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Mohamed Zommiti, Emeline Bouffartigues, Olivier Maillot, Magalie Barreau, Sabine Szunerits, et al.. Evaluation of probiotic and bacteriocinogenic potential of *Pediococcus pentosaceus* MZF16 isolated from artisanal Tunisian meat "Dried Ossban". ANTIMIC 2019, Jul 2019, Lille, France. . <hal-02362460>

HAL Id: hal-02362460

<https://normandie-univ.hal.science/hal-02362460v1>

Submitted on 16 Nov 2019

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Evaluation of probiotic and bacteriocinogenic potential of *Pediococcus pentosaceus* MZF16 isolated from artisanal Tunisian meat “Dried Ossban”



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Introduction

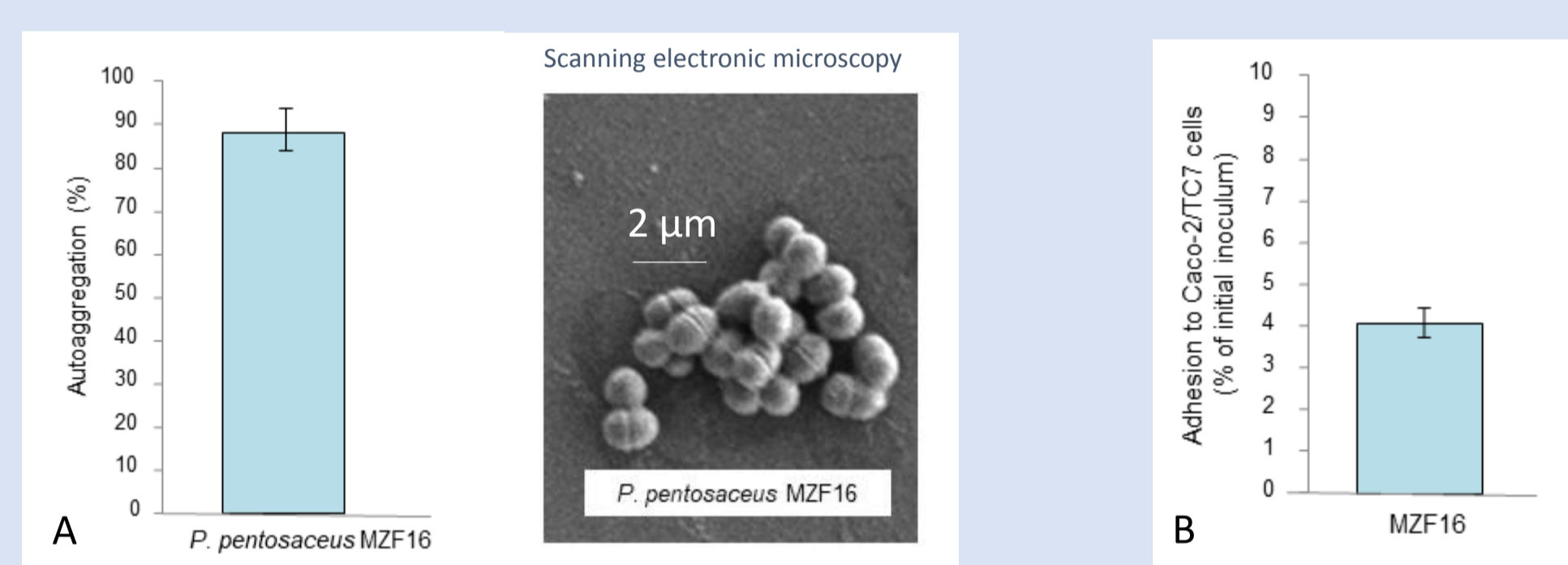
Thirty-seven samples of artisanal Tunisian meat “Dried Ossban” were collected from homemade production in different governorates of the Tunisian territory to isolate potential probiotics from the lactic acid flora (Zommiti et al, 2018). One of these isolates has been identified as *Pediococcus pentosaceus* MZF16, using MALDI-TOF mass spectrometry and 16SrDNA sequencing.

Pediococci are a group of coccus-shaped Gram positive lactic acid bacteria, frequently isolated from various food sources (ripened cheese, beverages, pickles, wine, dairy and meat products) and involved in the manufacturing of fermented foods. The best characterized species of this genus are *Pediococcus acidilactici* and *P. pentosaceus*.

Some strains of *Pediococcus* produce bacteriocins called pediocins, that are highly active against pathogenic bacteria. The most known pediocins are pediocin PA-1 from *P. acidilactici* PAC1.0 and pediocin Ach from *P. acidilactici* H, both belonging to the class IIa bacteriocins active against *Listeria monocytogenes*.

The aim of this study was to evaluate the probiotic properties and bacteriocinogenic potential of *P. pentosaceus* MZF16, a strain isolated from the original niche “Dried Ossban”, in order to find a new promising candidate for biopreservation of foods.

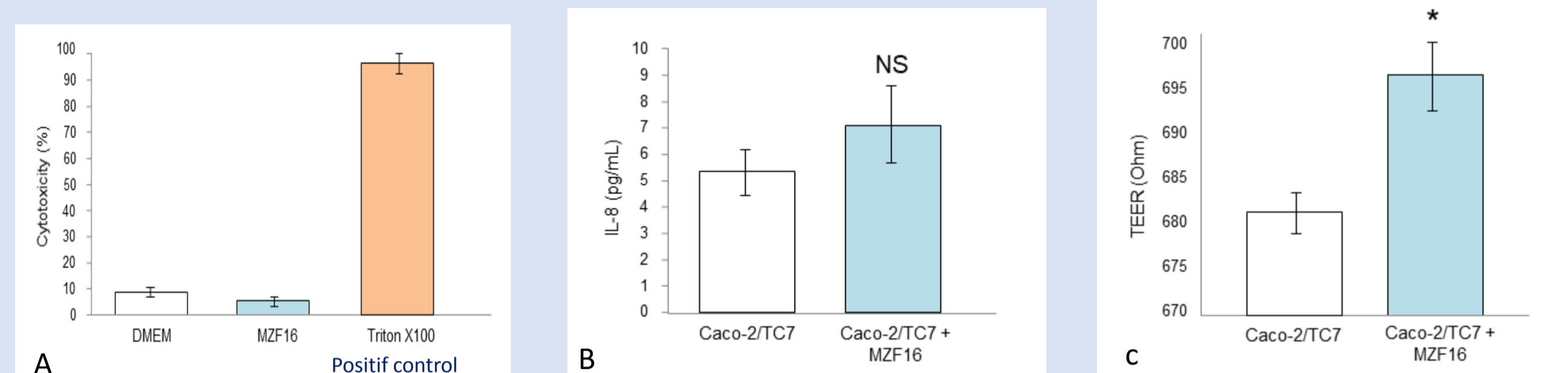
I) Autoaggregation and adhesion



A : MZF16 has a high level of autoaggregation potential (about 89%).
 B : MZF16 can adhere to the intestinal Caco-2/TC7 cells.

→ Capacity to colonize the gastrointestinal tract

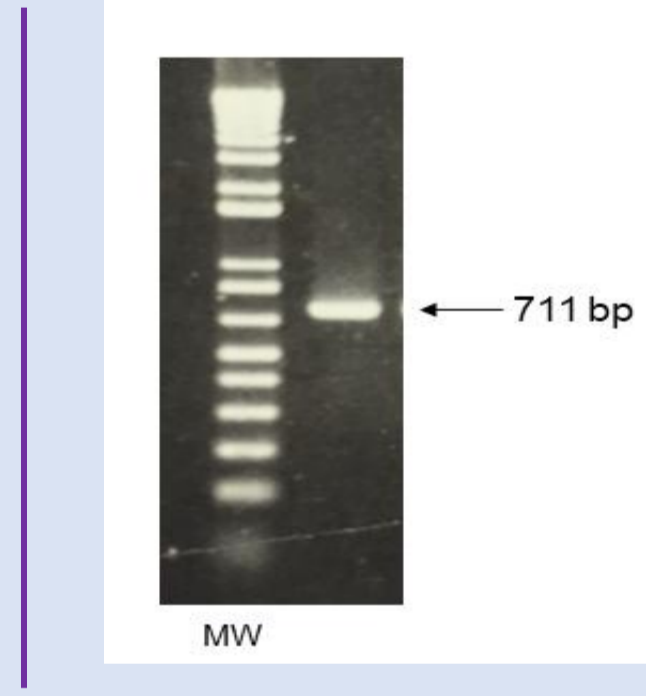
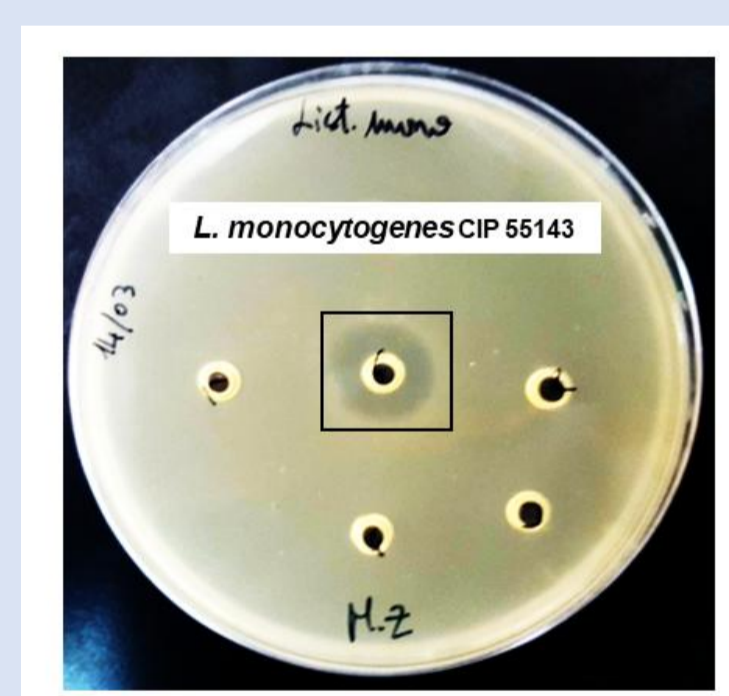
II) Cytotoxicity, inflammation and transepithelial resistance (TEER) analysis



A : MZF16 is not cytotoxic towards the intestinal Caco-2/TC7 cells.
 B : MZF16 does not induce an inflammatory response (no IL8 secretion).
 C : MZF16 can slightly enhance the intestinal barrier (↑ TEER) of differentiated Caco-2/TC7 cells.

→ Beneficial effects to the intestine

III) Antagonistic activity and characterization of MZF16 bacteriocin

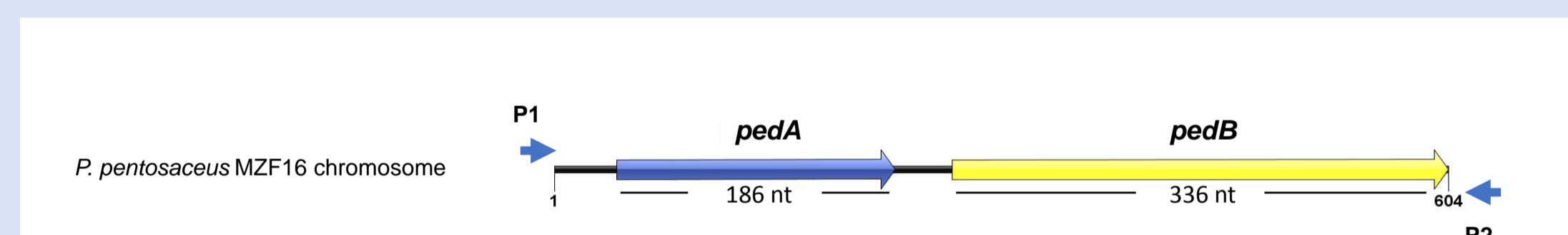


Pediocin MZF16 has been studied by PCR amplification using primers P1 and P2 designed to amplify the pediocin PA-1 operon of *P. acidilactici* PAC 1.0. (Marrug et al., 1992)

→ Sequencing of PCR product

MZF16 is active against *L. monocytogenes* CIP 55143. Activity was lost after treatment with proteinase K. The antimicrobial substance is bacteriocin in nature.

→ **Pediocin MZF16**



Sequencing of the PCR product showed a chromosomal organization similar to *P. acidilactici* with the presence of *pedA* gene encoding for the putative MZF16 pediocin, and *pedB* gene encoding for the putative immunity protein.

	Leader peptide	Pediocin
<i>P. acidilactici</i> PAC1.0	MKKIEKLTEKEMANIIGG	RY YGN GVTCGKHS ⁵⁵¹ CSVDWGKATT ⁵⁵² CIINNGAMAWATGGHQ ⁶⁰⁴ N ⁶⁰⁴ HKC
<i>P. pentosaceus</i> MZF16	MKKIEKLTEKEMANIIGG	RY YGN GVTCGKHS ⁵⁵¹ CSVDWGKATT ⁵⁵² CIINNGAMAWATGGHQ ⁶⁰⁴ T ⁶⁰⁴ HKC

=100% identity with coagulin A

Bioinformatic analysis (BlastX)

Peda ORF was translated to amino acid sequence using BlastX.

The putative MZF16 pediocin contains the **YGN**GV motif of class IIa bacteriocin, responsible for the anti-*Listeria* activity. MZF16 pediocin is 100% identical to coagulin A of *Bacillus coagulans*, and contains one mismatch compared to pediocin PA-1 from *P. acidilactici* PAC1.0 (T instead of N).

Conclusion

P. pentosaceus MZF16 passed successfully most of the *in vitro* tests recommended for probiotics by FAO/WHO. This strain produces a bacteriocin, the pediocin MZF16, which is the first pediocin present in a *Pediococcus* strain found to be 100% identical to the coagulin A of *Bacillus coagulans*. Due to this bacteriocin, MZF16 exhibited effective inhibition against the food spoilage and pathogenic bacterium *L. monocytogenes*.

According to these results, *P. pentosaceus* MZF16 can be proposed as a probiotic and bioprotective agent for fermented foods, including Tunisian dry meat and sausages. Further investigations will aim to study the behavior of this strain in meat products as a component of functional food.

References : Zommiti et al (2018), Front. Microbiol. 9, 1685.
 Marrug et al (1992), Appl. Environ. Microbiol. 58, 2360-2367.



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