THE NUTRITION OF PHYTOPATHOGENIC BACTERIA

I. Minimal Nutritive Requirements of the Genus Xanthomonas

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Study of the nutrition of phytopathogenic bacteria has lagged far behind research in comparable aspects of animal pathogens. In recent years the nutrition of a few species has received adequate treatment; for the remainder of the species, however, knowledge of this subject is limited to not more than casual and uncritical observations of growth in certain of the synthetic media recommended by Smith (1905). (In this connection, see the general literature review in Lewis' work, 1930.) Unfortunately, many of these data are of little value today because, with the exception of some recent investigations, no attention was devoted to refinements in methodology which present knowledge deems indispensable. Consequently, glassware and media components were often not unquestionably free of contamination with active traces of growth-affecting substances, nor was there usually any apparent attempt to control the carry-over of similarly active trace nutrients in the inoculum.

This lack is all the more regrettable when one considers the values pertaining to a thorough understanding of the nutrition of plant-pathogenic bacteria. Parasitism involves a nutritional interrelationship, for during its pathogenetic existence the phytopathogen derives its entire nourishment from the host's tissues. Therefore, information concerning the sort of food which the bacterium can use *in vitro* might indicate what is taken by the pathogen from the host plant. Moreover, such data would permit evaluation of any relationship which might exist between nutritional requirements and the high degree of host specificity exhibited by many species. It would be well to keep in mind, also, the potential value to the bacterial taxonomist of knowledge concerning exact nutritive needs, especially since nutritive differences or similarities have proved significant taxonomic characters in other groups of microorganisms. In addition, one should not lose sight of the important advances in biochemistry (cf. van Niel, 1944) which are deriving from the current general interest in microbial nutrition.

With these ultimate aims in mind, a comprehensive, comparative study of the nutrition of phytopathogenic bacteria was undertaken. A preliminary survey has been published (Starr and Weiss, 1943) devoted to the utilization of asparagine as sole carbon and nitrogen source by the heterogeneous phytopathogenic

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bacteria formerly lumped in the genus *Phytomonas* Bergey *et al.*[3] This work can be summarized by stating that, with a few exceptions, the green fluorescent *Pseudomonas* spp. and the tumor-inducing *Agrobacterium* spp. grow well upon serial transfer in a synthetic asparagine medium; whereas the yellow-pigmented, polarly flagellated, gram-negative *Xanthomonas* spp. and the gram-positive *Corynebacterium* spp. fail to grow. Subsequently, attention was focused on the nutrition of the genus *Xanthomonas* and of the phytopathogenic corynebacteria. The latter group will be the subject of a separate communication. The present discussion is limited to the minimal nutritive requirements of the genus *Xanthomonas*. To avoid misunderstanding it should be stated that the present study represents an attempt to obtain fair or good growth of *Xanthomonas* species in the simplest possible (i.e., minimal) media of known composition. It does not purpose to strive for the exact degree or rate of development which may be attained in complex media; merely a fair approximation of that ideal.

I

The validity of a comparative study is dependent to a great extent upon the authenticity of the cultures which are employed. In this connection the writer has been very fortunate in gaining the co-operation of Professor W. H. Burkholder, who very generously furnished the nucleus of the culture collection used in this and other studies. Additional authentic isolates were secured from various investigators; their assistance, also, is greatly appreciated.

The donor's identification of each culture was accepted if consistent with habitat and if the generic features matched those given for the genus *Xanthomonas* in the sixth edition of *Bergey's Manual of Determinative Bacteriology*.[4]

The purity of each culture was established by morphological examination and by repeated plating from dilute suspensions of cells. Particular care was necessary during plating to shake the cell suspensions thoroughly in order to insure separation of contaminants from the characteristic gummy *Xanthomonas* growth. Plating was repeated on various media and under different incubation conditions to provide every opportunity for contaminants to reveal themselves.

Inasmuch as the virulence of some phytopathogenic bacteria is lost or reduced during cultivation on laboratory media and this loss might be expected to have

[3] The heterogeneous nature of *Phytomonas* Bergey *et al.* has been discussed by Burkholder (1930, 1939). Rahn (1929) and others have pointed out the "practical impossibilities" of using plant pathogenicity as a prime taxonomic character. With the recognition (cf. Elliott, 1937) that, contrary to a resolution of the Second International Congress for Microbiology, *Phytomonas* is used homonymously for a group of protozoan flagellates and for the aforementioned bacterial phytopathogens, the bacterial genus was dropped and its species placed in somewhat more suitable genera: *Agrobacterium* (Conn, 1942), *Corynebacterium* (Jensen, 1934; Dowson, 1942), *Pseudomonas*, *Xanthomonas* (Dowson, 1939), with a few species in the genus *Bacterium* in the sense used by Breed and Conn (1936). Much of the synonomy is tabulated by Weiss and Wood (1943) and will be incorporated in the forthcoming sixth edition of *Bergey's Manual of Determinative Bacteriology*.

[4] The kindness of Professors R. S. Breed and W. H. Burkholder in making available the galley proof of the genus *Xanthomonas* in advance of publication, is gratefully acknowledged.

some influence on nutritive requirements, it was desirable to learn something about the pathogenicity of the cultures at the time they were used in the present study. Unfortunately, lack of facilities made it impossible to retest all the cultures, but through the co-operation of several plant pathologists,[5] a number of the isolates were tested on their usual host plants as indicated in table 2. From the results obtained with the tested cultures, it is likely that most of the isolates used in this study were virulent.

Stock cultures were maintained on glucose potato agar at 12 C, or on glucose yeast-extract $CaCO_3$ agar at room temperature, and transferred every 7 to 9 weeks. Duplicates of most cultures were preserved successfully by lyophilizing in the Flosdorf-Mudd (1935) apparatus; some for as long as 5 years.

TABLE 1

Composition of the NH_4Cl, glucose, and salts basal medium

	g
Glucose (added separately)	0.5
NH_4Cl	0.1
KH_2PO_4	0.2
$MgSO_4 \cdot 7H_2O$	0.02
	μg
$B[H_3BO_3]$	0.5
$Ca[CaCO_3]$	10.0
$Cu[CuSO_4 \cdot 5H_2O]$	1.0
$Fe[FeSO_4(NH_4)_2SO_4 \cdot 6H_2O]$	10.0 or 50.0
I[KI]	0.1
$Mn[MnSO_4 \cdot H_2O]$	1.0 or 2.0
$Mo[MoO_3]$	1.0
$Zn[ZnSO_4 \cdot 7H_2O]$	5.0
Distilled water to 100 ml	
pH adjusted to 6.8 with NaOH	

II

The nutritive requirements of the cultures were determined by conventional means. At first, experiments were conducted in test tubes until it was observed that the growth of these aerobic organisms was being limited by an insufficient air supply (cf. Rahn and Richardson, 1940, 1942). Heavier growth occurred in shallow flask cultures or in continuously shaken test tube cultures. Instructive in this connection is a comparison of the turbidities developed by unshaken 10-ml cultures of various *Xanthomonas* species in 50-ml conical flasks (surface diameter 40 to 45 mm) and in upright test tubes (surface diameter 14 mm). Typical of the results are those obtained with *X. campestris* grown from small inocula in the basal medium which is described in table 1: after 7 days' incuba-

[5] Thanks are due Drs. P. A. Ark, W. H. Burkholder, W. A. F. Hagborg, H. H. Thornberry, and J. R. Wallin for furnishing information regarding the virulence of these isolates.

tion at 28 C the average turbidities, expressed as "densities" (see below), of 10 replicate flask cultures was 0.25, that of 10 replicate tube cultures was 0.05, and that of the uninoculated medium was 0.

In view of the superiority of flask cultures for the purpose of the present study, in subsequent experiments 10-ml portions of media were distributed in 50-ml flasks. These were capped by suitable beakers or plugged with absorbent cotton. Cotton plugs were avoided, especially in experiments which might be affected by nutrilites introduced from the cotton (Sherwood and Singer, 1944); in general, it might be said that no difficulties attended the use of plugs made from absorbent, surgical cotton.

All glassware was cleaned by soaking in dichromate and sulfuric acid for not less than 24 hours, washing in tap water, and rinsing in distilled water. At times,

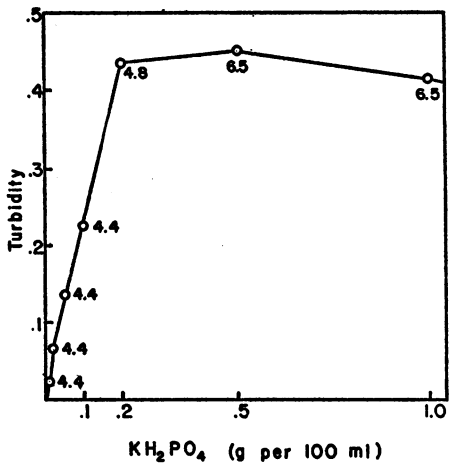

FIG. 1. EFFECT OF CONCENTRATION OF NEUTRALIZED KH_2PO_4, ADDED TO THE PHOSPHATE-FREE BASAL MEDIUM, ON THE GROWTH OF XANTHOMONAS CAMPESTRIS ISOLATE XC1

Turbidity measured after 15 days' incubation and expressed in "density" units (see text). Initial hydrogen ion concentration was pH 6.8; final pH's are shown on the graph.

an alkaline cleaning mixture similar to that of Gaddis (1942) was used with equally satisfactory results.

The composition of the basal medium used in these experiments is shown in table 1. In practice, the inorganic components were dissolved in about half the necessary volume of water, supplemented by test nutrients if any, made up to volume, distributed in flasks, and autoclaved. Sterile glucose was then added aseptically in the form of a 25 or 50 per cent solution prepared by treating glucose solutions twice at pH 3.0 with norit charcoal, filtering each time with the aid of super-cel, and autoclaving the resulting solution (Hutner, 1944).

Inasmuch as scanty growth in early experiments was traced to an inadequate buffer, it would be appropriate at this point to mention an experiment which led to the choice of a more suitable phosphate concentration. The basal medium minus phosphate was supplemented with various concentrations of neutralized

KH_2PO_4; after autoclaving, glucose was added in the manner described above. Series of these media were inoculated uniformly with 10 *Xanthomonas* isolates which are able to grow in the basal medium, and, after incubation, turbidities and hydrogen ion concentrations were determined. The results, such as those graphed in figure 1, definitely point to a need for adequate buffer if a heavy growth of *Xanthomonas* is to be attained in simple media. Sufficient buffer is needed, too, if one is to avoid so-called "growth-stimulating" effects which are actually due to the buffer capacity of the nutrient under test.

Casein hydrolyzate was prepared from alcohol-extracted, twice-reprecipitated casein by sulfuric acid hydrolysis, followed eventually by treatment with norit as mentioned above for glucose (Landy and Dicken, 1942). The purest available preparations of amino acids, vitamins, and other supplements were used; whenever possible, synthetic products were chosen. Inorganic chemicals were cp or equivalent. When storage was necessary, stock solutions were preserved with chloroform-toluene at 2 C.

Every effort was made to minimize the carry-over of growth-affecting substances in the inoculum. Test media were inoculated by means of Pasteur capillary pipettes with a drop of a light suspension in the basal medium of young agar-grown cells. Estimations by the plate count method showed that 10^4 to 10^5 viable cells were transferred to each flask containing 10 ml of the test medium. As a test of the efficiency of this dilution flask technique in eliminating any noticeable carry-over effects (see also section V, below), cultures in various test media were transferred serially four times into fresh flasks of the same medium. Inasmuch as results on the fourth transfer were usually identical with the first, or, if anything, the last transfers yielded better growth due to adaptation to the simple medium, serial transfers were later used only in critical or questionable experiments. The purity and viability of the inoculum were always checked by streaking from the dilution flasks at the time of inoculation.

Unless otherwise stated, all cultures were incubated at 28 C. Turbidity was estimated visually or measured by means of an Evelyn photoelectric colorimeter. The 620 mμ filter was used in these turbidity measurements to avoid possible interference by varying concentrations of cell pigment; the yellow *Xanthomonas* pigment transmits completely light of that wave length (Starr, 1944). Turbidity is expressed in terms of "density" or "L-value." These quantities were calculated from the Evelyn galvanometer readings by means of the relationship: $D = \log \frac{G_o}{G_t}$, where D is the "density"; G_o equals the galvanometer reading, ordinarily 100, corresponding to the uninoculated medium; and G_t indicates the galvanometer reading of the culture.

III

The data in table 2 show that many, but by no means all, of the tested *Xanthomonas* cultures grew in the NH_4Cl, glucose, and salts basal medium without supplement. Those cultures which grew in the basal medium ("nonexacting" strains) did so at a rate somewhat lower than in nonsynthetic media; although,

with sufficient buffer, the final crops were about the same. For example, the usual peptone or yeast extract media permitted moderate to heavy growth of

TABLE 2

Ability of Xanthomonas isolates to grow in the NH_4Cl, glucose, and salts basal medium and in the basal medium supplemented by glutamic acid and methionine

SPECIES	NUMBER OF ISOLATES	VIRULENCE*	YEAR ISOLATED OR RECEIVED	GROWTH IN BASAL MEDIUM PLUS			
				Nil	Glutamate plus methionine	Glutamate	Methionine
X. barbareae	2		1939	++†	++	++	++
X. begoniae	4		1939-1945	++	++	++	++
X. campestris	8	v+	1936-1944	++[2 +]‡	++	++	++
var. *armoraciae*	1		1939	++	++	++	++
X. carotae	1		1939	++	++	++	++
X. corylina	3		1935-1941	++	++	++	++
X. cucurbitae	1		1933	++	++	++	++
X. geranii	3		1936-1940	+[1 ++]	++	++	++
X. hederae	3	v+[1 v?]‡	1932-1944	0	++	0?	++
X. incanae	2		1940-1941	++	++	++	++
X. juglandis	9	v+	1940-1945	+[1 ++; 1 0?]	++	++	++
X. lespedezae	2	v+	1944-1945	++	++	++	++
X. maculafoliagardeniae	2	v+	1945	+[1 ++]	++	++	++
X. malvacearum	7		1941-1945	++[1 +; 1 0]	++	++	++
X. manihotis	6		1941	+[2 ++]	++	++	++
X. papavericola	2		1932-1943	+[1 ++]	++	++	++
X. pelargonii	4		1938-1942	+[1 0?]	++	++[1 +]	++[1 +]
X. phaseoli	5	v+	1941-1944	+[1 0?]	++	++[2 +]	++[2 +]
var. *fuscans*	3	v+	1944	0	++	+	+[1 0?]
var. *sojense*	6	v+	1930-1944	+	++	++	++
X. pruni	8	v+	1941-1945	0	0?§		
X. taraxaci	1		1942	+	++	++	++
X. translucens f. sp. *cerealis*	1	v+	1942	0	++	0?	++
f. sp. *cerealis* (?)	2	v+	1942	0	++	0?	++
f. sp. *cerealis* or *undulosa*	2	v+	1941-1942	0	++	0?	++
f. sp. *hordei*	1	v+	1938	0	++	0?	++
f. sp. *hordei-avenae*	3	v+	1942-1945	0[1 0?]	++	0?	++
f. sp. *undulosa*	4	v+	1936-1944	0[1 0?]	++	0?	++
X. vasculorum	1		1933	++	++	++	++
X. vesicatoria (from pepper)	7		1937-1939	+[3 ++; 1 0]	++	++[1 0]	++
(from tomato)	5		1932-1943	+[1 0?]	++	++[1 +]	++
var. *raphani*	1		1940	++	++	++	++
X. vignicola	3		1942	+	++	++	++

* Virulence determined on "usual" host at the time that nutritive requirements were studied. Legend: v+, distinctly virulent; v?, weakly or questionably virulent; v0, avirulent; no sign, virulence not determined at this time.

† ++ = moderate to heavy growth at a rate comparable to that observed in peptone media under similar conditions of inoculation and incubation; i.e., good growth within 3 to 6 days. + = subcultivable and eventually heavy growth, but at a distinctly lower rate than is indicated by "++". 0? = very slow development, starting only after 2 or 3 weeks' incubation, and possibly representing an adaptation to the medium rather than sufficient synthetic ability. 0 = no growth, even after prolonged incubation.

‡ The number of isolates deviating from the average reaction is shown in brackets together with the type of deviation.

§ An "average" value. Results in replicate experiments ranged from "0" to "+". See discussion in text.

most *Xanthomonas* cultures from small inocula within 2 or 3 days, whereas, in the synthetic medium, comparable development of the "nonexacting" isolates usually took 4 to 10 days or even longer. Moreover, *X. hederae*, *X. phaseoli* var.

fuscans, *X. pruni*, and *X. translucens*, as well as a few equally "fastidious" isolates of other species, failed to grow at all in the basal medium alone.[6]

Addition of 0.5 per cent "vitamin-free" casein hydrolyzate to the basal medium not only stimulated the growth of the "nonexacting" isolates, but also supplied the essential growth factors required for the prompt development of the "fastidious" cultures with the exception of *X. pruni*. Search for the stimulation factors and essential growth factors of casein hydrolyzate was facilitated by the observation that a mixture of amino acids produced identical effects. By systematic omission of amino acids from this mixture, individually and in groups, glutamic acid and methionine were inferentially identified as active ingredients for several typical cultures, since when both were omitted growth was again slow or nil as in the unsupplemented basal medium. This activity was verified by the prompt, luxuriant growth of every culture used in this study (except *X. pruni*, as discussed below) when synthetic *dl*-glutamic acid (0.1 per cent) and *dl*-methionine (0.02 per cent) were added to the basal medium.

The preceding statement is not to be interpreted as meaning that *both* glutamic acid and methionine were "needed" by *all* cultures. Indeed, reference to table 2 reveals that only a scant minority of the cultures (e.g., 3 *X. phaseoli* var. *fuscans*; 2 *X. phaseoli*) depended upon addition of both amino acids for prompt, full development.

Most of the cultures studied developed practically as well with either glutamic acid alone or methionine alone as the sole additional nutrilite. For the majority of this group, the single amino acid simply stimulated growth; however, there are a few examples of obligate requirement which could be satisfied by either of the amino acids. Preliminary experiments indicate that the nutrilite needs of at least some members of this class can also be met by substances other than these two amino acids.

In contrast to the foregoing categories, there are cultures which seem to depend primarily and apparently obligately on methionine; in this division are all available isolates of *X. hederae* and *X. translucens*[7] and rare exceptional strains of other species. Prompt growth of these cultures depended absolutely upon the presence of methionine in the medium. Occasionally, scanty development began in methionine-free media after incubating 10 or 20 days, possibly because of adaptation such as described for some species of marine luminous bacteria by Doudoroff (1942). However, most cultures of *X. hederae* and *X. translucens* did not grow at all unless methionine was present, and, moreover, in its presence there was *prompt* development of *all* isolates of these two species. Figure 2 demonstrates this relationship in the case of a typical isolate and indicates the range of active methionine concentrations. In the presence of sufficient methionine, there was little or no stimulation of growth of these species by

[6] Unquestionably, the dividing line between the more slowly developing "nonexacting" isolates and the "fastidious" isolates is indistinct. If observations had not been continued beyond 7 or 10 days, a number of the slower growing "nonexacting" cultures would have appeared as "fastidious."

[7] *X. translucens*, as used herein, includes the *formae speciales* of Hagborg (1942) as well as the pathogenetic forms of Wallin (1944*a*; 1944*b*).

glutamic acid. For many isolates of these two species, methionine could be replaced by homocysteine, or by homocysteine plus choline, but a reduced growth rate resulted from these substitutions.

As noted above, *X. pruni* did not grow in the unsupplemented basal medium. Even when "vitamin-free" casein hydrolyzate was added to the basal medium, this species did not grow, or began to develop only after 2 or 3 weeks' incubation, in sharp contrast to all other species of *Xanthomonas*, which grew luxuriantly and quickly with casein hydrolyzate. All available isolates of *X. pruni* grew well when Difco yeast extract was added to the casein hydrolyzate medium, and a mixture of B vitamins could be substituted for the yeast extract with no discernible loss of activity. By successively omitting one or two growth factors at a time from the vitamin mixture, nicotinic acid or nicotinamide was indicated

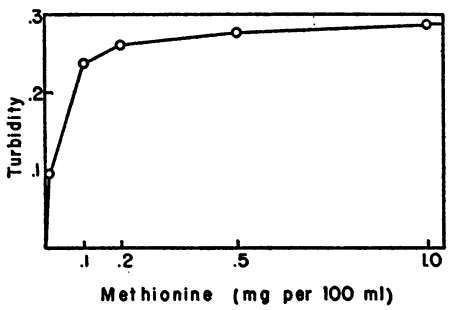

FIG. 2. EFFECT OF CONCENTRATION OF *dl*-METHIONINE, ADDED TO THE BASAL MEDIUM, ON THE GROWTH OF XANTHOMONAS TRANSLUCENS ISOLATE XT12

Turbidity measured after 5 days' incubation and expressed in "density" units. The turbidity at 2 to 100 mg of methionine per 100 ml was at the level shown at 1 mg; above 100 mg the growth fell off to practically none at 1,000 mg. These results are typical of those obtained with all available isolates of *X. translucens* and *X. hederae*.

as the active ingredient. All cultures of *X. pruni* grew well in the casein hydrolyzate medium plus nicotinic acid; figure 3 shows the quantitative response to the vitamin. Nicotinamide could replace the free acid.

In the presence of nicotinic acid, casein hydrolyzate could be substituted by glutamic acid and methionine as for other *Xanthomonas* species; somewhat slower growth occurred with either of the amino acids used individually, and there was no growth in the basal medium supplemented only by nicotinic acid.

Under certain conditions, there was growth of some *X. pruni* cultures in the supposedly nicotinic-acid-free glutamate and methionine medium—at times suggestive of a fluctuating contamination by nicotinic acid. After actual contamination from the glassware, medium components, and inoculum had been ruled out, certain cases of this seeming contamination were tentatively interpreted as the formation, during autoclaving of the media in the various experiments, of nicotinic acid or a substitute therefor, varying in concentration as a function of as yet undetermined conditions. This is probably similar to the phenomenon studied by Bovarnick (1943, 1944) in which a substance showing nicotinamide activity for *Shigella dysenteriae* (in one case, actually isolated and

identified chemically as nicotinamide) was formed by prolonged heating of glutamic acid, methionine, and other amino acids. Certain other cases of growth in the nicotinic-acid-free amino acid medium, not amenable to the foregoing interpretation, may represent synthesis of nicotinic acid from the amino acids; although, in this connection, it is puzzling why such a synthesis could proceed in the amino acid medium, but is not nearly so evident in the casein hydrolyzate medium, which, after all, does contain similar quantities of the two amino acids.

There can be no doubt that nicotinic acid was distinctly stimulatory for the growth of all *X. pruni* isolates used in this study; in certain cases the vitamin was indispensable. The requirement of *X. pruni* for nicotinic acid serves to distinguish this organism from the other species of *Xanthomonas*, since none of the latter were affected by nicotinic acid (see figure 3) or, for that matter, by a mixture of 12 water-soluble vitamins. From the determinative standpoint, it

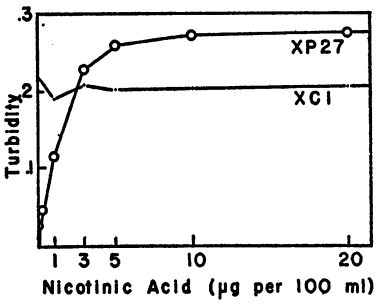

FIG. 3. RESPONSE OF A TYPICAL ISOLATE (XP27) OF XANTHOMONAS PRUNI TO NICOTINIC ACID IN A CASEIN HYDROLYZATE MEDIUM

For comparison, there is shown the "response" of an isolate of *X. campestris* (XC1), illustrating the indifference of all species other than *X. pruni* toward the vitamin. Turbidity measured after 3 days' incubation and expressed in "density" units.

would be best to test for the nicotinic acid requirement by using casein hydrolyzate media until such time as the anomalous behavior shown in the nicotinic-acid-free glutamate and methionine medium be clarified.

The inability of *Xanthomonas* species to grow with asparagine as the sole source of both carbon and nitrogen has been reported by Burkholder (1939) and confirmed by Starr and Weiss (1943). Because the majority of xanthomonads are now shown to be able to grow in simple media, it is of interest to examine the reason for the unsuitability of asparagine. By the study of several "non-exacting" isolates, it was learned that the addition of glucose to the asparagine medium resulted in growth at least as good as in the present NH_4Cl glucose basal medium. This might suggest that the incompetence of asparagine is due to its inappropriateness as a carbon or energy source, although it suffices as a nitrogen source.

IV

These findings on the nutrition of *Xanthomonas* may be useful in clarifying the taxonomy of the group. Since it is established that all the species studied

have comparatively simple nutritive requirements, a statement of these general needs might well be appended to the description of the genus. Perhaps, such information would eventually be helpful in establishing the affinities of the genus *Xanthomonas* in the family Pseudomonadaceae or elsewhere.

Minimal nutritive requirements may aid in distinguishing species within the genus; for example, the nicotinic acid requirement of *X. pruni*, and the methionine need of *X. hederae* and *X. translucens*. In using these properties in determinative bacteriology, one must bear in mind the experimental conditions under which these differences have been demonstrated. Only when small inocula, highly purified media components, and scrupulously cleaned glassware are employed can clear-cut results of the sort reported herein be expected. Furthermore, the well-known phenomenon of mutability of nutritive requirements must always be considered.

In this connection, however, the data in table 2 show relatively few instances in which individual isolates deviate qualitatively from the reaction characteristic of the species; nor is there any apparent trend toward less exacting requirements for the isolates which had been cultivated for the longest period on laboratory media. The latter is interesting in view of frequent references in the literature to the effect "that organisms as directly isolated from cases of disease have more exacting nutrient needs, and that in culture *in vitro* they become trained to grow in simpler media than they require when first isolated" (Knight, 1938, p. 152). In several cases in the present study, cultures just two or three transfers away from the host plant (the bare minimum considering the manipulations involved in isolating and purifying cultures) were shown to have precisely the same nutritive requirements as stock cultures of the same species which had been grown on artificial media for as long as 10 or 15 years. This was true of "nonexacting" species as well as of methionine- and nicotinic-acid-requiring species. The cause of this seeming stability does not lie in an inherent immutability, for it was possible to train some "fastidious" cultures to dispense with an initially "needed" growth factor; probably, it may be attributed to the fact that the stock culture media undoubtedly contained the few simple required nutrilites and, consequently, did not favor any regaining of synthetic ability.

V

It might not be amiss at this point to consider briefly the relationship of the exact nutritive requirements of *Xanthomonas* to the media used for determinative "fermentation" tests. As Elliott (1930) and Burkholder (1932) have emphasized, peptone basal media are unsuited for "fermentation" tests of *Xanthomonas* because of a prominent alkali production from the peptone with a consequent masking of any acid which may have been formed from the carbon source. Unfortunately, results with the suggested substitute, the synthetic carbohydrate medium of the *Manual of Methods for Pure Culture Study of Bacteria* (Comm. Soc. Am. Bact., 1923–1936), may be equally misleading for some species of *Xanthomonas*. Since the recommended synthetic basal medium does not provide the accessory growth factors required by the "fastidious" species, the failure of an organism to grow and produce a pH change in the synthetic medium plus

carbon source might result from lack of an essential growth factor rather than from an inability to dissimilate the carbon source.

Another source of confusion is the variable carry-over of growth factors in the inoculum, especially when transfers are made directly from stock cultures. Although this would have little effect on carbon source experiments made in peptone media, it is highly important in synthetic media, particularly with "fastidious" species. This fact is brought out by scores of observations in the present study which show that when the media were seeded heavily with a loopful of cells from stock cultures many of the "fastidious" *Xanthomonas* cultures grew in the unsupplemented basal medium. (This sort of experimentation quite likely led to the stated belief that *all* species of *Xanthomonas* grow in ammonium glucose media.) With much smaller inocula and thereby reduced carry-over of growth factors, such as is provided by inoculation via a dilution flask, there is no growth of these "fastidious" species. Between these extremes of inoculum size, growth would or would not occur depending upon the quality and quantity of growth factor carried over.

That the foregoing is not a purely rhetorical discussion is readily apparent from the discrepant results shown in the literature. One must guard against the lack of an essential growth factor in the basal medium, either by the somewhat uncertain method of using very large inocula, or preferably, by fortifying the basal medium with the proper accessory growth factor(s). If exact information on this subject be lacking, a small quantity of yeast extract (e.g., 0.02 to 0.05 per cent of Difco yeast extract) can usually be depended upon to supply the missing nutrients without interfering with the desired pH changes.

VI

From the preceding it is evident that the nutritive requirements of all tested *Xanthomonas* species could undoubtedly be met by the tissues of practically any plant. Why, then, are these phytopathogenic bacteria restricted to specific, or to a limited series of, host plants? The answer clearly does not lie in simple satisfaction of the minimal nutritive requirements of the pathogen. This is in agreement with Chester's (1933) analysis of the analogous mycological situation. Perhaps an explanation can be found in chemical inhibitors such as plant acids or phenols (Link and Walker, 1933). In view of recent reports concerning the occurrence of antibiotic substances in higher plants (Pederson and Fisher, 1944; Lucas and Lewis, 1944; Huddleson *et al.*, 1945), it is tempting to speculate on the existence in plants of *specific* antibiotics which are capable of inactivating phytopathogens other than the particular bacterial species which are able to infect that host.

SUMMARY

The minimal nutritive requirements of 113 isolates of phytopathogenic bacteria belonging to 30 species and varieties of the genus *Xanthomonas* were determined under conditions of controlled inoculum, carefully cleaned glassware, and pure medium components.

Under these experimental conditions, it was learned that most, but not all,

species of *Xanthomonas* can grow to some extent in the simplest medium used: NH_4Cl, glucose, and salts. Methionine, glutamic acid, and nicotinic acid in various combinations served to furnish the stimulation factors and essential growth factors necessary for the prompt development of the more exacting species.

Growth in these simple media shows promise of utility as a taxonomic character on the generic and specific levels.

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