A TAXONOMIC STUDY OF ERWINIA AMYLOVORA
(BURRILL 1882) WINSLOW ET AL. 1920

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SUMMARY. In the present report we give the results of our studies of the morphological, cultural, and biochemical properties of 49 strains of Erwinia amylovora. On the basis of our results, we propose a new description of this species. The following tests are most suitable for the differentiation of E. amylovora from other species of the genus Erwinia: pectinase, lactose, and the reduction of nitrates. We recommend that the strain BS 1114 (NCPB 683) be considered a neotype.

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The genus Erwinia is the last genus of the family Enterobacteriaceae which has been very insufficiently studied taxonomically. Whereas the other genera of the family Enterobacteriaceae have recently been studied intensively, the characterization of the genus Erwinia has received very little attention. The present description of the genus Erwinia is not satisfactory and the data on this genus do not appear in differentiation tables for the family Enterobacteriaceae. In monographs on the family Enterobacteriaceae we also find only sporadic data on this genus (e.g. Kauffmann 1954, Sedláč and Rische 1961).

The type species of the genus Erwinia is E. amylovora. This species was isolated by Burrill (1882) and described as Micrococcus amylovorus. Since that time this species has been placed in various genera. Trevisan (1889) classified it in the genus Bacillus, Chester (1901) in the genus Bacterium, and it was Winslow et al. (1920) who placed it in the genus Erwinia as the type species. The classification

of Winslow et al. has been accepted by most microbiologists (see Bergey's Manual 1957). Only a few authors keep to the old and unsuitable designation Bacterium amylovorum (e.g. Krasil'nikov 1949, Dowson 1949). The object of the present work was the study of 49 strains of E. amylovora with the purpose of complementing the characterization of this species.

MATERIAL AND METHODS

In the present work we studied the properties of 49 strains of Erwinia amylovora received from the National Collection of Plant Pathogenic Bacteria, Harpenden, England. These were the following strains:

100, 549, 595, 596, 597, 673, 674, 675, 676, 677, 678, 679,
681, 682, 683, 684, 686, 688, 689, 690, 691, 692, 693, 694,
695, 696, 697, 698, 699, 709, 710, 711, 713, 716, 717, 718,
725, 738, 744, 745, 749, 756, 772, 776, 777, 779, 817, 830.

One strain (409) was received from the Collection of Microorganisms of the Microbiological Institute, CAS in Prague.

In our studies on the characters of the strains of E. amylovora, we used current methods (Manual of Microbiological Methods 1957, Martinec and Kocur 1961). The production of pectinase was studied by the method recommended by Dye (1961).

RESULTS

Morphology. All the strains studied formed Gram-negative rods occurring singly or in clumps. They measured $0.6-1.2 \times 0.6-0.9 \mu$ in diameter. Out of the total of 49 strains, only 3 were motile; the remaining 46 strains were nonmotile.

Cultural characteristics. Most strains of E. amylovora formed round colonies with a straight or slightly undulate margin, slightly convex, smooth, greyish white. All strains grew well on Endo's agar. The form of the colonies in this medium was similar to that of colonies grown on a meat extract agar. All the strains formed a turbidity and a sediment in broth. Out of 49 strains studied, only 21 grew in Ushinski's medium.

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Cultural and physiological characteristics: The results of the studies of cultural and physiological characteristics are given in Table 1.

DISCUSSION

Our results showed that all the 49 strains studied agreed with the description of Erwinia amylovora. It was interesting to compare our results with the data in determinative manuals (Karsil'nikov 1949, Burkholder in Bergey's Manual 1957). In contrast to the authors mentioned above we have ascertained that none of the 49 strains studied formed acid from lactose, maltose, arabinose, and cellobiose in peptone water (Table 2). Further differences were ascertained in the studies of acetylmethylcarbinol production. It was shown that acetylmethylcarbinol was produced only by 37 strains out of 49; this means that its production is variable. On the other hand, Krasil'nikov states that E. amylovora fails to produce acetylmethylcarbinol, whereas Burkholder states the contrary. In contrast to the data of Burkholder that E. amylovora fails to grow at 37°C, we have ascertained that 30 strains of E. amylovora grow at this temperature. The other tests which we were able to compare agreed on the whole with the data of the authors mentioned above. The differences can be explained by the use of different methods and other strains, and by the fact that our description was made on the basis of studies of a great number of strains. Studying many strains of a certain species, we can ascertain which characters of the species are constant and which are variable. For example, in the case of E. amylovora, out of 71 biochemical tests, only 57 were relatively constant and the remaining 14 tests were variable (Table 1). From the relatively constant biochemical tests, there are some that are above all important for the differentiation of E. amylovora from the other species of erwiniae, namely: the inability to produce pectinase, to ferment lactose and to reduce nitrates.

Another matter of interest is the question of the relationship between E. amylovora and other representatives of the family Enterobacteriaceae. Elrod (1942a) thinks that the species of the genus Erwinia are aberrant coliform organisms. From our results it follows that E. amylovora is closely related to the genus Shigella.

Table 1. Results of cultural and physiological tests with 49 strains of Erwinia amylovora.

Test	No. positive	No. negative
Glucose	49	0
Gas from glucose	0	49
Lactose	0	49
Sucrose	49	0
Maltose	0	49
Galactose	49	0
Fructose	49	0
Rhamnose	0	49
Mannose	37	12
Inulin	0	49
Xylose	3	46
Arabinose	0	49
Cellobiose	0	49
Glycerol	0	49
Adonitol	0	49
Sorbitol	49	0
Mannitol	49	0
Dulcitol	0	49
Trehalose	49	0
Raffinose	27	22
Inositol	0	49
Esculin	49	0
Salicin	33	16
Starch hydrolysis	0	49
Insoluble starch (gas)	0	49
Cellulose	0	49
Sodium formate	49	0
Sodium acetate	5	44
Sodium lactate	44	5
Sodium succinate	49	0
Sodium benzoate	0	49
Sodium tartarate	0	49
Sodium malonate	0	49
Sodium citrate	20	29
Acetylmethylcarbinol	37	12
Methyl red	0	49

Table 1. (continued)

Test	No. positive	No. negative
Arginine dihydrolase	1	48
Lysine decarboxylase	0	49
Ornithine decarboxylase	0	49
Glutamic acid decarboxylase	38	11
Phenylalanine deaminase	0	49
Gelatin hydrolysis	38	11
Casein hydrolysis	0	49
Reduction of methylene blue	0	49
Nitrate reduction	0	49
Nitrite reduction	0	49
Ammonia production	0	49
Hydrogen sulfide	0	49
Indole	0	49
Braun's test (KCN)	0	49
Catalase	49	0
Pectinase	0	49
Cytochrome-oxidase	0	49
Lipase	0	49
Urease	0	49
Lecithinase C	0	49
Tyrosinase	0	49
Haemolysis	0	49
5% NaCl	0	49
7.5% NaCl	0	49
Growth at 37°C	28	21
Penicillin	0	49
Streptomycin	49	0
Chloramphenicol	49	0
Chlortetracycline	24	25
Tetracycline	49	0
Erythromycin	49	0
Neomycin	49	0
Tyrothricin	0	49
Nystatin	0	49
Bacitracin	0	49

Table 2. Comparison of various descriptions of *Erwinia amylovora*.

Test	Krasil'nikov (1949)	Burkholder (1957)	Own results
Morphology:	Rods single or in clumps	Rods in pairs and chains	Rods single or in clumps
Gram stain:	negative	negative	negative
Motility:	+	+	(e. +)
Colonies:	round, flat, white	round, smooth, slightly convex, white	round, smooth slightly convex, white
Nutrient broth:	turbidity, sediment	turbidity, thin pellicle	slight turbidity, sediment
Uchinsky's solution:	+	0	d
Glucose	+	+	+
Gas from glucose	-	-	-
Lactose	+	d	-
Sucrose	+	+	+
Maltose	+	+	-
Galactose	0	d	+
Fructose	0	+	+
Rhamnose	0	-	-
Mannose	+	+	d
Inulin	0	0	-
Xylose	0	-	-
Arabinose	+	+	-
Cellobiose	0	+	-
Glycerol	+	0	-
Adonitol	0	0	-
Sorbitol	0	0	+
Mannitol	0	0	+
Dulcitol	0	-	-
Trehalose	0	0	+
Raffinose	+	0	d
Inositol	0	0	-
Esculin	0	0	+
Salicin	+	+	d
Starch hydrolysis	-	0	-
Insoluble starch (gas)	0	0	-
Cellulose	0	0	-

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Table 2. (continued)

Test	Krasil'nikov (1949)	Burkholder (1957)	Own results
Sodium formate	0	0	+
Sodium acetate	0	0	- (e. +)
Sodium lactate	0	d	+ (e. -)
Sodium succinate	0	d	+
Sodium benzoate	0	-	-
Sodium tartrate	0	-	-
Sodium malonate	0	-	-
Sodium citrate	0	+	d (e. -)
Acetylmethylcarbinol	-	+	d
Methyl red	0	0	-
Arginine dihydrolase	0	0	-
Lysine decarboxylase	0	0	-
Ornithine decarboxylase	0	0	-
Glutamic acid	0	0	d
Phenylalanine deaminase	0	0	-
Gelatin hydrolysis	w	+	d
Casein hydrolysis	0	0	-
Methylene blue reduction	0	0	-
Nitrate reduction	-	-	-
Nitrite reduction	0	0	-
Ammonia production	0	+	-
Hydrogen sulfide	0	0	-
Indole	0	-	-
Braun's test (KCN)	0	0	-
Catalase	0	0	+
Pectinase	0	0	-
Cytochrome-oxidase	0	0	-
Lipase	0	0	-
Urease	0	-	-
Lecithinase C	0	0	-
Tyrosinase	0	0	-
Haemolysis	0	0	-
5% NaCl	0	0	-
7.5% NaCl	0	0	-
Growth at 37°C	0	-	d
0 = data not given w = weak d = different (e. +) = exceptionally positive (e. -) = exceptionally negative			

On the basis of our results, we have worked out the following description of Erwinia amylovora:

Erwinia amylovora (Burrill 1882) Winslow et al. 1920.

Micrococcus amylovorus Burrill 1882

Bacillus amylovorus Trevisan 1889

Bacterium amylovorus Chester 1897

Bacterium amylovorum Chester 1901

Gram-negative rods occurring singly or in clumps. They measure $0.6-1.2 \times 0.6-0.9 \mu$. Most strains nonmotile. Motile strains possess peritrichous flagella.

Colonies in agar (2—5 days): round, slightly convex, smooth, white.

Agar slant (24 hours): smooth, greyish white.

Broth (24 hours): slight turbidity, sediment.

Endo's agar (2 days): round colony with straight margin, flat, smooth.

Uschinski's medium (2 days): slight turbidity, sediment.

Peptone water with carbohydrates: acid, but no gas is produced from glucose, sucrose, galactose, fructose, sorbitol, mannitol, and trehalose. The production of acid from mannose, xylose, raffinose, and salicin may be variable. Acid is not produced from lactose, maltose, rhamnose, inulin, arabinose, adonitol, dulcitol, inositol, cellobiose, and glycerol.

Cellulose is not decomposed.

Esculine is not hydrolyzed.

Starch is hydrolyzed.

Gas is not produced from insoluble starch.

The salts of formic and succinic acids are utilized.

Utilization of salts of acetic, lactic, and citric acids is variable.

The salts of benzoic, tartaric, and malonic acids are not utilized.

Indole and hydrogen sulfide are not produced.

Acetylmethylcarbinol production is variable.

The methyl-red test is negative.

Nitrates and nitrites are not reduced.

Ammonia is not produced.

Gelatin hydrolysis: positive, exceptionally negative.

Casein is not hydrolyzed.

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Arginine dihydrolase is not produced.
 Decarboxylation of lysin is negative.
 Ornithine decarboxylase is not produced.
 Decarboxylation of glutamic acid is variable.
 Phenylalanine deaminase is not produced.
 Braun's test (KCN): negative.
 Urease, lipase, lecithinase C, tyrosinase, and cytochrome-oxidase are not produced.
 Catalase is produced.
 Haemolysis is not produced.
 Protopectinase and pectinase are not produced.
 Relation to oxygen: facultative aerobe.
 Tolerance to NaCl: no growth in 5% and higher concentrations of NaCl.
 Optimum pH: 6.8; minimum 4.0-4.4; maximum 8.8.
 Optimum temperature: 30°C; minimum between 3-8°C; maximum 37°C.
 Habitat: pathogenic for many species of the family Rosaceae.
 The strain BS 1114 (NCPFB 683) is proposed as neotype.

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