Genetic variability analysis of *Zymomonas mobilis* strains from the UFPEDA microorganisms collection

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ABSTRACT. *Zymomonas mobilis* is a Gram-negative bacterium that has drawn attention in the bioethanol industry. Besides bioethanol, this bacterium also produces other biotechnological products such as levans, which show antitumor activity. Molecular studies involving *Z. mobilis* have advanced to the point that allows us to characterize interspecies genetic diversity and understand their metabolism, and these data are essential for better utilization of this species. In this study, the genetic diversity of 24 strains from the Microorganisms Collection of Departamento de Antibióticos (UFPEDA) from Universidade Federal de Pernambuco were characterized. The methods used were amplified ribosomal DNA restriction analysis and diversity analysis of the internally transcribed *16S-23S rDNA*

spacer region (ISR). These analyses revealed low genetic variability of the *16S rDNA* gene. These data confirm that these isolates are, or are closely related to, *Z. mobilis*. Moreover, the analysis of the ISR confirmed the genetic variability of strains deposited in the UFPEDA collection of microorganisms and grouped these strains into ten ribotypes, which can be used in the future for breeding programs and for the preservation of biodiversity. Furthermore, this study characterized the genetic variability between the UFPEDA 205/ZAP, UFPEDA 98/AG11, and ZAG strains, which were obtained by spheroplast fusion among them. The data also indicate that there is genetic variability among the UFPEDA 202/CP4 and UFPEDA 633/ZM4 strains, demonstrating that these important *Z. mobilis* strains are distinct, as suggested in previous studies.

Key word: Amplified ribosomal DNA restriction analysis; Ribotypes; Microorganisms collection of the Departamento de Antibióticos; *Zymomonas mobilis*

INTRODUCTION

Zymomonas mobilis is a Gram-negative, facultative anaerobic bacterium that belongs to the α proteobacteria group. Colonies are white, lenticular, and have irregular borders (Swings and De Ley, 1977; Conway, 1992; Kang and Kang, 1998). This bacterium has received attention because it produces exopolysaccharides such as levan, and other metabolic products such as gluconate and sorbitol (Ernandes and Garcia-Cruz, 2009, 2011). Besides these characteristics, *Z. mobilis* also shows antagonistic activity against bacteria, fungi, and protozoa (Lima, 1958; Gonçalves de Lima et al., 1968).

Several techniques were developed with the goal of identifying genetic variability in *Z. mobilis*, including plasmid profiles, total protein analysis [sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)], rapid amplified polymorphic DNA (RAPD), and repetitive sequence-based polymerase chain reaction (rep-PCR) (Yablonsky et al., 1988; Silva, 2000; Lima, 2002). Furthermore, sequencing technologies have been employed to clarify *Z. mobilis* taxonomy (Seo et al., 2005; Kouvelis et al., 2011; Pappas et al., 2011; Câmara et al., 2013).

Amplified ribosomal DNA restriction analysis (ARDRA) has been used to differentiate *Z. mobilis* subspecies (Coton et al., 2005). Another technique used to evaluate the genetic variability is PCR-ribotyping, which is based on the genetic variability of the intergenic spacer region (ISR), a region of noncoding DNA situated between the small (16S) and large (23S) ribosomal subunit genes. The ISR provides sufficient variability to be able to clarify differences between strains within a species (Andrade, 2008).

Due to the importance of *Z. mobilis*, the Microorganisms Collection of Departamento de Antibióticos of Universidade Federal de Pernambuco (UFPEDA) maintains several strains of this bacterial species, which needed to be characterized from a molecular point of view. The aim of this study was to characterize the genetic diversity among strains of *Z. mobilis* from the UFPEDA collection using ARDRA and ribotyping.

MATERIAL AND METHODS

Bacterial strains and growth conditions

The strains were isolated from various habitats and are part of the UFPEDA (Table 1). The strains were grown at 30°C in 50-mL tubes with 30 mL SSDL medium (Swings and De Ley, 1977) containing 20 g/L glucose and 5 g/L yeast extract.

Table 1. Twenty-four strains of *Zymomonas mobilis* analyzed from the UFPEDA collection.

UFPEDA No.	Acronym	Authors	Location
98	AG -11	Gonçalves de Lima and Carmona, 1951	Mexico
199	CP1	Gonçalves de Lima et al., 1970	Recife/PE
200	CP2	Gonçalves de Lima et al., 1970	Recife/PE
201	CP3	Gonçalves de Lima et al., 1970	Recife/PE
202	CP4	Gonçalves de Lima et al., 1970	Recife/PE
203	Z-1-80	Falcão de Morais et al., 1993	Usina Massauassu, Escada/PE
204	Z-2-80	Falcão de Morais et al., 1983	São Paulo
205	ZAP	Falcão de Morais et al., 1993	Refinaria de Açúcar/PE
206	P3	Falcão de Morais et al., 1983	Engenho Pitu, Vitória de Santo Antão/PE
207	P4	Falcão de Morais et al., 1983	Engenho Pitu, Vitória de Santo Antão/PE
208	Z-1-81	Falcão de Morais et al., 1983	Usina Bulhões, Jaboatão/PE
210	T2	Falcão de Morais et al., 1993	Usina Massauassu, Escada/PE
238	ZAG-4	Calazans et al., 1989	-
240	ZAG-10	Calazans et al., 1989	-
241	ZAG-12	Calazans et al., 1989	-
353	Z-1-86A	de Morais et al., 1993	Usina Maravilhas, Goiana/PE
354	Z-1-86B	de Morais et al., 1993	Usina Maravilhas, Goiana/PE
355	Z-1-87	de Morais et al., 1993	Engenho de Alagoas/AL
356	Z-1-88	de Morais et al., 1993	Usina Laranjeiras, Vicência/PE
357	Z-2-88	de Morais et al., 1993	Destilaria GIASA, PB
362	Z-1-97	Sá, 2012	Destilaria PEMEL, Marataca/PB
363	Z-1-96	Sá, 2012	PE
388	ZAG-6	Calazans et al., 1989	-
633	ZM4	Yablonsky et al., 1988	Recife/PE

DNA extraction

After 24 h of incubation, each culture was centrifuged in a 50-mL tube for 10 min at 1000 *g*. The supernatant was discarded, and 500 µL TE buffer was added to resuspend the pellet, and then this step was repeated. Then, 0.10 g silica beads and 40 µL 10% SDS were added, and the solution was stirred with a "Bead-Beater" for 60 s at 3500-4200 beats/min and centrifuged for 10 min at 14,000 *g*. The supernatant was transferred to a clean microtube, and 450 µL chloroform:isoamyl alcohol (24:1) was added, mixed by inversion, and then the centrifugation was repeated under the same conditions.

The supernatant was transferred to a clean microtube and 20 µL 5 M NaCl and 120 µL isopropanol were added and mixed by inversion. After 10 min, the solution was centrifuged as previously described and 70% ethanol (cold) was added for precipitation followed by centrifugation for 5 min. The supernatant was discarded and the microtube was inverted in the oven at 37°C for 30 min. The samples were then resuspended in 50 µL TE buffer and stored at -20°C. To assess the quality of the DNA, 5 µL suspension was added to a microtube and mixed with 3 µL sample buffer (15% glucose, 25% bromophenol blue), which was run on a 1% agarose gel using the lambda marker *Hin*dIII (Q-Bio Gene; Irvine, CA, USA).

PCR

For molecular characterization of strains of *Z. mobilis*, specific primers were used for amplification of the 16S ribosomal RNA gene (*16S rDNA*) and the ISR of the *16S-23S rRNA*. The reaction mixture contained 1X buffer, 1.5 mM $MgCl_2$, 0.05 U/µL Taq polymerase from the GoTaq Flexi DNA Polymerase kit (Promega; Madison, WI, USA), 0.2 mM dNTPs (Promega), and 1.0 µM primers (Integrated DNA Technologies; Coralville, IA, USA) diluted in Ultrapure Water Type I (MilliQ; Merck KGaA; Darmstadt, Alemanha) sterile water. The total volume of the reaction was 25 µL, and 1.0 µL DNA was added to each reaction. PCR was performed in a Progene thermocycler (Techne; Cambridge, UK).

Amplification of *16S rDNA*

The *16S rDNA* gene was amplified using the following primers proposed by Weisburg et al. (1991): FD1 (AGAGTTTGATCCTGGCTCAG) and RD1 (AAGGAGGTGATCCAGCC). PCR was performed in a Progene thermocycler (Techne) programmed as follows: an initial cycle of 2 min at 40°C, as recommended by the manufacturer; an additional 5 min at 94°C followed by 30 cycles with each cycle a step consisting of denaturation (1 min at 94°C), a matching phase (30 s at 60°C), and an elongation step (2 min 72°C); and finally, one final extension step at 72°C for 7 min. After the amplification process, 5 µL of each PCR product was run on an agarose gel in a 1% running buffer, using the lambda *Hin*dIII marker. The gel was stained with ethidium bromide, observed on an ultraviolet transilluminator, and then photographed.

Restriction analysis of *16S rDNA* (ARDRA)

The products of the *16S rDNA* gene amplified by PCR were cleaved with the restriction enzymes *Taq*I or *Hae*III, using 20 µL amplification products from each sample. The *Taq*I enzymatic reaction occurred in the Progene thermocycler (Techne) at 40°C over 2 h, and the *Hae*III reaction occurred in an oven at 37°C over a period of 3 h. The DNA was then run on a 2% agarose gel in TAE buffer, along with the 100-bp molecular weight DNA ladder (Promega). The gel was then stained with ethidium bromide and photographed.

Profile amplification of the *16S-23S rDNA* region

We used the primers G1 (GAAGTCGTAACAAGG) and L1 (CAAGGCATCCACCGT) to amplify the ISR of *16S-23S rDNA* using the following protocol (Canstein et al., 2001): initial cycle of 2 min at 40°C, as recommended by the manufacturer; an additional 5 min at 94°C followed by 35 cycles, each cycle consisting of a denaturation step (94°C for 30 s), a matching phase (45°C for 1 min), and an elongation step (72°C for 2 min); and finally, one final extension step at 72°C for 10 min. The success of the reactions was verified by gel electrophoresis in 2.0% TAE buffer, along with the 100-bp molecular weight DNA ladder (Promega). The gel was then stained with ethidium bromide and photographed.

## Bioinformatic analysis of genetic polymorphisms

Amplification and cleavage data were analyzed using the NTSYS-pc version 2.02

software (Rohlf, 2008). The data were introduced in the form of binary variables, with 1 indicating band presence, and 0 indicating absence. Genetic similarities among *Zymomonas* UFPEDA accessions were calculated using Jaccard's similarity coefficient. The resulting similarity matrix was first subjected to cluster analysis by the unweighted pair-group method with arithmetic averages (UPGMA) and then to principal coordinate analysis (PCA).

RESULTS AND DISCUSSION

Restriction analysis of *16S rDNA* (ARDRA) and the 16S-23S ISR

The digestion of *16S rDNA* with the *Taq*I and *Hae*III restriction enzymes revealed a high degree of conservation when fragments larger than 100 bp were considered. The results presented in Figure 1 show the two profiles obtained, with three bands in each sample. The first profile consisted of fragments with molecular weights of approximately 200, 400, and 750 bp (Figure 1A-C), and the second profile, unique to strain UFPEDA 633/ZM4 (Figure 1C), contained fragments of approximately 200, 400, and 800 bp.
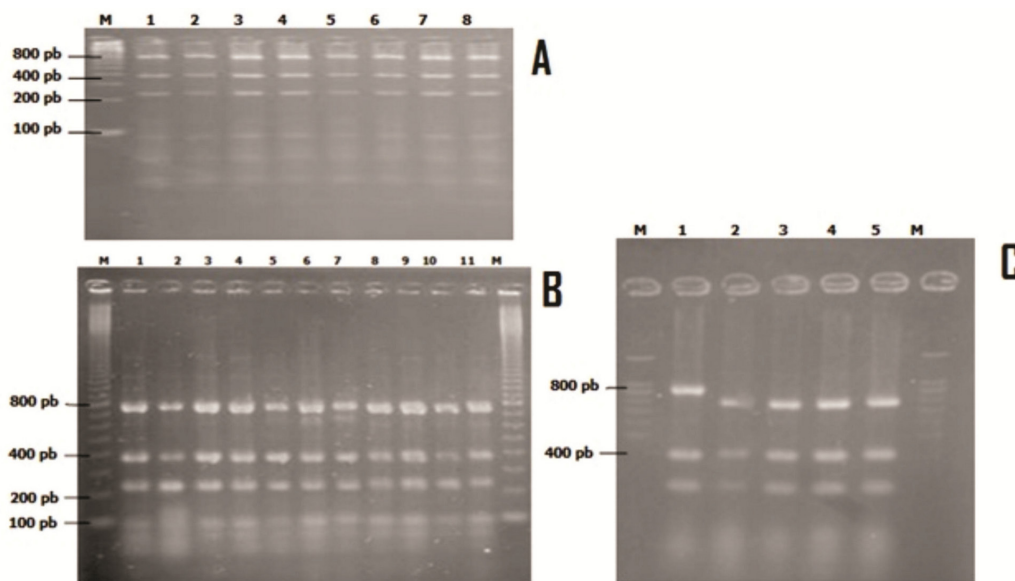

Figure 1. *16S rDNA* PCR products from strains of *Zymomonas mobilis* cleaved with *Taq* I. **A.** *Lane M* = 100-bp DNA ladder; *lane 1* = UFPEDA-198; *lane 2* = UFPEDA-199; *lane 3* = UFPEDA-200; *lane 4* = UFPEDA-201; *lane 5* = UFPEDA-202; *lane 6* = UFPEDA-203; *lane 7* = UFPEDA-204; *lane 8* = UFPEDA-205. **B.** *Lane M* = 100-bp DNA ladder; *lane 1* = UFPEDA-206; *lane 2* = UFPEDA-207; *lane 3* = UFPEDA-208; *lane 4* = UFPEDA-210; *lane 5* = UFPEDA-353; *lane 6* = UFPEDA-354; *lane 7* = UFPEDA-355; *lane 8* = UFPEDA-356; *lane 9* = UFPEDA-357; *lane 10* = UFPEDA-362; *lane 11* = UFPEDA-363. **C.** *Lane M* = 100-bp DNA ladder; *lane 1* = UFPEDA-633; *lane 2* = UFPEDA-238; *lane 3* = UFPEDA-388; *lane 4* = UFPEDA-240; *lane 5* = UFPEDA-241.

Moreover, cleavage with the enzyme *Hae*III was observed in only one restriction profile, which had five fragments: two between 100 and 200 bp, two between 200 and 300 bp,

and one around 500 bp. Coton et al. (2005) also used the restriction enzyme *Hae*III to classify strains of *Z. mobilis* subsp *mobilis*, *Z. mobilis* subsp *pomaceae*, and some new *Z. mobilis* isolates belonging to the Adria Normandie Collection. The authors concluded that the enzymes generated three restriction profiles: one corresponding to *Z. mobilis* subsp *mobilis*, a second corresponding to *Z. mobilis* subsp *pomaceae*, and another profile for the new isolates. When we considered the restriction patterns bands larger than 100 bp generated using *Hae*III, our results were similar to those observed for *Z. mobilis* subsp *mobilis* (Coton et al., 2005).

The banding patterns of the 16S-23S ISR of rDNA from *Z. mobilis* varied among strains. We observed one to five fragments with molecular weights ranging from 300 to 1000 bp (Figure 2A and B). These profiles were more variable than the ARDRA profiles.
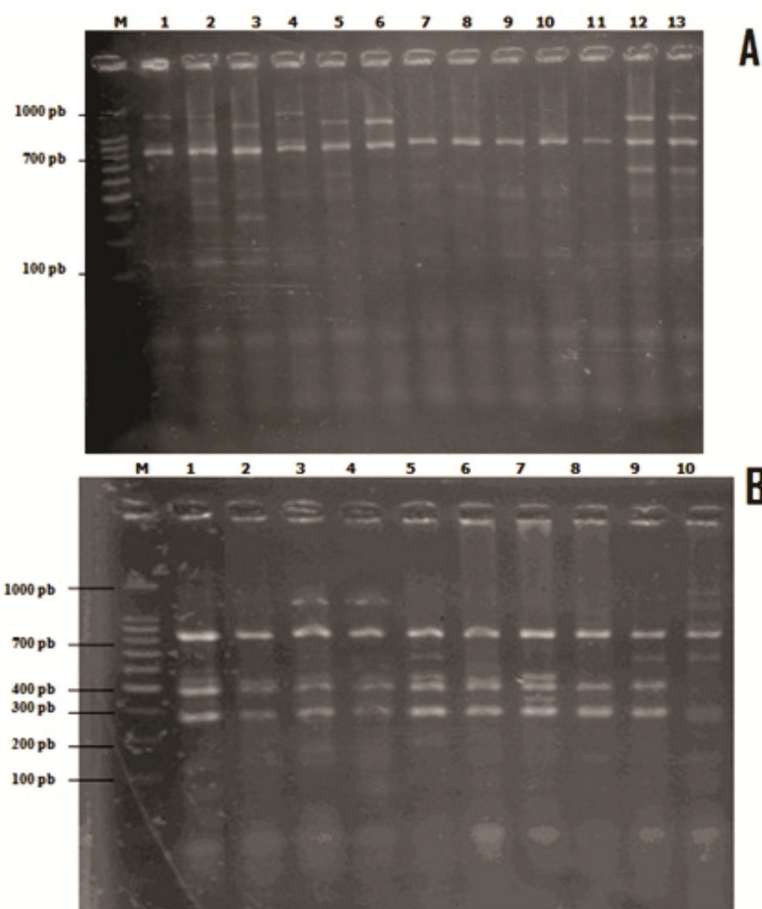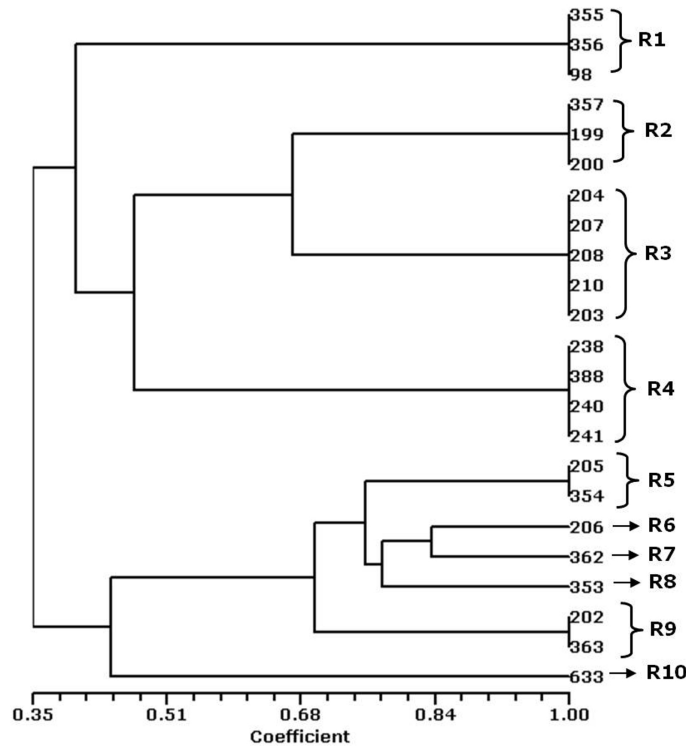

Figure 2. Amplification of the intergenic spacer region from *Zymomonas mobilis* UFPEDA strains. **A.** *Lane M* = 100-bp DNA ladder; *lane 1* = UFPEDA-355; *lane 2* = UFPEDA-356; *lane 3* = UFPEDA-357; *lane 4* = UFPEDA-98; *lane 5* = UFPEDA-199; *lane 6* = UFPEDA-200; *lane 7* = UFPEDA-203; *lane 8* = UFPEDA-204; *lane 9* = UFPEDA-207; *lane 10* = UFPEDA- 208; *lane 11* = UFPEDA- 210; *lane 12* = UFPEDA- 241; *lane 13* = UFPEDA-388. **B.** *Lane M* = 100-bp DNA ladder; *lane 1* = UFPEDA-205; *lane 2* = UFPEDA-206; *lane 3* = UFPEDA-240; *lane 4* = UFPEDA-241; *lane 5* = UFPEDA-353; *lane 6* = UFPEDA-354; *lane 7* = UFPEDA-362; *lane 8* = UFPEDA-363; *lane 9* = UFPEDA-633; *lane 10* = UFPEDA-202.

Ribotype genetic analysis

Lima (2002) and Lima et al. (2011) assessed the variability of the following strains of *Z. mobilis*: UFPEDA 202/CP4, UFPEDA 353/Z-1-86A, UFPEDA 354/ Z-1-86B, UFPEDA 355/Z-1-87, UFPEDA 356/Z-1-88, and UFPEDA 357/Z-2-88, using total protein analysis (SDS-PAGE), RAPD, and rep-PCR. In this study, analysis of total proteins (SDS-PAGE) showed no variation in banding pattern among strains, indicating that the proteins in these bacteria are fairly conserved, and that SDS-PAGE is insufficient for characterization of variability in these strains. In contrast, the techniques of RAPD and rep-PCR showed the genetic polymorphisms among these strains, and the level of similarity among strains was around 62%. In this study, ARDRA did not revel much genetic variability among strains in the UFPEDA collection, although at least one strain, UFPEDA 663/ZM4, was distinguished by ARDRA analysis. In contrast, the *16S-23S rDNA* ISR was variable among strains.

We built a dendrogram based on the amplification profiles of the ISR and ARDRA, which suggested that there are 10 ribotypes of *Z. mobilis* (Figure 3).

Figure 3. Dendrogram based on the amplification profiles of the intergenic spacer region and ARDRA, using the UPGMA clustering method and Jaccard's similarity coefficient. The ribotypes are labeled on the right.

The dendrogram revealed that there are two major groups that are 35% similar. The first group consisted of the R1, R2, R3, and R4 ribotypes and the second consisted of the R5, R6, R7, R9, and R10 ribotypes.

Moreover, the R4 ribotype members consist of spheroplast fusion strains between UFPEDA 205/ZAP, which belongs to the R5 group, and UFPEDA 98/AG11, which belongs to the R1 group. These studies were described by Calazans et al. (1989), in which strains of UFPEDA 205/ZAP (producing levan) and UFPEDA 98/AG11 (not producing levan) were used for spheroplast fusion. This fusion formed new ZAG lineages of *Z. mobilis* (represented in our analysis by the R4 group). The primary purpose of this experiment was to obtain clones that were tolerant to high temperatures (>38°C) and good producers of ethanol, such as UFPEDA 205/ZAP, and that do not produce levan, such as UFPEDA 98/AG11. The difference between the UFPEDA 205/ZAP, UFPEDA 98/AG11, and ZAG strains were already detected by Silva (2000).

In some collections of microorganisms, such as American Type Culture Collection, UFPEDA 202/CP4 and UFPEDA 633/ZM4 are considered to be the same. However, Yablonsky et al. (1988), who analyzed the plasmid profile of the UFPEDA 633/ZM4 and UFPEDA 202/CP4 strains, demonstrated that these strains have different plasmid profiles; UFPEDA 633/ZM4 has 3 plasmids (32.5, 34, and 40.5 kb) and UFPEDA 202/CP4 has 4 plasmids (31.5, 32.5, 33, and 35 kb). Our data also suggest that these strains are different, and that they belong to different ribotypes; UFPEDA 633/ZM4 belongs to the R10 group and UFPEDA 202/CP4 to the R9 group. Câmara et al. (2013) also found a difference between UFPEDA 633/ZM4 and UFPEDA 202/CP4. Recently, Kouvelis et al. (2014) described the difference between the UFPEDA 633/ZM4 and UFPEDA 202/CP4 genomes, and this is consistent with our data.

PCA demonstrated that UFPEDA 98/AG11 (R5, indicated with one arrow in Figure 4), UFPEDA 205/ZAP (R1, indicated with two arrows in Figure 4), and ZAG strains (R4, indicated with three arrows in Figure 4) do not form a consistent group. These data suggest that spheroplast fusion has increased genetic variability in the UFPEDA microorganism collection.

In addition, PCA indicated that the *Z. mobilis* UFPEDA collection includes a group of ribotypes, including R5, R6, R7, R8, R9, and R10 (indicated with a circle in Figure 4); these data suggest that it would be desirable to include new strains to improve the genetic variability of the collection.
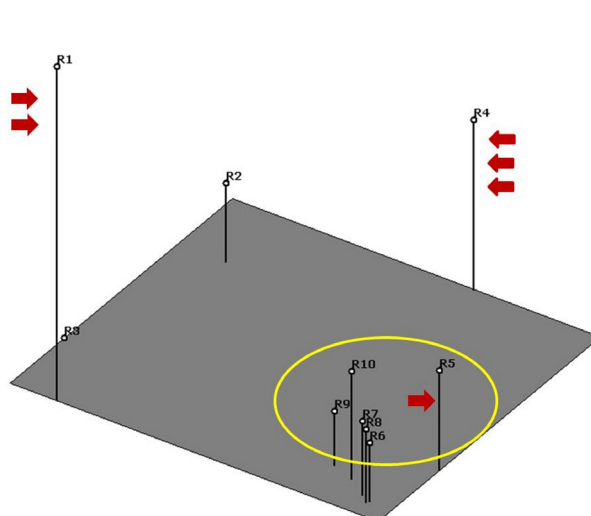
Figure 4. Principal coordinate analysis plot of Jaccard similarity values calculated from ribotype data. Note the positions of R5 (one arrow), R1 (two arrows), and R4 (three arrows). We also detected a group of related ribotypes: R5, R6, R7, R8, R9, and R10 (circled).

A technical analysis of the ribosomal DNA restriction digest (ARDRA) revealed a high degree of conservation in the *16S rDNA* gene of *Z. mobilis* strains of the UFPEDA Microorganisms Collection. The analysis of the amplification profile of the ISR *16S-23S rDNA* demonstrated the genetic variability of the collection and allowed subtyping of these lineages. It was possible to distribute the 24 strains analyzed among ten ribotypes, showing the genetic variation in these strains.

In addition, strains from spheroplast fusion between UFPEDA 98/AG11 and UFPEDA 205/ZAP increase the genetic variation of the UFPEDA Microorganisms Collection. We also observed differences between the UFPEDA 202/CP4 and UFPEDA 633/ZM4 strains, and these data also suggest that they are distinct strains.

ACKNOWLEDGMENTS

REFERENCES

Andrade MA (2008). Tipagem Molecular e Investigação dos Genes Toxigênicos em *Staphylococcus aureus* Isolados de Amostras Clínicas. Master's thesis, Centro de Pesquisa Aggeu Magalhães, Recife.

Calazans GMT, Rios EM, Falcão de Morais JO, Souza MFVQ, et al. (1989). Produção de etanol e levana por *Zymomonas mobilis* amostra ZAP, em meio de sacarose. *Arq. Biol. Tecnol.* 32: 631-636.

Canstein HF, Li Y, Felske A and Wagner-Dobler I (2001). Long-term stability of mercury-reducing microbial biofilm communities analyzed by 16S-23S rDNA interspacer region polymorphism. *Microb. Ecol.* 42: 624-634.

Câmara ALS, Silva LCN, Oliveira LMS, Araújo JM, et al. (2013). Variabilidade genética de linhagens de *Zymomonas mobilis* UFPEDA pelo sequenciamento do gene 16S rDNA. *Sci. Plena* 9: 1-10.

Conway T (1992). The Entner-Doudoroff pathway: history, physiology and molecular biology. *FEMS Microbiol. Rev.* 9: 1-27.

Coton M, Laplace JM and Coton E (2005). *Zymomonas mobilis* subspecies identification by amplified ribosomal DNA restriction analysis. *Lett. Appl. Microbiol.* 40: 152-157.

de Morais JO, Rios EM, Calazans GM and Lopes CE (1993). *Zymomonas mobilis* research in the Pernambuco Federal University. *J. Biotechnol.* 31: 75-91.

Ernandes FMPG and Garcia-Cruz CH (2009). *Zymomonas mobilis*: um microrganismo promissor para a fermentação alcoólica. *Cienc. Agrárias* 30: 361-380.

Ernandes FMPG and Garcia-Cruz CH (2011). Nutritional requirements of *Zymomonas mobilis* CCT 4494 for levan production. *Braz. Arch. Biol. Technol.* 54: 589-600.

Falcão de Morais JO, Araújo JM, Rios EM and Melo BR (1983). Isolamento de *Zymomonas mobilis* em mosto de caldo de cana de fermentações alcoólicas industriais. *Rev. Microbiol.* 14: 6-10.

Gonçalves de Lima O and Carmona L (1951). Aislamento y Propriedades Antagonistas de *Pseudomonas lindneri*. UNAM Congreso Científico Mexicano, Ciudad de México.

Gonçalves de Lima O, Schumacher IE and Araújo JM (1968). Novas observações sobre a ação antagonista de *Zymomonas mobilis* (Lindner) (1928), Kluyver e Van Niel, (1936). *Rev. Inst. Antibiot.* 8: 19-48.

Gonçalves de Lima O, Araújo JM, Schumacher IE and Cavalcanti da Silva E (1970). Estudos de microrganismos antagonistas presentes nas bebidas fermentadas usadas pelo povo do Recife. I. Sobre uma variedade de *Zymomonas mobilis* (Lindner) (1928). *Rev. Inst. Antibiot.* 10: 3-15.

Kang HL and Kang HS (1998). A physical map of the genome of ethanol fermentative bacterium *Zymomonas mobilis* ZM4 and localization of genes on the map. *Gene* 206: 223-228.

Kouvelis VN, Davenport KW, Brettin TS, Bruce D, et al. (2011). Genome sequence of the ethanol-producing *Zymomonas mobilis* subsp. *pomaceae* lectotype strain ATCC 29192. *J. Bacteriol.* 193: 5049-5050.

Kouvelis VN, Teshima H, Bruce D, Detter C, et al. (2014). Finished genome of *Zymomonas mobilis* subsp. *mobilis* strain CP4, an applied ethanol producer. *Genome Announc.* 2.

Lima GMS (2002). Ocorrência de Bacteriocina e Caracterização Molecular de Linhagens de *Zymomonas mobilis*. Master's thesis, Universidade Federal de Pernambuco, Recife.

Lima GMS, Souza LIO, Rosato YB and Araújo JM (2011). Detection of bacteriocins in *Zymomonas mobilis* and RAPD fingerprinting of the producer strains. *Afr. J. Pharm. Pharmacol.* 5: 2132-2139.

Lima OG (1958). Sobre uma interessante e pouco conhecida publicação de Paul Lindner acerca do emprego terapêutico de *Zymomonas mobilis* Kluyver e Van Niel, 1936 (*Pseudomonas lindneri*). *Rev. Inst. Antibiot.* 1: 119-124.

Pappas KM, Kouvelis VN, Saunders E, Brettin TS, et al. (2011). Genome sequence of the ethanol-producing *Zymomonas mobilis* subsp. *mobilis* lectotype strain ATCC 10988. *J. Bacteriol.* 193: 5051-5052.

Rohlf FJ (2008). NTSYSpc: Numerical Taxonomy System. Version 2.20. Exeter Publishing, Setauket.

Sá CBC (2012). Caracterização de Linhagens de *Saccharomyces cerevisae* e *Zymomonas mobilis* para Aplicação na Produção de Bioetanol. Master's thesis, Universidade Federal de Pernambuco, Recife.

Seo JS, Chong H, Park HS, Yoon KO, et al. (2005). The genome sequence of the ethanologenic bacterium *Zymomonas mobilis* ZM4. *Nat. Biotechnol.* 23: 63-68.

Silva JM (2000). Caracterização Molecular de Linhagens Selvagens de *Zymomonas mobilis* (Ag11 e ZAP) e suas Recombinantes. Master's thesis, Universidade Federal de Pernambuco, Recife.

Swings J and De Ley J (1977). The biology of *Zymomonas*. *Bacteriol. Rev.* 41: 1-46.

Weisburg WG, Barns SM, Pelletier DA and Lane DJ (1991). 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol.* 173: 697-703.

Yablonsky MD, Goodman AE, Stevnsborg N, Gonçalves de Lima O, et al. (1988). *Zymomonas mobilis* CP4: a clarification of strains via plasmid profile. *J. Biotechnol.* 9: 71-79.