JOURNAL OF BACTERIOLOGY, Aug. 1969, p. 496-502

Vol. 99, No. 2
Printed in U.S.A.

Tumor Growth Complementation Among Strains of *Agrobacterium*

JAMES A. LIPPINCOTT AND BARBARA B. LIPPINCOTT
Department of Biological Sciences, Northwestern University, Evanston, Illinois 60201

Received for publication 4 April, 1969

The ability of 31 strains of *Agrobacterium* to initiate the production of a tumor growth factor (TGF) which is associated with crown-gall tumors on primary pinto bean leaves was determined. Extracts from bean leaves inoculated with these bacteria were tested and they showed that 16 of the 19 strains that induced tumors on the leaves also initiated TGF production. The three strains for which no TGF was detected were of low infectivity and included two strains of *A. tumefaciens* and a strain of *A. rhizogenes*. Five of the 12 strains that did not induce pinto bean leaf tumors were found to initiate TGF production. Representatives of *A. tumefaciens*, *A. rhizogenes*, and *A. radiobacter* among these 12 strains were present in both categories. Mixed inocula composed of one of the three infectious TGF-negative strains and one of the five nontumorigenic TGF-positive strains resulted in increased growth of tumors induced by the former. These growth changes were not correlated with changes in tumor number. The ability of different strains to show these tumor growth complementation effects corresponded fully with their ability to initiate TGF, as determined by the assay of leaf extracts. The nontumorigenic TGF-positive strains also promoted the growth of tumors initiated by low concentrations of strain B6. These complementation effects were due, therefore, to the same TGF found in extracts of B6 inoculated leaves and of leaves inoculated with most tumorigenic as well as many nontumorigenic strains of *Agrobacterium*. Heat-inactivated cells of strain B6 failed to initiate sufficient TGF to be detected in extracts, and heat-inactivated cells of several strains failed to show tumor growth complementation, indicating bacterial viability to be one prerequisite for TGF initiation. Heat inactivated cells also inhibited TGF production by viable cells, similar to their ability to inhibit tumor initiation. Consequently, bacteria capable of attaching to the *A. tumefaciens* infection site may initiate one of four patterns of events: (i) TGF production only, (ii) tumor induction only, (iii) both, or (iv) neither. Suggestive evidence for a second tumor-associated growth factor is presented.

The positive correlation between the growth rate of individual crown-gall tumors on pinto bean leaves and the number of tumors on the leaves was proposed to result from a tumor growth factor (TGF) produced by the tumor tissue (11). Evidence to support this proposal came from the demonstration that tumor number and tumor size could be varied in parallel by certain treatments of leaves inoculated with a single concentration of *Agrobacterium tumefaciens* (4, 5) and from the apparent increase in the amount of TGF extractable from tumorous leaves with time after inoculation, coupled with its absence in either cultures of the bacterium or normal leaves (5). To more fully describe the source and role of this TGF in crown-gall etiology, the ability of many strains of *Agrobacterium* to initiate the production of TGF when inoculated on primary pinto bean leaves was determined.

The discovery of avirulent strains of *Agrobacterium* that initiate TGF production and of certain strains of low infectivity that do not led to experiments with inocula composed of positive and negative TGF strains that demonstrate tumor growth complementation in the absence of effects on tumor number. The results are consistent in showing that the TGF is a diffusible compound which is nonessential for tumor initiation but which is a secondary characteristic associated with tumor induction by most infectious *A. tumefaciens* strains. The initiation of TGF is not a simple result of the bacterium plus the wound

medium but requires the attachment of a viable bacterium to some site in the wound similar to that found essential for tumor induction (9).

MATERIALS AND METHODS

Bacterial cultures. Culture conditions, cell viability determinations, and cell-washing procedures were the same as those in previous studies (8, 9). All of the strains of *A. tumefaciens* and *A. radiobacter* were positive for 3-ketoglycoside production in the test of Bernaerts and De Ley (2). They also gave the typical litmus milk response for the group (13), and strains that induced pinto bean leaf tumors (including strain 13333) induced tumors on carrot root discs but failed to initiate significant root formation on this host. Those strains of *A. tumefaciens* and *A. radiobacter* that were unable to induce bean leaf tumors were also noninfectious on carrot root discs. The *A. rhizogenes* strains were all 3-ketoglycoside-negative, produced an acid response on litmus milk medium (13), and induced copious root formation on carrot root discs (1). With the exception of strain 13333 (originally classified as *A. rhizogenes*), all of the strains remain classified as received.

Tumor initiation and size determinations. Primary pinto bean leaves (*Phaseolus vulgaris* var. Pinto) were inoculated (10) for these determinations, and the methods described by Lippincott and Lippincott (9) and Lippincott, Lippincott, and El Khalifa (11) for tumor counts and the measurement of tumor size were followed. The interval between inoculation and the determination of both tumor number and tumor size in the complementation tests, however, was 5 days, except in Table 5 for which the interval was 6 days. Tumor size is reported in units of the ocular microscope scale (SU) employed in their measurement; 1 SU is equal to 0.033 mm. The per cent relative specific infectivities (tumors initiated per viable cell relative to strain B6 = 100%) reported for the different strains are a mean of five or more independent determinations. The standard error of the mean for these determinations averaged ±26% of the reported value. Strains reported as noninfectious induced no tumors in five or more tests and were estimated to have a relative specific infectivity of less than 0.05%.

Extraction and assay of TGF. Primary pinto bean leaves inoculated with undiluted stationary-phase cultures of bacteria were harvested 4 or 5 days after inoculation, washed, and then stored at −60 C. The frozen leaves were pulverized and extracts were prepared as described by El Khalifa and Lippincott (5). The extracts were tested for TGF by applying 0.1-ml samples to each of 16 leaves inoculated 3 days before with strain B6 at a concentration of about 10^8 cells per ml (5). Tumor counts on the whole leaf and tumor diameter measurements were made 6 days after inoculation. No more than three tumors were measured per leaf, and these were selected by starting at the same point on each leaf and searching the leaf in a prescribed pattern until up to three round discrete tumors were measured. Several blind tests were made to insure that this procedure did not prejudice the results.

Six samples of 16 leaves each were used in most TGF bioassay experiments; two served as controls and the others received leaf extracts. At least one of these extracts either was known to contain TGF or was strongly suspected to contain TGF. To be scored as positive for TGF, an extract had to promote the mean diameter of the tumors on treated leaves by at least 0.4 SU above that of the largest control. Of the extracts scored positive for TGF, 54 of 59 gave growth differences of 0.6 SU or greater. One of the 20 extracts tested from strains rated TGF-negative gave a tumor growth promotion of 0.2 SU, whereas the other 19 showed less or no positive activity. Assuming a spherical model for the tumors and a control diameter of 6.0 SU, an increase in diameter of 0.4 SU would represent an increase in tumor volume of about 22%. The standard error of the mean for these diameter measurements was between ±0.15 and 0.3 SU in nearly all determinations in which 20 or more tumors were measured. The duplicate or triplicate results reported for many strains were obtained from leaves inoculated at least 1 week apart with a different culture of the same bacterium. Many of the extracts were tested more than once and, although the results are not shown, they agreed with the original test results.

RESULTS

Of 19 strains of *Agrobacterium* tested that induced tumors on primary pinto bean leaves (Table 1), only three failed to give rise to detectable amounts of TGF when inoculated on the bean leaves. These three strains were all of very low infectivity and included one strain of *A. rhizogenes* and two strains of *A. tumefaciens*. Several noninfectious strains of these two species and of *A. radiobacter* also failed to give detectable amounts of TGF. Surprisingly, however, a number of strains that could not induce tumors on the bean leaves were still able to initiate TGF production. Strains from each of the three species showed this characteristic and, with the exception of the *A. rhizogenes* strains, they were also unable to induce tumors or roots when inoculated on carrot root slices. The production of TGF, therefore, requires little or possibly no actual tumor tissue. The presence or absence of a correlation between the production of TGF and the specific infectivity of the virulent strains that were TGF-positive, however, could not be determined with any accuracy because of the nature of the bioassay.

The discovery of noninfectious TGF-positive bacteria and of infectious TGF-negative bacteria through the direct assay of TGF extracted from inoculated leaves suggested that complementation effects on tumor growth should be observed when appropriate mixtures of these

TABLE 1. *Survey of Agrobacterium sp. for ability to initiate tumor growth factor production when inoculated on pinto bean leaves*

Species	Strain	Source[a]	Per cent relative specific infectivity on pinto bean leaves	Tumor growth factor[b]
TGF-positive				
A. tumefaciens	15955	ATCC	311	+(4:4)
A. tumefaciens	TT 107	De Ley (ICPB)	247	+(2:2)
A. tumefaciens	C-58-3	Hamilton	231	+(2:2)
A. tumefaciens	H-100	Stonier	111	+(1:1)
A. tumefaciens	B6	Stonier	100	+(19:19)
A. tumefaciens	B6[c]		0	−(0:2)
A. tumefaciens	T-37	Stonier	98	+(1:1)
A. tumefaciens	TT 9	De Ley (ICPB)	44	+(1:1)
A. tumefaciens	W-1	Manasse	41	+(1:1)
A. tumefaciens	A66B	Stonier	16	+(2:2)
A. tumefaciens	V-1	Hamilton	15	+(1:1)
A. tumefaciens	396	De Ley (NCPPB)	10	+(1:1)
A. tumefaciens	42 IV	De Ley (IP)	8	+(2:2)
A. tumefaciens	B2	Beardsley	6	+(2:2)
A. tumefaciens	Ade-14	Lippincott	1	+(1:1)
A. tumefaciens	IIBNV6	Stonier	0	+(2:3)
A. tumefaciens	SN	Manasse	0	+(2:3)
A. tumefaciens	B-48	Manasse	0	+(2:2)
A. rubi	13335	ATCC	198	+(2:3)
A. rhizogenes	TR 107	De Ley (ICPB)	1	+(2:2)
A. rhizogenes	TR 7	De Ley (ICPB)	0	+(2:2)
A. radiobacter	TR 1	De Ley (ICPB)	0	+(2:3)
TGF-negative				
A. tumefaciens	13333	ATCC (rhizogenes)	2	−(0:3)
A. tumefaciens	5	De Ley (NCPPB)	2	−(0:2)
A. tumefaciens	178	Manasse	0	−(0:1)
A. tumefaciens	5GlyFe	Beardsley	0	−(0:2)
A. rhizogenes	15834	ATCC	1	−(0:3)
A. rhizogenes	TR 101	De Ley (ICPB)	0	−(0:2)
A. rhizogenes	11325	ATCC	0	−(0:2)
A. rhizogenes	8196	De Ley (NCIB)	0	−(0:1)
A. radiobacter	4718	ATCC	0	−(0:2)
A. radiobacter	RV 3	De Ley (IP)	0	−(0:2)

[a] Investigator or culture collection that supplied the strain. Abbreviations: ATCC, American Type Culture Collection, Rockville, Maryland; ICPB, International Collection of Phytopathogenic Bacteria, Department of Bacteriology, University of California, Davis, Calif.; NCIB, National Collection of Industrial Bacteria, Torry Research Station, Aberdeen, Scotland; NCPPB, National Collection of Plant Pathogenic Bacteria, Ministry of Agriculture, Fisheries and Food, Hatching Green, Harpenden, England; IP, Institut Pasteur, Paris, France.

[b] Values in parentheses represent ratio of positive extracts to number of extracts tested.

[c] Culture was treated at 60 C for 20 min.

strains were inoculated. Table 2 (experiment 1) demonstrates that greater tumor growth was obtained with mixed inocula in the absence of significant changes in tumor number. Thus, noninfectious TGF-positive strains of *A. tumefaciens*, *A. rhizogenes*, and *A. radiobacter* increased the growth of tumors initiated by *A. tumefaciens* strain 13333.

Strains IIBNV6 and SN also promoted the growth of tumors initiated by strain 13333 (Table 2, experiment 2). TGF-negative strains of three species, however, failed to show this growth complementation, in complete agreement with the results obtained with extracts. Attempts to obtain similar tumor growth results with mixtures of strain 15834 (infectious, TGF-negative), and TGF-positive strains were confused by variations in tumor number and by complications due to inhibitory effects associated with products in the culture medium. Washed cultures, however, gave increased tumor growth which correlated with the concentration of the TGF-positive strain but not with tumor number.

The effect of varying the number of TGF-

TABLE 2. *Effect of avirulent TGF-positive bacteria on the growth of tumors induced by TGF-negative bacteria*

Experiment	Strains and mixtures inoculated (viable cells/ml × 10^8)[a]	T:L[b]	Mean no. of tumors per leaf	Mean tumor diameter (SU)
1. Promotion by avirulent TGF-positive strains	13333[c] (7)	34:14	6.8	6.6
	13333 (3.5) + B-48[d] (18)	29:11	6.4	8.7
	13333 (3.5) + TR 7[d] (5)	39:16	7.9	8.2
	13333 (3.5) + TR 1[d] (25)	25:14	3.5	7.8
2. Comparative action of TGF-positive and TGF-negative strains	13333[c] (20)	40:16	17.1	6.0
	13333 (20) + SN[d] (49)	46:16	13.2	6.6
	13333 (20) + IIBNV6[d] (75)	35:12	21.8	7.1
	13333 (20) + TR 7[d] (11)	45:16	14.4	7.0
	13333 (20) + 178[c] (27)	46:16	11.9	5.9
	13333 (20) + 5GlyFe[c] (25)	42:14	17.1	6.1
	13333 (20) + TR 101[c] (5)	43:16	13.7	6.1
	13333 (20) + 4718[c] (30)	46:16	16.3	5.9
3. Dependence on the number of TGF-positive bacteria	13333[c] (20)	38:16	8.5	5.7
	13333 (10)	24:16	2.9	5.4
	13333 (10) + B-48[d] (32)	32:16	4.1	6.6
	13333 (10) + B-48 (3.2)	35:16	4.2	6.0
	5[c] (8)	34:16	4.9	5.7
	5 (4)	28:16	2.1	5.2
	5 (4) + B-48[d] (32)	27:16	2.5	6.4
	5 (4) + B-48 (3.2)	26:16	1.9	6.0
4. Dependence on the number of TGF-negative bacteria	13333[c] (12)	30:16	3.7	5.7
	13333 (12) + B-48[d] (47)	41:16	4.2	6.8
	13333 (8) + B-48 (47)	22:16	2.3	6.3
	13333 (2.3) + B-48 (47)	13:16	0.8	6.2

[a] Final concentration of each strain is indicated in parentheses immediately after the strain designation.
[b] Number of tumors measured (T): number of leaves on which these tumors were located (L).
[c] TGF-negative strain.
[d] TGF-positive strain.

positive cells (B-48) on the growth of tumors initiated by TGF-negative strains 13333 and 5 is shown in Table 2, experiment 3. Greater tumor growth was observed in the presence of strain B-48 than in its absence, and the amount of growth obtained was related to the number of B-48 cells added and not to the number of tumors. The size of the tumors initiated by strains 13333 and 5 in the absence of B-48 cells, however, was correlated with the number of each bacterium applied to the leaves and consequently with tumor number.

Experiment 4 of Table 2 shows results obtained with different concentrations of strain 13333 cells in inocula that contained a relatively high concentration of B-48 cells. The B-48 cells promoted the growth of tumors induced by strain 13333 in each case without significantly changing tumor number. Despite the presence of these TGF-positive cells, tumor diameter also showed a direct correlation with both tumor number and the number of 13333 cells in the inoculum, even though tumor size was still greater on leaves inoculated with the mixture containing the lowest concentration of 13333 cells (and, correspondingly, fewest tumors) than in the control lacking B-48 cells.

The relation between the induction of TGF and one of the requirements for tumor initiation, site attachment (9), was studied to determine the extent of their interdependence (Table 3). The combination of nonviable cells with viable TGF-positive cells prevented tumor growth complementation by the latter on strain 13333-induced tumors. This was observed when the mixture was added either before or after the 13333 cells. Addition of TR 7 or TR 7 plus heat-inactivated cells prior to 13333 cells, however, inhibited tumor initiation, presumably because they prevented their attachment (9). Heat-inactivated IIBNV6 and B6 cells gave similar results to heat-inactivated TR 7 cells, indicating that each of these cell

TABLE 3. *Inhibition of tumor growth complementation by non-viable TGF-positive bacteria*[a]

Strains and mixtures inoculated (cells/ml × 10^8)[b]	T:L	Mean no. of tumors per leaf	Mean tumor diameter (SU)
13333 (36)	45:16	7.1	6.2
13333 (36)—TR 7 (1.9)	42:16	6.0	7.3
Order of addition reversed	18:16	1.1	6.9
13333 (36)—TR 7 (1.9) + TR 7 (95)	40:16	6.0	6.1
Order of addition reversed	8:16	0.5	6.0
13333 (36)—TR 7 (1.9) + IIBNV6 (650)	41:16	6.1	5.9
Order of addition reversed	8:16	0.5	6.0
13333 (36)—TR 7 (1.9) + B6 (550)	44:16	5.4	6.0
Order of addition reversed	12:16	0.8	6.1

[a] See Table 2 for an explanation of table headings.

[b] Cells of underlined strains were treated at 60 C for 20 min to destroy their viability. All cells were washed, and cell numbers were based on viable cell counts of the washed suspensions prior to any heat treatments. Dashes (—) indicate an elapsed time of about 15 min between the addition of strain 13333 cells and the addition of strain TR 7 or of one of the mixtures containing TR 7.

types was an effective inhibitor of both tumor initiation by strain 13333 and of growth complementation by TR 7. This latter result suggests that attachment of TR 7 to the same site essential for tumor initiation is required before TGF production can be initiated. Table 3 also provides additional confirmation of the result shown in Table 1, that viable bacteria are necessary for the initiation of TGF even though nonviable bacteria can attach to the infection site in the wound.

The relevance of the tumor growth complementation effects obtained with these noninfectious strains and with strains of species other than *A. tumefaciens* to the crown-gall tumor problem was checked by testing many of the avirulent strains shown in Table 1 for their ability to complement the growth of tumors induced by strain B6. TGF-positive strains promoted the growth of strain B6-induced tumors (Table 4) whereas TGF-negative strains did not, again indicating the direct correspondence between the results obtained with extracts added to leaves infected with strain B6 and the ability to obtain this complementation effect with mixed inocula. The increased

TABLE 4. *Growth of tumors induced by strain B6 inoculated with avirulentTGF-positive or avirulent TGF-negative Agrobacterium strains*[a]

Strains and mixtures inoculated (viable cells/ml × 10^8)	T:L	Mean no. of tumors per leaf	Mean tumor diameter (SU)
B6[b] (0.84)	42:14	10.0	5.8
B6 (0.84) + IIBNV6[b] (45)	47:16	14.1	6.3
B6 (0.84) + SN[b] (19)	39:14	7.9	6.7
B6 (0.84) + B-48[b] (27)	41:16	5.4	6.6
B6 (0.84) + TR 7[b] (1.5)	34:12	19.8	6.3
B6 (0.84) + TR 1[b] (21)	41:16	6.3	6.5
B6 (0.84) + 178[c] (17)	42:16	5.1	5.6
B6 (0.84) + 5GlyFe[c] (10)	41:16	4.1	5.7
B6 (0.84) + TR 101[c] (3.8)	42:16	5.5	5.3
B6 (0.84) + 4718[c] (22)	44:16	6.2	5.8

[a] See Table 2 for an explanation of table headings.

[b] TGF-positive strains.

[c] TGF-negative strains.

tumor number found with inocula containing low concentrations of strain B6 plus strain IIBNV6 cells was noted previously (9), and strain TR 7 may have similar effects.

DISCUSSION

These data clearly indicate that the production of TGF does not depend on tumor tissue per se, at least as it is normally detected on the pinto bean leaves or carrot root discs. The actual source of the TGF, the infecting bacterium or the affected plant cell, cannot be determined with certainty at present. The close correlation between tumor number and tumor size noted by varying tumor number through the addition of plant growth factors or of salts, or by removing parts of the plant while maintaining constant the number of bacteria applied to the leaves (4, 5, 11), led to the proposal that TGF was produced by the tumor tissue. Because these treatments occurred after the inoculation of the leaves, they presumably were not active via an influence on the attachment of the bacterium to the infection site but at some later stage in the tumor initiation process. Thus, the production of TGF and tumor initiation appear to be tightly coupled in infections with highly virulent strains. The results with nontumorigenic strains, however, show the two processes need not be related. We must assume, therefore, that something roughly equivalent to an "incipient tumor cell" may also be a source of TGF, or that in all cases the bacterium is the source of TGF, and that if the highly virulent bacteria proceed through the attachment and TGF production

stages they will also complete any remaining steps required for tumor initiation.

Although 3 of 19 infectious strains could not be shown to produce this TGF by assay of leaf extracts, the growth of tumors initiated by different concentrations of these TGF-negative bacteria was found to be related to tumor number, as in the case of highly infectious strains (11). These conflicting results may arise because the amount of TGF produced by these strains was too low to be detected under our extraction and assay conditions or because there are two or more growth factors produced by the bacterium-wound-tumor complex, only one of which occurs in our extracts. Titrations of TGF in extracts from B6- and 15955-infected leaves suggest that if the leaves inoculated with TGF-negative strains such as 13333 contained as little as 0.5% as much TGF, it would have been detected. On the other hand, the shape of the curves that relate tumor number with tumor growth (11) led to an earlier suggestion that more than one tumor growth factor might be present. At this time neither of these alternatives can be dismissed or proved, but we favor the proposal that two tumor growth factors are responsible for these results.

The tumor growth complementation effects noted between different strains of *Agrobacterium* probably stem from the same TGF detected in extracts of inoculated leaves. The direct correspondence between positive TGF extracts from noninfectious strains and their ability to increase tumor growth when mixed either with low concentrations of strain B6 or with infectious TGF-negative strains provides direct evidence for this conclusion. Also, TGF-negative strains still showed tumor growth differences related to the number of these bacteria in the inoculum (and, consequently, to the number of tumors) although combined with high concentrations of TGF-positive bacteria that could be expected to greatly elevate the level of TGF in the leaf. This response supports the concept of a second tumor growth factor but suggests that it is different from the extracted TGF and from the tumor growth complementation factor. Since this is the only other growth factor in evidence, this provides indirect evidence to suggest that TGF and the complementation factor are identical.

The results in Table 3, as well as many other observations, show that TGF production does not occur unless the bacterium successfully attaches to some site in the wound. A similar attachment was shown to be a requisite part of the normal tumor initiation process (9). Because heat-inactivated IIBNV6 and B6 cells can inhibit TGF production by nonvirulent cells, as well as tumor initiation by strain B6 cells, we presume the two attachment sites are identical. Attachment per se need not lead to TGF production, however, since (i) a few TGF-negative strains were able to initiate tumors and consequently may be assumed to attach to these sites, (ii) certain TGF-negative strains were previously shown to compete with strain B6 for the attachment site (9), and (iii) nonviable cells can compete for attachment sites but are unable to initiate TGF production.

The lack of significant promotion of tumor initiation by infectious TGF-negative strains when combined with TGF-positive strains as well as direct tests of TGF-containing extracts for effects on tumor initiation (5) indicate that this particular factor is not an active principle in the tumor initiation process. TGF production, therefore, must be considered as an event commonly associated with those involved in tumor initiation that occurs after bacterial attachment but which is not causally related or essential for tumor induction. The level of TGF in an infected plant, however, should affect the severity of the disease as it occurs in nature. Consequently, the degree of virulence assigned to different strains of the crown-gall bacterium based on such observations may be due in part to quantitative differences in their ability to induce TGF on a particular host.

Although the bacteria that induced TGF production include noninfectious strains of *Agrobacterium* and, in the case of *A. rhizogenes*, bacteria that induce a separate, though probably related, disease on certain hosts, all of these bacteria are closely related to the crown-gall-inducing organism (3, 6, 12). Heberlein et al. (7) have reported a high degree of deoxyribonucleic acid homology between these species. In addition to these similarities, our data show that certain strains of *A. radiobacter* and of *A. rhizogenes*, as well as strains of noninfectious *A. tumefaciens*, apparently can attach to the same infection site essential for crown-gall initiation (9) and can induce the same TGF that occurs in association with *A. tumefaciens* infections.

The concentrations of bacteria at which tumor growth complementation effects may be observed are sufficiently low in many instances to rule out the necessity of both kinds of bacteria at a single wound site to produce these effects (9). This also would be expected, since the tumor-initiating bacteria and the TGF-producing bacteria must both attach to elicit their respective actions. If significant competition for the same sites occurred, both tumor number and tumor growth would be less than maximum. This kind of result has been observed. Also, because tumor complementation can be obtained by adding the infectious strain first and by later applying the TGF-positive strain, an infectious aggregate composed of one

or more of each bacterium seems improbable. Thus, tumor growth complementation is due to a diffusible factor, in full confirmation of previous results (5, 11). It is probably produced at one wound site and detected at a second where a tumorigenic strain has established an infection.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service research grant CA-05387 from the National Cancer Institute.

We acknowledge the very able technical assistance of Shirley Fink.

LITERATURE CITED

1. Ark, P. A., and J. P. Thompson. 1961. Detection of hairy root pathogen, *Agrobacterium rhizogenes*, by the use of fleshy roots. Phytopathology 51:69–71.
2. Bernaerts, M. J., and J. De Ley. 1963. A biochemical test for crown gall bacteria. Nature 197:406–407.
3. De Ley, J., M. Bernaerts, A. Rassel, and J. Guilmot. 1966. Approach to an improved taxonomy of the genus *Agrobacterium*. J. Gen. Microbiol. 43:7–17.
4. El Khalifa, M. D., and J. A. Lippincott. 1968. The influence of plant growth factors on the initiation and growth of crown-gall tumours on primary pinto bean leaves. J. Exp. Bot. 19:749–759.
5. El Khalifa, M. D., and J. A. Lippincott. 1968. Extraction, assay and partial purification of a factor from tumorous leaves that promotes crown-gall tumor growth. Physiol. Plant. 21:742–753.
6. Graham, P. H. 1964. The application of computer techniques to the taxonomy of the root nodule bacteria of legumes. J. Gen. Microbiol. 35:511–517.
7. Heberlein, G. T., J. De Ley, and R. Tijtgat. 1967. Deoxyribonucleic acid homology and taxonomy of *Agrobacterium*, *Rhizobium*, and *Chromobacterium*. J. Bacteriol. 94:116–124.
8. Lippincott, B. B., and J. A. Lippincott. 1966. Characteristics of *Agrobacterium tumefaciens* auxotrophic mutant infectivity. J. Bacteriol. 92:937–945.
9. Lippincott, B. B., and J. A. Lippincott. 1969. Bacterial attachment to a specific wound site as an essential stage in tumor initiation by *Agrobacterium tumefaciens*. J. Bacteriol. 97:620–628.
10. Lippincott, J. A., and G. T. Heberlein. 1965. The quantitative determination of the infectivity of *Agrobacterium tumefaciens*. Amer. J. Bot. 52:856–863.
11. Lippincott, J. A., B. B. Lippincott, and M. D. El Khalifa. 1968. Evidence for a tumor-associated factor active in the promotion of crown-gall tumor growth on primary pinto bean leaves. Physiol. Plant. 21:742–753.
12. Moffett, M. L., and R. R. Colwell. 1968. Adansonian analysis of the *Rhizobiaceae*. J. Gen Microbiol. 51:245–266.
13. Riker, A. J., W. M. Banfield, W. H. Wright, G. W. Keitt, and H. E. Sagen. 1930. Studies on infectious hairy root of nursery apple trees. J. Agr. Res. 41:507–540.