Aminopeptidase Profiles of Virulent and Avirulent Erwinia amylovora and Erwinia herbicola

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ABSTRACT

Five isolates of *Erwinia amylovora*, two isolates of avirulent *E. amylovora* and three isolates of *E. herbicola*, an avirulent *Erwinia* sp., appear related on the basis of aminopeptidase profiles. The profiles were derived from the hydrolysis of 27 aminoacyl-β-naphthylamides and β-naphthylamides of phosphate, sulfate and acetate after incubation periods of 4 and 24 hours. Differences in hydrolysis were not apparent in replicate treatments and differences in hydrolysis by isolates within each of the two species were ⩽ 4% indicating that aminopeptidase profiles offer a rapid, reproducible means of microbial identification to supplement morphological and cultural criteria. Qualitative and quantitative differences in the hydrolysis of several aminoacyl-β-naphthylamides were evident among the three bacteria. Virulent *E. amylovora* hydrolyzed glycyl- and alanyl-β-naphthylamides, whereas neither avirulent *E. amylovora* nor *E. herbicola* hydrolyzed glycyl-β-naphthylamide. *Erwinia herbicola* hydrolyzed alanyl-β-naphthylamide less readily than did virulent *E. amylovora*. Avirulent *E. amylovora* did not hydrolyze alanyl-β-naphthylamide. Avirulent *E. amylovora* and *E. herbicola* hydrolyzed β-naphthylamides of phenylalanine and tyrosine more rapidly than did virulent *E. amylovora*.

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The identification of bacteria by classical microbiological techniques is a lengthy procedure involving the determination of morphological, cultural, physiological and pathogenic characters. Alternatives, such as phage typing (7) and utilization of gas chromatographic (16, 31), electrophoretic (12, 26), and phosphorescent (1) techniques are not sufficiently refined nor widely accepted. Deoxyribonucleic acid hybridization has been used to determine overall relationships between species (4), but sufficient guidelines are not available to correlate genetic and molecular information to taxonomic groupings.

Westley et al. (32) proposed using aminopeptidase profiles to identify *Bacillus* spp. and other bacteria. The procedure is based on the measurement of fluorescence of enzymatically released β-naphthylamine from β-naphthylamides. Fluorescence is linear with concentration allowing both quantitative and qualitative evaluations. Bacteria are distinguished by their ability to hydrolyze a series of β-naphthylamides which are recorded as specific "profiles" for each bacterium.

On the basis of aminopeptidase profiles, Huber et al. (17) rapidly distinguished race 1 from race 2 of *Pseudomonas phaseolicola*. Krawczyk and Huber (19) separated *Erwinia amylovora, Xanthomonas campestris, Pseudomonas tabaci,* and a saprophytic *Pseudomonas* sp. by their aminopeptidase profiles, and Mulczyk and Szewczyk (25, 28) distinguished various Enterobacteriaceae on the basis of their pyrrolidonyl peptidase activity.

Environmental factors affect aminopeptidase profiles (17, 32), and Krawczyk and Huber (19) standardized the procedure in terms of growth media, temperature, incubation time, inoculum density, and added cofactors. They concluded that there appeared to be sufficient latitude in all of these factors for this technique to be easily adapted for routine microbial identification.

MATERIALS AND METHODS.—Virulent *Erwinia amylovora* (Burr) Winslow et al. (W-LV-2, M-W-1) and *Erwinia herbicola* (Lohnis) Dye (M-Y-1), and avirulent *Erwinia* sp., were isolated from lyophilized, naturally infected apple buds (Purdue University, O'Neall Farm), and a change in the virulent *E. amylovora* isolate M-W-1 yielded avirulent isolate (M-Av-1). Avirulent isolate (E-8) of *E. amylovora* was obtained from R. N. Goodman, University of Missouri, and virulent streptomycin-resistant *E. amylovora* isolates (E. A. Smooth, 180 SR, 410 SR) from H. L. Keil, United States Department of Agriculture, Beltsville, Maryland. *Erwinia herbicola* isolates (ISO 57, ZP-1) were obtained from E. Klos, Michigan State University. Isolate ZP-1 was originally obtained from D. W. Dye, Auckland, N. Z. by E. Klos.

The identities of isolates were verified by comparison of their characteristics with descriptions in Bergey's Manual (3), Dowson (5) and Dye (6). Emphasis was placed on the fermentation of carbohydrates, as these tests are important in differentiation of the Enterobacteriaceae (3).

Aminopeptidase profiles of the isolates were determined by the method of Krawczyk and Huber (19). Bacteria were grown on nutrient agar slants for 36 hours, and suspended in 0.05 M tris-HCl buffer, pH 8.0. The bacterial concentration was standardized spectrophotometrically (540 nm) to approximately 3.7×10^7 cells/ml. One-tenth milliliter of bacterial suspension was dispersed into each substrate (Table 1) and maintained at room temperature (approximately 22 C) until hydrolysis of the β-naphthylamides was determined. Naphthylamides (obtained from Calbiochem, San Diego, California, The Nutritional Biochemicals Co., Cleveland, Ohio, and Sigma Chemical Co., St. Louis, Missouri) 2×10^{-5}M, were dissolved in 0.05 M tris-HCl buffer, pH 8.0, and

refrigerated until used. β-naphthylamine in tris-HCl buffer and buffer alone were also inoculated to determine the maximum fluorescence obtainable with complete hydrolysis and background fluorescence, respectively.

Aminopeptidase activity was determined fluorometrically by measuring the β-naphthylamine released using an Aminco fluoromicrophotometer with a Corning 7-60 narrow-band-pass primary filter and Wratten 47-B narrow-band-pass secondary filter. A 0.3 neutral-density filter was also used in the primary filter position to keep within limits of the photomultiplier tube.

To determine the effect of incubation time on hydrolysis, substrates were inoculated with virulent (W-LV-2) or avirulent *E. amylovora* (E-8) and percent hydrolysis determined 4, 8, 12, and 24 hours later. Hydrolysis was evident by 4 hours, and 4- and 24-hour incubation periods were used to determine the aminopeptidase profiles for all isolates. In all experiments, two replicates were used for both incubation periods for each isolate. There were no significant differences between replications of a single isolate. Variation between mean values for observations within each bacterial species were ± 1% for hydrolysis values ≤ 20% and ± 4% for values > 20%. Therefore, all data are presented as average percent hydrolysis by isolates of each species. Differences in the means of the quantities of β-naphthylamides hydrolyzed after 4- and 24-hour incubation periods by five isolates of virulent *E. amylovora,* two isolates of avirulent *E. amylovora,* and three isolates of *E. herbicola,* were determined by Duncan's multiple-range test.

RESULTS.—The characteristics of the virulent and avirulent isolates (Table 2) of *E. amylovora* were similar to those presented in Bergey's Manual (3) and by Dowson (5). The characteristics of the *E. herbicola* isolates (Table 2) were in accord with data by Dye (6).

Differences in hydrolysis of β-naphthylamides were evident after 4 hours of incubation, and these differences only changed quantitatively with increased incubation time (Fig. 1). Quantitative differences between values presented in Fig. 1 and those presented in Fig. 2 and 3 are due to the use of substrates at 10^{-4} M as compared to substrates at 2.5×10^{-5} M.

The profiles for these bacteria are similar; however, a few marked differences in the hydrolysis of some β-naphthylamides are evident (Fig. 2 and 3). Virulent *E. amylovora* readily hydrolyzed glycyl-and alanyl-β-naphthylamides, whereas neither avirulent *E. amylovora* nor *E. herbicola* hydrolyzed glycyl-β-naphthylamide. *E. herbicola* hydrolyzed alanyl-β-naphthylamide less readily than did virulent *E. amylovora.* Avirulent *E. amylovora* did not hydrolyze alanyl-β-naphthylamide. Avirulent *E. amylovora* and *E. herbicola* hydrolyzed β-naphthylamides of phenylalanine and tyrosine more rapidly than did virulent *E. amylovora.* The comparison of profiles by Duncan's multiple-range test (Table 3) shows greatest similarities in hydrolysis of substrates by avirulent *E. amylovora* and *E. herbicola,* and most differences are apparent in the hydrolysis of substrates by the virulent bacterium as compared to the avirulent bacteria. The decreasing order in hydrolysis of different amino-β-naphthylamides by the three bacteria is presented in Table 4.

DISCUSSION.—The aminopeptidase profiles (Fig. 2, 3) indicate that the three *Erwinia* are related. In general, virulent *E. amylovora* hydrolyzed greater amounts of each substrate than did avirulent *E. amylovora* or *E. herbicola.* No differences in aminopeptidase profiles were apparent between streptomycin-resistant and -susceptible isolates of virulent *E. amylovora.*

TABLE 1. β-Naphthylamides used to determine profiles of virulent and avirulent *Erwinia amylovora* and *Erwinia herbicola*

β-naphthylamides	Abbreviation
L-Alanyl	(ALA)
L-Arginyl	(ARG)
Benzyl-L-Arginyl	(BANA)
L-α-Aspartyl	(α-ASP)
L-β-Aspartyl	(β-ASP)
L-Cysteinyl	(CYS)
L-α-Glutamyl	(α-GLU)
L-γ-Glutamyl	(γ-GLU)
L-α-Glutamyl-L-Phenylalanyl	(GLU-PHE)
Glycyl	(GLY)
Glycyl-Glycyl	(Gly-Gly)
L-Histidyl	(HIS)
L-Hydroxyprolyl	(HPRO)
L-Leucyl	(LEU)
L-Isoleucyl	(ILEU)
L-Lysyl	(LYS)
L-Methionyl	(MET)
4-Methoxy-Leucyl	(4M-LEU)
L-Phenylalanyl	(PHE)
L-Prolyl	(PRO)
L-Pyrrolidonyl	(PYR)
L-Seryl	(SER)
L-Threonyl	(THR)
L-Tryptophyl	(TRY)
L-Tyrosyl	(TYR)
L-Valyl	(VAL)
β-Naphthylamine	(β-NAP)
Tris-HCl buffer blank pH 8.0	(T-BUF)
β-naphtylamide acetate	(ACE)
β-naphthylamide sulfate	(SUL)
β-naphthylamide phosphate	(PHO)

Statistical analysis of the data (Table 3) indicated significant differences in the hydrolysis of many substrates by the three bacteria. Comparison of these differences with the aminopeptidase profiles of the bacteria after both 4- and 24-hour incubation periods (Fig. 2, 3) indicates that most differences are quantitative, small, and that the general shape of the profiles for all three species are strikingly similar. Comparisons of these profiles with those of other bacterial genera (17, 19, 32) indicate that bacteria can be readily distinguished from each other on the basis of aminopeptidase profiles, and the similar profiles generated by the three bacteria used in these studies are indicative of a close relationship.

Billing and Baker (2), in the course of diagnostic work with fire blight, found a yellow-pigmented saprophytic organism which was similar to *E. amylovora.* They considered it to be a member of an ill-defined *Lathyris-herbicola* group within the genus *Erwinia.* Smith and Powell (27) also recovered yellow isolates from fire blight cankers which were non-pathogenic on apple and suggested that these isolates were probably the same species as those of Billing and Baker (2). The similarities that these workers observed between *E. amylovora* and the yellow isolate, and the classification of these yellow isolates as *E. herbicola* by Dye (6), with the

TABLE 2. Production of acid from carbohydrates by, and some other characters of, avirulent and virulent *Erwinia amylovora* isolates and isolates of *Erwinia herbicola*

		E. amylovora isolates[a]								*E. herbicola* isolates		
Test[b]	*E. amylovora*[c]	W-LV-2	M-W-1	E. A. Smooth	180SR	410SR	M-Av-1	E-8	*E. herbicola*[d]	M-Y-1	ZP-1	Iso 57
Production of acid from:												
Dextrose	A	A	A	A	A	A	A	A	A	A	A	A
Glycerol (Basal Broth)	A	A	A	A	A	A	A	A	A	A	A	A
Glycerol (Nut Broth)	A	A	A	A	A	A	A	A	A	A	A	A
Lactose	−	−	−	−	−	−	−	−	A	−	A	−
Mannose	A	A	A	A	A	A	A	A	A	A	A	A
Salicin	A	A	A	A	A	A	A	A	A	−	A	A
Starch	−	−	−	−	−	−	−	−	−	−	−	−
Sucrose	A	A	A	A	A	A	A	A	A	A	A	A
Xylose	−	−	−	−	−	−	−	−	A	A	A	A
Hydrolysis of gelatin	+	+	+	+	+	+	+	+	+	+	+	+
Production of NO_2 from NO_3	−	−	−	+	+	+	−	−	+	+	+	−
Lactose in an inorganic nitrogen medium with bromocresol purple	A, AG, or-	A	A	A	A	A	K	K	nt	K	K	K
Gram stain	−	−	−	−	−	−	−	−	−	−	−	−
Pigment	white	white	white	white	white	white	white	white	yellow	yellow	yellow	yellow

[a] *E. amylovora* isolates W-LV-2, M-W-1, E. A. Smooth, 180SR and 410SR are virulent, while M-Av-1 and E-8 are avirulent.
[b] A = Acid reaction; − = no reaction; K = Alkaline reaction; nt = not tested.
[c] From Bergey's Manual (3) and/or Dowson (5).
[d] From Dye (6).

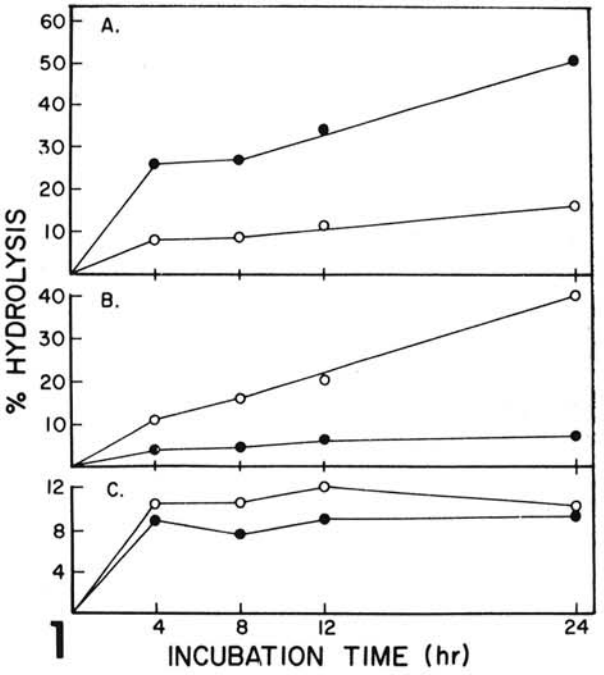

Fig. 1-(A to C). Hydrolysis of **A)** alanyl-, **B)** phenylalanyl-, and **C)** aspartyl- β-naphthylamides by virulent (•——•) and avirulent (*o*——*o*) *Erwinia amylovora* as compared to time of incubation. Values are averages of two replicate observations for each bacterium at each incubation period.

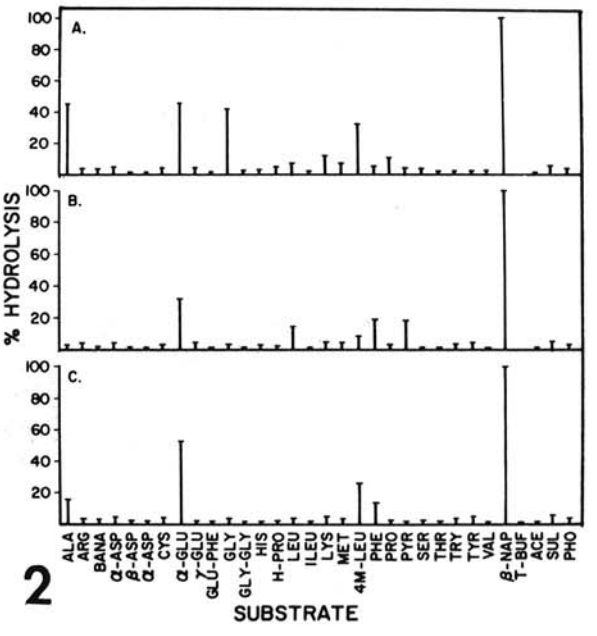

Fig. 2-(A to C). Aminopeptidase profiles of **A)** virulent and **B)** avirulent *Erwinia amylovora* and **C)** *Erwinia herbicola* after 4 hours of incubation. Values are averages of five (A), two (B) and three (C) isolates. The β-naphthylamide substrates are, left to right, L-Alanyl, L-Arginyl, Benzyl-L-Arginyl, L-α-Aspartyl, L-β-Aspartyl, L-α-Aspartyl, L-Cysteinyl, L-α-Glutamyl, L-γ-Glutamyl, L-α-Glutamyl-L-Phenylalanyl, Glycyl, Glycyl-Glycyl, L-Histidyl, L-Hydroxyprolyl, L-Leucyl, L-Isoleucyl, L-Lysyl, L-Methionyl, 4-Methoxy-Leucyl, L-Phenylalanyl, L-Prolyl, L-Pyrrolidonyl, L-Seryl, L-Threonyl, L-Tryptophyl, L-Tyrosyl, L-Valyl, β-Naphthylamine, Tris-HCl buffer blank pH 8.0, β-naphthylamide acetate, β-naphthylamide sulfate, β-naphthylamine phosphate.

acknowledgement that organisms in the *"herbicola"* group share many characters with those in both the *"amylovora"* and *"carotovora"* groups, suggests that *E. amylovora* and *E. herbicola* are closely related. This close relation between *E. amylovora* and *E. herbicola* is supported by the aminopeptidase profiles obtained from these bacteria. The strikingly similar profiles of avirulent *E. amylovora* and *E. herbicola* further suggests that differences in the profiles of virulent *E. amylovora* as compared to those of the two avirulent bacteria may relate to the virulence and/or avirulence of these organisms.

Several virulent *Agrobacterium* spp. lose virulence after successive passages on media containing glycine

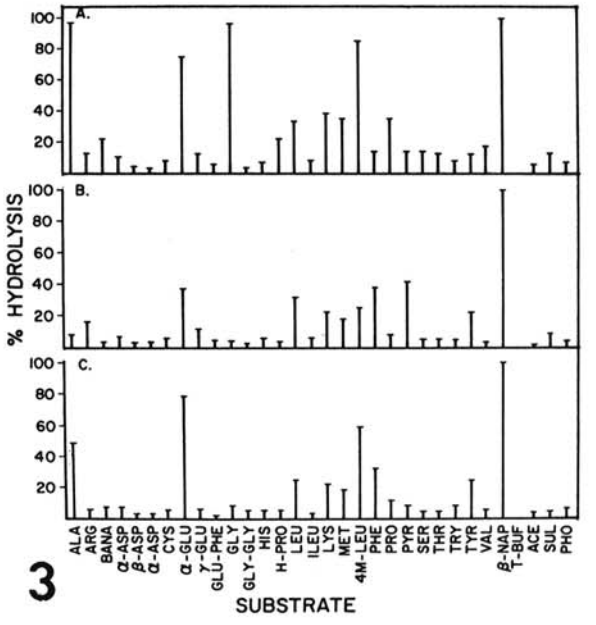

Fig. 3-(A to C). Aminopeptidase profiles of **A)** virulent and **B)** avirulent *Erwinia amylovora* and **C)** *Erwinia herbicola* after 24 hours of incubation. Values are averages of five (A), two (B) and three (C) isolates. The β-naphthylamide substrates are, left to right, L-Alanyl, L-Arginyl, Benzyl-L-Arginyl, L-α-Aspartyl, L-β-Aspartyl, L-α-Aspartyl, L-Cysteinyl, L-α-Glutamyl, L-γ-Glutamyl, L-α-Glutamyl-L-Phenylalanyl, Glycyl, Glycyl-Glycyl, L-Histidyl, L-Hydroxyprolyl, L-Leucyl, L-Isoleucyl, L-Lysyl, L-Methionyl, 4-Methoxy-Leucyl, L-Phenylalanyl, L-Prolyl, L-Pyrrolidonyl, L-Seryl, L-Threonyl, L-Tryptophyl, L-Tyrosyl, L-Valyl, β-Naphthylamine, Tris-HCl buffer blank pH 8.0, β-naphthylamide acetate, β-naphthylamide sulfate, β-naphthylamine phosphate.

TABLE 3. Differences in the means of the quantities of β-naphthylamides hydrolyzed after 4- and 24-hour incubation periods by five isolates of virulent *Erwinia amylovora*, two isolates of avirulent *E. amylovora* and three isolates of *E. herbicola* as determined by Duncan's multiple-range test

	Differences between mean hydrolysis values[a]					
	Four-hour incubation			Twenty-four-hour incubation		
	Virulent *E. amylovora*		Avirulent *E. amylovora*	Virulent *E. amylovora*		Avirulent *E. amylovora*
Napthylamide	Avirulent *E. amylovora*	*E. herbicola*	*E. herbicola*	Avirulent *E. amylovora*	*E. herbicola*	*E. herbicola*
L-Alanyl	**	**	*	**	**	**
L-Arginyl	–	–	–	**	**	**
Benzyl-L-Arginyl	**	*	–	**	**	–
L-α-Aspartyl	–	–	–	–	–	–
L-β-Aspartyl	**	*	–	*	*	–
L-α-Aspartyl	**	**	–	–	–	–
L-Cysteinyl	*	–	–	–	–	–
L-α-Glutamyl	–	**	**	*	–	*
L-γ-Glutamyl	–	*	–	–	**	**
L-α-Glutamyl-L-Phenylalanyl	**	*	*	–	**	**
Glycyl	**	**	–	**	**	–
Glycyl-Glycyl	**	**	–	–	–	–
L-Histidyl	**	–	*	–	**	**
L-Hydroxyprolyl	*	*	–	*	*	–
L-Leucyl	*	–	**	–	–	–
L-Isoleucyl	**	*	–	*	**	*
L-Lysyl	**	*	–	*	*	–
L-Methionyl	**	*	–	**	**	–
4-Methoxy-Leucyl	–	**	–	**	**	**
L-Phenylalanyl	–	–	–	*	*	–
L-Prolyl	**	**	–	**	*	–
L-Pyrrolidonyl	–	**	**	**	–	**
L-Seryl	**	**	–	*	*	–
L-Threonyl	**	–	*	*	**	–
L-Tryptophyl	–	–	–	–	–	–
L-Tyrosyl	–	–	–	*	*	–
L-Valyl	**	**	–	**	**	–
β-naphthylamide acetate	*	*	–	*	**	–
β-naphthylamide sulfate	*	–	–	–	–	–
β-naphthylamide phosphate	**	*	–	*	**	–

[a] ** = significant at $P = 0.01$; * = significant at $P = 0.05$; and – = no significant differences.

TABLE 4. Decreasing order in the means of the quantities of β-naphthylamide hydrolyzed[a] by virulent and avirulent *Erwinia amylovora* and *Erwinia herbicola* after 4- and 24-hour incubation periods

Incubation period (hour)	Bacteria	Decreasing order of and average percent hydrolysis of β-naphthylamides[b]						
		1	2	3	4	5	6	7	8
4	Virulent *E. amylovora*	ALA (48)	α-GLU (47)	GLY (42)	4M-LEU (35)				
	Avirulent *E. amylovora*	α-GLU (30)	PHE (21)	PYR (20)	LEU (15)				
	E. herbicola	α-GLU (55)	4M-LEU (29)	ALA (18)	PHE (17)				
24	Virulent *E. amylovora*	ALA (97)	GLY (96)	4M-LEU (90)	α-GLU (75)	LYS (40)	MET (39)	PRO (38)	LEU (34)
	Avirulent *E. amylovora*	PYR (42)	PHE (39)	α-GLU (38)	LEU (30)	4M-LEU (28)	LYS (23)	TYR (23)	MET (19)
	E. herbicola	α-GLU (79)	4M-LEU (60)	ALA (50)	PHE (35)	LEU (25)	TYR (23)	LYS (21)	MET (19)

[a]From most to least.
[b]ALA = L-Alanyl; α-GLU = L-α-glutamyl; GLY = Glycyl; 4M-LEU = 4-Methoxy-Leucyl; PHE = L-Phenylalanyl; PYR = L-Pyrrolidonyl; LEU = L-Leucyl; LYS = L-Lysyl; MET = L-Methionyl; PRO = L-Prolyl; TYR = L-Tyrosyl.

(22), alanine, and other amino acids or related compounds (30). Similar results have been obtained with other microorganisms (15, 18, 34). In comparing the aminopeptidase profile of virulent and avirulent isolates of *Pseudomonas glycinea,* Huber and Schmitt (*personal communication*) also observed that virulent isolates hydrolyzed alanyl-β-naphthylamide while the avirulent isolates did not, whereas avirulent isolates hydrolyzed β-naphthylamides of phenylalanine and tyrosine more rapidly than the virulent isolates. The ability to hydrolyze specific peptides by *E. amylovora* and *E. herbicola* may be related to virulence. In addition, the hydrolysis of phenylalanyl and tyrosyl peptide bonds by avirulent *E. amylovora* and *E. herbicola* may be related to the ability of the two bacteria to protect pear and apple against fireblight (8-11, 13, 14, 23, 24, 35). Chemotherapeutic effects of amino acids in various plant diseases (20, 21, 29), and reports where susceptibility or resistance may be related to the content of amino acids (33), suggest the possibilities for control of plant disease by the metabolic control of specific peptidases and hence virulence and avirulence.

LITERATURE CITED

1. ADELMAN, S. L., A. K. BREWER, K. C. HOERMAN, and W. SANBORN. 1967. Differential identification of microorganisms by analysis of phosphorescent decay. Nature 213:718-720.
2. BILLING, E., and L. A. E. BAKER. 1963. Characteristics of Erwinia-like organisms found in plant material. J. Appl. Bacteriol. 26:58-65.
3. BREED, R. S., E. G. D. MURRAY, and N. R. SMITH. 1957. Bergey's Manual of Determinative Bacteriology. 7th ed. Williams and Wilkins Co., Baltimore, Maryland. 1,094 p.
4. BRENNER, D. J., G. R. FANNING, K. E. JOHNSON, R. V. CITARELLA, and S. FALKOW. 1969. Polynucleotide sequence relationships among members of Enterobacteriaceae. J. Bact. 98:637-650.
5. DOWSON, W. J. 1957. Plant disease due to bacteria. 2nd ed. Cambridge University Press, Cambridge, England. 231 p.
6. DYE, D. W. 1969. A taxonomic study of the genus Erwinia. III. The "herbicola" group. N.Z.J. Sci. 12:223-236.
7. DYE, D. W., M. P. STARR, and G. STOLP. 1964. Taxonomic clarification of Xanthomonas vesicatoria based upon host specificity, bacteriophage sensitivity, and cultural characteristics. Phytopathol. Z. 51:394-407.
8. FARABEE, G. J., and J. L. LOCKWOOD. 1958. Inhibition or Erwinia amylovora by bacterium sp. isolated from fire blight cankers. Phytopathology 48:209-211.
9. GARBER, E. O. 1956. A nutrition-inhibition hypothesis of pathogenicity. Am. Nat. 90:183-194.
10. GARBER, E. O. 1960. The host as a growth medium. Ann. N.Y. Acad. Sci. 88:1187-1194.
11. GARBER, E. O. 1961. Wild fire diseases of tobacco. J. Bact. 81:974-978.
12. GLYNN, A. N., and J. REID. 1969. Electrophoretic patterns of soluble fungal proteins and their possible use as taxonomic criteria in the genus Fusaria. Can. J. Bot. 47:1823-1831.
13. GOODMAN, R. N. 1965. In vitro and in vivo interaction between components of mixed bacterial cultures isolated from apple buds. Phytopathology 55:217-221.
14. GOODMAN, R. N. 1967. The protection of apple stem tissue against Erwinia amylovora infection by avirulent strains and three other bacterial species. Phytopathology 57:22-24.

15. GREENE, M. R. 1945. The influence of amino acids on growth of Leptospira canicola. J. Bact. 50:39-45.
16. HENIS, Y., J. R. GOULD, and M. ALEXANDER. 1966. Detection and identification of bacteria by gas chromatography. Appl. Microbiol. 14:513-524.
17. HUBER, D. M., J. W. GUTHRIE, and O. BURNVIK. 1970. Identification of plant pathogenic bacteria with amino-peptidase profiles. Phytopathology 60:1534 (Abstr.).
18. HUTCHINGS, B. L., and W. H. PETERSON. 1943. Amino acid requirements of Lactobacillus casei. Proc. Soc. Exp. Biol. Med. 52:36-38.
19. KRAWCZYK, K., and D. M. HUBER. 1972. Standardization of aminopeptidase profiles for the identification of plant pathogenic bacteria. Proc. Indiana Acad. Sci. 82:98.
20. KUĆ, J., E. BARNES, A. DAFTSIOS, and E. B. WILLIAMS. 1959. The effect of amino acids on susceptibility of apple varieties to scab. Phytopathology 49:313-315.
21. KUĆ, J., E. B. WILLIAMS, and J. R. SHAY. 1957. Increase of resistance to apple scab following injection of host with phenylthiourea and β-phenylalanine. Phytopathology 47:21-22.
22. LONGLEY, B. J., T. O. BERGE, J. M. VAN LANEN, and I. L. BALDWIN. 1937. Changes in the infective ability of rhizobia and Phytomonas tumefaciens induced by culturing on media containing glycine. J. Bact. 33:29-30.
23. MC INTYRE, J. L., J. KUĆ, and E. B. WILLIAMS. 1972. Protection of pear against fire blight by bacteria and bacterial sonciates. Phytopathology 63:872-877.
24. MC INTYRE, J. L., and E. B. WILLIAMS. 1973. Protection of Bartlett pear by avirulent Erwinia spp. and Pseudomonas tabaci. Phytopathology 62:777 (Abstr.).
25. MULCZYK, M., and A. SZEWCZYK. 1970. Pyrrolidonyl peptidase in bacteria: A new colorimetric test for differentiation of Enterobacteriaceae. J. Gen. Microbiol. 61:9-13.
26. NEALSON, K. H., and E. D. GARBER. 1967. An electrophoretic survey of esterases, phosphatases, and leucine amino peptidase in mycelial extracts of species of Aspergillus. Mycologia 59:330-336.
27. SMITH, J. H., and D. POWELL. 1966. Characteristics of a yellow bacterium isolated from the tissue of Jonathan apple trees. Trans. Ill. State Acad. Sci. 59:358-363.
28. SZEWCZYK, A., and M. MULCZYK. 1970. Pyrrolidonyl peptidase in bacteria. The enzyme from Bacillus subtilis. Eur. J. Biochem. 8:63.
29. VAN ANDEL, O. M. 1966. Amino acids and plant diseases. Annu. Rev. Phytopathol. 4:349-368.
30. VAN LANEN, J. M., A. J. RIKER, and I. L. BALDWIN. 1952. The effect of amino acids and related compounds upon the growth, virulence, and enzyme activity of crown gall bacteria. J. Bact. 63:723-734.
31. VINCENT, P. G., and M. M. KULIK. 1970. Pyrolysis-gas liquid chromatography of fungi. Differentiation of species and strains of several members of Aspergillus flavus group. Appl. Microbiol. 20:957-963.
32. WESTLEY, J. W., P. J. ANDERSON, V. A. CLOSE, B. HALPERN, and E. M. LEDERBERG. 1967. Amino-peptidase profiles of various bacteria. Appl. Microbiol. 15:822-825.
33. WILLIAMS, B. J., and D. M. BOONE. 1963. Venturia inaequalis. XVI. Amino acids in relation to pathogenicity of two wild type lines to two apple varieties. Phytopathology 53:979-983.
34. WOLF, M., and I. L. BALDWIN. 1940. The effect of glycine on the rhizobia. J. Bact. 39:344.
35. WRATHER, J. A., J. KUĆ, and E. B. WILLIAMS. 1973. Protection of apple and pear fruit tissue against fireblight with nonpathogenic bacteria. Phytopathology 63:1075-1076.