Host Specificity in the Genus *Agrobacterium*

A. R. Anderson and L. W. Moore

Graduate research assistant and associate professor, respectively, Department of Botany and Plant Pathology, Oregon State University, Corvallis, 97331.

We wish to thank Barbara Parker and Jack Allen for technical assistance.
Oregon Agricultural Experiment Station Technical Paper 4848.
Accepted for publication 16 October 1978.

ABSTRACT

ANDERSON, A. R., and L. W. MOORE. 1979. Host specificity in the genus Agrobacterium. Phytopathology 69:320-323.

One hundred seventy-six *Agrobacterium* strains, principally from the USA and isolated from 26 host species in 11 plant families, were inoculated on 11 known crown gall hosts to determine host specificities. No strain was pathogenic on all hosts tested. Sixty-six percent of the pathogenic strains induced tumors or hairy root on six to eight of 11 host species tested. Twenty-seven strains (nonpathogenic on the initial 11 host species) were nonpathogenic on three additional host species. Three percent of the pathogens infected only the host species from which they were originally isolated, which suggests that host origin has a minimal influence on a strain's host range. In some instances, strains isolated from the same naturally occurring tumor infected different host species, but some strains failed to infect the host of origin. *Agrobacterium rubi* strains were indistinguishable from *A. tumefaciens* strains on the basis of pathogenicity, and five of eight *A. rhizogenes* strains formed tumors on some plants and hairy root on carrot. Tomato and *Datura stramonium* were infected by a greater number of pathogenic agrobacteria (81% each) than any other host species tested. However, all strains that infected tomato did not infect datura and vice versa. These data corroborate that speciation based on pathogenicity and host specificity is of little taxonomic or practical value.

Recent taxonomic analyses (5,15,24) and studies of the relatedness of agrobacteria by nucleic acid hybridization (9,10) have suggested that speciation based on phytopathogenicity does not reflect natural relationships. Despite these reports, classification of *Agrobacterium* in Bergey's Manual (1) is based essentially on pathogenicity. With the discovery of a plasmid (pTi) that confers oncongenicity to a strain of *Agrobacterium* (7,27), speciation based on pathogenicity becomes even more tenuous. For example, a pathogenic strain may become avirulent through complete or partial loss of the pTi (16), thus becoming *A. radiobacter*. Conversely, an avirulent strain can become virulent by receipt of a pTi via conjugation with a virulent strain of *Agrobacterium* (14), thus becoming *A. tumefaciens*. There also is new evidence that the determinants for host range are carried by the pTi (23, and L. W. Moore, *unpublished*).

Wormald's (25) early paper described 16 strains of *A. tumefaciens* that infected some but not all of the six plant species tested. In contrast, a recent review by DeCleene and DeLey (4) lists a large number of plant species that *A. tumefaciens* strain B_6 could infect. To infect so many species, the presence of a common mechanism for infectivity, or a common host receptor, or both, would be expeted. If true, is this "common" factor found extensively among the agrobacteria?

To explore this question, many *Agrobacterium* strains, isolated from diverse, naturally-galled plants, were tested for pathogenicity on selected host species. We sought to: (i) determine the extent of host specificity among strains, (ii) assess the impact of host specificity on current taxonomy, and (iii) catalog the strains according to the hosts infected for subsequent studies of plasmid relatedness.

MATERIALS AND METHODS

Cultures and inocula. One hundred seventy-six previously characterized *Agrobacterium* strains (3) were used in this study. Of the 176 strains, 19 were *A. rhizogenes* (including ATCC 11325, 13332, 13333, and 15834), and three were *A. rubi* (including ATCC 13334 and 13335). A complete list of the strains used in this study and their sources will be supplied upon request. Cultures were grown on potato dextrose agar (Difco) slants supplemented with 5.0 g $CaCO_3$/L.

Host plants tested. The following plants were inoculated with all 176 strains of *Agrobacterium*: sugar beet (*Beta vulgaris* monogerm cultivar); daisy (*Chrysanthemum frutescens* 'Paris'); datura (*Datura stramonium*); sunflower (*Helianthus annus*); bryophyllum (*Kalanchoë daigremontiana*); pea (*Lathyrus hirsutus* 'Austrian'); tomato (*Lycopersicon esculentum* 'Bonny Best'); green bean (*Phaseolus vulgaris* 'Spartan Arrow'); Myrobalan plum (*Prunus cerasifera*); and radish (*Raphanus sativus* 'Early Scarlet Globe').

Growth and inoculation of plants. In most instances, groups of two or three seedlings or cuttings of each species were inoculated in November, April, and August to compensate for any seasonal effects on symptom development. Apple seedlings were inoculated only in April. Plants were grown under artificial lights (16-hr photoperiod) in either a greenhouse (10,000 lux, winter) or growth chamber (10,000 lux) at 25 C day and 21 C night. In the summer, light intensity in the greenhouse reached a maximum of 22,000 lux. Herbaceous plant stems and leaves were wounded with six closely-spaced needle punctures; woody stems were wounded with a single scalpel cut. A loopful of inoculum from the slants immediately was applied to the wound with a sterile loop. Tumor formation was recorded after 4 wk for herbaceous plants and after 8 wk for woody plants. Small growths on datura were recorded as negative after 4 wk incubation because similar overgrowths were produced in response to wounding (11). The overgrowths on datura ceased enlargement after 2–3 wk while bacterial-induced tumors continued to grow. Strains were designated pathogenic if tumors developed on any of the inoculated plants. However, at least 50% of the plants inoculated by each strain were infected by 85 of 89 pathogens; at least 75% of the plants were infected by 78 of 89 pathogens. None of the wounded controls developed tumors.

Hairy root (Hr) induction was tested by the method of Lippincott and Lippincott (17) with carrot (*Daucus carota*) disks.

Because some of the *Agrobacterium* strains might not infect any of the 11 hosts, selected nonpathogenic *Agrobacterium* strains were inoculated to several new host species or to the host species from which they were first isolated. In the following list, the numbers in brackets indicate the number of strains tested on a given host: cucumber (*Cucumis sativus* 'Chicago Pickling', 25 strains); incense

00031-949X/79/000059$03.00/0

cedar (*Libocedrus decurrens*, 3 strains); lippia (*Lippia canesens*, 35 strains); gernaium (*Pelargonium* sp. hybrid 'Snowball', 102 strains); apple (*Malus domestica* 'Rome' open-pollinated seedlings, 179 strains); red raspberry (*Rubus idaeus*, 7 strains); boysenberry (*Rubus ursinus* var. *loganobaccus boysen* 'Thornless Young', 7 strains); and zinnia (*Zinnia elagans* 'Envy', 27 strains).

RESULTS

Eighty-nine of the 176 *Agrobacterium* strains tested were pathogenic, and all 89 exhibited host specificity. None of the pathogens infected all 11 host species (Fig. 1, Table 1). Two strains, M2/73 isolated from birch and N2/73 isolated from red raspberry, exhibited the widest host range, infecting 9 and 10 of the 11 host species, respectively (Table 2). Fifty-nine strains infected six to eight hosts, and the remaining 28 strains induced tumors on five or fewer hosts. The agrobacteria examined could be grouped by the total number (but not kind) of hosts they infected (Fig. 1). For example, eight of the 17 strains nonpathogenic on tomato were nonpathogenic on datura, showing that some strains that infect datura will not infect tomato. Under the conditions of these experiments, tomato and datura plants were susceptible to the largest number of *Agrobacterium* strains (72 of 89), followed closely by sunflower (70 of 89) and bryophyllum (69 of 89). The probability of verifying the pathogenicity of an unknown strain was 92% if both tomato and datura plants were inoculated versus 81% if only one of the host species was used. The probability of detecting a pathogenic strain was increased only an additional 3% by inoculating each strain to tomato, datura, sunflower, and bryophyllum.

Twenty-seven of the 85 strains that were nonpathogenic on the 11 hosts tested were screened further for pathogenicity on lippia, cucumber, and zinnia; none infected these additional host species. However, three of these strains (AB2/73, S5/72, and AA17/73) were pathogenic when inoculated to their homologous host (the plant species from which the strain was isolated originally).

The host specificity of a strain was not determined by the plant species from which that strain was isolated. For example, strains AB1/73, AB2/73, AB11/73, and AB12/73 were all from the same tumor, but they had different patterns of host specificity (Table 3). Strains AB1/73 and AB11/73 were not pathogenic on lippia (homologous host). Similarly, strains S1/73, S2/73, and S9/73 were isolated from a single lippia tumor but infected different hosts (Table 3). Therefore, strains of the same origin differed in host specificity.

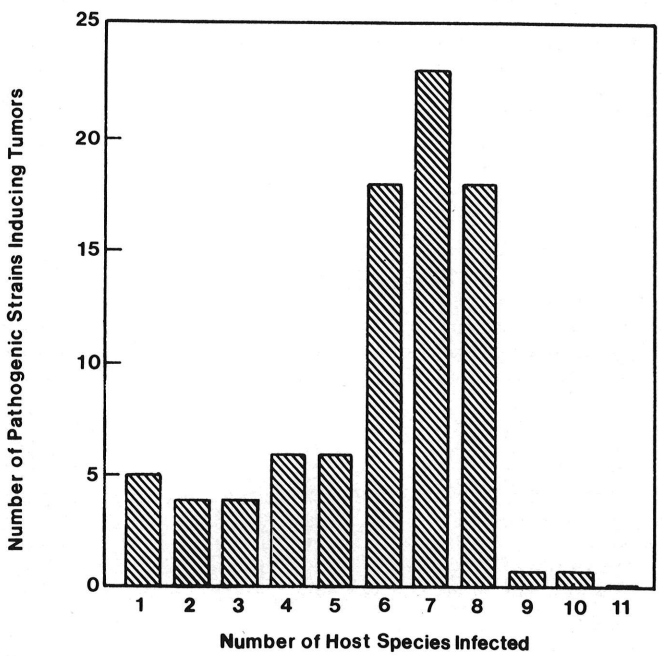

Fig. 1. Frequency of tumor induction in 11 host species inoculated individually with 89 pathogenic *Agrobacterium* strains.

Each of the three *A. rubi* strains was pathogenic on at least seven hosts other than *Rubus* spp.; conversely, several *A. tumefaciens* strains from other hosts were pathogenic on *Rubus* spp. No morphological differences in the tumors incited by *A. rubi* and *A. tumefaciens* were noted on any of the hosts.

Of 19 strains labeled *A. rhizogenes* from other laboratories and the American Type Culture Collection (2), only eight induced hairy root (Hr) symptoms on carrot disks. Five of these eight Hr strains also formed tumors on at least four of the 11 hosts tested. Even though each strain was cloned by single-colony isolation (three times) on solid agar media before testing, those strains causing tumors and Hr symptoms may have been mixed (26).

DISCUSSION

The genus *Agrobacterium* is noted for its broad host range (1,4,8) and researchers have been inclined to view individual strains in a similar manner. However, under our experimental conditions, host specificity was the rule and not the exception for individual pathogens. Our experimental conditions included a 4 or 8 wk incubation in either the greenhouse or growth chamber following inoculation. More of the inoculated hosts might have developed galls had the incubation time been increased (4,22) or the host's growing conditions varied (6,20). Even so, this study demonstrates notable differences of infectiousness among the *Agrobacterium* strains for a particular host species. These differences may reflect differences in susceptibility of the host, the virulence of the pathogen, or the interaction of the two.

The majority (66%) of these pathogens infected six to eight of 11 plant species tested. The number of strains that infected less than six or more than eight host plants dropped sharply, suggesting that some factor limits the number of species a strain can infect. If host specificity is controlled genetically, the genes could be located either on the bacterium's pTi plasmid, shown to confer oncogenicity (7,27) or on its chromosome. An argument can be developed in favor of the chromosome based on Kerr's demonstration that in planta plasmid transfer occurs in nature (14). Thus, strains isolated from the same naturally occurring tumor, but infecting different plants, could have a common pTi plasmid but a different chromosomal background. On the other hand, we have demonstrated that an in planta cross (14) between donor strain AB2/73 (infects only lippia) and a plasmidless recipient strain A136 produces transconjugants that infect only lippia, suggesting that the pTi plasmid may confer host specificity (Moore, *unpublished*). Sonoki et al (23) also reported that a narrow host range strain, ID1151, was converted into a broad host range pathogen upon receipt of the pTi from a broad host range donor strain, 15955.

It is interesting that several *A. rhizogenes* strains induced tumors on hosts other than carrot and only hairy roots on carrot slices. The

TABLE 1. Pathogenicity of 89 *Agrobacterium* sp. strains on each of 11 host species

Hosts	Strains inducing tumors (no., of 89)	Strains pathogenic on each host species (%)
Tomato	72	80.9
Datura	72	80.9
Sunflower	70	78.7
Bryophyllum	69	77.5
Pea	65	73.0
Radish	55	61.8
Plum	43	48.3
Bean	33	37.1
Daisy	23	25.8
Sugar beet	6	6.7
Apple	2	2.2

TABLE 2. Host specificity patterns of 18 *Agrobacterium* sp. strains on 11 host species

Strain	Host species of origin	Apple	Bean	Beet	Bryophyllum	Plum	Daisy	Datura	Pea	Radish	Sunflower	Tomato
W2/73	Euonymus	–[a]	+	–	+	–	–	+	–	+	+	+
K6/73	Willow	–	–	–	+	+	–	+	+	+	+	+
AR5K/71	Apple	–	–	–	–	–	–	–	–	–	–	+
30	Peach	–	–	–	+	–	–	–	–	–	–	+
T3/73	Rose	–	–	–	+	–	–	+	+	–	–	+
B1/74	Tomentosa cherry	–	–	–	+	+	–	+	+	–	+	+
RR 5	Red raspberry	–	+	–	+	+	–	+	+	+	+	+
H7/73	Crab apple	–	–	–	+	–	–	+	+	+	+	+
A25/75	Mazzard cherry	–	–	–	–	+	–	–	–	–	–	–
M2/73	Birch	+	+	–	+	+	–	+	+	+	+	+
B3/73	Norway maple	–	+	–	+	–	–	+	+	+	+	+
N2/73	Raspberry	–	+	+	+	+	+	+	+	+	+	+
S7/73	Lippia	–	–	–	–	–	–	+	+	–	–	–
C5/73	Mountain ash	–	–	–	+	+	–	+	+	+	+	+
E8/73	Dahlia	–	+	–	+	–	+	+	–	+	+	–
CG1C	Pear	–	–	–	+	–	+	+	+	–	+	+
IIBV7	Chrysanthemum	–	–	–	+	+	+	+	+	–	+	+
T37	Walnut	–	–	–	+	–	–	–	+	–	–	–

[a]Symbols: – = no tumors formed on four to six inoculated plants, and + = tumors formed on one or more of four to six inoculated plants. Controls for all host species tested were negative.

TABLE 3. Tumorigenicity of *Agrobacterium* sp. strains isolated from *Lippia canescens* galls

Common names of host species tested[b]	Agrobacterium strains[a]							
	AB1/73	AB2/73	AB11/73	AB12/73	S1/73	S2/73	S7/73	S9/73
Apple	–[c]	–	+	–	–	–	–	–
Bean	–	–	+	–	–	–	–	–
Beet	–	–	–	–	+	–	–	–
Bryophyllum	–	–	–	–	–	–	–	–
Plum	–	–	+	–	–	–	–	–
Daisy	–	–	–	–	–	–	–	–
Datura	+	–	+	+	+	+	+	–
Pea	–	–	–	–	–	–	+	–
Radish	–	–	+	–	–	–	–	–
Sunflower	+	–	–	–	+	–	–	–
Tomato	–	–	–	–	–	–	–	–
Lippia	–	+	–	+	+	+	+	–

[a]Strains AB1/73, AB2/73, AB11/73, and AB12/73 were all isolated from a single tumor. Similarly, strains S1/73, S2/73, and S9/73 were all isolated from the same tumor. Strain S7/73 was isolated from a third tumor.
[b]Controls for all host species tested were negative.
[c]Symbols: – = no tumors formed on four to six inoculated plants, and + = tumors formed on one or more of four to six inoculated plants.

genes for hairy root induction also are plasmid-borne (21), but are these genes different from the pTi genes for tumor induction, or are they the same? Possibly the pTi genes are modified by other gene products coded on the plasmid or chromosome, such as cytokinin production (12).

The use of strains with varied host specificity patterns as tools in genetic and ecological studies should facilitate our understanding of host-specific mechanism(s) in agrobacteria.

The low degree of host specificity observed among all agrobacteria that were studied again amplifies the fact that speciation in *Agrobacterium* based on pathogenicity is of little taxonomic value. For example, *Agrobacterium rubi* and *A. tumefaciens* strains infected the same plant species and therefore are indistinguishable by pathogenicity tests, as also reported by McKeen (19). Anderson (3) and others (5,10,13,18,24) also have concluded that *A. rubi* is indistinguishable biochemically and physiologically from *A. tumefaciens*.

LITERATURE CITED

1. ALLEN, O. N., and A. J. HOLDING. 1974. Agrobacterium. Pages 264-267 in R. E. Buchanan and N. E. Gibbons, ed. Bergey's Manual of Determinative Bacteriology. The Williams and Wilkins Co., Baltimore. 1268 pp.
2. AMERICAN TYPE CULTURE COLLECTION. 1978. Catalogue of Strains I, 13th ed. American Type Culture Collection, Rockville, MD. 556 pp.
3. ANDERSON, A. R. 1977. Taxonomy and host specificity of the genus Agrobacterium. Ph.D. Thesis, Oregon State University, Corvallis. 61 pp.
4. DeCLEENE, M., and J. DeLEY. 1976. The host range of crown gall. Bot. Rev. 42:389-466.
5. DeLEY, J., M. BERNAERTS, A. RASSEL, and J. GUILMOT. 1966. Approach to an improved taxonomy of the genus Agrobacterium. J. Gen. Microbiol. 43:7-17.
6. DHANVANTARI, B. N. 1976. Biological control of crown gall of peach in southwestern Ontario. Plant Dis. Rep. 60:549-551.

7. DRUMMOND, M. H., M. P. GORDON, E. W. NESTER, and M.-D. CHILTON. 1977. Foreign DNA of bacterial plasmid origin is transcribed in crown gall tumors. Nature 269:535-536.
8. ELLIOTT, C. 1951. Pages 3-12 in Manual of Bacterial Plant Pathogens. Chronica Botanica Co., Waltham, Mass. 186 pp.
9. GIBBINS, A. M., and K. F. GREGORY. 1972. Relatedness among Rhizobium and Agrobacterium species determined by three methods of nucleic acid hybridization. J. Bacteriol. 111:129-141.
10. HEBERLEIN, G. T., J. DeLEY, and R. TIJTGAT. 1967. Deoxyribonucleic acid homology and taxonomy of Agrobacteria, Rhizobium and Chromobacterium. J. Bacteriol. 94:116-124.
11. HILDEBRAND, D. C., J. P. THOMPSON, and M. N. SCHROTH. 1966. Bacterial enhancement of self-limiting outgrowth formation on Datura. Phytopathology 56:365-366.
12. KAISS-CHAPMAN, R. W., and R. O. MORRIS. 1977. Trans-zeatin in culture filtrates of Agrobacterium tumefaciens. Biochem. Biophys. Res. Commun. 76:453-459.
13. KEANE, P. J., A. KERR, and P. B. NEW. 1970. Crown gall of stone fruit. II. Identification and nomenclature of Agrobacterium isolates. Aust. J. Biol. Sci. 23:585-595.
14. KERR, A. 1969. Transfer of virulence between isolates of Agrobacterium. Nature 223:1175-1176.
15. KERSTERS, K., J. DeLEY, P. H. A. SNEATH, and M. SACKIN. 1973. Numerical taxonomic analysis of Agrobacterium. J. Gen. Microbiol. 78:227-239.
16. LIN, B. and C. I. KADO. 1977. Studies on Agrobacterium tumefaciens. VII. Avirulence induced by temperature and ethidium bromide. Can. J. Microbiol. 23:1554-1561.
17. LIPPINCOTT, J. A., and B. B. LIPPINCOTT. 1969. Tumour-inducing ability and nutrition in the genus Agrobacterium. J. Gen. Microbiol. 59:57-75.
18. LIPPINCOTT, J. A., and B. B. LIPPINCOTT. 1975. The genus Agrobacterium and plant tumorigenesis. Annu. Rev. Microbiol. 29:377-405.
19. McKEEN, W. E. 1954. A study of cane and crown galls on Vancouver Island and a comparison of the causal organisms. Phytopathology 44:651-655.
20. MOORE, L. W. 1976. Latent infections and seasonal variability of crown gall development in seedlings of three Prunus species. Phytopathology 66:1097-1101.
21. MOORE, L. W., G. WARREN, and G. STROBEL. 1978. Plasmid involvement in the hairy root infection caused by Agrobacterium rhizogenes. Proc. IVth Int. Conf. Phytopathogenic Bacteria, Angers, France (In press).
22. MUNNECKE, E. E., P. A. CHANDLER, and M. P. STARR. 1963. Hairy root (Agrobacterium rhizogenes) of field roses. Phytopathology 53:783-799.
23. SONOKI, S., C. R. IRELAND, J. LOPER, M. BARAKA, and C. I. KADO. 1978. New genetic determinants of the virulence plasmid of Agrobacterium tumefaciens. Proc. IVth Int. Conf. Phytopathogenic Bacteria, Angers, France (In press).
24. WHITE, L. O. 1972. The taxonomy of the crown-gall organism Agrobacterium tumefaciens and its relationship to Rhizobia and other Agrobacteria. J. Gen. Microbiol. 72:565-574.
25. WORMALD, H. 1945. Physiologic races of the crown gall organism in Britain. Trans. Br. Mycol. Soc. 28:134-146.
26. WRIGHT, W. H., A. A. HENDRICKSON, and A. J. RIKER. 1930. Studies on the progeny of single-cell isolations from the hairy-root and crown-gall organisms. J. Agric. Res. 41:541-547.
27. ZAENEN, I., N. VAN LAREBEKE, H. TEUCHY, M. VAN MONTAGU, and J. SCHELL. 1974. Supercoiled circular DNA in crown-gall inducing Agrobacterium strains. J. Mol. Biol. 86:109-127.