GRASAS Y ACEITES, 58 (2),
ABRIL-JUNIO, 136-141, 2007,
ISSN: 0017-3495

Plasmid DNA studies in *Lactobacillus plantarum* strains isolated from olive fermentations: production of and immunity to plantaricin OL15 is associated to a 9.6 Kb plasmid (pOL15)

By **Kacem Mourad**

Département de Biotechnologie, Faculté des Sciences, Université d'Es-Senia,
Oran BP.1524 ORAN EL M'Naouar (31000), ORAN - Algérie
E-mail: mourad.kacem1@caramail.com

RESUMEN

Estudios de ADN plásmido en cepas de *Lactobacillus plantarum* aisladas de aceitunas fermentadas: La producción de y la inmunidad a plantaricina OL15 está asociada a un plásmido de 9.6 Kb (pOL15).

En un estudio previo, se aislaron 12 cepas de *Lactobacillus plantarum* a partir de aceitunas fermentadas. Entre ellas, solo *L. plantarum* OL15 produjo bacteriocinas (plantaricin OL15). En este estudio, se examinó el contenido de AND plásmido en las 12 cepas citadas. Entre ellas, 9 cepas han mostrado de una a tres bandas de plásmido con tamaños en el rango de 5.4 a 12.2 kb. *L. plantarum* OL15 exhibió un plásmido (9.6 kb) que se denominó pOL15. Después del curado con novobiocina y bromuro de etidio, la pérdida del plásmido pOL15 asociada a la pérdida de su facultad para producir plantaricin OL15, sugiere que la producción de plantaricina OL15 está ligada al plásmido. La plantaricin OL15 no se inactivó por amilasa ni por lipasa sugiriendo que su actividad no es dependiente de la presencia de carbohidratos o lípidos. La plantaricina OL15 mostró actividad frente a bacterias lácticas de diferentes especies y también frente a residuos de aceitunas y bacterias fitopatogénicas, incluyendo *Pseudomonas* y *Erwinia*

PALABRAS-CLAVE: Aceitunas – Agentes de curado – Bacteriocinas – Lactobacillus plantarum – Plásmido.

SUMMARY

Plasmid DNA studies in *Lactobacillus plantarum* strains isolated from olive fermentations: production of and immunity to plantaricin OL15 is associated to a 9.6 Kb plasmid (pOL15).

Previously 12 *Lactobacillus plantarum* strains were isolated from fermented olives. Among these, only *L. plantarum* OL15 produced bacteriocin (plantaricin OL15). In this study, the 12 strains were examined for plasmid DNA content. Of these, 9 strains have shown one to three plasmid bands ranging in size from 5.4 to 12.2 kb. *L. plantarum* OL15 exhibited one plasmid (9.6 kb) which was named pOL15. After curing with novobiocin and ethidium bromide, the plasmid profile analysis of non producing derivatives, showed that the 9.6 kb plasmid pOL15 harbored by the parental strain had been lost in all cases and none of them regained the ability to produce plantaricin OL15 suggesting that the production of plantaricin OL15 is plasmid linked. Plantaricin OL15 was not inactived by amylase and lipase suggesting that plantaricin OL15 activity was not dependent on the presence of either a carbohydrate or lipid moiety. Plantaricin OL15 showed activity against lactic acid bacteria of different species and also against olive spoilage and phytopathogenic bacteria, including *Pseudomonas* and *Erwinia*

KEY-WORDS: Bacteriocin – Curing agents – Lactobacillus plantarum – Olives – Plasmid.

1. INTRODUCTION

Lactobacillus plantarum is considered to be the most frequent and the most important specie in the process of lactic fermentation in olives (Jimenez-Diaz *et al.,* 1993 and Kacem and Karam, 2006). Some strains of this specie produce bacteriocins (Maldonado *et al*., 2002). Bacteriocins are proteins or protein complexes displaying a bactericidal mode of action towards sensitive closely related species (Klaenhammer, 1988).

Several reports of plasmid-associated bacteriocin production in lactic acid bacteria have been reported (Ray *et al*., 1989; and Mørtvedt and Nes, 1990). It is well known that the association between plasmids and bacteriocin production is generally, determined by the use of chemical curing agents, including novobiocin, ethidium bromide, acriflavin, and others, which eliminate plasmid (s) in the tested strains (Bringel *et al*., 1989 and Floriano *et al*., 1998).

In previous studies, *L. plantarum* strains were isolated from fermented green olives (Kacem *et al*., 2004). Only *L. plantarum* OL15 produced bacteriocin (plantaricin OL15) which showed a bactericidal mode of action towards Gram negative and positive bacteria (Kacem *et al.,* 2005). The aims of this work were to determine (*i*) the plasmid profiles of these *L. plantarum* strains, and (*ii*) whether the genetic determinant for plantaricin OL15 is plasmid associated.

2. MATERIALS AND METHODS

2.1. Bacterial strains and media

Lactobacillus plantarum strains used in this study (OL2, OL7, OL9, OL12, OL15, OL16, OL23, OL33, OL36, OL40, OL53 and OL54) were isolated

from fermented green olives by Kacem *et al.* (2004). *L. plantarum* OL15 is a bacteriocin (plantaricin OL15) producer strain (Kacem *et al.,* 2005). *Lactococcus lactis* BO8, also isolated from fermented green olives (Kacem *et al.,* 2004), was used as indicator strain for the evaluation of plantaricin OL15 activity.

The bacterial strains were maintained as a frozen stock at −20 °C in distilled water plus 20% (v/v) glycerol and propagated twice in Man Rogosa Sharpe (MRS) broth (Oxoid Ltd., UK) (de Man *et al.*, 1960) at 30°C before use.

2.2. Plasmid DNA isolation

Plasmid DNA was isolated based on the method described by Anderson and Mc Kay (1983). DNA preparations were analysed by electrophoresing (3.5 V cm^{-1} for 5 h) on 0.7% agarose gels. Plasmids size were estimated by using commercial covalently closed circular (CCC) DNA markers containing plasmid species of known molecular weigh (18.2, 14.2, 12.1, 10.1, 8.1, 4.0, and 3.0 Kb) (Bathesda Research Laboratory, Product No 5639, USA).

2.3. Plasmid curing

Novobiocin (Sigma, Mo, USA) and ethidium bromide (Sigma, Mo, USA) were used to cure the indigenous plasmid (s) of the wild-type *L. plantarum* OL15 according to Ruiz-Barba *et al*. (1991) and Floriano *et al*. (1998) methods with minor modifications.

Prior to curing, we determined the sublethal concentrations of the two curing agents for the *L. plantarum* OL15 strain. The sublethal concentration was defined as the highest concentration allowing for the detectable, albeit reduced, growth of a *L. plantarum* OL15 strain (OD_{600}=0.1-0.4). The sublethal concentrations of novobiocin and ethidium bromide for *L. plantarum* OL15 strain were found to be 1.8 µg/ml and 2.5 µg/ml, respectively. Overnight cultures (approximately 10^6cfu/ml) of the wild-type *L. plantarum* OL15 were inoculated into fresh MRS broth containing a sublethal concentration of the respective curing agent. At appropriate intervals (7, 14, and 21 days), the cultures were serially diluted, then, plated onto MRS agar to yield individual colonies. After 18 h at 30 °C, MRS soft agar (0.6% agar) containing the indicator strain *Lactococcus lactis* BO8 (10^5 cfu/ml) was poured onto the plates, which were incubated at 30 °C for 24 h.

Colonies without clear zones of inhibition, indicating loss of bacteriocin activity, were isolated on MRS agar and repeatedly transferred into MRS broth. Crude extracts (CE) from these liquid cultures were essayed for bacteriocin activity as described below. As controls, MRS broth cultures of *L. plantarum* OL15 that had not been treated with novobiocin and ethidium bromide as well as those treated cultures which didn't lack the antagonistic phenotype on MRS agar were processed at the same time. The immunity of the nonproducing variants to plantaricin OL15 was examined by spotting active *L. plantarum* OL15 MRS culture supernatants on lawns of these derivatives (Floriano *et al*., 1998). Both producing and nonproducing variants of *L. plantarum* OL15 were analysed for plasmid DNA content.

2.4. Bacteriocin assays

The producer and nonproducing variants of *L. plantarum* OL15 were propagated in 500 ml MRS broth for 24 h at 30 °C. Then, the culture extract samples, partial purification, ultrafiltration and titre determination of bacteriocin were done by the same methods described by Kacem *et al.* (2005) for plantaricin OL15. After these steps, the resulting material was referred to as "crude extract" (CE). The CE activity was determined by the agar well diffusion assay (Schillinger and Lücke, 1989). Pre-poured MRS agar plates were overlaid with 7 ml MRS soft agar containing 0.2 ml of indicator culture (*Lactococcus lactis* BO8). In order to standardize the assay, the inoculum was approximately 10^6 indicator cfu/ml. Wells of 5 mm in diameter were cutted into the agar plate by using a cork borer and aliquots of 50 µl from each CE solution samples were placed into the wells. Plates were incubated at 30 °C for 18 h, and examined for the presence of 1.5 mm or larger clear zones of inhibition around the wells. Each assay was performed in duplicate.

2.5. Search for bacteriocin spectrum

For the determination of the inhibition spectrum of CE, several lactic acid bacteria and non-lactic acid bacteria strains were used (Table 1) with the agar diffusion test, using the appropriate agar media and incubation conditions for their growth: lactococci, streptococci, enterococci and lactobacilli were tested in MRS agar at 30 °C for 18 h, *Propionibacterium* strains in YGL agar medium at 37 °C for 48 h, *Pseudomonas aeruginosa* in Brain Heart Infusion agar at 32 °C for 48 h, and *E. coli* and *Erwinia* in Nutrient agar at 37 °C for 3 days.

3. RESULTS AND DISCUSSION

In this study, 12 strains of *L. plantarum* from fermented olives were examined for plasmid DNA content. Of these, 9 strains have shown one to three plasmid bands ranging in size from approximately 5.4 to 12.2 Kb (Figure 1, lane 1 to 12). It was found that some strains showed the same plasmid DNA profiles. Among these, 3 strains (*L. plantarum* OL2, OL53 and OL54) showed two bands of similar size (5.4 and 11.4 Kb), 3 strains (*L. plantarum* OL16, OL33 and OL40) had three plasmids (5.4, 10.9 Kb and 12.2 Kb), *L. plantarum* OL12 and OL36 showed a single band of 5.4 Kb, while *L. plantarum* OL15 exhibited a quite distinct single band corresponding

Table 1
Antimicrobial spectrum of plantaricin OL15 crude extracts (CE) against a wide range of indicator strains.

Source	Indicator species	Strain	Diametre of[1] inhibition (mm)
Cow's milk (Kacem *et al*., 2003)	*L. lactis* subsp bv. *diacetylactis*	LVA24	12
	L. lactis subsp bv. *diacetylactis*	LVA8	13
	L. lactis subsp bv. *diacetylactis*	LVA9	9
	L lactis subsp bv. *diacetylactis*	LVA10	9
	L. lactis subsp bv. *diacetylactis*	LVA11	9
	L. lactis subsp. *lactis*	LVA7	11
	L. lactis subsp. *lactis*	LVA25	11
	L. lactis subsp. *lactis*	LVA26	12
Sheep's milk (Kacem *et al*., 2003)	*Lc. lactis* subsp. *cremoris*	BA8	15
	Lc. lactis subsp. *cremoris*	BA9	12
	Lc. lactis subsp. *cremoris*	BA10	15
	Sc. thermophilus	BA45	0
	Sc. thermophilus	BA46	0
	Enterococcus sp.	BA47	11
	Enterococcus sp.	BA48	12
Goat's milk (Kacem *et al.,* 2003	*Lc. lactis* subsp. bv.*diacetylactis*	LCH9	11
	Enterococcus sp.	LCH10	11
Fermented olives (Kacem *et al*., 2004)	*Enterococcus* sp.	0L32	0
	Enterococcus sp.	0L98	0
	L. plantarum	OL16	8
	L. plantarum	OL23	12
	L. plantarum	OL12	15
	L. plantarum	OL33	15
	L. plantarum	OL36	12
	L. plantarum	OL40	11
	L. plantarum	OL53	12
ATCC	*L. gasseri*	9857	0
	L. fermentum	4931	0
	L. fermentum	23271	0
	L.delbrueckii subsp. *delbrueckii*	9649	8
	L. delbrueckii subsp. *lactis*	4749	8
	L. jensenii	25258	0
	L. casei subsp. *casei*	27139	11
	L. acidophilus	4356	10
	E. coli	25922	0
	P. aeruginosa	10145	13
	S. aureus	25923	13
ATCC	*P. freudenriechii shermanii*	9619	13
	P. freudenriechii shermanii	1367	13
	P. freudenriechii shermanii	8262	13
ATCC	*E. chrysanthemi*	11663	11
CIP	*E. chrysanthemi*	82.99	12
NCPPB	*E. chrysanthemi*	402	11
NCPPB	*E. chrysanthemi*	2547	11
NCPPB	*E. chrysanthemi*	426	15
NCPPB	*E. chrysanthemi*	2541	11
PDDCC	*E. chrysanthemi*	5703a	11
LBMB*	*E. chrysanthemi*	M88	12
LBMB*	*E. chrysanthemi*	C23	12
LBMB*	*E. chrysanthemi*	C26	13

ATCC: American Type Culture Collection. 12301 Parklawn Drive, Rockville, Maryland 20852, EE.UU.
CIP: Collection of the Institut Pasteur. Rue du Dr. Roux. París 15 France.
NCPPB: National Collection of Plant Pathogenic Bacteria. Plant Pathology Laboratory, Hatching Green, Harpenden, England, U.K
PDDCC: Culture Collection of Plant Diseases Division. New Zealand Department of Scientific and Industrial Research, Auckland. New Zealand.
LBMB: Laboratoire de Biologie des Microorganismes et Biotechnologie, Faculté des Sciences, Université d'Oran, Algeria.
* : Our strains collection.
[1] : Inhibition essays were done according to the well agar diffusion test (Schillinger and Lücke, 1989)

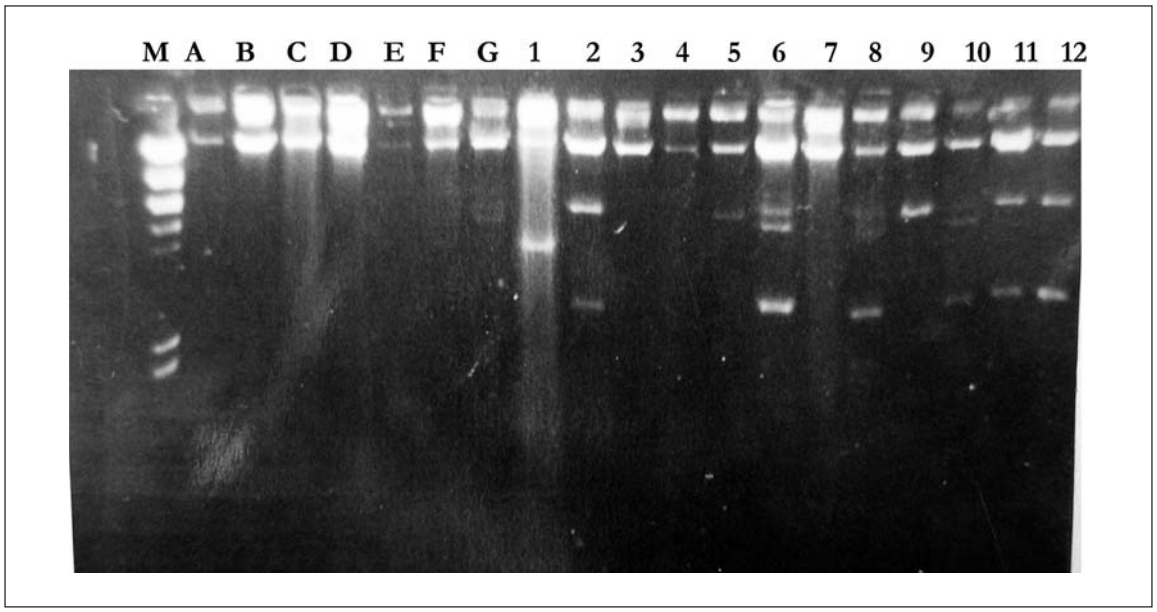

Figure 1
Gel electrophoresis. DNA preparations were analysed by electrophoresing on 0.7% agarose gels, stained with ethidium bromide and then photographed. M: Molecular weigh marker (18.2, 14.2, 12.1, 10.1, 8.1, 4.0 and 3.0 Kb), Lanes 1 to 12: *Lactobacillus plantarum* strains (OL15, OL2, OL7, OL9, OL12, OL16, OL23, OL33, OL36, OL40, OL53 and OL54), Lanes A to G: non producing derivatives from the wild type *L. plantarum* OL15 isolated after novobiocin and ethidium bromide treatment.

to 9.6 Kb which was named pOL15 (Figure 1, lane 1). In contrast, no plasmid DNA was observed in *L. plantarum* OL9, OL7 and OL23 strains.

Our results were in accordance with those of Ruiz-Barba *et al*. (1991) who showed that 35 *L. plantarum* strains, isolated from different green olive fermentors, contained a large number of plasmids in the CCC form (from 5 to 16) ranging from 2.0 to 68 kb in size. Xanthopoulos *et al.* (2000) also have found different plasmids in several *L. plantarum* strains isolated from Feta cheese. Delgado and Mayo (2003) have reported that *L. plantarum* LL441 strain harbours seven plasmids and produces a bacteriocin. Similar results were obtained by Kanatani and Oshimura (1994) with *L. plantarum* strains, isolated from fermented sausage. However, our results may not reflect the actual number of plasmids in our *L. plantarum* strains, as we know; plasmid DNA in lactic acid bacteria is not always easily detected. This may be due to growth temperature, copy number and isolation procedure (Casey and Jimeno, 1989).

L. plantarum OL15 was treated by combination of sublethal concentrations of novobiocin and ethidium bromide in attempts to free this strain of its plasmid. Of 632 treated colonies screened for bacteriocin production, 16 (2.5%) of them produced no zone of inhibition in the overlays containing the sensitive strain (*Lactococcus lactis* BO8). When these suspected non-bacteriocin (plantaricin OL15) producers were isolated on MRS agar and repeatedly subcultured in MRS broth (at least three transfers in MRS broth), none of them regained the ability to produce bacteriocin.

L. plantarum OL15 producer culture was not inhibited by its own CE, while all bacteriocin deficient derivatives cultures were sensitive to CE from the wild type *L. plantarum* OL15 (results not shown). This indicated the presence of an immunity mechanism.

Plasmid profile analysis of seven non producing derivatives, isolated after novobiocin and ethidium bromide treatment, showed that the 9.6 Kb plasmid pOL15 harbored by parental strain OL15 had been lost in all cases (Figure 1, lane A to G). This result indicates that the loss of the bacteriocin-producing trait could be correlated with a loss of the plasmid (pOL15), and therefore, that the bacteriocin produced by *L. plantarum* OL15 is plasmid encoded. This result is in agreement with those reported by other authors (Scherwitz *et al*., 1983 and Mørtvedt and Nes, 1990) who reported that bacteriocins production is plasmid-encoded. On the contrary, in other cases, bacteriocins production in *L. plantarum* strains has been linked to chromosomal DNA (Kato *et al*., 1994 and Franz *et al.,* 1998).

In order to further characterize plantaricin OL15 as described in Kacem *et al*. (2005), we tested the CE sensitivity (loss of activity) to another enzymes (lysozyme, ficin, proteinase K, α-amylase and lipase). The antimicrobial activity was inactived only with proteinase K, thus indicating his proteinaceous nature. The fact that the inhibitory compound was not inactivated by treatment with lipase or α-amylase, suggests that activity was not dependent on the presence of either a carbohydrate or lipid moiety.

Bacteriocin produced by *L. plantarum* OL15 exhibited similar biochemical properties to other bacteriocins produced by lactic acid bacteria, including producer strains isolated from fermenting olives such as *L. plantarum* LB17.2b (Delgado *et al*., 2001) or *L. plantarum* NC8 (Maldonado *et al*., 2003). On the other hand, plantaricin OL15 production differs from plantaricin B (West and Warner, 1988) and plantaricin F (Fricourt *et al.,* 1994) since this bacteriocin is produced either on solid medium or in liquid culture, while the latter two are produced on solid medium only. In addition, plantaricin OL15 differs from plantaricin B (West and Warner, 1988), as the later appears to require a carbohydrates moiety for activity. Plantaricin OL15 differs from plantaricin F because it is active at pH values of 3 to 8, whereas plantaricin F is active only at pH 4.5 or lower (Fricourt *et al.,* 1994). Plantaricin S is inactivated by lipolytic and glycolytic enzymes but not by proteinase K (Jimènez Diaz *et al.,* 1993), and thus differs in these characteristics from plantaricin OL15. Similar result was reported by (Gonzàlez *et al*., 1994) for plantaricin C.

Plantaricin OL15 (CE of *L. plantarum* OL15) showed inhibition against a wide range of lactic acid bacteria strains of different species and other non-lactic acid bacteria (Table 1). In addition, inhibitory activity was directed against the natural flora present in olive fermentations, including *L. plantarum* and *L. lactis* strains and also against olive spoilage and phytopathogenic bacteria organisms, including, *Pseudomonas*, *Erwinia*, and *Propionilbacterium*. This type of result was also described by Visser *et al.* (1986), Floriano *et al.* (1998) and Kacem *et al*. (2004).

In conclusion, further work will be necessary to test the efficiency of plantaricin OL15 against more undesirable bacteria in olive fermentation and in the biological control of phytopathogenic bacteria *in vivo*. Finally, further experiments concerning physical mapping of the plasmid pOL15 are in progress.

ACKNOWLEDGEMENTS

We are grateful to Pr J. B. Millier of the "Département de Génie Biologique Agro-Alimentaire, Nancy, France" for correction and critical reading of manuscript.

REFERENCES

Anderson DG, and McKay LL. 1983. Simple and rapid method for isolating large plasmid DNA from lactic streptococci. *Appl Environ Microbiol*. **46**, 549-552.

Bringel F, Frey L, Hubert JC. 1989 Characterization, cloning, curing, and distribution in lactic acid bacteria of pLP1, a plasmid from *Lactobacillus plantarum* CCM 1904 and its use in shuttle vector construction. *Plasmid*. **22**, 193-202.

Casey MG, Jimeno J.1989. *Lactobacillus delbruckii* subsp *lastis* plasmids. *Neth. Milk Dairy J.* **43**, 279-286.

De Man JC, Rogosa M, Sharpe EM. 1960. A medium for the cultivation of lactobacilli. *J. Appl. Bacteriol.* **23**, 130-135.

Delgado S, Mayo B. 2003. Development of *Lactobacillus plantarum* LL441 and its plasmid-cured derivatives in cheese. *J. Industr. Microbiol. Biotechnol*. **30**, 216-219.

Delgado A, Brito D, Fevereiro P, Marques JF. 2001. Antimicrobial activity of *L. plantarum*, isolated from a traditional lactic acid fermentation of table olives. *Lait* **81**, 203-215.

Floriano B, Ruiz-Barba JL, Jiménez-Diáz, R.1998. Purification and genetic characterization of enterocin I from *Enterococcus faecim* 6T1a, a novel antilisterial plasmid-encoded bacteriocin with does not belong to the pediocin family of bacteriocins. *Appl. Environ. Microbiol*. **64**, 4883-4890.

Franz CM, Du Toit M, Olasupo NA, Schillinger U, Holzapfel WH. 1998. Plantaricin D, a bacteriocin produced by *Lactobacillus plantarum* BFE 905 from ready-to-eat salad. *Lett. Appl. Microbiol.* **26**, 231-235.

Fricourt BV, Barefoot SF, Testin RF, Hayasaka SS. 1994. Detection and activity of plantaricin F, an antibacterial substance from *Lactobacillus plantarum* BF001 isolated from processed channel catfish. *J. Food Protect.* **57**, 698-702.

Gonzàlez BA, Mayo P, Suarez JE. 1994. Detection, purification and partial characterization of plantaricin C, a bacteriocin produced by *Lactobacillus plantarum* strain of dairy origin. *Appl. Environ. Microbiol.* **60**, 2158-2163.

Jiménez-Diáz, R, Rios-Sanchez RM, Desmazeaud M, Ruiz-Barba JL, Piard J. 1993. Plantaricins S and T, two new bacteriocins produced by *Lactobacillus plantarum* LPCO10 isolated from a green olive fermentation. *Appl. Environ. Microbiol.* **59**, 1416-1424.

Kacem M. and Karam N. 2006. Microbiological study of naturally fermented Algerian green olives: isolation and identification of lactic acid bacteria and yeasts along with the effects of brine solutions obtained at the end of olive fermentation on *Lactobacillus plantarum* growth. *Grasas Aceites* **57**, 292-300.

Kacem M., Zadi-Karam H. and Karam N. 2005. Detection and activity of plantaricin OL15, a bacteriocin produced by *Lactobacillus plantarum* isolated from Algerian fermented olives. *Grasas Aceites* **56**, 192-197.

Kacem M, Zadi-Karam H, Karam N. 2004. Isolation of lactic acid bacteria for its possible use in the fermentation of green algerian olives. *Grasas Aceites* **55**, 385-393.

Kacem M, Zadi-Karam H, Karam N. 2003. Lactic acid bacteria of western Algeria. Characteristics of strains isolated from raw milk and olive oil, *Sci. Technol.* (Algeria), **20**, 45-50.

Kanatani K, Oshimura M.1994 Plasmid-associated bacteriocin production by a *Lactobacillus plantarum* strain. *Biosci. Biotechnol. Biochem*. **58**, 2084-2089.

Kato T, Matsuda T, Ogawa E, Ogawa H, Kato H, Doi U and Nakamura R. 1994. Plantaricin-149, a bacteriocin produced by *L. plantarum* NRIC 149. *J. Ferment. Bioeng.* **77**, 277-282.

Klaenhammer TR. 1988. Bacteriocins of lactic acid bacteria. *Biochimie* **70**, 337-349.

Maldonado A, Ruiz-Barba, JL, Jiménez-Díaz R. 2003. Production of plantaricin NC8 by *Lactobacillus plantarum* NC8 is induced in the presence of different types of gram-positive bacteria. *Arch. Microbiol.* **181**, 8-16.

Maldonado A, Ruiz-Barba JL, Floriano B, Jiménez-Díaz R. 2002. The locus responsible for production of plantaricin S, a class IIb bacteriocin produced by *Lacto-*

bacillus plantarum LPCO10, is widely distributed among wild-type *L. plantarum* strains isolated from olive fermentations. *Int. J. Food Microbiol.* **77**, 117-124.

Mørtvedt CI, Nes TE. 1990. Plasmid-associated bacteriocin production by a *Lactobacillus sake* strain. *J. Gener. Microbiol*. **136**, 1601-1607.

Ray SS, Johnson MC, Ray B. 1989. Bacteriocin plasmids of *Pediococcus acidilactici*. *J. Ind. Microbiol.* **4**, 163-171.

Ruiz-Barba J L, Piard JC, Jimenez-Diaz R. 1991. Plasmid profiles and curing of plasmids in *Lactobacillus plantarum* strains isolated from green olive fermentations. *J. Appl. Bacteriol*. **71**, 417-421.

Scherwitz KM, Baldwin KA, Mckay LL. 1983. Plasmids linkage of bacteriocin-like substance in *Streptococcus lactis* subsp. *diacetylactis* strain WM4: transferability to *Streptococcus lactis*. *Appl. Environ. Microbiol.* **45**, 1506-1512.

Schillinger U, Lüke FK. 1989. Antibacterial activity of *Lactobacillus sake* isolated from meat. *Appl. Environ. Microbiol.* **55**, 1901-1906.

Visser R, Holzapfel WH. Bezuidenhout J. J and Kotze J. M. 1986. Antagonism of lactic acid bacteria against phytopathogenic bacteria. *Appl. Environ. Microbiol*. **52**, 552-555.

West CA, Warner JP. 1988. Plantaricin B, a bacteriocin produced by *Lactobacillus plantarum*. NCDO1193. FEMS *Microbiol. Lett.* **49**, 163-165.

Xanthopoulos V, Hatzikamari M, Adamidis T, Tsakalidou E, Tzanetakis N, Litopoulou-Tzanetaki E. 2000. Heterogeneity of *Lactobacillus plantarum* isolates from Feta cheese throughout ripening. *J. Appl. Microbiol*. **88**, 1056-1064.

Recibido: 12/12/05
Aceptado: 25/1/07