

## BACTERIOCIN ASSOCIATED GENES IN FRENCH AND UKRAINIAN FERMENTED VEGETABLE ISOLATES OF *LACTIPLANTIBACILLUS PLANTARUM*

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### ABSTRACT

*Lactiplantibacillus plantarum* species is known for the high diversity of the loci encoding bacteriocin (plantaricin) production. The aim of this work was to study the distribution of *pln* genes in French and Ukrainian isolates of *L. plantarum* originated from fermented plant material. PCR with the primers to *plnA*, *plnB*, *plnC*, *plnD*, *plnEF*, *plnI*, *plnK*, *plnJ*, *plnG*, *plnN*, *plnW* genes was performed to find out the peculiarities of *L. plantarum* strains. French and Ukrainian isolates shared none of the identical combinations of genes from plantaricin regulon indicating the diversity of *pln* loci in *L. plantarum* of fermented plant origin from the distant geographical regions. Certain combinations of genes from plantaricin regulon could be proposed as indicators marking the geographical origin of *L. plantarum* strains. Among the studied isolates, some resembled the described in the literature *L. plantarum* strains, and others contained unique combinations of *pln* genes.

**Keywords:** *Lactiplantibacillus plantarum*, plantaricin, *pln* loci, diversity, geographical areas

### INTRODUCTION

*Lactiplantibacillus plantarum* species is characterized by the high diversity of the strains (Martino *et al.*, 2016; Choi *et al.*, 2018; Araujo *et al.*, 2020) including genetic organization of *pln* loci encoding bacteriocin production (Basa *et al.*, 2020; Choi *et al.*, 2021; Wu *et al.*, 2021). As the result, bacteriocins belong to the most variable sub-categories of secretome (Martino *et al.*, 2016). All *pln* loci are similar in the presence of a two-component regulatory system consisting of a histidine protein kinase and response regulators, in encoding inducible peptides, proteins of an ABC transport systems and some other products (Saenz *et al.*, 2009; Diep *et al.*, 2009). Synthesis of plantaricins is mediated by quorum-sensing (Kwak *et al.*, 2016).

Phylogenetic analysis of 54 *L. plantarum* strains from different geographical zones including 22 strains of plant origin, showed the presence of three groups encompassing strains with various combinations of *pln* genes (Choi *et al.*, 2021). Martino *et al.* (2016) analyzed draft and complete genomes of 54 *L. plantarum* strains and found that the strains with the same geographical origin could share similar orthologous groups in their genomes as it was described for *L. plantarum* isolated from Vietnamese fermented fruits, vegetables and pickled meat products. At the same time, no correlation between the habitat origin of *L. plantarum* strains (animal or plant organisms, products) and genetic specificity was found (Martino *et al.*, 2016; Choi *et al.*, 2018).

We have previously described the absence of association between geographical origin of the *L. plantarum* strains isolated from grape must and pickles in France and Ukraine by means of their RAPD-profiles (Limanska *et al.*, 2019).

The aim of this work was to study the distribution of *pln* genes in French and Ukrainian isolates of *L. plantarum*.

### MATERIAL AND METHODS

#### Bacterial strains

*L. plantarum* originated from grape collected in France (13 isolates) and from grape and pickles collected in Ukraine (17 isolates, Tab 1) were studied for the presence of *pln* genes.

**Table 1** Origin of *L. plantarum* strains

Strain	Source isolation	Country of origin
<i>L. plantarum</i> ONU 351, ONU 352, ONU 353, ONU 354, ONU 355, ONU 356, ONU 357, ONU 359, ONU 362, ONU 363, ONU 364, ONU 365, ONU 471	Grape must	France
<i>L. plantarum</i> ONU 12, ONU 311, ONU 312, ONU 313, ONU 333, ONU 335, ONU 337, ONU 339, ONU 340, ONU 342, ONU 345, ONU 348, ONU 349, ONU 350	Grape must	Ukraine
<i>L. plantarum</i> ONU 472, ONU 475	Sauerkraut	Ukraine
<i>L. plantarum</i> ONU 476	Fermented pickled tomatoes	Ukraine

#### PCR amplification of the genes from *pln* loci

DNA from overnight cultures of *L. plantarum* was isolated using Qiagen DNA purification kit (Germany). PCR was carried out with the primers to *plnA*, *plnB*, *plnC*, *plnD*, *plnEF*, *plnI*, *plnK*, *plnJ*, *plnG*, *plnN*, *plnW* genes as described and reviewed in Ben Omar *et al.* (2008) according to the wide distribution of these genes in fermented vegetable material (Ben Omar *et al.*, 2008; Rojo-Bezares *et al.*, 2008; Sáenz *et al.*, 2009; Diep *et al.*, 2009; Choi *et al.*, 2021).

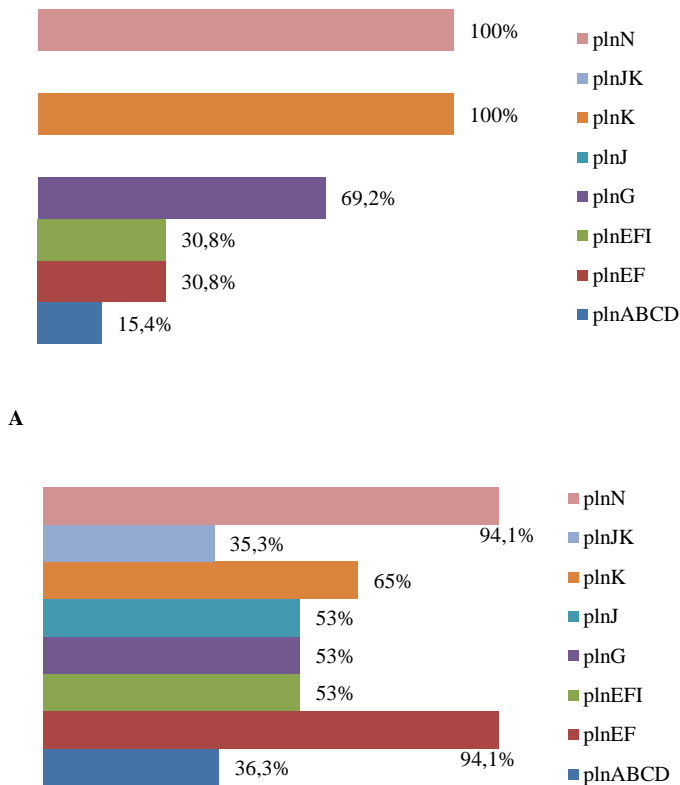
#### RESULTS AND DISCUSSION

Our results are in agreement with the data indicating that *pln* loci are widespread in oenological *L. plantarum* (Sáenz *et al.*, 2009; Knoll *et al.*, 2008). However, French and Ukrainian isolates shared none of the identical combinations of genes from plantaricin regulon indicating the diversity of *pln* loci in *L. plantarum* strains of fermented plant origin from distant geographical regions (Tab 2).

**Table 2** Presence of *pln* genes in genomes of the French and Ukrainian isolates of *L. plantarum*

Strains and their origin	<i>plnA</i>	<i>plnB</i>	<i>plnC</i>	<i>plnD</i>	<i>plnEF</i>	<i>plnG</i>	<i>plnI</i>	<i>plnJ</i>	<i>plnK</i>	<i>plnN</i>	<i>plnW</i>
<b>France</b>											
351, 352, 357, 471	-	+	-	+	-	-	+	-	+	+	-
353, 354	-	+	-	+	-	+	+	-	+	+	-
355	+	+	+	+	-	+	+	-	+	+	-
356, 365	-	+	+	+	-	+	+	-	+	+	-
359	-	+	-	+	+	+	+	-	+	+	-
362	+	+	-	+	+	+	+	-	+	+	-
363	-	+	+	+	+	+	+	-	+	+	-
364	+	+	+	+	+	+	+	-	+	+	-
<b>Ukraine</b>											
12, 311, 313	-	-	-	+	+	+	+	+	-	+	-
312	-	-	-	+	+	+	+	+	+	+	-
333	+	+	+	+	+	-	-	-	+	+	-
335	-	+	+	-	+	-	-	-	+	+	-
337	-	+	+	-	+	-	-	-	+	-	-
339	-	+	+	-	-	-	-	-	+	+	-
340, 472, 475, 476	-	+	+	+	+	-	-	-	+	+	-
342, 345, 348, 349, 350	+	+	+	+	+	+	+	+	+	+	-

Thus, 16 unique combinations of *pln* genes were found among French and Ukrainian isolates – 8 in each geographical group. Within each geographical group, 2-5 strains could share identical set of *pln* genes; however, no combinations were identical in *L. plantarum* from distant geographical areas. The complete *plnABCD* locus was detected in 15.4% of the French isolates, and in 36.3% of Ukrainian *L. plantarum* (Fig 1).



**Figure 1** Percentage of *L. plantarum* strains originated from France (a) and Ukraine (b)

The regulatory operon *plnABCD* encodes an inducing pheromone peptide PlnA, a histidine protein kinase PlnB and the response regulators PlnC and PlnD (Anderssen et al., 1998; Diep et al., 2009; Havarstein et al., 1995; Kleerebezem et al., 2003).

Our strain *L. plantarum* ONU 362 containing *plnA*, *plnB*, *plnD* genes but missing *plnC* by the combination of the studied genes was identical to *L. plantarum* EC80 strain from fermented maize described by Ben Omar et al. (2008).

Gene *plnN* indicating the presence of *plnMNOP* operon was found in 100% of French, and in 94.1% of Ukrainian strains (Fig 1).

Strains *L. plantarum* ONU 355 and ONU 364 from France and ONU 333, 340, 345, 348, 349 and ONU 350 from Ukraine contained the complete *plnABCD* operon and the gene *plnN* which allowed to characterize them as C11- and WCFS1-like strains. Strains WCFS1 and C11 with an exception of two genes are similar in *pln* loci (Diep et al., 2009; Kleerebezem et al., 2003). Sáenz et al. (2009) investigated strains isolated in Spain from must and wines for the presence of 27 *pln* genes and clustered them in groups with well described strains C11, WCFS1, J51, J23 and NC8. The group of the strains resembling C11 and WCFS1 in composition of *pln* regulon was the largest – 60.6%.

Strain *L. plantarum* ONU 337 with the absent *plnN* gene could resemble the strain *L. plantarum* J51 described in the literature (Diep et al., 2009; Navarro et al., 2008).

*Pln* E/F was found in 30.8% of the French strains and in 94.1% of Ukrainian isolates. Complete operon *pln* EFI was detected in 30.8% of the French isolates and 53% of the Ukrainian strains.

*plnK* was detected in 100% of French strains, and in 65% of Ukrainian isolates. Strains ONU 12, ONU 311, ONU 312 and ONU 313 could resemble the described *L. plantarum* J23 strain (Rojo-Bezares et al., 2008) by absence of *plnABCD* and *plnK*.

Gene *plnJ* was present in 53% of Ukrainian isolates, among them both genes of *plnJKLR* operon – *plnJ* and *plnK* – were found in 35.3%. In French isolates *plnJ* genes were absent (Fig 1). Loss of the sequences of some genes resulting in negative PCR result could probably happen due to integration of mobile genetic elements found in the *pln* clusters (Evanovich et al., 2019; Choi et al., 2021).

The absence of *plnJ* in ONU 333, 355 and 364 was unusual for the WCFS1 and C11 types of plantaricin regulons. The similar situation was described in Sáenz et al. (2009) for an oenological *L. plantarum* strain missing *plnJ* and moreover, missing *plnD* but with the similarity > 89% ranged in one group with C11 and WCFS1. Two strains from fermented vegetables isolated in Korea were also characterized by the presence of *plnK* and absence of *plnJ* (Choi et al., 2021).

Operons *plnEFI* and *plnJKLR* encode two-peptide bacteriocins, PlnEF and PlnJK and their immunity proteins (Anderssen et al., 1998; Diep et al., 2009; Havarstein et al., 1995; Kleerebezem et al., 2003). Among Ukrainian strains, 76.4% carried genes encoding two bacteriocins – *plnEF* and *plnJK*. Strains with the potential synthesis of two types of bacteriocins are more adaptable to the environment and indeed, they are more abundant among the strains of fermented food origin (Choi et al., 2021). Use of such strains is the most perspective for probiotic and food production. *plnG* from *plnGHSTUVW* operon was found in 69.2% of the French strains, and in 53% of Ukrainian isolates (Fig 1).

Five from all the tested *L. plantarum* strains – ONU 342, 345, 348, 349 and 350 (of Ukrainian origin) carried all the tested genes except the plantaricin W (Tab 2).

None of the studied French and Ukrainian strains encoded the *plnW*. The plantaricin W structural gene encoding two-peptide bacteriocin PLW was initially described for the strain *L. plantarum* LMG 2379 isolated from fermenting wine in the USA (Holo et al., 2001). Our results coincide with the previously reported rare occurrence of this gene in *L. plantarum* (Diep et al., 2009; Ben Omar et al., 2008), although strains encoding *plnW* were also described (Sogandi et al., 2019).

The results of our study describing various combinations of *pln* genes confirm the data of the previous investigators about the high genetic diversity of *L. plantarum* species (Saenz et al., 2009; Diep et al., 2009; Ben Omar et al., 2008; Sogandi et al., 2019).

Although we could not find differences between *L. plantarum* originated from distant geographical areas in our previous work based on RAPD-analysis (Limsanska et al., 2019), study of distribution of *pln* genes allowed us to distinguish the unique genetic combinations in French and Ukrainian isolates.

## CONCLUSION

French and Ukrainian strains of *L. plantarum* of plant origin shared no identical combinations of *pln* genes showing the high diversity of *pln* loci in isolates from distant geographical regions.

Certain combinations of genes from plantaricin regulon could be proposed as indicators marking the geographical origin of *L. plantarum* strains.

Among the studied isolates, some resembled those described in the literature for *L. plantarum* strains, while others encoded the unique combinations of *pln* genes.

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