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Standards of Teran oenology: control of malolactic fermentation

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WHICH MICROBES ARE IMPORTANT IN WINE PRODUCTION?

The main microorganisms in wine production are the yeasts, responsible for alcoholic fermentation, and the lactic acid bacteria responsible for malolactic fermentation, which is desirable as it leads to biological de-acidification, provides microbial stability and enhance aroma complexity.

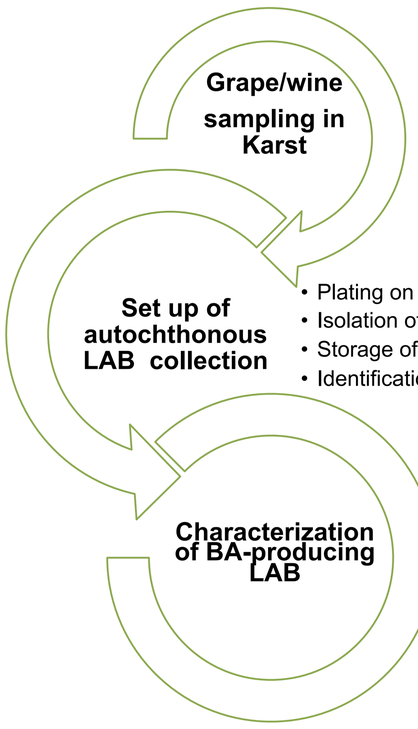
WHY IS IMPORTANT TO CONTROL MALOLACTIC FERMENTATION (MLF)?

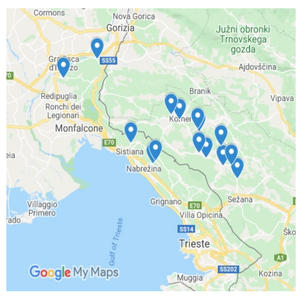
Lactic acid bacteria (LAB) can also form unwanted volatile compounds and biogenic amines (BA), such as histamine, which can cause disturbances in sensitive subjects (headache, gastrointestinal disorders…).

RESEARCH QUESTION & RELEVANCE

Since it is of primary importance for wine producers to end malolactic fermentation on time and to stabile the wine as soon as possible to prevent the formation of unwanted compounds, we controlled this process during the production phases (from grape to wine).

EXPERIMENTAL APPROACH



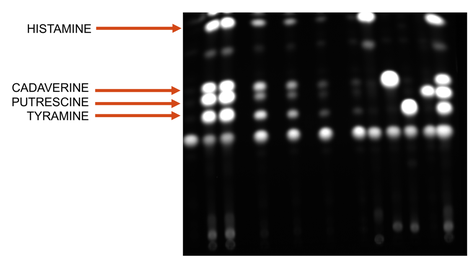


- Plating on MRS + 2% TJ + cycloheximide (pH 4.8)
- Isolation of pure LAB cultures
- Storage of cryo-cultures in 30% glycerol on -80 °C
- Identification (species-specific primers, 16S rRNA gene)

- decarboxylase screening medium (the plate/ microtiter plate procedure)
- Detection of biogenic amine genes by multiplex PCR
- Chromatographic analysis (HPLC), thin-layer chromatography (TLC)
- Enzymatic determination of histamine with horseradish peroxidase and diamine oxidase
- Determination of BA in sampled wine by HPLC analysis

BA gene distribution of the BA-positive strains on decarboxylase screening medium

		putrescin-positive		tyramin-positive	histamin-positive
		odc+	*odc+/agdi+*	*tyrdc+*	*hdc+*
2018	*O. oeni*	1	1	0	1
	other MLB	7	1	1	2
2019	MLB	17	9	4	8



TLC: Mixed standards of biogenic amines

EXPERIMENTAL RESULTS

LAB strain	Source	Decarboxylase screening medium AADM	PCR	TLC	HPLC
0006	Positive control, IOEB, France	/	*hdc+*	Histamine positive (HIS+)	HIS+
MKBT-282	Sampled in 2018, Karst		*odc+*	Putrescin positive (PUT+)	PUT+
MKBT-307	Sampled in 2018, Karst		*neg.*	n.d.	n.d.
MKBT-325	Sampled in 2018, Karst		*hdc+*	HIS+	HIS+
MKBT-523	Sampled in 2019, Karst	/	*hdc+*	HIS+/ Tyramin positive (TYR+)	HIS+/ TYR+

CONCLUSIONS & PERSPECTIVES

- Different methods (using differential screening medium, chromatography as HPLC and TLC, molecular methods as PCR multiplexs, enzymatic methods) were compared for biogenic amine (BA) determination in order to characterize native lactic acid bacteria from Karst region.
- Although biogenic amine-producing bacteria have been identified in both grapes and wine, we have not detected histamine in the wine itself. Our results show that Teran's quality and safety improved in comparison to previous years.
- During AGROTUR II collection of native lactic acid bacteria was set-up which could be used for future explotaion of LAB starters for using them wine production.

CREDITS, ACKNOWLEDGEMENTS & CONTACTS

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AGROTUR II web: http://www.agrotur2.si/it/; https://www.ita-slo.eu/it/AGROTURII CONTACT: Lorena Butinar. E-mail: lorena.butinar@ung.si

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