NOTES

Taxonomic Position of Additional *Zymomonas mobilis* Strains

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The strains CP3 and CP4, isolated from fermenting sugar-cane juice in Brazil, and the strain Ag11, isolated from fermenting agave juice in Mexico, belong in *Zymomonas mobilis* subsp. *mobilis*. The existence of a separate *Zymomonas mobilis* subsp. *recifensis* for the former two is not justified. The strains NCIB 8777 and NCIB 10565 from sick cider and apple pulp, respectively, in England, belong in *Zymomonas mobilis* subsp. *pomaceae*. Protein electropherograms allow a quick and accurate identification in *Zymomonas*.

In previous papers (2, 4, 5, 6) we examined the genotype and phenotype of the genus *Zymomonas* and concluded that the type and sole species *Zymomonas mobilis* consists of two subspecies *mobilis* and *pomaceae*.

We report here on the taxonomic position of five additional strains, received as *Z. mobilis* var. *recifensis* CP3 and CP4, *Z. mobilis* Ag11, and *Z. anaerobia* var. *pomaceae* NCIB 8777 and NCIB 10565. It is the purpose of the present communication to identify and name the above strains correctly and to locate them in our nomenclatural system (1). In previous papers (2, 5) we demonstrated that the taxonomy of *Zymomonas*, as described in *Bergey's Manual of Determinative Bacteriology* (1), is not satisfactory. The methods used have been described elsewhere (2, 4, 6). The main phenotypic features and the protein electropherograms of each strain were determined and compared with known data (2, 6). Two type strains were included for comparison.

Correct taxonomic position of *Zymomonas* strains CP3 and CP4. *Z. mobilis* var. *recifensis* has been proposed as a new subspecies by Gonçalves de Lima et al. (3; personal communication) for the strains CP1, CP2, CP3, and CP4 isolated from fermenting sugar-cane juice in Recife, Brazil. These authors compared them to *Z. mobilis* strain Ag11 (see below). They differentiated *Z. mobilis* var. *recifensis* by: (i) a better growth yield, (ii) growth at 37°C and the ability of fermentation at 42°C, (iii) greater aerotolerance; (iv) no H₂S formation, and (v) a characteristic antagonistic pattern towards 41 microorganisms. We found that both strains CP3 and CP4 were phenotypically identical (Table 1) and that their protein electrophero-

grams were indistinguishable (Fig. 1). We conclude that both isolates are but subcultures of the same organism. We found no significant differences in growth yields between CP3 and CP4 and more than 40 other strains from our collection. Both strains still grew at 38°C but so did 28 other strains from the *Z. mobilis* subsp. *mobilis* (2). Both strains grew well aerobically, reaching a colony diameter of 3 mm after 7 days at 30°C. The high aerotolerance was not restricted to these strains since, for example, the centrotypic strain ATCC 29191 from *Z. mobilis* subsp. *mobilis* grew equally well aerobically. H₂S formation occurred rarely (and only in traces) in *Zymomonas* (2). The phenotypic diagnosis of *Z. mobilis* subsp. *recifensis* as given by Gonçalves de Lima et al. (3) is thus very equivocal and not subspecific. Furthermore, its protein electropherograms are very similar to those of *Z. mobilis* subsp. *mobilis* (6). For all of these reasons we conclude that the existence of *Z. mobilis* var. *recifensis* Gonçalves et al. is not justified and that it is but a subjective synonym of *Z. mobilis* subsp. *mobilis* De Ley and Swings 1976, 146.

Correct taxonomic position of *Zymomonas* strain Ag11. *Zymomonas* strain Ag11 was isolated from fermenting agave juice in Mexico (3). Its protein electropherogram indicated that it belongs in *Z. mobilis* subsp. *mobilis*. It was characterized by a strong P band at the bottom of the gel, resembling strain 1.1 (Fig. 1). Phenotypically, strain Ag11 differed from CP3 and CP4 and/or from the centrotypic ATCC 29191 in a few features only, namely, pleomorphism, no fermentation of sucrose, formation of H₂S, growth in standard medium + 0.0075% KCN, and no growth at 38°C. These differences should

TABLE 1. Phenotypic characteristics of some new *Zymomonas* strains^a

Phenotypic characteristic	Zymomonas mobilis subsp. mobilis ^b				Zymomonas mobilis subsp. pomaceae ^b		
	ATCC 29191 (Z. mobilis subsp. mobilis)	CP3 (Z. mobilis var. recifensis)	CP4 (Z. mobilis var. recifensis)	Ag11 (Z. mobilis)	ATCC 29192 (Z. mobilis var. pomaceae)	NCIB 10565 (Z. mobilis var. pomaceae)	NCIB 8777 (Z. mobilis var. pomaceae)
Colony diam (mm) after aerobic growth for 7 days at 30°C	1.5	1.5	1.5	1.5	Microcolonies	Microcolonies	Microcolonies
Colony diam (mm) after anaerobic growth for 7 days at 30°C	3-4	3	3	3	1.5 or less	4	1 or less
Shape of the cells	Rod	Rod	Ovoid and short rod	Rod	Rod	Rod	Irregular rod or ovoid
Cell width (μm)	1.0-1.2	1.2-1.4	1.4	1.1	1.0	1	0.8
Cell length (μm)	2.0-6.0	1.4-3.5	1.4-2.8	1.4-3.5	1.5-3.5	1.4-2.8	1.4-4.2
Pleomorphism	+	-	-	-	+	+	-
Formation of rosettes of cells	+	+	+	+	-	-	+
Motility	-	-	-	-	-	+	+
Cell deposit in liquid medium	Compact	Compact	Compact	Compact	Compact	Flocculent	Flocculent
Growth on and fermentation of sucrose.	+	+	+	-	-	-	-
Growth in standard medium at 38°C	-	-	-	+	-	+	+
Formation of H ₂ S	-	-	-	-	-	-	-
Indole production. Gelatin liquefaction.	-	-	-	-	-	-	-
Growth in yeast extract. Growth in nutrient broth. Growth in standard medium at 40°C. Nitrate reduction	-	-	-	-	-	-	-
Growth in standard medium + 0.5% NaCl. Growth in standard medium at 34°C	+	+	+	+	-	+	-
Growth in standard medium + 0.0075% KCN	+	-	-	-	-	-	-
Growth in standard medium at 36°C	+	+	+	+	-	-	-
Final pH after growth for 7 days at 34°C in standard medium	5.15	5.0	5.05	5.1	4.65	4.85	5.7

^a The standard medium consists of 2% glucose + 0.5% yeast extract in distilled water. Strains ATCC 29191 and ATCC 29192 were included as reference type strains (2).

^b Name in parentheses is that under which the organism was received.

FIG. 1. Protein electropherograms of some new *Zymomonas* strains. From left to right: normalized protein patterns of the strains *Z. mobilis* subsp. *mobilis* 1.1 (a), Ag11 (b), CP3 (c), CP4 (d), and *Z. mobilis* subsp. *pomaceae* NCIB 10565 (e), NCIB 8777 (f), and ATCC 29192 (g). The strains 1.1 (a) and ATCC 29192 (g) were included as references. These results must be compared with Fig. 2 of Swings and De Ley (5).

be regarded as strain specific. We conclude that strain Ag11 belongs in *Z. mobilis* subsp. *mobilis* (2).

Correct taxonomic position of *Zymomonas* strains NCIB 8777 and NCIB 10565. *Zymomonas* strains NCIB 8777 and NCIB 10565 were both received as *Z. anaerobia* var. *pomaceae*. The former strain was isolated from "sick cider" and the latter was isolated from apple pulp in England. The protein patterns and phenotypic features of both strains were nearly identical with those of strain ATCC 29192 (Fig. 1, Table 1), the proposed type strain of *Z. mobilis* subsp. *pomaceae* (2). Phenotypically there are some minor differences between these strains (Table 1). Obviously these tests are only useful for

strain differentiation and not for subspecies differentiation. Both strains NCIB 8777 and NCIB 10565 thus belong in *Z. mobilis* subsp. *pomaceae* together with strain ATCC 29192.

We thus conclude the following: (i) the correct taxonomic position of the five new *Zymomonas* strains has been established; (ii) the existence of *Z. mobilis* subsp. *recifensis* is not justified; (iii) the most useful phenotypical test to differentiate between *Z. mobilis* subsp. *pomaceae* and *Z. mobilis* subsp. *mobilis* is the growth in 2% glucose + 0.5% yeast extract at 36°C; and (iv) after a rapid tentative identification by a few characteristic phenotypical tests of a new specimen as *Zymomonas*, the easiest way to achieve a prompt and accurate identification is by comparison of protein electropherograms.

REPRINT REQUESTS

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