POLYPHASIC APPROACH TO THE TAXONOMY OF THIRTEEN *Clostridium* STRAINS BY MEANS OF MULTIVARIATE DATA ANALYSIS: STRATEGY FOR DESCRIBING A NEW SPECIES

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Introduction

Polyphasic taxonomy is aiming at the integration of different kinds of data (i.e. phenotypic, genotypic and phylogenetic) on microorganisms and essentially indicates a consensus type of taxonomy (1,2). In this work *multivariate data analysis* was used as a tool for determining the taxonomy of thirteen solventogenic strains of Clostridia isolated from Colombian soils (3). This strategy was selected due to its ability for establishing relationships between individuals (strains) based on its characteristics (variables).

Materials and methods

1. Thirteen Colombian clostridia strains and ten reference strains were characterized by phenotyping (3) and genotyping techniques (4):

- Biochemical tests *
- Solvent production **
- Cellulolytic activity **
- 16S rRNA sequencing
- PFGE macro restriction profiles *
- AFLPs *
- DNA-DNA hybridization **

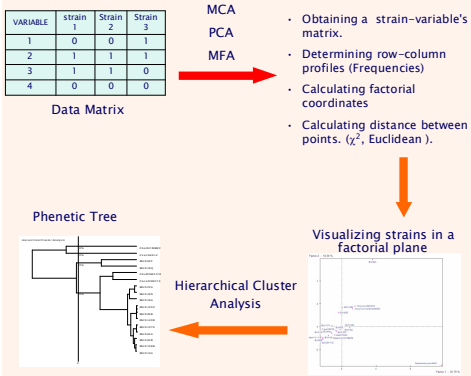
(*categorical data, ** numerical)

2.The data so obtained was then grouped into different sets according to the kind of variable (i.e. categorical or continuous).

3.Multiple correspondence analysis (MCA) was used for categorical variables whereas principal component analysis (PCA) was used for numerical variables, by using SPAD 5.6.2 software (Decisia, France).

4.Each analysis was followed by a hierarchical clustering analysis for visualizing the strains on a tree.

5.Multiple factor analysis (MFA) provided factorial coordinates; hierarchical cluster analysis was then applied to the combined set of data.



Results

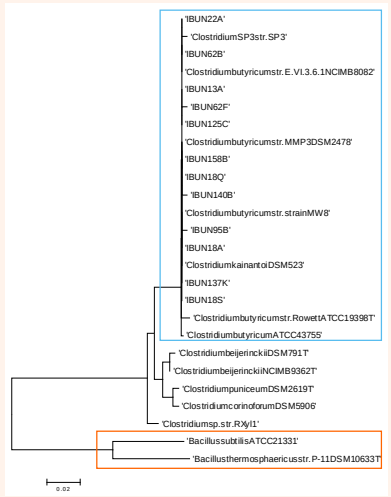


FIGURE 1.16SrRNA based phylogenetic dendogram obtained by maximum likelihood analysis. This criterion was used to include reference strains in multivariate data analysis

The results revealed a 10-native-strain-cluster clearly separated from reference strains in all types of analysis. This suggests that native strains may constitute a new species within the *Clostridium* genus. The strategy developed in this study may be the departure point for describing a new bacterial species having great biotechnological potential.

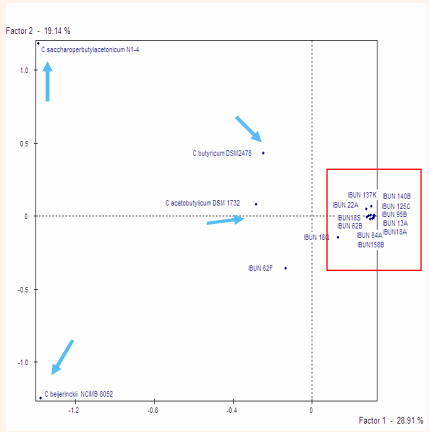


FIGURE 2. Factorial plane based on multiple correspondence analysis for AFLP-PFGE profiles. Note that Colombian strains (IBUN, red square) are clearly separated from reference strains included in this study (blue arrows).

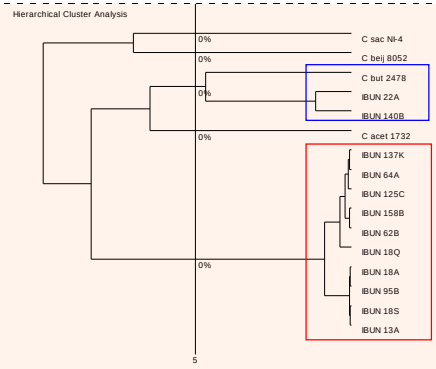


FIGURE 2. Hierarchical Cluster analysis Tree based on Multiple Factor analysis of the Molecular and Biochemical Variables considered (DNA-DNA hybridization, AFLP, PFGE and biochemical tests). Note that 10 Colombian strains (IBUN, red) are separately clustered from reference strains included. IBUN 22A and IBUN 140B were shown to be closely related to *C. butyricum*.

Conclusions

1.It was determined that 10 of the native strains (IBUN 125C, IBUN 158B, IBUN 137K, IBUN 64A, IBUN 18Q, IBUN 18A, IBUN95B, IBUN18S, IBUN 62B and IBUN 13 A) could be proposed as being a new specie from the Clostridium genus after comparing them to other solventogenic species from the genus.

2.IBUN 140B and IBUN 22A native strains were seen to be highly related to representative strains from the *C. butyricum* specie by virtue of the results arising from combining their phenotypical and genotypic characteristics.

Literature cited

1.Vandamme P *et al.* 1996. Microbiol .Mol .Biol. Rev. 60:407-438.

2.Roselló-Mora R & Amann R.2001.FEMS Microbiol. Rev. 25:39-67.

3.Montoya D. *et al.* 2000. J. Biotechnol.79:117-126

4.Montoya D *et al.* 2001.J. Ind. Microbiol. & Biotechnol. 27:309-335

Acknowledgments

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For further information:

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