BACTERIAL VIRUSES SPECIFIC FOR THE YELLOW CHROMOGENIC BACTERIA OF WHEAT

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A Major Thesis submitted to

the Committee of Graduate

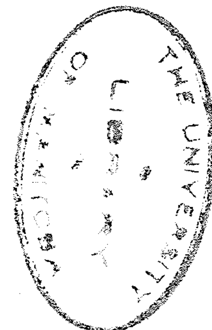
Studies and Research of

The University of Manitoba

in candidacy for the degree of

Master of Science.

1955.



ACKNOWLEDGMENT

The writer expresses his sincere gratitude to Dr. Norman James, Chairman, Department of Microbiology, University of Manitoba, for his assistance and advice throughout the course of this investigation and the preparation of the manuscript.

Bacterial Viruses Specific for the Yellow Chromogenic Bacteria of Wheat.

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ABSTRACT.

Twenty bacterial viruses specific for the yellow chromogenic bacteria of wheat were isolated from wheat seed. In each case the virus lysed the homologous culture and certain other cultures, but was too specific for identifying these bacteria as a group. A composite virus was prepared by mixing equal portions of four viruses, selected because these viruses lysed the complete range of cultures from which individual viruses had been obtained. The composite virus lysed all cultures of the chromogen, but none of 18 cultures of *Xanthomonas translucens*, nor *X. carotae*, nor *X. campestris*, nor any of 22 other species. Likewise, the composite virus lysed 100% of 20 fresh isolates from wheat and 82% of 160 isolates from other cereal and forage seeds. On the basis of these results it is evident that the yellow chromogens constitute a distinct group of bacteria.

Evidence is presented which indicates that one virus did not exert an inhibitory effect on another when mixed, since the composite virus invariably lysed all cultures lysed by the single viruses. In fact, there was evidence of a phenomenon analogous to synergism in bacterial cultures, since the composite virus, in one case at least, lysed a culture not lysed by any of the single viruses used in the composite.

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INTRODUCTION

The occurrence of appreciable numbers of yellow chromogenic bacteria on wheat, up to 89% of the bacterial count (29), as well as on other cereal and forage seeds has been observed by many investigators during the last half century. James et al (14) studied the bacterial flora of wheat and found this type predominant on all samples. Since then a detailed study of these organisms has been carried out in this laboratory by a number of investigators.

Wilson (28) investigated 22 isolates and found that culturally, morphologically and physiologically they resembled the pathogen, *Phytomonas translucens* var. *undulosa*. (now *Xanthomonas translucens* f. sp. *undulosa*, Hagborg).

Stark (21) studied 38 isolates of these bacteria from wheat, oats, barley and flax. Slight physiological differences among these cultures were noticed, but these were not of sufficient importance to justify considering them to belong to different species.

Goldstein (11) carried out a comparative study on 13 cultures of yellow bacteria isolated from wheat and nine known cultures of the genus *Xanthomonas*. This investigator concluded these two groups to be so much alike as to warrant placing them in the same genus.

Steel (22) conducted a study on 31 isolates and seven *Xanthomonas* cultures. He confirmed the morphological, cultural and physiological findings of Wilson, Stark, and Goldstein. The main difference between these two groups was found to be in the utilization

of asparagine as the sole source of carbon and nitrogen. Tests for pathogenicity were made on 500 plants known to be susceptible to the different varieties of *Xanthomonas translucens*. None of the isolates produced disease, whereas under the same conditions the pathogens produced characteristic symptoms of disease. The pigments of the two groups were found to be similar with regard to the lipocyan reaction, extracting solvents, chromatographic adsorption, partition and color tests.

The identification of bacteria involves morphological, cultural and physiological procedures, and in case of pathogens host inoculations. These are time consuming. Specific sera have been used successfully for the quick identification of certain bacteria, but their development is complicated and often impracticable. In the past two decades the lytic reactions of specific bacterial viruses have proven helpful to a number of investigators in identifying bacteria.

Accordingly, this investigation was undertaken in the hope that a bacterial virus specific for the yellow chromogenic bacteria of wheat could be isolated and that it could be used for the rapid identification of these bacteria.

HISTORICAL

In 1915 Twort observed peculiar changes in colonies of organisms grown on solid media from vaccine lymph. After 24 hours these became transparent (7). D'Herelle, 1917, found that a filtrate from faeces of Shiga dysentery (now Shigella dysenteriae) cases, emulsified in broth and then incubated for 18 hours, inhibited the growth of a young culture of Shiga (7).

Ever since the lytic reactions of bacterial viruses were observed by these two scientists, their possible value as an aid in identifying bacteria has been considered. Among the first to investigate this possibility was Laird (17) who isolated a number of bacterial viruses for the purpose of identifying Rhizobium strains. Because of great variation in susceptibility of the strains to the viruses he did not consider this method promising. Evans (5), only a year later, found bacterial viruses useful in the identification of certain hemolytic streptococci. According to this investigator (5) Lancefield identified 47 of 56 strains of streptococci with "Clark phage".

Conn et al (2) used bacterial viruses as a criterion in classifying soil bacteria. Viruses developed from each of the six strains of Agrobacterium radiobacter lysed all the six strains, but were incapable of lysing any of the 33 Rhizobium cultures and practically none of the 14 organisms showing the morphology of Bacterium globiforme, nor seven miscellaneous soil types. Of the 14 bacterial viruses isolated

from cultures "showing the morphology typical of B. globiforme" six showed complete cross-lysis. Three of the six cultures showing complete lysis were subcultures of one strain. This, according to the authors, indicated that the bacterial virus method is a satisfactory means of identifying strains. Further, on the basis of cultures showing partial lysis, the authors justify Lochhead's observations that there were many types of bacteria in soil with "globuliform-like" morphology. The viruses isolated for Rhizobium strains of alfalfa, soy and lima beans were found strain specific and incapable of cross-lysis. Thus, viruses could be successfully used for their differentiation. But, as Laird (17) had found, these investigators were not successful in developing specific viruses for Rhizobium strains isolated from clover, peas and beans. The bacteria associated with the latter group were found to produce identical races of viruses capable of cross-lysis.

Fulton (10) isolated two bacterial viruses from Pseudomonas tabaci and the closely related P. angulatum. One virus lysed P. tabaci, P. angulatum and P. lachrymas, the other virus was found to lyse P. angulatum, P. tabaci, P. coronafaciens, P. phaseolicola and P. syringae. The viruses differed in thermal inactivation exposures and in morphology. Similar results were obtained by Sutton and Katznelson (20) with P_{26} and P_{11} viruses isolated from Pseudomonas pisi strains. The P_{26} virus lysed both P_{26} and P_{11} strains of P. pisi and 4 species of the same genus. The P_{11} virus was specific for the host culture only. But the viruses isolated for certain strains of P. coronafaciens lysed other strains

also and were found inactive against a number of other Pseudomonas species. Fifty nine bacterial viruses isolated from wheat from different parts of Canada, for avirulent strains of P. atrofaciens, were found to be specific to the virulent strains only. None of the 14 cultures belonging to 11 different species of Pseudomonas and the three virulent strains of P. atrofaciens were susceptible. The viruses isolated from three form species of Xanthomonas translucens reacted with host cultures only and, therefore, were regarded as too specific for species identification. Katznelson and Sutton (15) isolated specific viruses for Pseudomonas phaseolicola and Xanthomonas phaseoli. P. phaseolicola virus acted on all cultures of X. phaseoli and was completely inactive against X. phaseoli var. fuscus and var. sojensis. The latter two differ from X. phaseoli only in respect to host pathogenecity.

In later work these two investigators (16) reported the isolation of polyvirulent viruses for the identification of X. translucens. Twenty four of 28 cultures were lysed by the polyvirulent virus KPg 34. Thus, 91% of the cultures tested were assigned to X. translucens. Attempts to use form specific viruses to identify cultures of X. translucens f. spp. hordei, hordei-avenae and undulosa were unsuccessful. However, the use of specific viruses for the detection of homologous cultures of X. translucens f. sp. secalis gave encouraging results.

Thornberry et al (25) reported the use of bacterial viruses for the identification of Xanthomonas pruni. The virus was isolated in 1927, and was one of the first bacterial viruses recognized for plant

pathogenic bacteria. After 20 years storage, the 10^{-1} dilution of the virus lysed all cultures of X. pruni isolated from peaches, plums and apricots in various regions of Illinois, whereas cultures of X. campestris, X. lactucae-scariole, distinguishable from X. pruni by pathogenecity only, were not lysed.

Thornberry et al (24) found that the reactions of specific bacterial viruses for X. pruni were more reliable than cross-agglutination tests. Six isolates of X. pruni and 54 other species of the same genus were tested for susceptibility to the virus of X. pruni. Only six cultures of X. pruni were lysed. None of the other 54, including X. corylina, X. phaseoli var. sojensis and X. lespedezae, which according to cross-agglutination reactions should be placed into one serological group with X. pruni, were lysed.

Toshach (26) used bacterial viruses for typing Corynebacterium diphtheriae. This investigator was unable to obtain a definite pattern of susceptibility of C. diphtheriae strains as to type or source of these organisms, except in two instances where epidemiologically related strains showed uniform susceptibility to one of the viruses.

Fahey (6) undertook a detailed study in order to investigate the possibility of classifying C. diphtheriae strains by this method. From four original bacterial viruses a number of "host range mutants" were developed, and, by their reactions 68 strains of C. diphtheriae were grouped into nine distinct "phage types".

Fisk (8) reported that the lytic reactions of bacterial viruses were not altered by environment or time, and, therefore, this

method might be successfully used for typing Staphylococcus aureus (now Micrococcus pyogenes var. aureus) and for tracing the origin of infections. Ninety five cultures from related sources in different patients were typed with 27 viruses. Of special interest is the fact that a number of strains isolated from members of the same household were found susceptible to the same viruses, though, in one case, the bacterial strain differed in type of pigment. Fisk and Mordvin (9) confirmed the practical use of bacterial viruses for identifying strains of Staphylococcus aureus. Seventy eight cultures, isolated from different sources (throat, urine, furuncles, etc.) from 30 patients during a period of three years, were studied in detail. Thirty three mono- and two polyvalent bacterial viruses were employed. Cultures isolated from sources which presupposed their identity were lysed by the same bacterial viruses, while most of the cultures isolated from different sources reacted with homologous viruses. Only some of the cultures isolated from related sources were found to be of the same "phage type", though they differed in some other respects. However, several cultures isolated from similar sources were found to be different "phage types" and were considerably unlike in other respects. These investigators found this method of typing of S. aureus quite reliable and suggested its wider use.

Craigie and Yen (4) employed 18 bacterial viruses for typing B. typhosus (now Salmonella typhosa). The "Type II phages" exhibited high selective affinity for particular strains of B. typhosus. On the basis of susceptibility of cultures to particular bacterial viruses a

number of distinct types of B. typhosus were recognized.

Gunther (12) reported that the number of bacterial viruses originally used by Craigie and Yen (4) was reduced from 18 to eight when the modified method of isolation was employed. With eight viruses 65.7% of the 67 strains of typhoid bacilli were typed. The virus typing method also corroborated the epidemiological findings during the 1945 outbreak in Philadelphia, where 29 of 65 cases and the carrier were found to excrete Type F typhoid bacilli.

Wassermann and Seligmann (27) reported the isolation of four bacterial viruses for strains of Serratia marcescens. The viruses exhibited fair specificity, only three of 68 possible cross-lysings with 17 cultures occurred. One of the four viruses was found to be specific to its homologous culture only. The species specificity was checked on about 100 cultures of Enterobacteriaceae. Only few of them showed any degree of lysis when the first undiluted filtrate was used, but on dilution of the filtrate no lysis could be observed, whereas all S. marcescens strains were lysed.

Thomas (23) isolated viruses for nine species of plant pathogenic bacteria and found that each virus reacted best with the species from which it was isolated. A group of eight human pathogens was tested against a similar number of viruses for plant pathogens. All reactions were negative. Likewise when viruses for human pathogens were tested against bacteria isolated from plants, complete lysis was not observed in any case.

Hunter (13) used specific bacterial viruses in order to clarify the division of lactic streptococci into two species. A number of microbiologists had not recognized Streptococcus cremoris as a separate species and included it with S. lactis. This worker isolated a number of cultures and according to their characteristics divided them into two groups, S. lactis and S. cremoris, and then isolated bacterial viruses from representatives of each group. About 7000 tests for susceptibility were carried out. None of the cultures of S. cremoris was lysed by the bacterial viruses isolated from S. lactis. Three cultures of the S. lactis group reacted with viruses of both groups, and three others were susceptible to viruses of the S. cremoris group, but did not react with any other S. lactis virus. None of the viruses was found to attack cultures of S. fecalis and D (Lancefield) streptococci.

Further work on lactic streptococci classification was carried out by Nichols and Hoyele (18). In this investigation 375 strains of S. lactis and S. cremoris and their reactions with 78 bacterial viruses, involving some 60,000 tests, were studied. Strains of both species were grouped into 11 distinct "phage types". These investigators were of the opinion that, at the present time, this method provides the best available means of differentiating strains within these two species.

Reilly et al (19) reported that bacterial virus "M", obtained from Merck and Company, when tested against cultures of Streptomyces bikiniensis, S. violaceus and 12 strains of S. griseus, was found active against seven streptomycin producing strains of S. griseus only.

PROCEDURE

Thirty two cultures of typical yellow chromogenic bacteria were isolated from samples picked at random from 120 samples of six varieties of wheat collected in Western Canada. These cultures were used to isolate a bacterial virus specific for the yellow chromogenic bacteria of wheat. The method for isolation of bacterial viruses from cereal seeds employed by Katznelson and Sutton (16) was modified slightly. The method follows.

Twenty five g. ground wheat was placed in a sterile 250 ml. Erlenmyer flask containing 75 ml. nutrient broth* and inoculated with a 24 hr. nutrient broth culture of one of the isolates. This was incubated at 25°C. for about 24 hr., and the mixture was filtered twice, the first time through filter paper and the second through a sterile Seitz filter. One ml. of the bacteriologically sterile filtrate was added to 8 ml. nutrient broth, inoculated with one ml. of the culture, incubated as before, and filtered. Usually after two to four such passages, one drop of the filtrate inhibited one ml. of the culture. At that time each of one ml. of the filtrate, and of serial dilutions of it, was mixed with two ml. nutrient agar* heavily seeded with the culture and poured into a plate containing a sterile layer of solidified agar. After incubation at 25°C. for 24 hr. a plaque was fished from a plate showing discrete plaques into 8 ml. nutrient broth inoculated lightly with the culture. This enrichment process was continued through two to six passages, or

* Difco nutrient broth plus 0.1% Merk's dextrose, and Difco nutrient agar were used throughout this investigation. Other sugars and yeast extract did not support growth of the yellow chromogenic bacteria as well as did dextrose.

until one drop of the filtrate inhibited one ml. of the culture.

By this method twenty viruses were obtained by using different cultures of the yellow chromogen. Three cultures were discarded because after a period of time they exhibited certain abnormal characters. The nine remaining cultures failed to yield viruses that completely lysed homologous cultures, or their titers could not be raised to reach the accepted range.

Katznelson and Sutton (15) suggested that the difficulty in isolating a species specific phage might be reduced to some extent by combining various strain-specific phages. In this investigation a composite virus was prepared by mixing equal portions of four virus filtrates. This particular combination was found necessary in order to cover the cross-lysing range of the 20 viruses.

The bacterial virus filtrates were stored in tightly closed glass bottles at 4°C. in a dark room. A number of duplicates was stored in frozen state in darkness and at room temperature exposed to light.

In testing for susceptibility two nutrient broth tubes were used for each culture of the yellow chromogenic bacteria. The same amount of inoculum was added to each tube from an 18-24 hr. culture. One tube received one drop of bacterial virus filtrate, the other was the control. The virus reactions were read after incubation for 18 to 24 hr. In testing for susceptibility in other than the yellow chromogenic bacteria three test tubes were employed for each culture. Two test tubes received equal amounts of inoculum from a young culture of the bacteria to be tested, the third was inoculated with equal amounts

from the virus-homologous culture of yellow chromogenic bacteria. One of the two and the third received one drop of the virus. Thus two controls were used. These were incubated at optimum temperature for growth as indicated in Bergey's Manual (1) and the results were read after the "virus-free" control showed satisfactory growth. Each test for susceptibility was carried out in duplicate.

Degrees of lysis were designated by +++ for complete lysis, ++ for lysis with slight growth, + for partial lysis and - for no visible effect of the virus filtrate. Three plus and two plus lysis were grouped together and regarded as positive in the interpretation of results.

RESULTS

1. Virus Reactions.

Wheat seeds were found to harbour bacterial viruses capable of lysing the yellow chromogenic bacteria. From 29 cultures tested bacterial virus isolation was successful 20 times. Most of the virus isolates from single cultures were found to lyse certain other cultures of the yellow chromogenic bacteria but in no case all other cultures from which viruses were obtained.

Each of the 20 viruses was tested against the nine cultures from which virus isolation was not successful in this study. Eleven viruses lysed one to five cultures each, whereas nine viruses did not lyse any of these cultures. The results with the two groups of cultures are shown in Tables I and II.

TABLE I.

Cross-lysis by viruses from cultures

from which viruses were obtained.

Bacterial Viruses

Cultures	III	IV	VI	X	XI	XII	XIII
3	+++	-	-	-	-	-	+++
4	-	+++	-	+++	-	-	-
6	-	+++	+++	+++	-	-	-
10	-	+++	-	+++	-	-	-
11	-	+	-	-	+++	-	-
12	-	+++	-	-	-	+++	+++
13	++	-	-	-	-	-	+++
14	-	+++	+++	+++	+++	-	-
16	-	+++	-	+++	-	-	-
17	-	++	-	+++	-	-	-
18	-	+++	-	+++	-	-	-
19	-	+++	-	-	-	-	-
20	-	+++	-	+++	-	-	-
21	-	+++	-	+++	-	-	-
22	-	+++	-	+++	-	-	-
24	-	+++	-	+++	-	-	-
28	-	+++	-	++	-	-	-
30	-	+++	-	+++	-	-	-
31	+++	-	-	-	-	-	+++
32	-	+++	-	+++	-	-	-

TABLE I (Cont.)

Cultures	XIV	XVI	XVII	XVIII	XIX	XX	XXI
3	-	-	-	-	-	-	+++
4	+++	-	-	-	-	+++	++
6	+++	-	-	-	-	+++	-
10	-	-	-	-	-	+++	-
11	+++	-	-	-	-	-	-
12	+++	-	-	+++	-	-	+++
13	-	-	-	-	-	-	-
14	+++	-	+	-	+++	+++	-
16	-	+++	-	-	-	+++	-
17	+++	-	+++	-	+++	+++	+++
18	+++	-	-	+++	-	+++	-
19	+++	+++	-	-	+++	+++	+++
20	++	-	-	-	-	+++	+++
21	+++	-	-	-	-	+++	+++
22	+++	-	-	-	-	+++	+++
24	+++	-	-	-	-	+++	-
28	-	-	-	-	+++	++	-
30	+++	-	-	-	-	-	+++
31	-	-	-	-	-	-	++
32	+++	-	-	+++	-	+++	-

TABLE I (Cont.)

Cultures	XXII	XXIV	XXVIII	XXX	XXXI	XXXII
3	-	-	-	-	+++	-
4	-	+++	+++	-	-	++
6	-	-	-	-	-	-
10	-	-	+++	-	-	+++
11	-	-	-	-	-	-
12	-	-	+++	-	+++	+++
13	-	-	-	-	+++	-
14	-	-	-	-	-	-
16	-	-	+	-	-	-
17	-	-	+++	-	-	-
18	-	+++	+++	-	-	+++
19	+++	-	+++	-	-	-
20	+++	+++	+++	+++	-	+++
21	-	++	+++	-	-	+++
22	+++	+++	+++	+++	-	+++
24	-	+++	-	-	-	+++
28	-	+++	+++	-	-	+++
30	+++	+++	-	+++	-	+++
31	-	-	++	-	+++	-
32	-	+++	+++	-	-	+++

TABLE II.

Lysis by viruses from cultures
from which viruses were obtained with cultures
from which viruses were not obtained.

Bacterial Viruses.

Cultures	III	IV	VI	X	XI	XII	XIII	XIV	XVI	XVII
7	-	-	-	+++	-	-	-	+++	-	-
8	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-
15	-	+++	-	++	-	-	-	+	-	-
23	-	++	-	+++	-	-	-	+++	-	+++
25	-	+++	-	+++	-	-	-	++	-	-
26	-	+++	-	++	-	-	-	+++	-	-
27	+++	-	-	-	-	-	++	-	-	-
29	-	-	-	-	-	-	-	+++	-	-

TABLE II (Cont.)

Cultures	XVIII	XIX	XX	XXI	XXII	XXIV	XXVIII	XXX	XXXI	XXXIII
7	-	-	+++	-	-	+++	-	-	-	++
8	-	-	-	-	-	-	+++	-	-	-
9	-	-	-	-	-	-	+++	-	-	-
15	-	-	+++	+++	-	+++	+++	-	-	-
23	-	-	+++	+++	-	-	+++	-	-	-
25	-	-	+++	+++	-	+++	+++	-	-	++
26	-	-	+++	+++	-	+++	+	-	-	+++
27	-	-	-	-	-	-	-	-	+	-
29	-	-	-	-	-	-	-	-	-	-

The titer of the virus filtrates, one drop of which lysed one ml. inoculum of the homologous culture, was found to vary from 200 to 4 billion virus-particles per ml. These data were based on numbers of plaques produced on nutrient agar incubated at 25°C. for 24 hr. The titer bears no relation to the number of susceptible cultures as shown in Table III. Virus XXIV, with 200 particles per ml., lysed nine of the 20 cultures; whereas virus XIX with 4 billion particles per ml. lysed four cultures only. The number of serial transfers required to raise the titers of the original filtrates to the accepted standard was not related to the number of serial transfers required to raise the titers of the single-plaque filtrates. Also, the numbers of transfers from the original, and from the single plaque likewise, were related neither to numbers of susceptible cultures nor to numbers of virus particles per ml.

TABLE III

Relation between titer and complete lysis.

Virus	Titer	Cultures lysed	Original*	Single Plaque*
III	2000	3	3	5
IV	40000	16	2	2
VI	120000	2	3	2
X	400000	14	4	2
XI	100000	2	2	2
XII	500	1	2	3
XIII	280000	4	3	6
XIV	400000	14	2	2
XVI	4800	2	4	4
XVII	60000	1	4	4
XVIII	400	3	2	6
XIX	4000000000	4	3	3
XX	60000	14	2	5
XXI	400	10	3	2
XXII	40000	4	2	2
XXIV	200	9	3	2
XXVIII	300000	12	2	4
XXX	520000	3	2	2
XXXI	120000	4	4	3
XXXII	40000000	11	2	2

* Number of serial transfers from the wheat and from the single plaque to get complete lysis.

Because of the close relationship between the yellow chromogenic bacteria of wheat and *Xanthomonas translucens* (11) (22) (28), each of the cultures from which a virus was obtained was tested for pathogenicity* on a number of plants known to be susceptible to strains of *X. translucens*. None of the 20 cultures produced disease, whereas under the same conditions the pathogens did. For the same reason, each of the 20 viruses was tested against a number of strains of *X. translucens*. Eighteen cultures of *X. translucens*, including five pathogenic to each of wheat, barley and rye and three pathogenic to *Agropyron*, were not lysed by any of the viruses.

As stated above, none of the viruses isolated from single cultures was found to lyse the whole group of the yellow chromogenic bacteria listed in Tables I and II. In order to overcome this difficulty, a composite virus was tried. This was obtained by mixing equal portions of virus-filtrates of four cultures, X, XIV, XXVIII and XXXI, selected because these viruses lysed the complete range of cultures from which individual viruses had been obtained. The composite virus was found to lyse the 20 cultures and also the nine cultures from which viruses were not isolated. The individual viruses showed no inhibitory effect on the lytic action of each other when mixed. For example, cultures eight and nine, susceptible to virus XXVIII only and culture 29, susceptible to virus XXIV only, yielded ╪╪╪ lysis with the

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* The 18 cultures of *X. translucens* were supplied by Dr. W. A. F. Hagborg, Plant Pathology Laboratory, Department of Agriculture, Government of Canada, Winnipeg, Manitoba. Tests for pathogenicity were carried out by Dr. Hagborg.

composite virus. Of special interest is the fact that culture 27 was lysed +++ by the composite virus, even though it was not lysed by any of the single viruses used in preparing the composite.

The composite virus did not lyse any of the 18 cultures of *Xanthomonas translucens* previously tested with the 20 individual viruses, nor *Xanthomonas carotae*[*] nor *X. campestris*. Neither *Sarcina lutea*, the pigment of which is similar to that of the bacteria under investigation (28), nor *Micrococcus citreus*[*], another yellow chromogen, nor any of the following:

Agrobacterium radiobacter

Aerobacter aerogenes

Alcaligenes viscosus

Bacillus cereus

B. fusiformis **

B. megatherium

B. mycoides **

B. niger **

B. panis **

B. subtilis

Escherichia coli

Micrococcus pyogenes var. *albus*

M. pyogenes var. *aureus*.

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* One culture of each of *X. carotae* and *M. citreus* was supplied by Mr. A. R. Yates, Department of Bacteriology, Ontario Agricultural College, Guelph, Ontario.

** According to the 6th ed. of Bergey's Manual of Determinative Bacteriology these are listed as, *B. sphericus* var. *fusiformis*, *B. cereus* var. *mycoides*, *B. subtilis* var. *niger*, and *B. subtilis* morphotype *panis*.

M. roseus

Protens vulgaris

Pseudomonas aeruginosa

P. fluorescens

Salmonella typhosa

Shigella dysenteriae

Serratia marcescens, were lysed by the composite virus.

In order to determine whether the lytic action of the composite virus applied to other isolates of the yellow chromogenic bacteria, seed samples of wheat, barley, oats, flax, birds foot trefoil, brome grass, sweet clover, *Poa* and wild oats[*] were plated. Twenty cultures of yellow bacteria were obtained from each and, without further study or purification, used in this investigation. All 20 cultures isolated from wheat were lysed +++ by the composite virus. Nine of the 20 cultures isolated from barley were lysed +++, seven ++, three + and one was not lysed. Nine of the 20 isolated from oats were lysed +++, six ++, four + and one was not lysed. Fourteen of the 20 isolated from flax were lysed +++, one + and four were not lysed. Eighteen of the 20 isolated from birds foot trefoil were lysed +++, one ++, and one was not lysed. Eleven of the 20 isolated from brome grass were lysed +++, one ++, one +, and seven were not lysed. Nineteen of the 20 isolated from sweet clover were lysed +++, and one was not lysed. Twelve cultures isolated from

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* The samples of wheat, barley, flax and oats seeds were supplied by the Cereal Breeding Laboratory, Department of Agriculture, Government of Canada, Winnipeg, Manitoba. The samples of forage seeds were obtained from the Department of Plant Science, The University of Manitoba, Winnipeg, Manitoba.

Poa were lysed +++, three ++, and five were not lysed. Fifteen of the 20 isolated from wild oats were lysed +++, three ++, one + and one was not lysed. Thus, considering +++ and ++ lysis as positive, 100% of the wheat isolates, 95% of the birds foot trefoil, 95% of the sweet clover, 90% of wild oats, 80% of barley, 75% of each of oats, flax and Poa and 60% of the isolates from brome grass were lysed. Thus on the average 82.8% of the cultures tested were lysed by the composite virus, even though conceivably some of the cultures could not have been true to type in some respects.

It may be of interest to note that by chance a plate prepared in the student laboratory was found to harbour colonies that appeared to be identical with those under investigation. Raw milk from an unidentified source had been plated in tryptone-glucose-extract milk agar. The milk could have been contaminated by the yellow chromogenic bacteria from any of the above plant materials. Ten of the 11 of these cultures were lysed by the composite virus.

2. Cross-lysis and Cross-susceptibility.

In this investigation the term cross-lysis is used to designate cultures lysed by a single virus, and cross-susceptibility, lysis of a single culture by viruses obtained from different cultures.

No consistent relationship between cross-susceptibility and cross-lysis was found in this study except in the case of culture 11 and virus XI. Culture 11 was susceptible to viruses XI and XIV and virus XI lysed cultures 11 and 14. In 10 of the 20 cases a culture was

susceptible to a greater number of viruses than was the number of cultures lysed by it's virus. For example, culture 12 was susceptible to the following nine viruses IV, XII, XIII, XIV, XVIII, XXI, XXVIII, XXXI and XXXII, respectively. Whereas virus XII lysed culture 12 only. In the remaining nine cases each culture was susceptible to a smaller number of viruses than was the number of cultures lysed by it's virus. For example, culture 4 was susceptible to eight viruses but virus IV lysed 16 cultures. Thus, when a culture is lysed by a single virus it must not be exactly similar to the culture from which the virus was isolated. But when a number of cultures are cross-susceptible to the same viruses it is more likely that they are identical. According to cross-susceptibility a number of cultures could be considered identical. For example, cultures 4 and 21 were susceptible to the following viruses: IV, X, XIV, XX, XXI, XXIV, XXVIII and XXXII. Also cultures 18 and 32, cultures 20 and 22, and cultures 8 and 9 were susceptible to the same viruses, respectively. The data are presented in Tables I, II and IV.

TABLE IV.

Cross-lysis by viruses from single cultures
and cross-susceptibility of single cultures to the viruses.

Culture	Cross-Susceptibility	Virus	Cross-lysis
3	4	III	3
4	8	IV	16
6	5	VI	2
10	5	X	14
11	2	XI	2
12	9	XII	1
13	3	XIII	4
14	7	XIV	14
16	4	XVI	2
17	8	XVII	1
18	8	XVIII	3
19	8	XIX	4
20	10	XX	14
21	8	XXI	10
22	10	XXII	4
24	6	XXIV	9
28	7	XXVIII	12
30	8	XXX	3
31	5	XXXI	4
32	8	XXXII	11

Figures in column (2) represent numbers of viruses and in column (4) numbers of cultures.

3. <u>Effect of Storage on the Titer of the Virus Filtrate.</u>

Tests on the effect of storage on bacterial virus filtrates at 4°C. and in a frozen state in a dark room were carried out. The periods of storage varied from 22 to 35 weeks. One drop of each bacterial virus filtrate was tested against one ml. (the standard method), one drop and one loop inoculum of the homologous culture. Nine of the 20 virus filtrates did not lose their standard titers, eight viruses lysed ++, and three +. Eleven viruses lysed one drop of culture +++ and nine ++. Sixteen viruses lysed one loop of inoculum +++ and four lysed ++. As a further check on storage, the composite virus was tested against one culture lysed by the smallest number of single viruses and one culture lysed by many viruses. An additional test on the composite virus exposed to laboratory temperatures and light was carried out. No significant differences were found between lysis of viruses stored in a frozen state and those at 4°C. However a difference was found between the composite virus stored at 4°C. in a dark room and the same virus exposed to light at room temperature. The data appear in Table V.

TABLE V.

Effect of storage of virus on titer.

Virus	Weeks Stored	A	B	C
III[x]	27	+++	+++	+++
IV	28	+++	+++	+++
VI	27	+++	+++	+++
X	27	+++	+++	+++
XI	31	++	+++	+++
XII	35	+	++	++
XIII	22	++	++	+++
XIV	31	+++	+++	+++
XVI	28	+++	+++	+++
XVII	28	+	++	++
XVIII	35	++	++	++
XIX	22	++	++	+++
XX	27	+++	+++	+++
XXI	32	+++	+++	+++
XXII	32	++	+++	+++
XXIV	32	++	++	+++
XXVIII	34	+++	+++	+++
XXX	32	++	++	+++
XXXI	35	++	++	+++
XXXII	35	+	++	++
Composite	20[a]	+++	+++	+++
	20[b]	+++	+++	+++
Composite[xx]	20[a]	+	++	+++
	20[b]	+	+++	+++
VI[xxx]	27	+++	+++	+++
XII	35	+	++	+++
XIX	22	++	+++	+++

x Stored in dark at 4°C.
xx " " light at 20°C.
xxx " " dark at -15°C.
a Culture 11
b " 20
A One drop virus and one ml. culture.
B " " " " one drop " .
C " " " " one loop " .

DISCUSSION

This method is a much simpler method of identifying the yellow chromogenic bacteria than a detailed study of their morphology, cultural characteristics and physiology. The composite virus lysed all the cultures of the yellow chromogenic bacteria isolated from wheat and 82% of the cultures isolated from other sources, and tested without further study or purification. One hundred percent of the wheat isolates and 82% of the isolates from other sources were identified by this procedure.

On the basis of this investigation it is evident that the yellow chromogenic bacteria constitute a distinct group that logically could represent a species. None of the 20 individual viruses, nor the composite, lysed 18 cultures of *Xanthomonas translucens*, different from the yellow chromogenic bacteria with respect to pathogenicity and utilization of asparagine as the sole source of carbon and nitrogen. In addition, none of 24 different species, including *X. campestris* and *X. carotae* and two other yellow pigmented species, was lysed by the composite virus. Thus, this method of identifying may be regarded quite reliable.

It is apparent from the findings that there are minor differences among the yellow chromogenic bacteria, and, it is probable, that one could establish subspecies within the species on the basis of virus reactions. In support of this statement no two viruses isolated from different cultures lysed exactly the same group of cultures; and,

in most cases, no two cultures were lysed by exactly the same group of viruses against which they were tested. However, in a few cases, two cultures were lysed by the same viruses and on this basis could be considered identical.

When single viruses were mixed in the composite virus there was no indication that one virus was inhibited by another (3). In every case the composite virus lysed all the cultures lysed by single viruses. In addition, the composite virus lysed at least one culture that was not lysed by any of single viruses used in the composite. This would appear to indicate a phenomenon somewhat similar to synergism in bacterial cultures.

The fact that all of the isolates of yellow chromogenic bacteria from wheat seeds were lysed by the composite virus and the isolates from seeds other than wheat were lysed in 82% of the isolates only indicates that the host plant in some measure might be responsible for the small differences in the cultures.

Further, no consistent relationship between cross-lysis and cross-susceptibility was found in this investigation. Thus, when a culture was lysed by a virus it must not necessarily be exactly identical with the culture from which that virus was obtained. But there must be a very close relationship between such cultures.

According to the results it is believed that the number of virus-particles per ml. must not be related directly to lytic capacity of the virus, as no relationship could be found between titer and the

number of susceptible cultures nor the number of serial enrichment transfers necessary for complete lysis. The virus filtrates with only a few hundred particles per ml. lysed homologous cultures as completely as those containing millions of virus-particles per ml.

The loss in titer seems to be related to some property of the virus rather than the number of virus-particles per ml. Considerable loss in titer, both in storage at 4°C. and in the frozen state, was found in a filtrate containing 4 billion virus-particles per ml. as was the case also with the one containing 200 particles per ml. only.

SUMMARY

1. Wheat seeds were found to harbour bacterial viruses specific for the yellow chromogenic bacteria under investigation. From 29 cultures tried the isolation of a virus was successful 20 times. The nine remaining cultures either failed to produce a virus or the virus titer could not be raised to the accepted standard by the method used.

2. Each of the 20 bacterial viruses isolated from single cultures was found too specific for identifying the yellow chromogenic bacteria as a group.

3. The composite virus, obtained by mixing equal portions of four viruses isolated from single cultures, was used to identify the yellow chromogenic bacteria. This virus lysed all of the 49 cultures of the yellow chromogenic bacteria isolated from wheat and about 82% of isolates from other sources.

4. In no case did a single virus isolated from each of the 20 cultures of the yellow chromogen, nor the composite virus, lyse any of the 18 cultures of *Xanthomonas translucens*. Neither did the composite virus lyse one culture of each of *X. campestris* and *X. carotae*, two other yellow pigmented species, nor any of the 20 additional bacterial species tested. Accordingly, the composite virus is rather specific

for the yellow chromogenic bacteria and their identification by this method is simple and fairly reliable.

5. On the basis of the virus reactions obtained in this investigation it is evident that the yellow chromogenic bacteria constitute a distinct group of bacteria.

6. According to the reaction of bacterial viruses isolated from single cultures the yellow chromogenic bacteria under investigation must include a large number of strains or subspecies. A number of identical cultures was recognized in this study and, it is believed that on further investigation a certain number of subspecies could be established by this method.

7. On mixing of single virus filtrates no inhibitory effect of one virus on the lytic reaction of another was found, on the contrary a phenomenon similar to bacterial synergism was observed in this study.

8. The titer of a bacterial virus filtrate was related neither to the number of susceptible cultures nor to the number of serial transfers to obtain the titer necessary for complete lysis.

9. A number of bacterial viruses isolated from single cultures was found to lose titer on storage for 22-35 weeks, both at 4°C. and in the frozen state in a dark room.

10. The composite virus stored at 4°C. in a darkroom for 20 weeks did not show loss in titer whereas a portion of the same filtrate kept at room temperature exposed to light had a lower titer.

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