PROTEOMIC APPROXIMATION TO POZOL FERMENTATION

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Introduction. Pozol is an acid fermented beverage of prehispanic origin which has survived time and sustained cultural change in Mexico (1). Pozol is consumed by the inhabitants of southeastern Mexico who consider it more than an important part of the daily diet, since it is used in traditional medicine and as a ceremonial element.
Pozol presents physicochemical features different from that of other food fermentation, such as a high content of starch in addition to low protein content. To understand this fermentation, many studies have been conducted mainly to describe the associated microbiota (2, 3, 4, 5). A wide variety of microorganisms have already been isolated: fungi, yeasts, lactic acid bacteria, and non-lactic acid bacteria but none has shown high amylolytic capacity. Given these results, we wondered how grows such a rich and abundant microbiota with so few free sugars and so poor amylolytic activity? a is otherwise the starch actually used?
The aim of this study was to obtain a proteomic view of the different groups of proteins within the pozol at different fermentation times, with the purpose of identify the enzymes that allow the development of the complex microbiota and to understand fermentation dynamics.

Methods. To achieve our goal we had to design a protein extraction method suitable for fermented maize dough. Two important difficulties has to been solve, the partial gelatinization of starch that form nets which encapsulate proteins and the high abundance proteins such as zeins that mask low-abundance proteins.
Finally we obtain a methodology to extract the metaproteome of pozol. Repeatability of the extraction was verified by analysis of spots in one and two dimensional electrophoresis. Sequencing was done by LC/MS/MS, and protein identification was done in NCBInr database using databases from: bacteria, fungi, yeast and green plants.

Results.

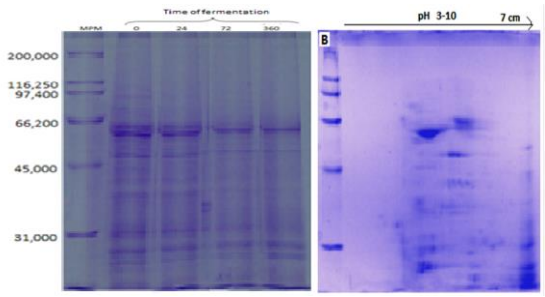

Fig.1 Left, SDS-PAGE gel of different time of fermentation pozol metaproteome. Right, 2-D gel of 24 h of fermentation

In pozol we identify the enzymes related to the utilization of starch and its derivates (Table 1). Several enzymes were identified specially glycoside-hydrolases involved in starch degradation and related oligosaccharide. Interestingly, several identified enzymes come from maize. These activities appear since the beginning of the fermentation suggesting that the grain amylases are responsible of drive the beginning of the fermentation. However, to have a complete knowledge of the system of fermentation it should be explore the use of other substrates.

Table 1. The following table shows the principal activities found in each time of fermentation

Enzyme activity \ Time of Fermentation	0	24	72	360
Catalytic/hydrolase *Zea mays*	X	X	X	X
Pullulanase-type starch debraching enzyme 1 *Zea mays*	X	X		
Starch branching enzyne IIb *Zea mays*	X	X	X	X
Amylose extenderstarch-branching enzyme *Zea mays*		X	X	X
Starch branching enzyme1 *Zea mays*	X			X
Glycosylhydrolase family 38 protein *Zea mays*		X		
Glycosil hydrolase family 28 *Zea mays*	X			
Beta-amylase *Zea mays*			X	

Conclusions.
The corn glycoside hydrolases are responsible for the initial release of soluble sugars which allow the beginning of fermentation.

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