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CHARACTERISTICS OF *ERWINIA HERBICOLA*

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Taxonomic studies were carried out on yellow-pigmented bacteria which were found widely in paddy rice, fruit and other related plant materials, and they were included in *Erwinia herbicola* on the basis of flagellation and biochemical characteristics. Determination, nomenclature and relation to other allied bacteria of this species were discussed. Taxonomic position of the strains of *Pseudomonas perlurida* and *Ps. trifolii*, which were previously reported by the authors, was corrected.

Since the work of DÜGGELI (*1*), a large number of gram-negative, yellow-pigmented and rod-shaped bacteria have been found in a wide variety of plant materials. Previously, the present authors (*2—4*) reported the isolation of *Pseudomonas perlurida* and *Ps. trifolii* from paddy rice. These pseudomonads had been considered to be unique species because they metabolized carbohydrates fermentatively. Re-examination of these bacteria revealed, however, peritrichous flagellation not in conformity with the previous investigation. Therefore, taxonomic comparison was made of the strains employed previously and related bacteria. This paper deals with the correction of taxonomic position of pseudomonads mentioned above and characteristics of *Erwinia herbicola* which they were newly identified.

MATERIALS AND METHODS

Microorganisms. Strains employed in this study were those reported previously and freshly isolated from vegetables and fruit using nutrient agar plate with incubation at 30°. Gram-negative, yellow-pigmented, fermentative and rod-shaped bacteria were screened from the isolates. Sources of isolation are shown in Table 1. The other related bacteria listed were used as controls.

Determination Methods. Determination techniques were mainly those described in the "Manual of Microbiological Methods" (*5*), "Manual of the Identification of Medical Bacteria" (*6*), and in the previous papers (*3*). Flagellation was ascertained by TODA's staining method (*7*) and by electron

Table 1. Sources of tested bacteria.

Ps. perlurida	
Y-4-1 (IAM 1567, AJ 2186), 2Y-4 (IAM 1589, AJ 2187), 2Y-5 (IAM 1600, AJ 2189), Y-5 (IAM 1610, AJ 2190), Y-6 (IAM 1619, AJ 2191), Y-9 (IAM 1627, AJ 2192).	paddy rice
Ps. trifolii	
L-10 (IAM 1531, AJ 2193), PY-5 (IAM 1543, AJ 2194), PY-7 (IAM 1555, AJ 2196).	paddy rice
Isolates	
AJ 2669, AJ 2670, AJ 2671, AJ 2672, AJ 2676, AJ 2677.	banana
AJ 2673.	water-melon
AJ 2674, AJ 2675.	paddy rice
AJ 2678, AJ 2680.	apple
AJ 2679.	Chinese cabbage
AJ 2188.	segregant from AJ 2187
AJ 2195.	segregant from AJ 2194
Ps. aeruginosa	
ATCC 10145.	ATCC
Ps. trifolii	
IAM 1309 (AJ 2134).	IAM
X. trifolii	
ATCC 12287 (AJ 2803).	ATCC
Flavobact. harrisonii	
No. 1161 (AJ 2681, ATCC 14589).	soil
Erw. amylovora	
CCM 1114[a]	CCM
Erw. aroideae	
NARI No. 16	NARI
Erw. carotovora	
NARI No. 2	NARI
Erw. milletiae	
NARI Em-2 (AJ 2721).	NARI
Enterobact. aerogenes	
ATCC 13048[a], ATCC 13882.	ATCC
Enterobact. cloacae	
ATCC 13047[a]	ATCC
Enterobact. liquefaciens	
ATCC 14460[a]	ATCC
Aerobact. cloacae	
2Y-1 (IAM 1562, AJ 2663), 2Y-2 (IAM 1573, AJ 2664), Y-1 (IAM 1584, AJ 2665), Y-7 (IAM 1595, AJ 2666), Py-3 (IAM 1606, AJ 2667).	paddy rice

Table 1. Sources of tested bacteria. (continued)

S. marcescens	
IAM 1105	IAM
Cit. freundii	
ATCC 8090[a]	ATCC
Cit. intermedium	
ATCC 6750	ATCC
E. coli	
ATCC 11775[a]	ATCC

[a] Type or neotype culture.
IAM: The Institute of Applied Microbiology, Tokyo, Japan.
ATCC: American Type Culture Collection, Rockville, U.S.A.
NARI: National Agricultural Research Institute, Tokyo, Japan.
CCM: Czechoslovak Collection of Microorganisms, Brno, Czechoslovakia.
AJ: Central Research Laboratories, Ajinomoto Co., Inc., Kawasaki, Japan.

microscopy. Nitrate respiration and nucleoside phosphotransferase (NPTase) were tested by the methods reported previously (*8*, *9*). Deoxyribonuclease (DNase) was detected by using Difco DNase medium (*10*), and pectolytic activity (pectinase) was tested by maceration of potato slice (*11*). Color of colonies was determined according to the Color Standard (*12*). All the tests were carried out at 30° but pectinase was tested at 25°. Base composition (GC content) of deoxyribonucleic acid (DNA) was calculated by determining the melting temperature of DNA (*13*, *14*).

Computer Analysis. According to the method of SNEATH (*15*) computer analysis was made on the bacteria and similarity was obtained by the following formula:

$$S = \frac{N_s}{N_s + N_d}$$

where N_s=numbers of positive features shared; N_d=numbers of features positive in one strain and negative in the other. As shown in Table 2, 69 features were used.

RESULTS

General Characteristics.

Strains tested were all gram-negative and rod-shaped. Cells were straight rods measuring 0.4 to 0.6 by 1.2 to 1.8 microns in average size. Diversity of the general characteristics was found among the strains as shown in Table 3. Twenty-nine strains presented in Table 3 were composed of 6 strains of *Ps. perlurida* (*4*), 3 strains of *Ps. trifolii* (*4*), 14 strains of the fresh

Table 2. Features employed for computer analysis.

Rod		Acid from lactose,	O
Gram reaction		//	C
Motility		Acid from starch,	O
Peritrichous flagellation		//	C
Polar flagellation		Acid from adonitol,	O
Production of water-insoluble		//	C
yellow pigment		Acid from dulcitol,	O
Production of water-soluble		//	C
yellow pigmet		Acid from mannitol,	O
Growth on glutamate agar		//	C
Acid in B.C.P. milk		Acid from inositol,	O
Alkaline in B.C.P. milk		//	C
Coagulation in B.C.P. milk		Gas from carbohydrates	
Peptonization in B.C.P. milk		Glucose assimilation	
Liquefaction of gelatin		Gluconate assimilation	
Nitrate reduction		Citrate assimilation	
Nitrate respiration		Succinate assimilation	
Indole		*p*-Hydroxybenzoate assimilation	
MR		Protocatechuate assimilation	
V-P		Growth on desoxycholate agar	
H_2S on KLIGLER agar		Malonate utilization in LEIFSON's medium	
Acid from glycerol,	O[a]	Citrate utilization in SIMMONS agar	
//	C[b]	Gluconate oxidation	
Acid from xylose,	O	KCN resistance	
//	C	Lysine decarboxylase	
Acid from arabinose,	O	Arginine dihydrolase	
//	C	Ornithine decarboxylase	
Acid from glucose,	O	DNase	
//	C	NPTase	
Acid from fructose,	O	Production of 3′-nucleotide	
//	C	Production of 5′-nucleotide	
Acid from sucrose,	O	Maceration of potato slice (pectinase)	
//	C	Cytochrome oxidase	
Acid from maltose,	O	Catalase	
//	C	Growth at 42°	
		Growth at pH 5.0	

[a] Open; oxidative production of acid from carbohydrates.
[b] Closed; fermentative production of acid from carbohydrates.

Table 3. Diversity of characteristics of *Erw. herbicola*.

	Erw. herbicola AJ 2671 (typical strain)	No. of strains showed positive reaction	Per cent of strains showed positive reaction
Rod	+	29[a]	100
Gram-negative	+	29	100
Motility	+	25	86
Peritrichous flagellation	+	25	86
Production of water-insoluble yellow pigment	+	29	100
Growth on glutamate agar	+	22	76
Acid in B.C.P. milk	+	23	79
Alkaline in B.C.P. milk	−	6	21
Coagulation in B.C.P. milk	+	28	97
Peptonization in B.C.P. milk	−	3	10
Liquefaction of gelatin	+	29	100
Nitrate reduction[b]	+	26	90
Nitrate respiration	−	0	0
Indole	+	12	41
MR	+	28	97
V-P	+	25	86
H_2S in KLIGLER agar	−	0	0
Hydrolysis of starch	−	0	0
Acid from carbohydrates			
glycerol, O	+	24	83
// C	+	14	48
xylose, O	+	28	90
// C	+	28	90
arabinose, O	+	29	100
// C	+	29	100
glucose, O	+	29	100
// C	+	29	100
fructose, O	+	29	100
// C	+	29	100
sucrose, O	+	26	90
// C	+	25	86
maltose, O	+	29	100
// C	+	29	100
lactose, O	+	26	90
// C	+	18	62
starch, O	−	0	0
// C	−	0	0

Table 3. Divisity of characteristics of *Erw. herbicola*. (continued)

	Erw. herbicola AJ 2671 (typical strain)	No. of strains showed positive reaction	Per cent of strains showed positive reaction
adonitol, O	+	10	34
// C	+	8	28
dulcitol, O	−	4	14
// C	−	0	0
mannitol, O	+	29	100
// C	+	29	100
inositol, O	+	25	86
// C	+	21	72
Gas from carbohydrates	−	5	17
Assimilation			
glucose	+	26	90
gluconate	+	28	97
citrate	+	23	76
succinate	+	28	97
p-hydroxybenzoate	−	0	0
protocatechuate	+	14	48
Growth on desoxycholate agar	+	21	72
Malonate utilization	−	13	45
Citrate utilization on SIMMONS agar	+	26	93
Gluconate oxidation	−	9	31
KCN resistance	−	0	0
Lysine decarboxylase	−	4	14
Arginine dihydrolase	−	4	14
Ornithine decarboxylase	−	4	14
Phenylalanine deamination	−	0	0
DNase	−	0	0
NPTase			
3′-nucleotide	−	0	0
5′-nucleotide	+	29	100
Maceration of potato slice	−	0	0
Cytochrome oxidase	−	0	0
Catalase	+	29	100
Growth at 42°	−	4	14
Growth at pH 5.0	+	19	65

[a] Numbers include 6 strains of *Ps. perlurida*, 3 strains of *Ps. trifolii*, 14 strains of isolates, 4 strains of *Aerobact. cloacae*, and 1 strain of *Flavobact. harrisonii* reported by present authors, respectively; and 1 strain of *Ps. trifolii* IAM 1309.

[b] Nitrate reduction in succinate-nitrate broth containing 0.02% yeast extract.

isolates, 4 strains of *Aerobacter cloacae* (*16*), 1 strain of *Flavobacterium harrisonii* (*17*) and 1 strain of *Ps. trifolii* IAM 1309. The reason why such kinds of bacteria were included together will be described below. Of the 29 strains, 25 were motile and exhibited peritrichous flagellation consistently, but single lateral flagellum was also found in the stained preparation, as shown in Fig. 1. The strains of *Ps. perlurida* 2Y-4 and 2Y-5, and *Ps. trifolii*

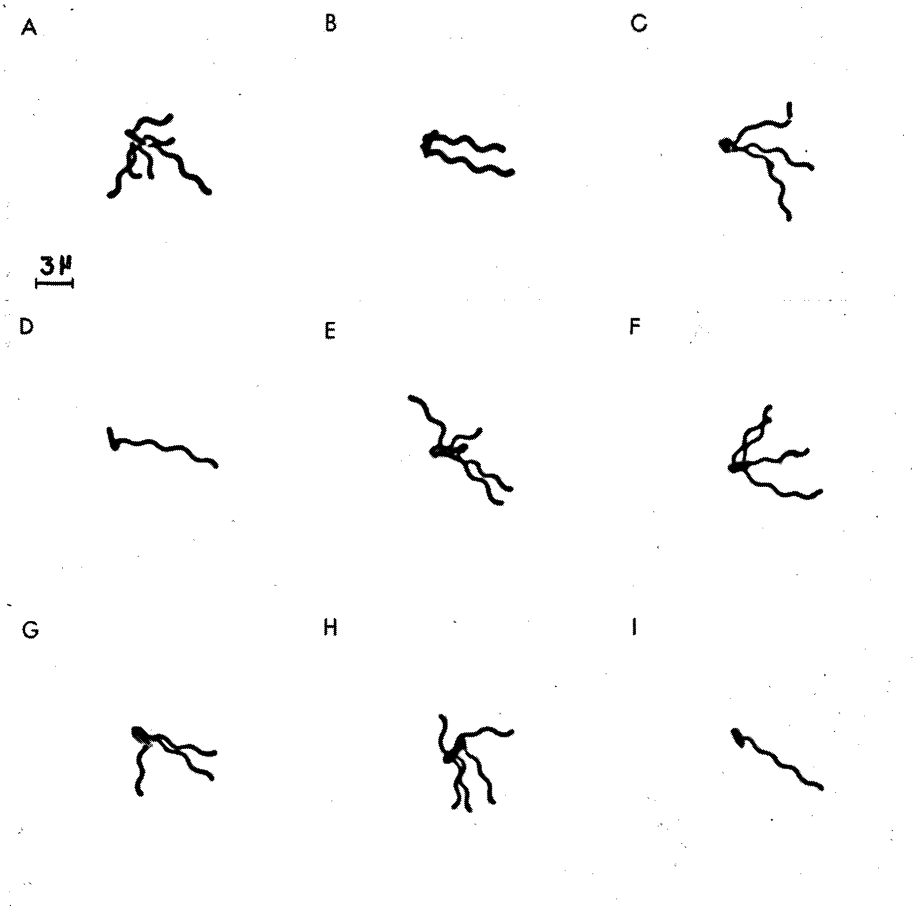

Fig. 1. Flagellation of *Erw. herbicola*.

Cells grown on nutrient agar slant for 18 hr at 25°. Stained by TODA's method.

A. Strain AJ 2196 (*Ps. trifolii* PY-7)
B. Strain AJ 2196 (*Ps. trifolii* PY-7)
C. Strain AJ 2672
D. Strain AJ 2194 (*Ps. trifolii* PY-5)
E. Strain AJ 2680
F. Strain AJ 2673
G. Strain AJ 2669
H. Strain AJ 2803
I. Strain AJ 2803 (*X.trifolii* ATCC 12287) (*X. trifolii* ATCC 12287)

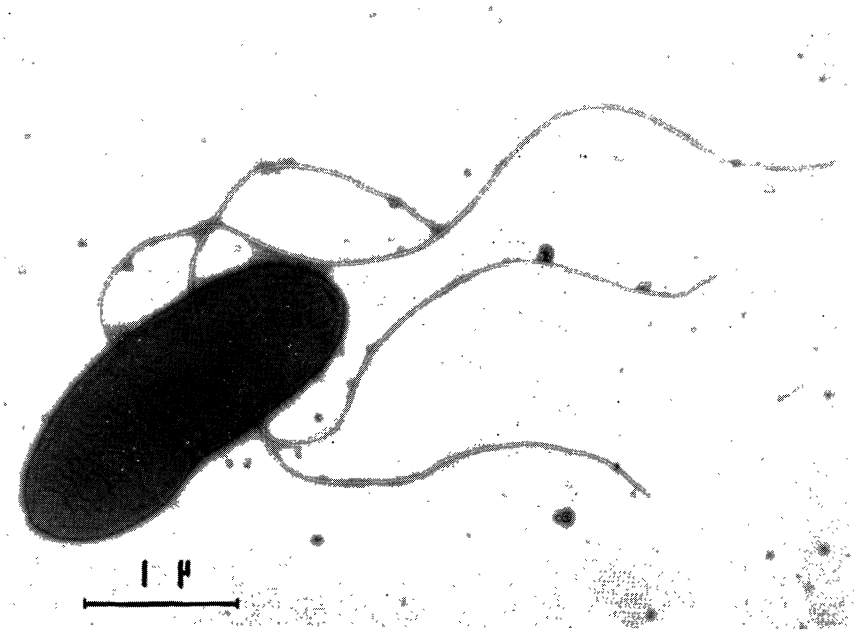

Fig. 2. Electron micrograph of *Erw. herbicola* AJ 2677.
Cell grown on nutrient agar slant for 18 hr at 25°. Negatively stained.

L-10 had been motile when isolated in 1957, but motility was not found in 1967. The segregant AJ-2188 from *Ps. perlurida* 2Y-4 also did not exhibit motility. Colonies on nutrient agar were circular, smooth and convex, and texture was butyrous. Color of colonies was versatile from dark yellow to pale yellowish brown as shown in Table 4. The isolates of AJ-2188 and AJ-2195 were color segregants from *Ps. perlurida* 2Y-4 and *Ps. trifolii* PY-5, respectively, and their color was slightly lighter than those of the original strains. Colonies on yeast extract-peptone agar were almost the same as those on nutrient agar, but the color was somewhat lighter than those on nutrient agar. On glutamate agar metallic sheen and slimy appearance were observed. Gelatin was slowly liquefied. B.C.P. milk was acidified and coagulated, but was alkaline in some cases. All the strains reduced nitrate to nitrite in succinate-nitrate broth but some showed scanty growth. Much less number, 15 strains, reduced nitrate to nitrite in nitrate broth, and 27 strains did in succinate-nitrate broth supplemented with 0.02% yeast extract. This may be ascribed to selective assimilation of nitrogenous compounds by these bacteria. All the strains failed to grow anaerobically by nitrate respiration. Almost all the strains gave a positive reaction on V-P test, and about half produced indole. Hydrogen sulfide was not produced on KLIGLER agar.

Table 4. Versatility of coloration of colonies.

	No. of strains	%
Dark yellow	6	20
Dull yellow	9	31
Pale yellow	5	17
Reddish yellow	7	24
Yellowish gray	1	3
Pale yellowish brown	1	3

According to HUGH and LEIFSON's method, they all produced acid fermentatively from various carbohydrates. They could develop anaerobically in the presence of glucose. Some strains produced reducing substance from gluconate. Starch was not hydrolyzed. Glucose, gluconate, citrate, succinate and protocatechuate were utilized as the sole source of carbon with ammoniacal nitrogen, though some exceptions were found. Of the 29 strains, 21 grew on desoxycholate agar, 13 utilized malonate and 26 assimilated citrate on SIMMONS agar. All the strains produced 5'-isomer of nucleotide by NPTase. Cytochrome oxidase and urease were negative. DNase was not found within 24 hr but indefinite zones were seen after long incubation in some cases. On the contrary, *Serratia marcescens* gave a large clear zone within 24 hr. Catalase was positive in all the strains.

Computer Analysis.

All the strains resemble one another with similarity of 60% and were divided into distinct 3 clusters as shown in Fig. 3. Clusters of I and II were closely related, but cluster III was somewhat different from other two. Members of cluster I were biochemically more active than those of clusters of II and III. These bacteria also showed similarity to the members of Enterobacteriaceae. *Erw. carotovora* NARI No. 2, *Erw. amylovora* CCM 1114, *Erw. milletiae* NARI Em-2 and *Aerobact. cloacae* 2Y-2, Y-1, Y-7 and Py-3 were included in cluster III, but *Ps. aeruginosa* ATCC 10145 differed distinctly from clusters of I, II and III. *Aerobact. cloacae* 2Y-2, Y-1, Y-7 and Py-3 produced gas from glucose, grew at 42°, and showed positive reactions of lysine decarboxylase, arginine dihydrolase and ornithine decarboxylase. However, they produced 5'-nucleotide by NPTase and reduced nitrate to nitrite but could not grow by nitrate respiration.

DISCUSSION

The strains of *Ps. perlurida* and *Ps. trifolii* reported previously by the present authors (*2—4*) exhibited the same bacteriological characteristics as

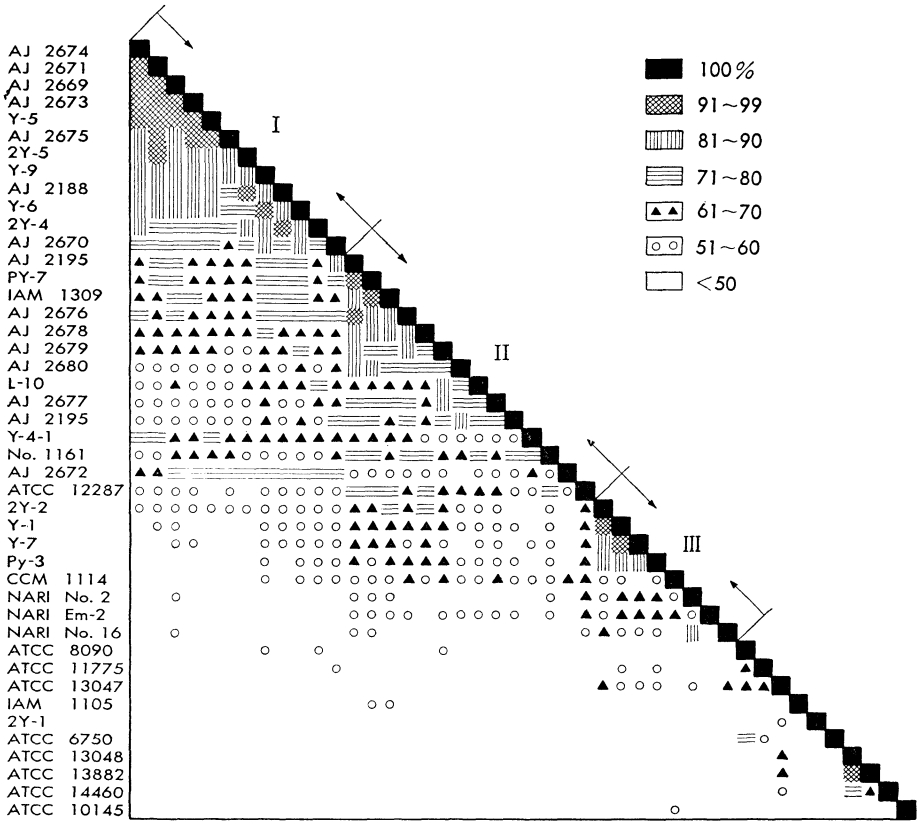

Fig. 3. Diagram of S value.

described, and they were included in a single species pattern on the basis of computer analysis. However, they should be removed from the genus *Pseudomonas* and transferred to Enterobacteriaceae because of peritrichous flagellation and of fermentative cleavage of carbohydrates. Furthermore, base composition of DNA of the isolate AJ-2195 and *Aerobact. cloacae* 2Y-2 were 52.0 and 52.2, respectively. These correspond to those of the members of Enterobacteriaceae (*18*). They should be excluded from the genera of animal origin because they are widely distributed in plant materials.

Meanwhile, these bacteria appear to be quite similar to *Bacterium herbicola aureum* Düggeli, 1904, by comparison with the original description. After the work of DÜGGELI (*1*), several bacteria taxonomically related to *Bact. herbicola aureum* have been reported as presented in Table 5 and the nomenclature of this species has been complicated as shown in Table 6. HUSS (*19*) isolated *Ps. trifolii* from plant materials, and MACK (*20*) concluded that

both species of *Bact. herbicola aureum* and *Ps. trifolii* were identical with each other by a comparative study, and proposed *Bact. herbicola* or *Flavobact. herbicola* according to Bergey's system. JAMES (*21*) isolated a similar bacterium from wheat and proposed a new combination, *Xanthomonas trifolii*, on the coloration of colonies and flagellation. Further, BILLING and BAKER (*22*) found *Erwinia*-like organisms in plants.

A reason for the complicated nomenclature of these bacteria might be ascribed to the description of flagellation. DÜGGELI (*1*) reported motility of *Bact. herbicola aureum* but not its flagellation. HUSS (*19*) described flagellation of *Ps. trifolii* as polar with a sketch. MACK (*20*) also reported polar flagellation of *Bact. herbicola* but micrograph revealed lateral flagellation. JAMES (*21*) described motility of *X. trifolii* as follows: "In nutrient broth culture they were actively motile. Most cells had one polar flagellum, but a few had two and a few four near one pole". The strain used in his work (*X. trifolii* ATCC 12287), however, exhibited peritrichous flagellation on the observation of the present authors as shown in Fig. 1. Electron micrograph of *Bact. herbicola* taken by HOUWINK and VAN ITERSON (*23*) revealed peritrichous flagellation. HOLDING (*24*) described flagellation of *Bact. herbicola* as peritrichous. Flagellation of *X. uredovorans* which is biochemically similar to *Bact. herbicola aureum* was reported as peritrichous by HAYWARD and HODGKISS (*25*). From the report of HOUWINK and VAN ITERSON (*23*) that young cells of *Bact. herbicola* may possess smaller numbers of flagella compared with old ones and from the observation of the present authors, the workers in early days must have misinterpreted the flagellation of such bacteria. Biochemical characteristics of bacteria that appeared in the past literature were almost the same as shown in Table 5. From comparison, the authors consider that the strains used by DÜGGELI (*1*), HUSS (*19*), MACK (*20*), JAMES (*21*), and by BILLING and BAKER (*22*), and employed in the present study should be included together in the same species pattern, though some intraspecific differences could be found.

Another complication in this nomenclature has been the specific epithet, and both "herbicola" and "trifolii" have been used as shown in Table 6. DÜGGELI (*1*) reported the species in trinominal but did not describe in binominal. JAMES (*21*) pointed out the inadequateness of "herbicola" because the trinominal had meant the variety of bacteria, and stressed the misuse of MACK (*20*) that she was not concerned about dropping the third word of trinominal. DYE (*26*) concluded that *X. trifolii* reported by JAMES should be placed in the genus *Erwinia* on the basis of peritrichous flagellation and proposed a new combination, *Erw. herbicola* (Düggeli). However, BUCHANAN *et al.* (*27*) pointed out the invalid publication of *Bact. herbicola aureum* because of trinominal naming and valid publication of *Bact. herbicola* by GEILINGER (*28*). They described a correct combination of *Erw. herbicola* (Geilinger) Dye. The genus *Erwinia* has been well known as pathogens to many kinds of plants, and the pathogenicity has been concerned with

Table 5. Comparison of *Bact. herbicola*

	Bact. herbicola aureum Düggeli (1904)	*Ps. trifolii* Huss (1907)
Form	rod	small rod
Size	0.6—0.7×1.5—2.0 μ	0.5—0.7×0.75—2.1 μ
Motility	+	+
Flagellation	not described	polar
Gram reaction	−	−
Pigmentation	yellow	yellow
Milk	unchanged or acid coagulated	coagulated
Gelatin	liquefied	liquefied
Nitrate reduction	+	+
Indole production	+	+
V-P		
Acid from		xylose
		arabinose
		glucose
		sucrose
		mannitol
No acid from		lactose
Gas from sugar		
Oxidative or fermentative		
Utilization		
Slime formation	+	+
Temperature relation	33—35°	18—30°
Aerobiosis		aerobic
Source	plant	clover hay

aureum and related bacteria.

Bact. herbicola Mack (1936)	*X. trifolii* James (1955)	*Erwinia*-like organism Billing *et al.* (1963)	*Erw. herbicola* Dye (1964)
small rod	rod to coccoid	small rod	rod
0.7×2—3 μ	0.7×0.7—2.5 μ		
+	+	+	+
polar	polar	peritrichous	peritrichous
−	−	−	−
yellow	yellow	yellow	yellow
coagulated	alkaline		acid, coagulated
liquefied	liquefied	liquefied	liquefied
+	+	+	+
−	−	−	−
+		+	+
glucose	maltose	xylose	xylose
galactose	salicin	arabinose	arabinose
mannose		glucose	glucose
sucrose		galactose	mannose
lactose		fructose	galactose
(rarely)		sucrose	fructose
		maltose	sucrose
		mannitol	maltose
		salicin	mannitol
		glycerol	salicin
	lactose	lactose	lactose (variable)
		dulcitol	dulcitol
trace			
		fermentative	fermentative
		succinate	succinate
		malate	malate
		citrate	citrate
			lactate
+		+	
		22—37°	37—39°
facultatively anaerobic		aerobic	facultatively anaerobic
plant	wheat	apple stem and pear blossom	leaves, seed and fruit of plants

Table 6. Nomenclature of *Erw. herbicola.*

1904	*Bact. herbicola aureum* [DÜGGELI][a] (*1*)	
1907		*Ps. trifolii* [HUSS] (*19*)
1921	*Bact. herbicola* [GEILINGER] (*28*)	
1923		*Flavobact. trifolium* (Huss) [BERGEY *et al.*] [Bergey's Manual, 1 st ed.] (*31*)
1927	*Ps. herbicola* (Geilinger) [DE'ROSSI] (*29*)	
1934		*Flavobact. trifolii* (Huss) Bergey *et al.* [Bergey's Manual, 4 th ed.] (*32*)
1936	*Bact. herbicola* [MACK] (*20*)	
1938		*Ps. trifolii* Huss [Bergey's Manual, 5 th ed.] (*33*)
1948		*Ps. trifolii* Huss [Bergey's Manual, 6 th ed.] (*34*)
1955		*X. trifolii* (Huss) [JAMES] (*21*)
1957		*Ps. trifolii* Huss [Bergey's Manual, 7 th ed.] (*35*)
1959	*Ps. herbicola* (Burri et Düggeli) [KRASSILNIKOV's Manual] (*30*)	
1960	*Bact. herbicola* [HOLDING] (*24*)	
1961		*X. trifolii* (Huss) James [PRÉVOT's Manual] (*36*)
1963	*Erwinia*-like organisms [BILLING and BAKER] (*22*)	*Ps. trifolii* Huss [IIZUKA and Komagata] (*4*)
1964	*Erwinia herbicola* (Düggeli) [DYE] (*26*)	
1966	*Erwinia herbicola* (Geilinger) Dye [Index Bergeyana] (*27*)	
1967	*Erw. herbicola* (Geilinger) Dye [KOMAGATA *et al.*]	

[a] Names in parentheses indicate the investigators.

identification of the species of this genus. As a strain of *Erw. milletiae* pathogenic to Japanese wisteria seems to be included in the species pattern of the tested bacteria, as shown in Fig. 3 and Table 7, plant pathogenicity of these bacteria is expected. Therefore, all the strains employed in the present work were inoculated into Japanese wisteria, but the symptoms of disease were not recognized. Further, JAMES (*21*) and DYE (*26*) did not find pathogenicity in *X. trifolii* and *Erw. herbicola,* respectively, in spite of inoculation test.

From the viewpoint of determination, it is probably reasonable to conclude that these bacteria should be placed in the genus *Erwinia* on the basis of flagellation, biochemical characteristics and habitat regardless of plant patho-

Table 7. Comparison of *Erw. herbicola* and related bacteria.

	Erw. herbicola AJ 2671 (typical strain)	*X. trifolii* ATCC 12287	*Erw. milletiae* NARI Em-2	*Erw. amylovora* CCM 1114	*Erw. carotovora* NARI No. 2	*S. marcescens* IAM 1105	*Enterobact. cloacae* ATCC 13047	*Enterobact. aerogenes* ATCC 13048	*E. coli* ATCC 11775
Gram-negative rod	+	+	+	+	+	+	+	+	+
Peritrichous flagella	+	+	+	+	+	+	+	−	+
Yellow pigmentation	+	+	+	−	−	−	−	−	−
Coagulation of milk	+	+	−	−	+	+	+	+	+
Liquefaction of gelatin	+	+	+	+	+	+	+	−	−
Nitrate reduction	+	+	+	−	+	+	+	+	+
Nitrate respiration	−	−	−	−	+	+	+	+	+
Indole	+	−	−	−	−	−	−	−	+
V-P	+	+	+	+	+	+	+	+	−
Citrate	+	+	+	−	+	+	+	+	−
Glucose, O.	+	+	+	+	+	+	+	+	+
C.	+	+	+	+	+	+	+	+	+
Lactose, O.	+	−	−	−	+	−	+	+	+
C.	+	−	−	−	+	−	+	+	+
NPTase test									
3′-nucleotide	−	−	−	−	−	−	+	+	+
5′-nucleotide	+	+	+	−	−	+	−	−	−
DNase	−	−	−	−	−	+	−	−	−
Pectinase	−	−	−	−	+	−	−	−	−
Growth at 42°	−	−	−	−	+	+	+	+	+

genicity. From such a consideration and nomenclature described by BUCHANAN *et al.* (*27*), the authors identified the following strains with *Erw. herbicola*: *Ps. perlurida* Y-4-1, 2Y-4, 2Y-5, Y-5, Y-6 and Y-9; *Ps. trifolii* L-10, PY-5 and PY-7; and the isolates AJ-2669, -2670, -2671, -2672, -2676, -2677, -2673, -2674, -2675, -2678, -2680, -2679, -2188 and -2195. Further, the following strains were also included in *Erw. herbicola* on the flagellation and biochemical characteristics: *Ps. trifolii* IAM 1309, *X. trifolii* ATCC 12287, *Flavobact. harrisonii* No. 1161 (AJ 2681, ATCC 14589) and *Erw. milletiae* NARI Em-2. The strains of *Aerobact. cloacae* 2Y-2, Y-1, Y-7 and Py-3 were also included

in *Erw. herbicola* because of production of 5'-nucleotide by NTPase and of inability to grow by nitrate respiration, although they were somewhat different from the bacteria mentioned above in respects to production of gas from carbohydrates, growth at 42° and positive reactions of lysine decarboxylase, arginine dihydrolase and ornithine decarboxylase. The strains of *Erw. herbicola* can be easily differentiated from the other related bacteria in Enterobacteriaceae on the basis of inability to grow by nitrate respiration, NPTase and other biochemical characteristics as shown in Table 7. The detailed study concerning the differentiation among the species of the genus *Erwinia* will be reported in the following paper.

DESCRIPTION

Erwinia herbicola (Geilinger) Dye

Synonyms:

Bacterium herbicola aureum Düggeli, 1904 (*1*).

Pseudomonas trifolii Huss, 1907 (*19*).

Bacterium herbicola Geilinger, 1921 (*28*).

Flavobacterium trifolium (Huss) Bergey *et al.*, 1923 (*31*).

Pseudomonas herbicola (Geilinger) de'Rossi, 1927 (*29*).

Bacterium herbicola (Düggeli) Mack, 1936 (*20*).

Xanthomonas trifolii (Huss) James, 1955 (*21*).

Pseudomonas herbicola (Burri et Düggeli) Krassilnikov, 1959 (*30*).

Erwinia herbicola (Düggeli) Dye, 1964 (*26*).

Rods, 0.4 to 0.6 by 1.2 to 1.8 microns. Occurring singly or in pairs, not in chain. Motile with peritrichous flagella. Non-motile varieties are found. Gram-negative. Spore not formed.

Nutrient agar colonies: Circular, smooth, entire, raised, glistening, opalescent, dark yellow, butyrous. (Variation: Dull yellow, pale yellow, reddish yellow, yellowish gray or pale yellowish brown.)

Yeast extract-peptone agar colonies: Circular, smooth, entire, raised, glistening, opalescent, reddish yellow, butyrous. (Variation: Dull yellow, dark yellow, pale pellow, yellowish brown or yellowish gray; irregular form; slightly rough surface; and erose margin.)

Nutrient agar slant: Growth moderate, filiform, glistening, opalescent, dull yellow. (Variation: Dark yellow, yellowish orange or pale yellowish brown.)

Yeast extract-peptone agar slant: Growth moderate, filiform, glistening, opalescent, reddish yellow. (Variation: Dull yellow, pale yellowish brown, yellowish gray or pale yellow.)

Glutamate agar slant: Growth moderate, filiform, glistening, metallic sheen, dull yellow. (Variation: Scanty or no growth; rough or pitted surface; viscid or fluid texture; yellowish gray, reddish yellow, pale yellow or white.)

Nutrient broth: Fragile pellicle, slightly turbid. (Variation: No surface growth, moderately turbid.)

Glutamate broth: Moderately turbid. (Variation: Flocculent pellicle; or no growth.)

Nutrient gelatin stab: Slow liquefaction.

B.C.P. milk: Acid coagulation. (Variation: Alkaline or neutral, soft coagulum; peptonization.)

Nitrite produced from nitrate in nitrate broth and succinate-nitrate broth. (Variation: No production in nitrate broth.)

Nitrate respiration: Negative.

Indole produced. (Variation: No production.)

Hydrogen sulfide not produced in KLIGLER agar.

Starch not hydrolyzed.

MR test: Positive. (Variation: Negative.)

V-P test: Positive. (Variation: Negative.)

Acid but no gas is produced from glycerol, xylose, arabinose, glucose, fructose, sucrose, maltose, lactose, mannitol and inositol in both aerobic and anaerobic conditions, but not from dulcitol and starch according to HUGH and LEIFSON's method. (Variation: No anaerobic production of acid from glycerol, lactose and inositol; no production of acid from glycerol, sucrose, xylose and inositol in both aerobic and anaerobic conditions; production of acid from dulcitol; production of gas from xylose, arabinose, glucose, fructose, maltose and sucrose.)

Reducing substance is not produced from gluconate. (Variation: Production of reducing substance.)

Glucose, gluconate, citrate, succinate and protocatechuate are utilized as the sole carbon source with ammoniacal nitrogen but *p*-hydroxybenzoate is not. (Variation: No utilization.)

Malonate is not utilized in LEIFSON's medium. (Varition: Utilization.)

Citrate is utilized in SIMMONS agar. (Variation: No utilization.)

Desoxycholate agar: Growth. (Variation: No growth.)

Nucleoside phosphotransferase: Positive. (5'-Nucleotide is produced.)

Lysine decarboxylase: Negative. (Variation: Positive.)

Arginine dihydrolase: Negative. (Variation: Positive.)

Ornithine decarboxylase: Negative. (Variation: Positive.)

Cytochrome oxidase: Negative.

Catalase: Positive.

Maceration of potato slice: Negative.

Good growth between 20° and 35°. No gowth at 15° and 42°. (Variation: Growth at 15° and 42°.)

Growth at pH 5.0: Growth. (Variation: No growth.)

Sources: Paddy rice, fruit, soil, etc. Widely distributed in plant materials.

The typical strain, *Erw. herbicola* AJ 2671, has been deposited with The Institute of Applied Microbiology, University of Tokyo.

After completion of this manuscript, GRAHAM and HODGKISS (*37*) reported the identity of gram-negative, yellow-pigmented, fermentative bacteria isolated from plants and animals, and included such bacteria in *Erw. herbicola.*

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