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Mohamed Zommiti, Mélyssa Cambrone, Olivier Maillot, Magalie Barreau, Khaled Sebei, et al.. Evaluation of probiotic properties and safety of *Enterococcus faecium* isolated from artisanal Tunisian meat ‘Dried Ossban’. SFM 2018, Oct 2018, Paris, France. 10.3389/fmicb.2018.01685 . hal-02362520

HAL Id: hal-02362520

<https://normandie-univ.hal.science/hal-02362520>

Submitted on 13 Nov 2019

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Evaluation of probiotic properties and safety of *Enterococcus faecium* isolated from artisanal Tunisian meat 'Dried Ossban'



Mohamed Zommiti¹, Mélyssa Cambrone², Olivier Maillot², Magalie Barreau², Khaled Sebei¹, Marc Feuilloley², Mounir Ferchichi¹, Nathalie Connil²



- Unité de Protéomique Fonctionnelle et Potentiel Nutraceutique de la Biodiversité de Tunisie, Institut Supérieur des Sciences Biologiques Appliquées de Tunis, Rue Z. Essafi, 1006 Tunis, Université Tunis el Manar, Tunisie.
- Laboratoire de Microbiologie Signaux et Microenvironnement (LMSM) EA 4312, Université de Rouen, 27000 Évreux, France.



INTRODUCTION

Contacts: mohamed.zommiti@hotmail.fr, nathalie.connil@univ-rouen.fr

Enterococci belong to the large group of lactic acid bacteria (LAB), the phylum of Firmicutes, and family of Enterococcaceae (de Vos et al. 2009). *Enterococcus* species are widely present in traditional fermented foods (Giraffa, 2002).

In this work, the lactic microbiota of "Dried Ossban" has been studied, and diverse biochemical approaches, molecular tools and cellular microbiology have been used to investigate the probiotic properties of the highly active isolates against *Listeria spp.* and *Enterococcus faecalis* ATCC 29212.

LAB strains that may be found in meat and meat products are mainly (Hugas, 1998): *Lactobacillus curvatus*, *L. sakei*, *L. casei*, *L. brevis*, *L. plantarum*, *Carnobacterium piscicola*, *C. divergens*, *Leuconostoc mesenteroides*, *Enterococcus faecium*, *Pediococcus pentosaceus*.

Numerous studies have been published on bacteriocin-producing enterococci in association with various food ecosystems including meat and meat products (Cintas et al. 1995), and these bacteria are increasingly investigated as potential probiotic candidates. However, there is not enough data to make any firm conclusions on the efficiency of enterococci as probiotics for humans and their use in foods must be based on case-by-case examinations (Ogier and Serror, 2008).

Keywords: *Enterococcus faecium*, Probiotics, Dried Ossban, Safety, Antibiotic Resistance, Virulence.

AIM of the STUDY

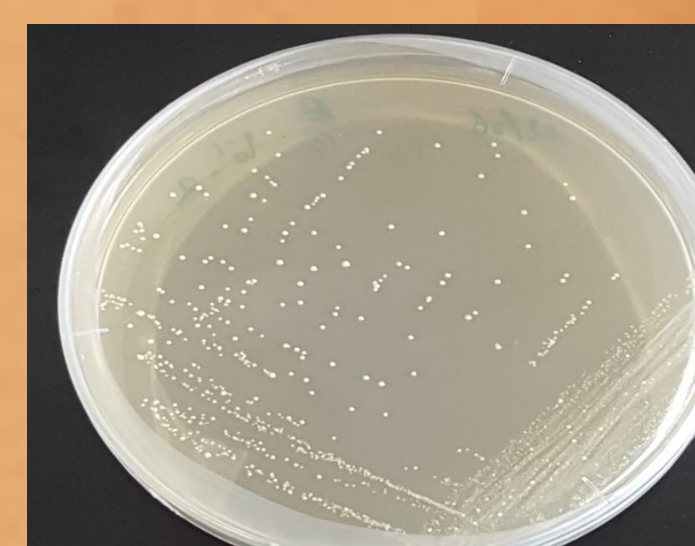
- ❖ Isolate and characterize the lactic microbiota of an artisanal Tunisian dried meat a.k.a « Dried Ossban ».
- ❖ Investigate the safety, antimicrobial properties and probiotic potential of five enterococci isolates.

MATERIALS and METHODS

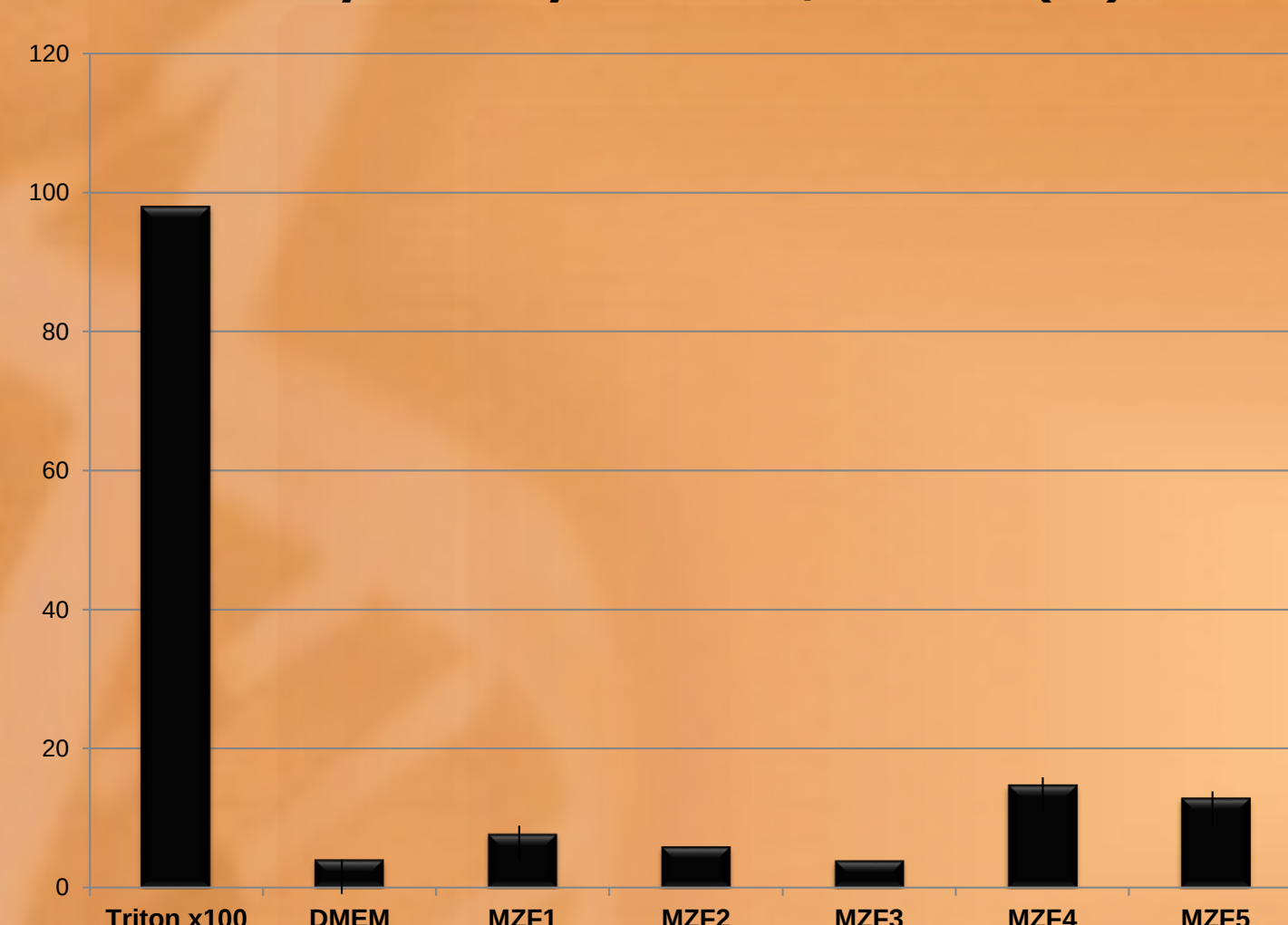
- Collection of about 40 «Dried Ossban» samples, covering all the Tunisian territory (LAB isolation on MRS agar 24h at 37°C).
- Identification of the enterococci by MALDI-TOF biotyper, and by PCR and sequencing of the 16S Ribosomal RNA gene.
- Characterization of the antimicrobial activity of five enterococci isolates (MZF1, MZF2, MZF3, MZF4 and MZF5) (Todorov and Dicks, 2005).
- Investigation of virulence genes and potential bacteriocins by PCR.
- Study of the safety aspect and the probiotic properties of the five strains.

RESULTS and DISCUSSION

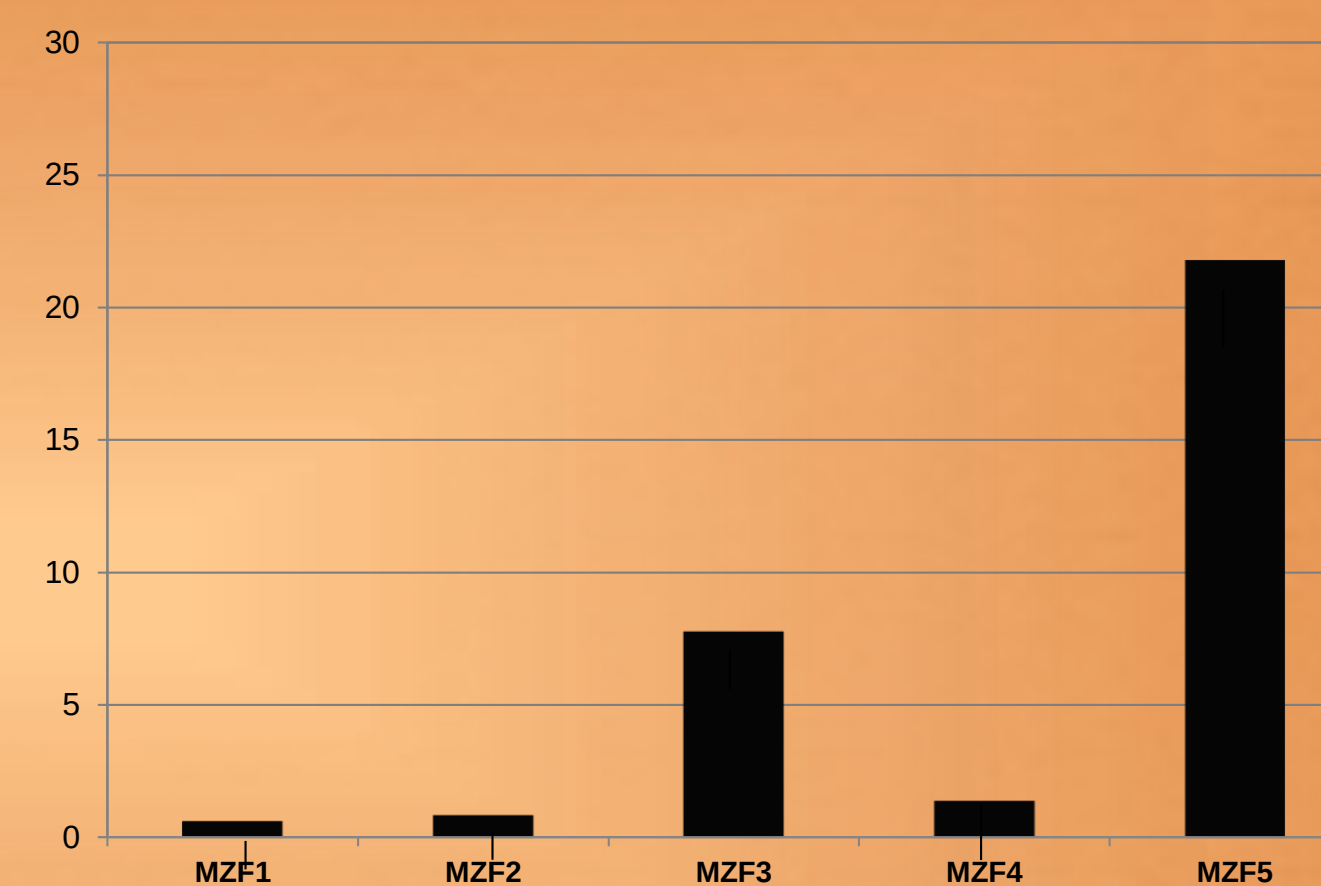
- Among 200 isolates of LAB, five enterococci were found to be highly active against *Listeria spp.* and *E. faecalis* ATCC 29212
- MALDI-TOF biotyper and sequencing of the 16S Ribosomal RNA gene → five *Enterococcus faecium* strains.
- The strains were susceptible to Vancomycin (Van), Ampicillin, Chloramphenicol and Tetracyclin.
- They were highly resistant to harsh conditions including acidity (pH 3) and bile salts (0.3% of Ox-bile).
- They were not cytotoxic towards the intestinal Caco-2/TC7 cells.



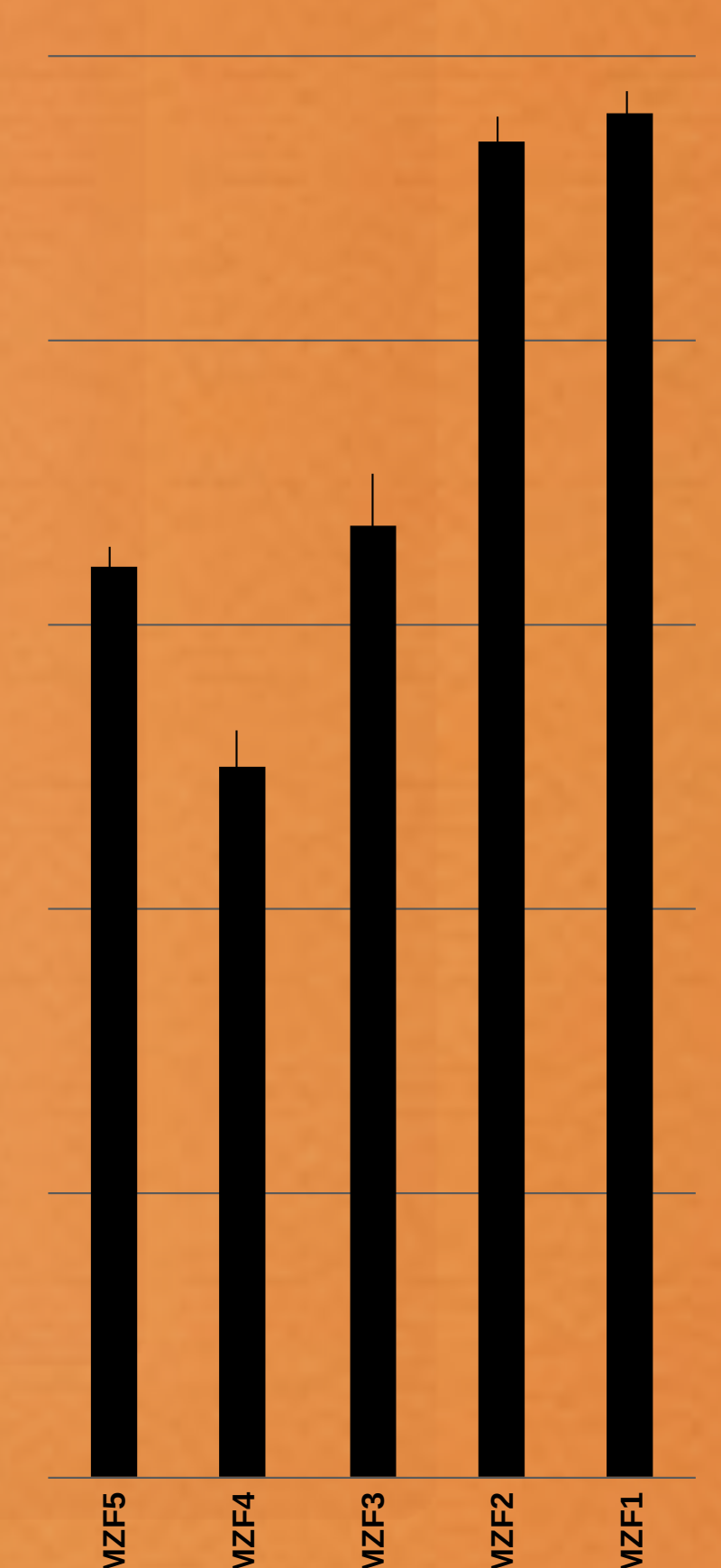
Cytotoxicity on Caco-2/TC7 cells (%)



Adhesion to Caco-2/TC7 cells (%)



Autoaggregation (%)



PCR detection of virulence and van resistance genes

<i>E. faecium</i> isolates	Genotype profile
MZF1	agg ⁺ gelE ⁺ esp ⁺ ace ⁻ vanA ⁻ vanB ⁻
MZF2	agg ⁻ gelE ⁻ esp ⁺ ace ⁻ vanA ⁻ vanB ⁻
MZF3	agg ⁻ gelE ⁻ esp ⁺ ace ⁻ vanA ⁻ vanB ⁻
MZF4	agg ⁻ gelE ⁺ esp ⁺ ace ⁻ vanA ⁻ vanB ⁻
MZF5	agg ⁻ gelE ⁻ esp ⁺ ace ⁻ vanA ⁻ vanB ⁻

PCR detection of the Enterocin A, B and P genes and anti-*Listeria spp* activity

Bacteriocin genes	MZF1	MZF2	MZF3	MZF4	MZF5
Enterocin A	+	+	-	+	+
Enterocin B	+	+	+	+	+
Enterocin P	+	+	-	+	+
Anti- <i>Listeria spp.</i> activity	+++	+++	+	+++	+++

Conclusion

* Enterococci in 'Dried Ossban' do not present a risk to human health and are interesting candidates for use as probiotic or protective culture.

** Further tests are needed to evaluate the technological characteristics of the five isolates and their safety profile before use in food fermentations.

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Résultats publiés dans Frontiers in Microbiology
Zommiti et al, 2018, Front Microbiol, 9:1685. doi: 10.3389/fmicb.2018.01685.