these are two distinctive carotenoids. Although the lower zone (pigment 1) is spectrally homogeneous, a spectral gradient is apparent when the upper zone is mechanically divided into sections. This gradient (Fig. 3) ranges from pigments with bathochromic (high-wavelength) peaks and hypsochromic (low-wavelength) shoulders of equal height through pigments with bathochromic peaks much lower in absorbance than the corresponding hypsochromic shoulders. In extreme examples of this latter case, the bathochromic peak actually disappears, and the hypsochromic shoulder blends into the principal

TABLE 1. *Absorption maxima of the carotenoids of Xanthomonas*

Species	Culture designation[a]	Adsorption behavior[b]		Pigment no.[c]	Absorption maxima (mμ)[d]		
		Zone	Color		Petroleum ether	Benzene	Methanol
X. badrii	ICPB-XB103	I	Red	5	(415) 433 459	(433) 451 479	(419) 438 (462)
		II	Orange	1	(418) 437 465	(435) 454 481	(420) 441 (468)
X. begoniae	ICPB-XB8	I	Red	2	(418) 437 463	(435) 454 481	(420) 441 (468)
		II	Orange	2_1	(418) 437 463	(435) 454 481	(420) 441 (468)
X. beticola	ICPB-XB109	I	Orange	2	(418) 437 463	(435) 454 481	(420) 441 (468)
	Sm^r	II	Yellow	1	(418) 437 463	(435) 454 481	(420) 441 (468)
X. carotae	ICPB-XC139	I	Red	3	(401) 420 445	(415) 436 460	(425) (445) —
		II	Orange	4	(398) 417 442	(412) 431 457	(425) (443) —
		III	Yellow	1	(418) 437 463	(435) 454 481	(420) 441 (468)
X. cassiae	ICPB-XC141	I	Red	2	(418) 437 463	(435) 454 481	(420) 441 (468)
		II	Orange	2_1	(418) 437 463	(435) 454 481	(420) 441 (468)
X. corylina	ICPB-XC12	I	Orange	2	(418) 437 463	(435) 454 481	420) 441 (468)
X. desmodii-gangeticii	ICPB-XD106	I	Red	2	(418) 437 463	(435) 454 481	(420) 441 (468)
		II	Orange	1	(418) 437 464	(435) 454 481	420 441 (468)
X. geranii	ICPB-XG10	I	Red-orange	2	(418) 437 463	(435) 454 481	(420) 441 (468)
		II	Orange-yellow	1	(418) 437 463	(435) 454 481	(420) 441 (468)
X. holcicola	ICPB-XH3	I	Red	2	(418) 437 463	(435) 454 481	(420) 441 (468)
		II	Orange	1	(418) 437 463	(435) 454 481	(420) 441 (468)
X. hyacinthi	ICPB-XH110	I	Orange	1_2	(418) 437 463	(435) 454 481	(420) 441 (468)
X. juglandis	ICPB-XJ103	I	Red	2	(418) 437 463	(435) 454 481	(420) 441 (468)
		II	Orange	1	(418) 437 463	(435) 454 481	(420) 441 (468)
X. lespedezae	ICPB-XL2	I	Red	2	(418) 437 463	(435) 454 481	(420) 441 (468)
		II	Orange	1	(418) 437 463	(435) 454 481	(420) 441 (468)
X. manihotis	ICPB-XM12	—	—				
X. nakatae-olitorii	ICPB-XN101	I	Orange	2	(418) 437 463	(435) 454 481	(420) 441 (468)
		II	Yellow	1	(418) 437 463	(435) 454 481	(420) 441 (468)
X. oryzae	ICPB-XO101	I	Red-orange	2	(418) 437 463	(435) 454 481	(420) 441 (468)
		II	Pale orange	1	(418) 437 463	(435) 454 481	(420) 441 (468)
X. pelargonii	ICPB-XP8	I	Red	3	(401) 420 443	(415) 436 461	(426) (445) —
		II	Orange	1	(418) 437 464	(435) 454 482	(420) 441 (468)
X. phaseoli	ICPB-XP104	I	Orange	2	(418) 437 463	(435) 454 483	(420) 441 (468)
		II	Yellow	1	(418) 437 463	(435) 454 481	(420) 441 (468)
X. taraxaci	ICPB-XT11	I	Red	2	(418) 437 463	(435) 454 483	(420) 441 (468)
		II	Orange	1	(418) 437 463	(435) 454 483	(420) 441 (468)
X. vasculorum	ICPB-XV24	I	Orange	1_2	(418) 437 463	(435) 454 483	(420) 441 (468)
		II	Pale yellow	1	(418) 437 465	(435) 454 483	(420) 441 (468)
X. vesicatoria	ICPB-XV3	I	Red	1_2	(418) 437 463	(435) 454 483	(420) 441 (468)
		II	Orange	1	(418) 437 463	(435) 454 483	(420) 441 (468)

[a] ICPB = International Collection of Phytopathogenic Bacteria, Department of Bacteriology, University of California, Davis (M. P. Starr, Curator).

[b] Zones are numbered in the order in which they appear on the columns, from top to bottom. Based upon partition tests of crude pigments as explained in the text, all pigments could be categorized—somewhat arbitrarily as is the custom in carotenoid research—as "alcohols."

[c] The numbers given to the pigments serve to identify the specific carotenoids in the text.

[d] Parentheses around an absorption maximum indicate a shoulder or broad central peak centered around that approximate wavelength.

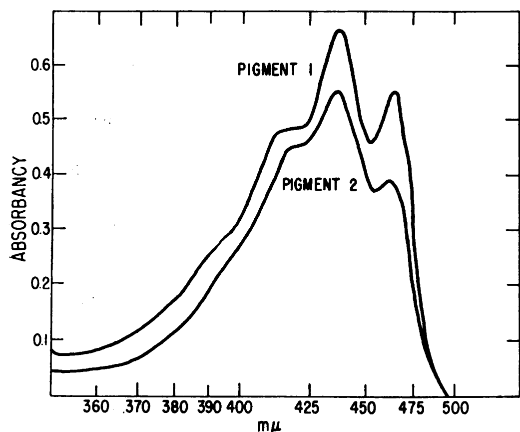

FIG. 2. *Absorption spectra in petroleum ether of pigments types 1 and 2 of Xanthomonas.*

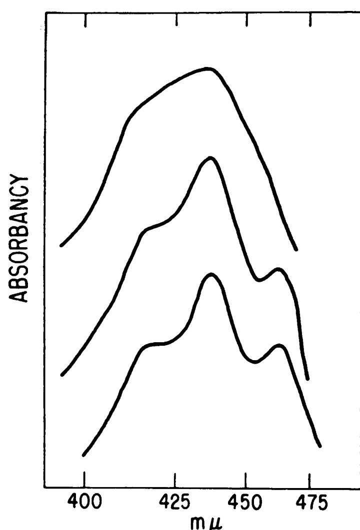

FIG. 3. *Diagrammatic representation of the spectral gradient which is apparent when a single chromatogram zone containing pigment type 2 is mechanically divided into successive sections. The different spectral configurations (in petroleum ether) are arranged in the order in which they occur in the chromatogram zone, from top to bottom.*

peak, thus presenting a single broad-band spectrum. The pigment designated type 1 has a bathochromic peak relatively high in absorbance compared to its hypsochromic shoulder.

Under certain conditions, pigment type 1 can be converted into pigment type 2. When this conversion takes place, there is an accompanying change in chromatographic behavior. Upon development with benzene, pigment 1 moves down a MgO-Celite column, while pigments of type 2 remain at the top of the column. Occasionally, intermediate types are found: those forms which have a spectral configuration like pigment 1, but remain at the top of the chromatograph column like pigment 2, are designated pigment 1_2; those forms which have the spectral configuration of pigment 2, but chromatograph like pigment 1, are designated pigment 2_1.

Table 1 shows that *X. geranii* yielded two bands, when chromatographed on MgO-Celite and developed with benzene. The top band held a carotenoid alcohol with a pigment 2 spectral configuration, while the lower band contained pigment 1. This was the most common pattern encountered in the survey. Other species containing only pigments 1 and 2 were *X. beticola*, *X. desmodii-gangeticii*, *X. holcicola*, *X. juglandis*, *X. lespedezae*, *X. nakatae-olitorii*, *X. oryzae*, *X. phaseoli*, and *X. taraxaci*.

Chromatograms of *X. corylina* always contained a single band, which proved to be a carotenoid alcohol with the pigment 2 configuration. *X. carotae* forms three carotenoid alcohols, one with the spectral configuration and chromatographic behavior of pigment 1, and the other two, pigments 3 and 4, with absorption maxima at much lower wavelengths. *X. begoniae* and *X. cassiae* each yielded two bands when chromatographed on MgO-Celite; the pigments in both top and bottom bands had the spectral configuration of pigment 2. The top bands actually contained pigment 2; because the pigment in the bottom bands chromatographed like pigment 1, it was considered to be pigment 2_1. *X. hyacinthi* had a single pigment with type 1 spectral configuration, but which remained (like pigment 2) at the top of a MgO-Celite column after development with benzene; hence, it was pigment 1_2. *X. vasculorum* and *X. vesicatoria* contained pigment 1_2 and, in addition, they each had pigment 1. *X. pelargonii* contained two carotenoid alcohols; namely, pigment 1 and a carotenoid very similar to the pigment 3 found in *X. carotae*. *X. badrii* had two carotenoid alcohols; besides pigment 1, there was found pigment 5, with absorption maxima at wavelengths slightly lower than those of pigments 1, 1_2, 2, and 2_1.

Chromatograms and absorption maxima of

nonxanthomonads. Table 2 contains the results of the chromatograms and the absorption maxima of all pigments found in the survey of yellow-pigmented species from the genera *Cellulomonas*, *Erwinia*, *Flavobacterium*, and *Pseudomonas*, and in three doubtful species of *Xanthomonas*: *X. stewartii*, *X. trifolii* (including a strain received as *Bacterium herbicola*), and *X. uredovorus*. The numbers given to the pigments in this table are arbitrary and are used merely to label the several carotenoids. Zones are numbered in the order in which they appear on the columns from top to bottom. Parentheses around an absorption maximum indicate a shoulder or a broad central peak centered around that approximate wavelength.

These 20 cultures of yellow nonxanthomonads each contained carotenoids, but none of them contained the "*Xanthomonas*-carotenoid." The partition behavior of all these carotenoids in crude lipid extracts was like that of carotenoid alcohols, which was true also for the *Xanthomonas* pigments. However, the carotenoids of the nonxanthomonads move into the diethyl ether phase from alkaline aqueous methanol much more rapidly than does the *Xanthomonas* pigment. The fine details of the spectral absorption curves of the xanthomonad and nonxanthomonad pigments differ. Furthermore, the "*Xanthomonas*-carotenoid" has a relatively greater sharpness of the absorption peaks in petroleum ether rather than in methanol; most of the pigments from nonxanthomonads reverse this pattern and have the sharper resolution in methanol. Although all the pigments described here partition in crude lipid extracts like carotenoid alcohols, other types of carotenoids were detected in additional control cultures treated in the same manner. Carotenoids which partition like hydrocarbons and alcohols were found in *Rhodospirillum rubrum*; pigments which partition like carotenoid hydrocarbons, alcohols, and esters were found in an unidentified fruiting myxobacterium.

Since the primary purpose of this survey was to determine whether the *Xanthomonas* pigments could be found outside that genus, these other pigments are not described here in any detail. The partition and adsorption behavior, and absorption maxima of each pigment, are presented in Table 2. It is quite possible that the same pigment was isolated from more than one of the examined strains; however, no attempts were made to check duplication, and the pigments from each organism were given separate numbers. The most significant information gained from this survey of nonxanthomonads was the absence of the "*Xanthomonas*-carotenoid" from yellow bacteria other than *Xanthomonas*.

DISCUSSION

In everyday phytobacterial practice, the yellow color of *Xanthomonas* colonies is the key discriminating feature which—rationally or not—is used to distinguish the xanthomonads from other phytopathogenic or saprophytic bacteria isolated from plants (Burkholder and Starr, 1948). This pigmentation can now be attributed to formation of presumably oxygenated carotenoids with absorption-spectrum maxima at 418, 437, and 463 mμ in petroleum ether. All 19 yellow *Xanthomonas* spp. examined in the present study were found to produce this distinctive pigment, which exists in a cluster of related forms. An alcoholic carotenoid with these characteristic properties has hitherto not been reported in the literature (Fox, 1953; Goodwin, 1955; Karrer and Jucker, 1950; Zechmeister, 1934). This unique "*Xanthomonas*-carotenoid" is not produced by any of the other yellow nonxanthomonad bacteria examined in the present study (*Cellulomonas*, *Erwinia*, *Flavobacterium*, *Pseudomonas*, and a few incorrectly designated "*Xanthomonas*" spp.), nor by any of the yellow phytopathogenic corynebacteria previously examined by our group (Starr and Saperstein, 1953; Saperstein, Starr and Filfus, 1954).

Insofar as our present study is representative, the demonstration that distinctive carotenoids occur universally in the genus *Xanthomonas*—and, moreover, that they are restricted to that group—has considerable taxonomic significance. First of all, one could conclude that the formation of a carotenoid with absorption maxima at 418, 437, and 463 mμ (petroleum ether)—by a gram-negative, polarly-flagellated, oxidative, rod-shaped bacterium—would strongly suggest placement in the genus *Xanthomonas*. Certainly our experience to date makes it likely that possession solely of other carotenoids might provide a practicable basis for excluding yellow bacteria from the genus *Xanthomonas*. Arguing in another

TABLE 2. *Absorption maxima of the carotenoids of the nonxanthomonads*

Species[a]	Culture designation[b]	Adsorption behavior[c]		Pigment no.[d]	Absorption maxima (mμ)[e]		
		Zone	Color		Petroleum ether	Benzene	Methanol
Cellulomonas biazotea	2074	I	Orange	33	(415) 435 465	(425) 450 480	(417) 437 467
		II	Pale yellow	34	413 436 466	423 449 480	414 436 466
C. fimi	2544	I	Yellow	35			(414) 436 467
		II	Orange	36		(423) 450 478	(415) 436 465
		III	Pale yellow	37	413 435 465	423 449 479	414 437 467
Erwinia ananas	ICPB-EA133	I	Orange	6	— (445) —	(435) 459 (485)	(425) 446 (467)
		II	Orange	7	(425) 445 (473)	(435) 460 (487)	(425) 445 (473)
E. lathyri	ICPB-EL102	I	Orange	8		(433) 457 (488)	(425) 444 (473)
		II	Pale orange	9	(420) 443 471	(435) 457 (490)	(425) 443 (471)
Flavobacterium acidificum	ATCC-8366	I	Orange	10	(420) 443 (475)	(435) 456 (487)	(427) 445 (477)
		II	Orange	11	(425) 443 (467)	(435) 459 (485)	(425) 445 (470)
F. ferrugineum	ATCC-13524	I	Dark red	12	(445) 467 497	(459) 483 515	(444) 469 499
F. heparinum	ATCC-13125	I	Orange	13	(425) 447 473	(435) 461 490	(425) 448 474
F. meningisepticum	ATCC-13253	I	Pale orange	14	(423) 447 (470)	(435) 457 (488)	(425) 448 (475)
		II	Yellow	15		(435) 459 (490)	(425) 445 (471)
F. resinovorum	ATCC-12524	I	Orange	16	(425) 443 469	(437) 461 488	(426) 447 472
Pseudomonas rimaefaciens	ICPB-PR107	I	Orange	17		(400) 418 (440)	— (425) —
		II	Yellow	18		(398) 420 (440)	408 (425) —
P. syringae	ICPB-PS169	I	Orange	19		— (435) —	
Pseudomonas sp.	622-11-1	I	Pale yellow	20		(435) 457 (488)	(425) 443 (477)
"Xanthomonas" stewartii	ICPB-SS1	I	Yellow-orange	21	— (445) —	(440) 461 (488)	(427) 448 (469)
		II	Pink-orange	22	(425) 443 (467)	437 457 (485)	— 445 (470)
"X." stewartii	ICPB-SS11	I	Yellow-orange	23	— (445) —	(440) 461 490	(427) 448 473
		II	Pink-orange	24	(425) 443 (470)	(438) 460 490	(427) 448 473
"X." stewartii	ICPB-SS12	I	Yellow	25	— (445) —	(435) 457 (490)	(425) 447 (473)
		II	Orange	26	(415) 440 (470)	(435) 457 (490)	(425) 445 (470)
"X." stewartii	ICPB-SS18	I	Orange	27	— (445) —	(437) 459 (487)	(425) 446 (470)
		II	Orange	28	(425) 443 (470)	(435) 458 (485)	(425) 444 (470)
"X." trifolii	ICPB-XT109	I	Orange	29		(435) 458 (487)	(425) (444) (473)
		II	Pale orange	30	(425) 446 (473)	(437) 458 (490)	(425) (446) (477)
"X." trifolii	*"Bacterium herbicola"* 2554	I	Yellow	38	(423) 443 (468)	(435) 456 (487)	(427) 448 475
		II	Orange	39	(420) 443 470	(437) 460 487	(427) 447 473
"X." uredovorus	ICPB-XU103	I	Orange	31		(437) 461 (490)	(425) 447 (475)
"X." uredovorus	ICPB-XU103	I	Orange	32		(437) 461 (490)	(425) 447 (475)

direction, the fact that all yellow *Xanthomonas* "species" form the same carotenoids might be used to support the contention (Starr, 1959) that many of the present "species" are indeed phytopathogenic *formae speciales* of a limited number of true species. We must note that unpigmented mutant strains of normally yellow *Xanthomonas* spp. have been encountered occasionally. Breed, Murray, and Smith (1957) assigned seven normally achromogenic species to *Xanthomonas*; we have already shown that one of these—*X. manihotis*—forms no carotenoids. Colorless xanthomonads, whether naturally occurring or artificially induced, pose the usual systematic dilemma which emerges whenever too great reliance is placed upon a single determinative trait; however, this quandary should not detract from the basic utility of the foregoing taxonomic recommendation.

This basic utility has already been demonstrated. Through the years, yellow bacteria of uncertain systematic position have been placed in the genus *Xanthomonas*. *X. stewartii*, *X. trifolii*, and *X. uredovorus* are three examples of what we and others consider to be erroneous placement. In the present study, all strains of these three species were found to be devoid of the "*Xanthomonas*-carotenoid," thus supporting their expulsion from the genus *Xanthomonas* which had been advocated on other bases (Burkholder and Starr, 1948; Hayward and Hodgkiss, 1961; Dye, 1962, 1963; Starr and Stephens, 1963).

ACKNOWLEDGMENT

This study was supported in part by research grant AI-01067 from the National Institutes of Health, U.S. Public Health Service.

LITERATURE CITED

BREED, R. S., E. G. D. MURRAY, AND N. R. SMITH. 1957. Bergey's manual of determinative bacteriology, 7th ed. The Williams & Wilkins Co., Baltimore.

BURKHOLDER, W. H., AND M. P. STARR. 1948. The generic and specific characters of phytopathogenic species of *Pseudomonas* and *Xanthomonas*. Phytopathology **38**:494–502.

DOWSON, W. J. 1939. On the systematic position and generic names of the gram negative bacterial plant pathogens. Zentr. Bakteriol. Parasitenk. Abt. II **100**:177–193.

DYE, D. W. 1962. The inadequacy of the usual determinative tests for the identification of *Xanthomonas* spp. New Zealand J. Sci. **5**:393–416.

DYE, D. W. 1963. The taxonomic position of *Xanthomonas uredovorus* Pon *et al.* 1954. New Zealand J. Sci. **6**:146–149.

FOX, D. 1953. Animal biochromes and structural colors. Cambridge University Press, Cambridge, England.

GOODWIN, T. W. 1955. Carotenoids. Ann. Rev. Biochem. **24**:497–522.

HAYWARD, A. C., AND W. HODGKISS. 1961. Taxonomic relationships of *Xanthomonas uredovorus*. J. Gen. Microbiol. **26**:133–140.

KARRER, P., AND E. JUCKER. 1950. Carotenoids. Elsevier Publishing Co., Inc., New York.

KUHN, R., AND H. BROCKMANN. 1932. Bestimmung von Carotinoiden. Z. Physiol. Chem. **206**:41–64.

SAPERSTEIN, S., M. P. STARR, AND J. A. FILFUS. 1954. Alterations in carotenoid synthesis accompanying mutation in *Corynebacterium michiganense*. J. Gen. Microbiol. **10**:85–92.

SOBIN, B., AND G. L. STAHLY. 1942. The isolation and absorption spectrum maxima of bacterial carotenoid pigments. J. Bacteriol. **44**:265–276.

STARR, M. P. 1944. Studies of phytopathogenic bac-

[a] The quotation marks indicate our belief that these three species ("*X.*" *stewartii*, "*X.*" *trifolii*, and "*X.*" *uredovorus*) are not correctly assigned to the genus *Xanthomonas*.

[b] ICPB = International Collection of Phytopathogenic Bacteria, Department of Bacteriology, University of California, Davis (M. P. Starr, Curator). ATCC = American Type Culture Collection, Washington, D.C. Cultures 2074, 2544, and 2554 are part of the general collection maintained by the Department of Bacteriology, University of California, Davis. Culture 622-11-1 was received from D. C. Erwin, University of California, Riverside.

[c] Zones are numbered in the order in which they appear on the columns, from top to bottom. Based upon partition tests of crude pigments as explained in the text, all pigments could be categorized—somewhat arbitrarily as is the custom in carotenoid research—as "alcohols."

[d] The numbers given to the pigments serve merely to identify the specific carotenoids for possible future reference. The pigments from each organism in this table were given separate numbers; however, it is quite possible that the same pigment was isolated from more than one of the examined strains.

[e] Parentheses around an absorption maximum indicate a shoulder or broad central peak centered around that approximate wavelength.

teria. Cornell University, Abstracts of theses, 1943, p. 349–350.

STARR, M. P. 1958. The blue pigment of *Corynebacterium insidiosum*. Arch. Mikrobiol. **30**:325–334.

STARR, M. P. 1959. Bacteria as plant pathogens. Ann. Rev. Microbiol. **13**:211–238.

STARR, M. P., AND S. SAPERSTEIN. 1953. Thiamine and the carotenoid pigments of *Corynebacterium poinsettiae*. Arch. Biochem. Biophys. **43**:157–168.

STARR, M. P., AND W. L. STEPHENS. 1963. Pigmentation and taxonomy of the genus *Xanthomonas*. Bacteriol. Proc., p. 11.

STEPHENS, W. L., AND M. P. STARR. 1963. Localization of carotenoid pigment in the cytoplasmic membrane of *Xanthomonas juglandis*. J. Bacteriol. **86**:1070–1074.

STRAIN, H. H. 1938. Leaf xanthophylls. Carnegie Inst. Wash. Publ. 490, 1–147.

ZECHMEISTER, L. 1934. Carotenoide. Julius Springer, Berlin, Germany.