



BIODIVERSITY OF YEASTS IN FERMENTATIONS OF AGAVE MUSTS

Anne Gschaedler

CIATEJ A.C., Av. Normalistas # 800, 44270 Guadalajara, Jalisco, Mexico
fax: 3333455245; e-mail: agschaedler@ciatej.net.mx

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Tequila, Mezcal, Bacanora, Sotol and Raicilla are the principal spirits obtained from different species of *Agave* in Mexico. The general stages of the process of production are: harvest of raw material, cooking, milling, fermentation, distillation and in some case maturation. The general process is similar for all of these beverages however some practices differ in each region or sometimes in each distillery. The raw material in some case is cultivated (*A. tequilana* for tequila o *A. angustifolia* for mezcal) or in others the plants are directly collected in the nature (*A. salmiana*, *A. cupreata*, *A. duranguensis*, *A. potatorum* for mezcal, *A. maximiliana* for raicilla).

The cooking process is carried out in rustic ovens, in brick ovens heated with steam injection or in steel autoclaves. In some parts of Guerrero and Michoacan the milling is still carried out with wood or steel mallets and the juice is collected in a "canao" (recipient made-up in the trunk of a tree). The most common method consists of a rudimentary mill (named "tahona") which is composed by a circular stone of about 1.5 m in diameter rotating in a circular pit where the cooked agave is placed. The stone in general is drawn by a horse. When the milling process is carried out with a "tahona", two different methods are used. In San Luis Potosi, during the milling process water is added and only juice is collected by gravity. In Oaxaca or Guerrero no water is added and all the crushed agave is used for the fermentation process. The mills used in the tequila industry are similar to those used in the sugarcane industry, which combine water extraction and milling. Whereas in the tequila industry most of the fermentation tanks are constructed in stainless steel in the mezcal, raicilla or bacanora distilleries a wide variety of designs of fermentation vessels are used.

All of these practices should have an important impact on the microbiota (particularly on the yeasts) present during the fermentation. In spontaneous fermentations the few studies available pointed out the great diversity of yeasts involved in the

fermentation process, and that *Saccharomyces cerevisiae* is not the only predominant yeast (like in wine), some non-*Saccharomyces* are still present at the end of the fermentation. Lachance (1995) found in tequila fermentation as dominant yeasts, *S. cerevisiae*, *Zygosaccharomyces bailii*, *Candida milleri* and *Brettanomyces anomala*, and secondary yeasts: *B. bruxellensis*, *Hanseniaspora guilliermondii*, *H. vineae*, *Pichia membranaefaciens*, *Torulaspora delbrueckii* and *Kluyveromyces marxianus*. In mezcal elaborated with *A. salmiana*, Escalante-Minakata et al. (2008) reported the identification of *K. marxianus*, *Pichia fermentans* and *Clavispora lusitaniae*. With this same raw material we found *S. cerevisiae*, *K. marxianus*, *P. kluyveri*, *S. exiguus*, *T. delbrueckii*, *Z. bailii*, *C. lusitaniae* and *C. ethanolica*. In mezcal obtained with *A. angustifolia* the biodiversity is higher, 23 different species where found in two distilleries. These species are: *S. cerevisiae*, *C. apicola*, *C. boidinii*, *C. parapsilosis*, *Citeromyces matritensis*, *Cryptococcus albidus*, *H. osmophila*, *Issatchenkia orientalis*, *K. marxianus*, *P. anomala*, *P. galeiformis*, *P. guilliermondii*, *P. kluyveri*, *P. membranaefaciens*, *Rhodospodium fluviale*, *Rhodotorula mucilaginosa*, *Schizosaccharomyces pombe*, *Sporidiobolus salmonicolor*, *T. delbrueckii*, *Trigonopsis sp.*, *Z. bailii*, *Z. bisporus*, *Z. rouxii*.

Presently, in some distilleries (mainly in tequila) the must is directly inoculated with fresh packages of baker's yeasts, with commercial dried yeast (originally prepared for wine, beer, or run production) or with a yeast strain (*S. cerevisiae*) isolated from a natural fermentation. This kind of practice is achieved mainly in order to improve the productivity, but the risk consists in the elimination of the non-*Saccharomyces* present in spontaneous fermentations. So it is important to study these artisanal fermentations in order to characterize the microbiota and to conserve these non-*Saccharomyces* strains which could have some new biotechnological applications.