IOCFE 2025 Conference
The 1st International Online Conference on Fermentation
12–13 November 2025 | Online
MDPI

Pulque Microbiota Core: Stability and Functional Role Across the Fermentation Process

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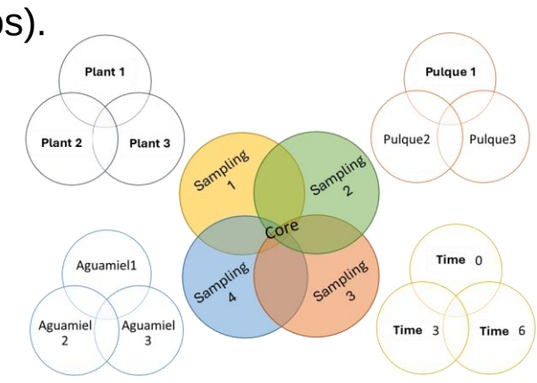
INTRODUCTION & AIM

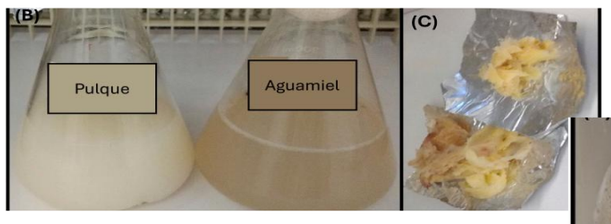
Pulque is a traditional Mexican beverage produced from the sap extracted from different Agave species. Previous studies on the microbial diversity associated with this beverage have applied various experimental strategies, including both culture-dependent and independent methodologies. Omics-based approaches have expanded our understanding of the microorganisms involved in pulque production, revealing novel species of bacteria, fungi, and even viruses.

In this study, we identified a core group of microorganisms that persisted throughout the entire pulque production process from the plant tissue of agaves to the final fermented product and evaluated the stability of this core over the course of one year in a single geographic region (Huitzilac, Morelos).



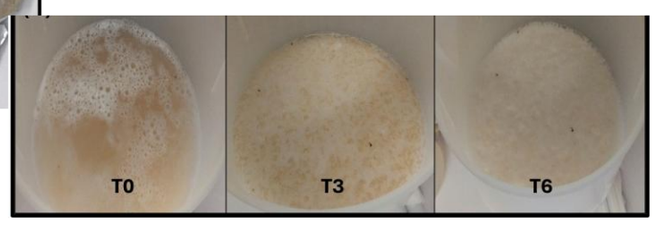
METHOD

A total of 90 biological samples were analyzed, including independent triplicates of plant tissue from metzal (obtained by scraping the fermentation cavity), aguamiel (sap), and commercial pulque, as well as three fermentation time points (T0, T3, T6). Of these, 18 samples were used to determine the core microbiota, while the remaining 72 were used to evaluate its temporal stability.



Pulque / Aguamiel Metzal

Fermentation times



Collection and type of samples vegetable tissues (metzal), (sap) aguamiel, pulque (24h ON) and fermentation times

Quantification using HPLC and UPLC techniques

Sucrose, fructose, glucose, ethanol, lactic acid and acetic acid

16S gene sequencing and ITS1

Libraries of the 16S gene regions V3-V4 and the ITS "ITS1"

Massive sequencing (NovaSeqP250)

Taxonomic identification

Establishment of the "core" of pulque

Analysis and discussion of results

RESULTS & DISCUSSION

Sampling season	Sample	pH	Sugar content (g/L)		
			Sucrose	Glucose	Fructose
May–Jun 2021	AM	4.5 ± 0.2	21.8 ± 13.4[c]	11.6 ± 13.8	13.6 ± 4.0
	PQ	3.9	0	6.7 ± 4.6	9.2 ± 3.9
Aug–Sep 2021	AM	5.2 ± 0.7	44.4 ± 10.8[c]	7.1 ± 17.7	8.4 ± 4.1
	PQ	4.0 ± 0.1	3.0 ± 2.4	8.0 ± 5.0	11.8 ± 3.3
Nov 2021	AM	4.9 ± 0.4	58.8 ± 20.5[c]	14.6 ± 25.1	8.4 ± 4.1[b]
	PQ	4.2 ± 0.1	0.5 ± 0.7	6.9 ± 3.8	11.8 ± 3.3
Jan–Feb 2022	AM	5.1 ± 0.4	85.0 ± 10.1[c]	36.4 ± 19.0	19.0 ± 6.0
	PQ	4.1 ± 0.1	0.6 ± 1.0	9.9 ± 5.2	9.4 ± 2.4

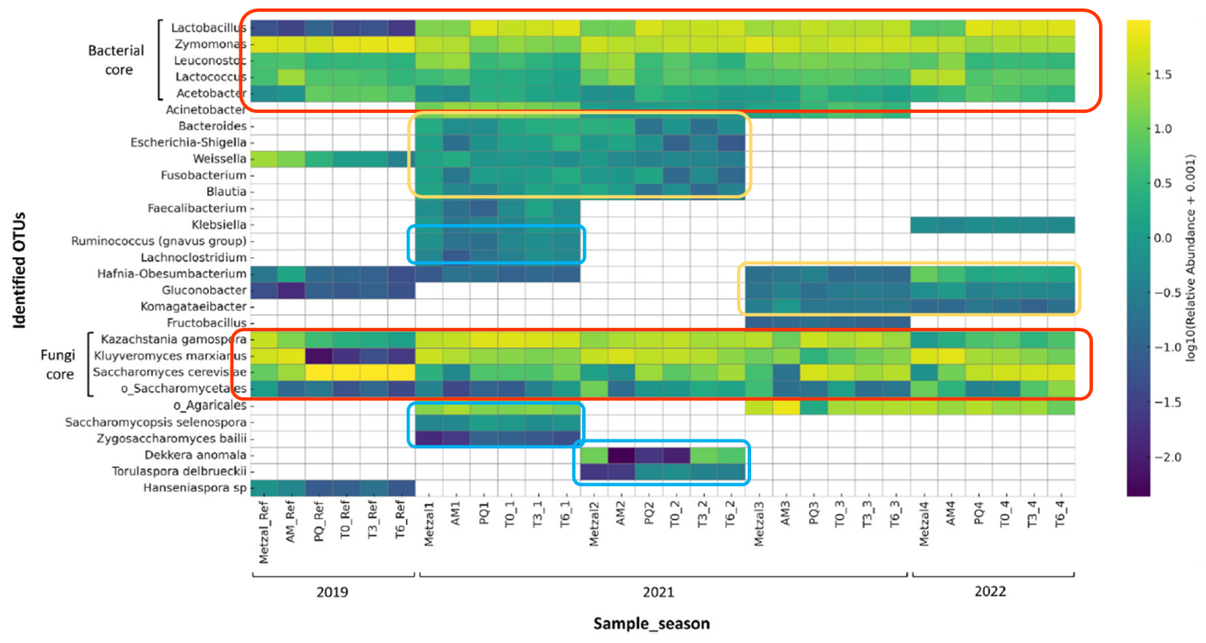
Sucrose was the most abundant sugar in Aguamiel samples.
Glucose and fructose concentration were around 14 g/L

Organic acids concentration less than 4 g/L.
Ethanol concentration was around 28 g/L.

Sampling season	Sample	Organic acid content (g/L)		
		Ethanol	Lactate	Acetate
May–Jun 2021	T0	12.2 ± 6.6	3.46 ± 0.8	1.4 ± 0.2
	T3	21.5 ± 6.7	3.88 ± 1	1.5 ± 0.2
	T6	17.1 ± 7.6	4.09 ± 1.1	1.6 ± 0.3
Aug–Sep 2021	T0	22.7 ± 3	2.83 ± 0.7	1.5 ± 0.6
	T3	24.4 ± 3.5	2.98 ± 0.7	1.5 ± 0.6
	T6	31.1 ± 6.9	3.26 ± 0.8	1.7 ± 0.7
Nov 2021	T0	25.8 ± 2.2	2.13 ± 0.1	1.1
	T3	31.4 ± 3.2	2.68 ± 0.2	1.4 ± 0.1
	T6	32.8 ± 2.6	2.58 ± 0.1	1.3
Jan–Feb 2022	T0	23.4 ± 6.6	2.66 ± 0.5	1.6
	T3	24.6 ± 5.7	2.81 ± 0.5	1.6 ± 0.1
	T6	28.7 ± 5.2	2.95 ± 0.4	1.8 ± 0.1

In the first phase of the analysis, the core microbiota present throughout the entire pulque production process was found to consist of eight bacterial and five fungal genera.

During the assessment of core stability, the loss of three bacterial genera and one fungal genus was observed relative to the original core, indicating a 70% stability rate.



CONCLUSION

These results demonstrate the existence of a microbial core that is maintained throughout the entire pulque production process from the walls of the fermentation cavity (cajete) in the *Agave* plant to the final product. This core exhibits at least 70% stability in terms of microbial presence, though abundance varies among samples..

FUTURE WORK / REFERENCES

Astudillo-Melgar, F., et al. (2023). Analysis of the microbial diversity and population dynamics during the pulque fermentation process. Fermentation, 9, 342. https://doi.org/10.3390/fermentation9040342.

Astudillo-Melgar, F., et al. (2025). Evaluating the temporal stability of the core microbiota in traditional Mexican pulque fermentation. *Food Bioscience*, *71*. https://doi.org/10.1016/j.fbio.2025.107306