Differentiation of *Kluyveromyces marxianus* strains isolated from Mezcal fermentations by MALDI-TOF MS

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Introduction

Since it development in the late 1980s, Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been applied for the determination of molecular masses of organic compounds. During the last decade, it has been shown that this technique possesses the capacity to identify microorganisms at the genus and specie levels directly from the biomass of isolated colonies generating a phenotypic profile or “molecular fingerprint”. MALDI TOF MS is rapidly gaining popularity for this purpose in microbial ecology studies due to its time and cost effectiveness as compared to morpho-physiological or other molecular biology based methods.

In the present work, the capacity of MALDI-TOF MS for the differentiation of isolates of *Kluyveromyces marxianus* from different mezcal production regions was evaluated. In order to exam the validity of this approach the resulting dendogram was compared with that obtained by rep-PCR, a commonly applied gene-based fingerprinting technique.

Material & Methods

Yeast Strains

19 strains of *K. marxianus* isolated from different mezcal fermentation processes (Figure 1) were used in this study. The identification of yeasts was performed by MALDI-TOF MS (Bruker Daltonics, Bremen, Germany) and 26S region sequence analysis.

DNA extraction

Pure colonies of each strain were grown in YPD Agar at 30 ºC for 18 hours. Genomic DNA of the samples was extracted using the protocol reported by Tapia-Tussell et al. (2006). The quality of the extracted DNA was analyzed on agarose gels (1% w/v) and quantified spectrofotometrically.

rep-PCR

Fingerprinting using the primers $(GTG)_5$ was realized according to Ramírez-Castrillón et al. (2014). The PCR products were separated on 1.5% (w/v) agarose geles run in 1X TAE buffer. Gels were stained with GelRed (Biotium) for visualization under UV light and digital image capturing was done using the Quantity one (BIO-RAD). The resulting fingerprints were analyzed using the software PhyElph (Pavel and Vasile. 2012).

Cluster analysis

The genetic similarity among all the isolates was estimated by measuring the proportion of identical bands of two isolates and assigning a value ranging from 0 to 1. The matrix generated was used to obtain an unweighted pair group method with arithmetic mean (UPGMA) dendogram.

MALDI-TOF

Protein extracts were generated from yeast cells according to the formic acid extraction protocol (Bruker Daltonics, Bremen, Germany). 1 µl of the supernatants was spotted in duplicate onto a MALDI target MSP 96 target polished steel plate (Bruker Daltonics, Bremen, Germany) and air-dried at room temperature. Then, each spot was overlaid with 1 µL of HCCA (α-cyano-4-hydroxy cinnamic acid) matrix solution and air-dried completely before measurement. The spectra were automatically acquired with the AutoXecute command using the MBT_FC run method. PCA dendogram clustering was carried out with the standard settings of MALDI Biotyper 3.0 (distance measure: correlation; linkage: average).

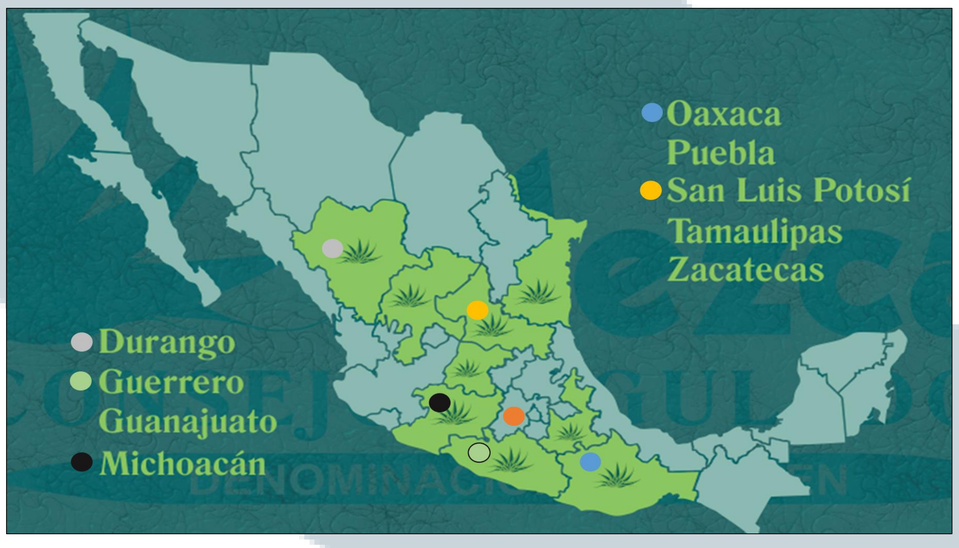

Figure 1: Mezcal production regions

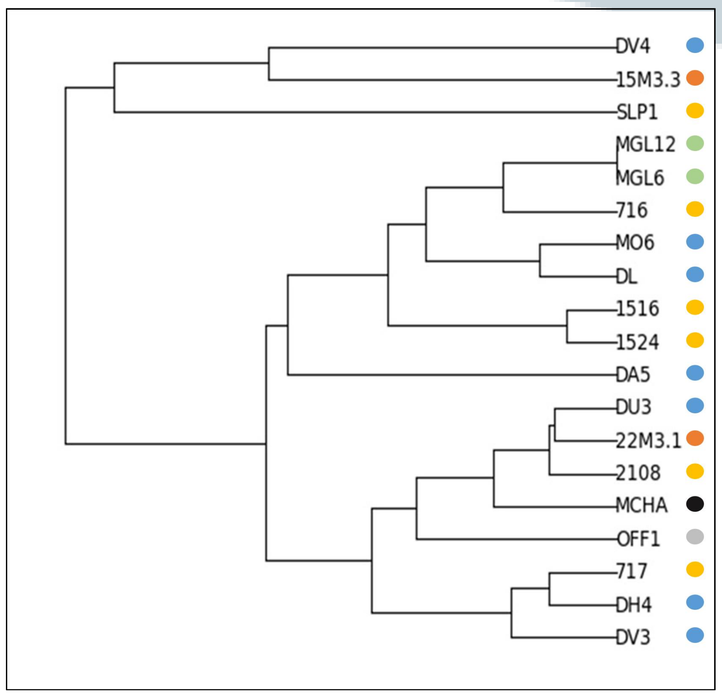

Figure 2: Dendogram based on rep-PCR

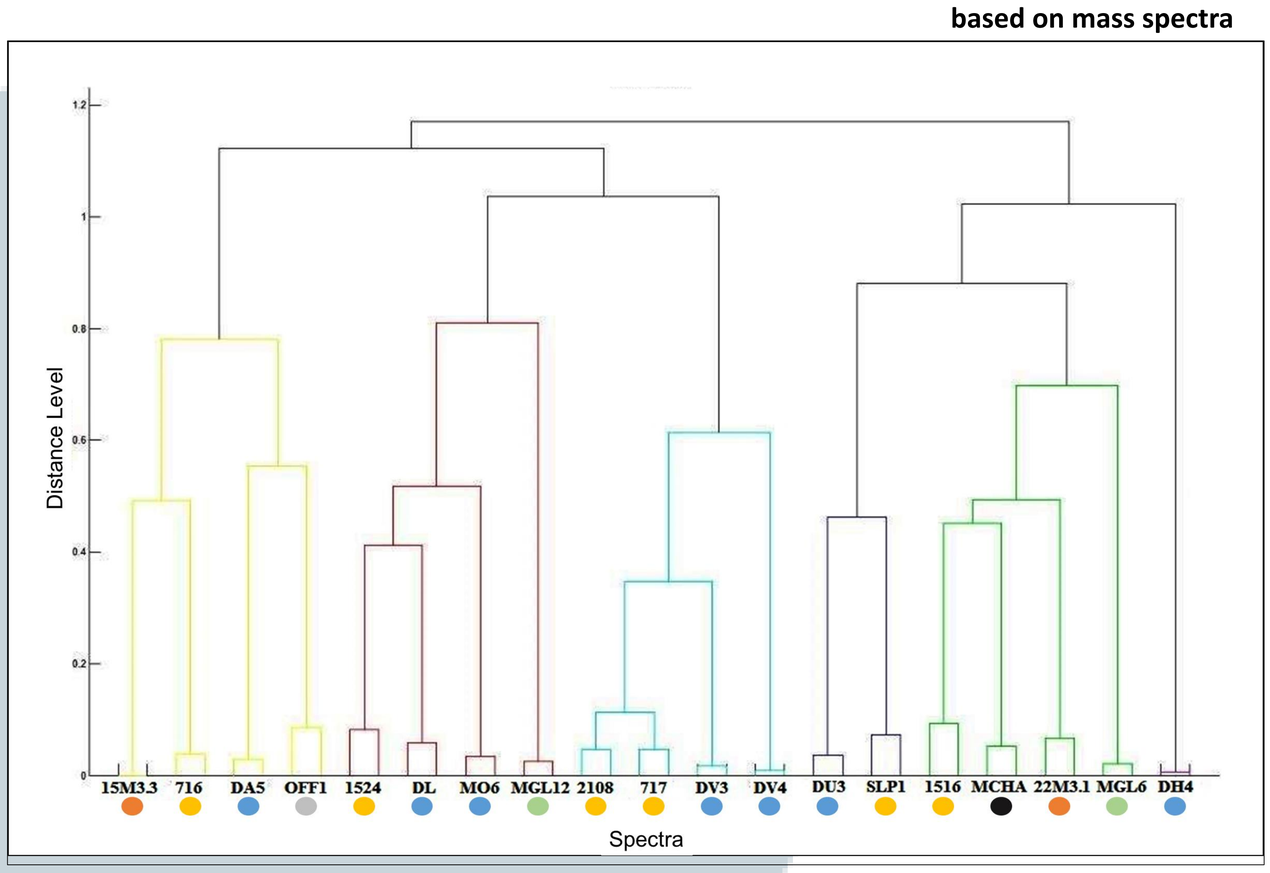

Figure 3: Dendogram based on mass spectra

Results:

The dendogram generated by rep-PCR grouped some *K. marxianus* strains which were isolated from the same production region as for example MGL6 and MGL12, isolated from Guerrero state, or 1516 and 1524, isolated from San Luis Potosi state. Nevertheless other strains from the same production regions were separated in different branches.

The dendogram obtained by MALDI-TOF MS showed similarities of mass spectra of strains 2108 and 717, isolated from San Luis Potosi state, but failed to group the strains based on production regions. When comparing both dendograms, clear differences where observed between both techniques.

Discussion:

Exist several reports discussing the succesfull use of MALDI-TOF MS as a rapid and reliable tool for the identification of yeasts and bacteria to the species level. On the strain level, however, only few reports stressed the capacity of this technique, differentiating for example between older and newer pathogenic *C. parapsilosis* strains (Pulcrano et al. 2012).

In the present study, both methods, rep-PCR and MALDI-TOF MS, did not achieve a clear differentiation between *K. marxianus* strains isolated from different Mezcal production regions, which could be explained by too little differences on the genetic or proteomic level between the analyzed strains from these processes.

Conclusion:

Both, rep-PCR and MALDI-TOF cluster analysis failed to group *K. marxianus* strains according to mezcal production regions. Other strains isolated from distinct fermentation processes and habitats should be included in the analysis in order to evaluate the use of MALDI-TOF MS for strain categorization rather than strain differentiation.

Aknowledgements:

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