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Antimicrobial activities of *Rhizobium* sp. strains against *Pseudomonas savastanoi*, the agent responsible for the olive knot disease in Algeria

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RESUMEN

Actividades antimicrobianas de cepas de *Rhizobium sp.* contra *Pseudomonas savastanoi*, el agente causante de la tuberculosis del olivo en Argelia.

En la presente investigación, seis cepas de *Rhizobium* aisladas de suelos argelinos fueron estudiadas para conocer su actividad antimicrobiana contra *Pseudomonas savastanoi*, el agente causante de la tuberculosis del olivo. *Rhizobium* sp. ORN 24 y ORN 83 produjeron actividad antimicrobiana contra *Pseudomonas savastanoi*. La actividad antimicrobiana producida por *Rhizobium* sp. ORN 24 precipitó con sulfato amónico, tuvo un peso molecular entre 1000 y 10000 KDa, fue resistente al calor pero sensible a proteasas y detergentes. Estas características sugieren que la sustancia antimicrobial producida por *Rhizobium* sp. ORN 24 es la bacteriocina natural conocida como rizobiocina 24. Por el contrario, la actividad antimicrobiana producida por *Rhizobium sp*. ORN83 no fue precipitable con sulfato amónico, y tuvo un peso molecular menor de 1000 KDa, fue lábil al calor y resistente a detergentes y proteasas. Estas características podrían indicar una relación de la sustancia antimicrobiana producida por *Rhizobium sp*. ORN83 con la "pequeña" bacteriocina descrita en otros *Rhizobium*.

PALABRAS-CLAVE: Aceitunas – Antagonista – Argelia – Bacterocina – Biocontrol – Pseudomonas savastanoi – Rhizobium.

SUMMARY

Antimicrobial activity of *Rhizobium* sp. strains against *Pseudomonas savastanoi*, the agent responsible for the olive knot disease in Algeria.

In the present investigation, six *Rhizobium* strains isolated from Algerian soil were checked for their antimicrobial activity against *Pseudomonas savastanoi*, the agent responsible for olive knot disease. *Rhizobium* sp. ORN 24 and ORN 83 were found to produce antimicrobial activities against *Pseudomonas savastanoi*. The antimicrobial activity produced by *Rhizobium* sp. ORN24 was precipitable with ammonium sulfate, between 1,000 and 10,000 KDa molecular weight, heat resistant but sensitive to proteases and detergents. These characteristics suggest the bacteriocin nature of the antimicrobial substance produced by *Rhizobium* sp. ORN24, named rhizobiocin 24. In contrast, the antimicrobial activity produced by *Rhizobium sp*. ORN83 was not precipitable with ammonium sulfate; it was smaller than 1,000 KDa molecular weight, heat labile, and protease and detergent resistant. These characteristics could indicate the relationship between the antimicrobial substance produced by *Rhizobium* sp. ORN 83 and the "*small"* bacteriocins described in other rhizobia.

KEY-WORDS: Algeria – Antagonism – Bacteriocin – Biocontrol – Olives - Pseudomonas savastanoi – Rhizobium

1. INTRODUCTION

The Mediterranean region is the world's leading olive-growing area, accounting for almost 98 per cent of global olive production. As with all the countries in the Mediterranean region, olive trees are cultivated in Algeria. With an area of 2,400,000 km^2, olive groves occupy a total area of 160,800 hectares (3% of world production) distributed essentially in mountainous areas (Bartolini and Petrucelli, 2002,). More than 30% of the olives produced are conserved and consumed traditionally as a base for soup or as a pickle (Kacem *et al*., 2006). The remaining 70% are used for oil.

Because olives can be grown in marginal, saline and waste land where the soil is unsuitable for other crops, their cultivation increases the land value, contributes to soil conservation and helps to combat problems of environmental degradation and desertification. The Algerian government (Ministry of Agriculture of Algeria) has established tree plantations and launched a top-working program of new olive grove plantations (Anonymous, 2006)

Today, olive groves have started to influence the arid and semi arid areas of Algeria, reaching as far as the Sahara area, which was considered unsuitable a few years ago. In all these regions olive groves are planted as intercropping with legumes of forage, legume crops or those associated with other fruit species.

Olive trees are constantly faced with the risk of being attacked by pathogenic bacteria and fungi which can cause severe damage in olive groves. For example, olive nodes or tuberculosis caused by *Pseudomonas savastanoi* (olive knot) is observed in many areas of Sig, Mascara, Relizan, Mohammadia

and Remechi of western Algeria. This bacterial disease is characterized by hyperplasia formation on the stems and branches of olive plants and occasionally on the leaves and fruits (Surico, 1986; Lavermicocca *et al*., 2002; Young *et al*., 2004).

As we know, the control of olive knot disease is very difficult and the use of copper compounds is one of the conventional practices employed to reduce symptoms. However, the diffuse resistance to copper bactericides among the pathovars of *Pseudomonas savastanoi* (Wilson and Backman, 1999) requires the development of alternative control methods for bacterial pathogens, such as the use of biological products to lower toxic pesticide residues on fruits and vegetables and to avoid environmental accumulation of chemicals and the consequent development of resistance among pathogens.

Recent studies reveal that most, if not all, major lineages of bacteria produce one or more antimicrobial agents (Hu and Young, 1998; Kacem *et al*., 2005; Montesinos, 2007; Holtsmark *et al*., 2006). An increasing number of studies reveal the potential for these active agents to serve as the next generation of antibiotics for use in human health and agricultural settings (van Sluys *et al*., 2002; Whitehead *et al*., 2002). Among these antimicrobial agents is a bacteriocin produced by bacteria. Bacteriocins are all ribosomally synthesized proteinaceous compounds and are active against bacteria closely related to the producing bacteria (Tagg *et al*. 1976; Riley and Wertz, 2002).

Several studies were reported on the bacteriocins active against phytopathogens (Osnat *et al*., 2005; Kacem, 2007; Holtsmark *et al*., 2008). The potential of Gram-negative produced bacteriocins as a mean of biological control in fighting plant pathogens has been investigated, influenced by the prevalence of antibiotic resistant phytopathogenic bacteria (McManus, 2002) and growing health concerns associated with chemical pesticides (Cook, 1993). For example, a bacteriocin-producing strain of *Pseudomonas solanacearum* prevented tobacco wilt infection (Chen and Echandi, 1984). The incidence and severity of bacterial blight infection that causes leaf streak in rice was reduced by treatment with a bacteriocin-producing strain of *Xanthomonas-oryzae pv oryzae* (Sakthivel and Mew, 1991). *S. plymithicium* produces a colicin-like bacteriocin, which is active against *Erwina amylovora*. This pathogen is the causative agent of fire blight, a costly disease to the apple and pear industry (Jabrane *et al*., 2002). A bacteriocin produced by *Pseudomonas* inhibited the multiplication of *Pseudomonas savastanoi*, the agent responsible for olive knot disease as well as lesion on a carob tree. (Lavermicocca *et al*., 2002).

In addition, rhizobial bacteria, which have the capacity to induce the formation of root nodules in the host plant and increase nitrogen fixation (Lerouge *et al*., 1990; Vargas and Graham, 1989**;** Pedrosa *et al*., 2002), have been shown to produce bacteriocins characterized as small, medium or large on the basis of their assumed sizes, diffusion characteristics, and sensitivity to heat and proteolytic enzymes (Schwinghamer and Brockwell, 1978; Gross and Vidaver, 1978; Schripsema *et al*., 1996). The first description of bacteriocin production by a number of species within the economically important genus *Rhizobium* was published by Roslycky (1967). Many rhizobial species (bacteriogenic strains) produce bacteriocins, designated as rhizobiocins (Hirsch, 1979; Goel *et al*., 1999; Sridevi and Mallaiah, 2008). However, the rhizobiocin properties and their effects on phytopathogenic bacteria remain poorly documented in the literature and until now and the antibacterial produced by *Rhizobium* genus has not yet been explored.

The production of bacteriocins and the competition between species seem tightly connected; it is also possible that future bacteriocin research will reveal new pathways, thus yielding new targets for alternative approaches for plant disease control.

In previous studies (Merabet *et al.,* 2006, Merabet, 2007), rhizobial strains were isolated from root nodules of *Medicago ciliaris* and *Medicago polymorpha*, two legumes of forage and ecological importance in Algeria, especially in saline soil regions. By partial sequencing of the gene coding for the 16S ribosomal RNA, they were found to be affiliated to *Rhizobium* and *Sinorhizobium* genera. In this study we report a bacteriocin like substance produced by *Rhizobium* sp. ORN 24 and ORN 83 strains against *Pseudomonas savastanoi* and other phytopathogen bacteria. Our investigation includes partial purification and preliminary characterization of the bacteriocins produced.

2. MATERIALS AND METHODS

2.1. Bacterial strains and culture media

Rhizobium sp. (ORN83, ORN24 and ORN26) and *Sinorhizobium* sp. (ORN16, ORN88, and ORN89) strains were previously isolated in saline soils in the region of Oran (Algeria) (Merabet *et al*., 2006; Merabet 2007). *Pseudomonas savastanoi* CFBP 2074 came from CFBP «Collection Française de Bactéries Phytopathogènes, Angers, France ». It was obtained from INRA (Institut National de la Recherche Agronomique, Algers, Algeria). This strain (*Pseudomonas savastanoi* CFBP 2074) was isolated from olives cultured in Algeria (Iacobellis *et al*., 1994) and was chosen on the basis of its proven pathogenicity to olives (Iacobellis *et al*., 1994). All strains were maintained as a frozen stock at −20 °C in distilled water plus 20% (v/v) glycerol and propagated twice in YEM broth (van Brussel *et al.*, 1977) at 28 °C before use. The other strains used as indicators were tested as follows: lactobacilli (*Lactobacillus plantarum*) were tested in MRS agar at 30 °C for 18 h, *Propionibacterium* strains in YGL agar medium at 37 °C for 48 h, *Pseudomonas* 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 (Kacem, 2007).

2.2. Bacterial interaction and detection of antimicrobial activity

Rhizobium sp. (ORN83, ORN24 and ORN26) and *Sinorhizobium* sp. (ORN16, ORN88, and ORN89) strains were tested for their antagonistic activity against *Pseudomonas savastanoi* CFBP 2074 by the well diffusion method as previously described by Kacem *et al*. (2005). Briefly, each strain was propagated in 200 ml YEM broth for 24 h at 28 °C. Culture was centrifuged (10.000 rpm for 20 min. at 4 °C) and the supernatant was adjusted to pH 7.0 with 3M NaOH to exclude the antimicrobial effect of organic acid, followed by filtration of the supernatant through a 0.22-µm pore size filter (Gelman Acrodisc 13, Pall Corp., Ann Arbor, USA). **This solution was designated as Faction FI*** and then *Pseudomonas savastanoi* CFBP 2074 was tested for sensitivity to this fraction as follows: prepoured YEM agar plates were overlaid with 7 ml YEM soft agar containing 0.2 ml (10^7 cells/ml suspension of a 2-day YEM broth culture at 28 °C) of *Pseudomonas savastanoi* CFBP 2074. Wells measuring 5 mm in diameter were cut into the agar plate by using a sterile Durham tube and 100 µl of Fraction I was placed into each well. The plates were incubated at 28 °C for 18 h and examined for the appearance of 10 mm or larger clear zones of inhibition around the wells. Each assay was performed in duplicate.

2.3. Concentration and partial purification of the inhibitory agent

Fraction FI* (100 ml) was treated with solid ammonium sulphate to 0, 30, 35, 40, 45, 50, 55, 60 and 75% (w/v) saturation. The mixtures were stirred for 2 h at 4 °C and later centrifuged at 20.000 rpm for 1 h (4 °C). The precipitate (Fraction FI) was re-suspended in 25 ml of 0.05 M potassium phosphate buffer (pH 7.0). Dialysis was followed in a tubular cellulose membrane (Specrapor, 1000 dalton MWco, Fisher Scientific Pittsburgh, PA USA) against the same buffer for 12 h in spectrapor dialysis tubing (**Fraction FII**). The assay of the inhibitory agent activity was tested on *Pseudomonas savastanoi* CFBP 2074 and titer was determined in both the precipitate and supernatant to know which one actually contained the inhibitory agent.

Several aliquots of FII (1ml) were ultrafiltered through various filtron membranes (Filtron Technology Corp; Northborough, Mass), including 1.000.000, 100.000, 10.000 and 1.000 KDa molecular exclusion sizes. Inhibitory activity was determined in retained and eluted fractions. For the quantification of protein concentration in the different fractions a modification of the Bradford (1976) method has been used, according to manufacturer's instructions for micro-assay procedure (Bio-rad Protein Assay, Bio-Rad Laboratories, Hercules-CA, USA).

2.4. Determination of the titer of the inhibitory agent

The titers of the inhibitory agent were quantified in fraction FII by the critical dilution method as described by Schillinger and Lucke (1989). Two fold serial dilutions of each fraction were made in saline solution. Aliquots of 50 µl from each dilution were placed in wells in plates seeded with *Pseudomonas savastanoi* CFBP 2074 strain. These plates were incubated at 28 °C for 24 h, and the diameters of the inhibition zones were measured. Each assay was performed in duplicate. The antimicrobial activity of the inhibitory agent was defined as the reciprocal of the highest dilution showing inhibition of the indicator (*Pseudomonas savastanoi* CFBP 2074) lawn and was expressed in arbitrary units per ml (AU/ml).

2.5. Characterization of the inhibitory agent

Fraction FII at pH 7.0 was treated with trypsin, -chymotrypsin or protenase K. Enzymes were dissolved in phosphate buffer (0.1M, pH 6) and mixed with FII (1:1) to a final concentration of 1mg/ml. To demonstrate the effect of pH, the fraction FII was adjusted to pH values from 2-12 with 4M HCl and 4M NaOH. To test the effect of temperature, the fraction FII was assessed by heating at 100 °C for 10, 15, 20 and 60 min in a water bath. Samples were withdrawn at different time intervals and the activity was tested on *Pseudomonas savastanoi* CFBP 2074. Heat resistance was also checked after autoclaving Fraction FII at 121 °C for 15 minutes. Fraction FII was also treated with Tween 20, Tween 80, SDS (Sodium Dodecyl Sulfat) and Triton X-100 to a final concentration of 1%.

2.6. Search for the bactericidal activity of the inhibitory agent

From inhibitory assays conducted with *Pseudomonas savastanoi* CFBP 2074, samples were taken from inhibition zones (the clear surface of agar) and streaked onto fresh medium agar plates and incubated for 48 h at 28 °C. Growth or no growth was recorded respectively as bacteriostatic and bactericidal activities of fraction FII (Toba *et al*., 1991).

2.7. Search for inhibitory spectrum

For the determination of the inhibition spectrum of fraction FII, several groups of bacteria were used (Table 1) with the agar diffusion test, using the appropriate agar media and incubation conditions for their growth as indicated in the "Bacterial strains and culture media" section.

3. RESULTS

Two, *Rhizobium* sp. ORN 24 and ORN83, out of the six *Rhizobium* strains screened for their antagonistic activity against *P. savastanoi* were found to produce inhibition halos of 10 and 25 mm on lawns of this strain, respectively. Figure 1 shows the typical antagonism produced by cell free supernatants (fraction FI) from *Rhizobium* sp. ORN24 (designated FI24) and ORN83 (designated FI83). We can observe that the zone of inhibition produced by FI83 on indicator plates is extremely clear and large compared to the zone of inhibition produced by FI24. Based on the quality and size of the zones of inhibition, *Rhizobium* sp. ORN83 and ORN24 were therefore considered in this study as antibacterial agent-producing strains.

Concerning the result of the concentration procedure, in the case of *Rhizobium* sp. ORN83, no activity was detected in the precipitate phase after treatment of the fraction FI83 (up to 75%) with solid ammonium sulphate, while activity was only found in the supernatant (designated FII83), suggesting that the inhibitory agent (s) present in FI83 do not have the ability to precipitate by "salting-out". In addition, the inhibitory activity was not retained when FII83 was ultrafiltered through filtron membranes (1,000-molecular weight cut-off).

In the case of *Rhizobium* sp. ORN24, table 1 show that the activity recovery was achieved by including ammonium sulphate (55%) and dialysis. Each step resulted in a considerable loss of protein concentration while, specific activity increases. The antibacterial agent (s) in this fraction (designated FII24) was able to pass through cellulose membranes with a 10,000-molecular weight cut-off but was retained with a 1, 000-molecular weight cut-off. The antibacterial activity of FII24 or FII83 was found to be 1600 and 800 AU/ml respectively.

Since bacteriocins are by definition proteinaceous substances they must be sensitive to at least one proteolytic enzyme. Consequently, protease sensitivity is a key criterion in their characterization. In our case, α-chymotrypsin, trypsin or proteinase K had no effect on the activity of FII83, whereas the inhibitory activity of FII24 was completely lost by the same enzymes, reflecting the proteinaceous nature of the inhibitory agent produced by *Rhizobium* sp.ORN24. Additionally, complete inactivation of FII83 was observed when it was exposed to 100 °C for 20 min, while FII24 showed resistance to heat, retaining all activity after exposure to 100 °C for 60 min. *Results* appear to indicate that *Rhizobium sp.* ORN83 produces a small, non-proteinaceous antimicrobial compound and that *Rhizobium sp.* ORN24 produces a bacteriocin-like peptide.

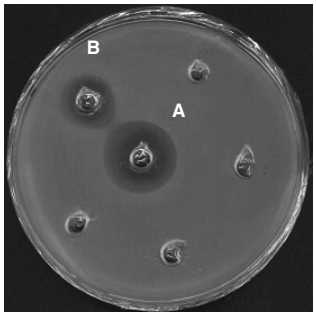

Figure 1.
Agar well diffusion assay showing the antibacterial activity of FI83 from Rhizobium sp. ORN83 and FI24 from Rhizobium ORN24 strains (A) and FI83 from Rhizobium sp. ORN24 (B) strain on Pseudomonas savastanoi CFBP 2074 strain.

The other physico-biochemical tests showed that both FII83 and FII24 retained their antibacterial activity within the range of pH 4 to 7 and maintained full stability after storage for 60 days at 4 °C, 0 °C and at −20 °C, while no activity was detected after storage for 80 to 120 days at 37 °C. Tween 80, Tween 20, SDS or Triton X-100 seems to have a marked effect on FII24; in contrast the activity of FII83 was not reduced when treated with these agents.

As described in Materials and methods, samples were taken from inhibition zones and surface staked to medium agar plates. After the incubation of plates, the growth of *Pseudomonas savastanoi* CFBP 2074 was not observed. This result suggests that the antibacterial agents of both FII83 and FII24 are bactericidal toward the target strain. Additionally, strains were resistant to their own cell free supernatants. This may be due to an immunity substance simultaneously being produced by the producer strain along with its product, as in the case of some bacteriocins (Kacem, 2007). The inhibitory spectrum of FII24 is shown in Table 2.

4. DISCUSSION

Research on bacteriocins from bacteria has expanded during the last decades, to include the use of bacteriocins or the antibacterial producer organisms as natural food preservatives (Kacem *et al*., 2005; Kacem, 2007) and also as biocontrol of many plant pathogens (Holtsmark *et al*., 2008).

Olive knot are considered to be diseases caused by distinct pathogenic populations in *Pseudomonas savastanoi* (Young *et al*., 2004).

Results showed that inhibitory agents are present in cell free supernatants obtained from *Rhizobium* sp. ORN83 and ORN 24 strains isolated in saline soils from the region of Oran located in western Algeria.

Among the six strains screened for their antagonistic activity, FII83 and FII24 from *Rhizobium* sp. ORN24 and ORN83 showed an inhibition zone of 10 mm and 25 mm in diameter respectively on *Pseudomonas savastanoi* CFBP 2074 strain. Similar results have been reported by Hirsch (1979) and Rodelas *et al.* (1998). These authors delineated two types of inhibitions zones produced by *Rhizobium* genus on indicator plates; the first one was wide (10 to 30 mm) and clear due to the production of small bacteriocin, while the

Table 1
Concentration and partial purification of FI24 produced by *Rhizobium* sp. ORN24 strain

Purification Stages	Volume (ml)	Activity (AU/ml)[1]	Total protein (μg/ml)[2]	Specific activity[3] (AU/μg)
Fraction FI24	100	400	208	1.6
FractionFII24	10	1,600	141	5.8

Membrane molecular weight cut-off	AU (% Initial activity)[4]	
	Retentate (%)	Eluted fraction (%)
1,00,000	200 (12.5)	800 (50.0)
10,000	800 (50.0)	400 (25.0)
1,000	1,600 (100.0)	0 (0.0)

[1]Antimicrobial activity of Fraction FI24 against *Sinorhizobium* ORN16 strain (AU/ml).
[2]Determined by the Bradford method.
[3]Activity (AU/ml) (column 2) divided by the protein concentration (μg/ml) (column 3)
[4]Initial bacteriocin activity was 1600 AU/ml.
Fraction FI24: Culture was centrifuged and the supernatant was adjusted to pH 7.0 followed by filtration of the supernatant through a 0.22-μm pore size filter.
FractionFII24: Fraction FI24 was treated with solid ammonium sulphate. The mixtures were centrifuged and re-suspended potassium phosphate buffer (pH 7.0). Dialysis was followed in a tubular cellulose membrane.

second one was smaller and turbid (5 to 10 mm) due to the production of medium bacteriocin.

FII83 was heat labile, showed resistance to proteolytic enzymes, and produced wide zones of inhibition (25 mm) on *Pseudomonas savastanoi* CFBP 2074 strain. However, The FII24 was heat resistant and showed sensitivity to proteases, suggesting that the substance (s) is proteinaceous, or at least has a proteinaceous activator. FII24 produced small zones of inhibition (10 mm) on the same *Pseudomonas savastanoi* CFBP 2074 strain. Inhibitory agents in Both FII83 and FII24 were able to pass through cellulose membranes (10,000-molecular weight cut-off), suggesting a low molecular weight. Some of the characteristics of our antibacterial substances exhibited properties similar to those of bacteriocins of several rhizobia (Wijffelman *et al*., 1983; Leroy and De Vuyst, 1999). Hirsch (1979) has described two types of bacteriocins production by *Rhizobium leguminosarum*; these are designated small and medium bacteriocins. The small bacteriocin is heat labile and resistant to proteolytic enzymes, whereas the medium one is also heat labile but sensitive to proteolytic enzymes. Also, in another study, Schwinghamer and Brockwell (1978) have described the production of bacteriocin by *Rhizobium trifolii* which is sensitive to heat and proteolytic enzymes. At present, the proteinaceous nature was confirmed for medium bacteriocins and the gene was isolated (Rodelas *et al*., 1998; Wisniewski, 2002). van Brussel *et al*., 1985 have reported that the *small* bacteriocins produced by rhizobia are chloroform soluble and are not required for effective nodulation. Schripsema *et al.* (1996) described the production of an antimicrobial compound by *Rhizobium leguminosarum* which they named "bacteriocin small" and resulted to be an N-acyl-Homoserin Lactone molecule. However, classical definition of bacteriocin only includes "peptides or proteins with antimicrobial activity directed against closely related species (Tagg *et al*., 1976; Jack *et al*., 1995). Now small bacteriocins have been identified as *N*-acyl homoserine lactone (Gray *et al.* 1996; Schripserna *et al.* 1996) and are therefore structurally related to the quorum sensing factors produced by bacteria.

From these findings and in accordance with the guidelines outlined by several authors (Hirsch, 1979; Wijffelman *et al.,* 1983; Leroy and De Vuyst, 1999) the antibacterial substances present in FII83 or FII24 can be attributed to a small or medium bacteriocin like respectively.

FII24 showed inhibition against some *Rhizobium* sp. and *Sinorhizobium* sp. strains among the 14 strains tested. As reported by Hafeez *et al*. (2005), the activity spectrum in *Rhisobium* genera varied from strain to strain. An auto-antagonism relationship was not observed; no tests strain inhibited its own growth which is characteristic of a bacteriocin producer (Hardy, 1975; Nirmala and Gaur, 2000; Sridevi and Mallaiah, 2008).

Inhibitory activity was also directed against the natural flora present in soil, including *Pseudomonas (Pseudomona. aeruginosa* 25923), and also against some *Erwinia chrysanthemi* strains. As we know, these two phytopathogen bacteria can affect many vegetables and cause diseases in plants, including vegetables, root and tuber crops, tree crops and other industrial crops (Toth *et al*., 1997; Yap *et al*., 2004), and which result in enormous economic losses annually.

Antimicrobial substances produced by *Rhizobium* sp. ORN 24 and ORN 83 exhibited inhibitory activity against the phytopathogen with a view to improving the hygiene and safety of food products and vegetable products. Further experiments concerning the effect (*in vivo*) of this novel bacteriocin on other phytopathogen and the spoilage bacteria are in progress. Finally, all these

Table 2
Antimicrobial spectrum of FII24 produced by *Rhizobium* sp. ORN24 strain against a wide range of indicator strains

Source	Indicator species	Diameter of[1] inhibition (mm)
Medicago ciliaris and *Medicago polymorpha* nodules roots (Merabet, 2007) (Merabet *et al.,* 2006)	*Rhizobium* sp. ORN20,	19
	Rhizobium sp. ORN08	0
	Rhizobium sp. ORN24	0
	Rhizobium sp. ORN *25*	0
	Rhizobium sp. ORN 31	0
	Rhizobium sp. ORN 38	23
	Sinorhizobium sp. ORN11	22
	Sinorhizobium sp .ORN16	17
	Sinorhizobium sp .ORN20	0
	Sinorhizobium sp. ORN41	0
	Sinorhizobium sp. ORN42	0
	Sinorhizobium sp. ORN49	0
	Sinorhizobium sp. ORN51	20
	Sinorhizobium sp. ORN55	0
Fermented olives (Kacem *et al*., 2004) (Kacem, 2007)	*Lactobacillus plantarum* OL16	0
	Lactobacillus plantarum OL12	0
	Lactobacillus plantarum OL33	12
	Lactobacillus plantarum OL36	15
	Lactobacillus plantarum OL53	0
	Lactobacillus plantarum OL40	0
	Lactobacillus plantarum OL23	0
ATCC (Kacem, 2007)	*E. coli* 25922	0
	Pseudomona. aeruginosa 25923	24
CFBP (Iacobellis et al., 1994)	*Pseudomonas savastanoi* CFBP 2074	25
ATCC (Kacem, 2007)	*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* M88	11
LBMB*	*E. chrysanthemi* 5703a	12
LBMB*	*E. chrysanthemi* C23	12
LBMB* (Kacem, 2007)	*E. chrysanthemi* C26	13

CFBP : Collection Française de Bactéries Phytopathogènes» (Angers, France). 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.

properties obtained in this study identify *Rhizobium* sp. ORN 83 and ORN 24 as potential candidates for use in the biocontrol of many plant pathogens, including olives, potatoes, carrots, and cucumbers as well as other vegetable products produced in Algeria.

5. CONCLUSION

The exploitation of antimicrobial compounds produced by *Rhizobium* can constitute an attractive strategy for the targeted combat of bacterial infections, e.g. in plant disease control. The antibacterial substance present in FII24, which we assigned to medium bacteriocin, appear to have the properties commonly associated with "true" bacteriocins and we propose referring to it as rhizobiocin 24, according to the suggestion of Roslycky (1967). More purification to homogeneity of the novel rhizobiocin 24 should be carried out in order to conduct a comprehensive physical characterization of the protein. SDS-PAGE analysis and gel filtration may be used to estimate the size

of the bacteriocin. The results of the current study open several perspectives for further investigation on this novel bacteriocin including its structure and its possible role during bacterial competition and plant-rhizobial interactions. These findings prompt new interest in bacteriocins produced by plant pathogens that can be considered an alternative bio-control system useful in reducing the hazard associated with the use of synthetic pesticides. Valid formulations to ensure adequate effectiveness of the bactericide under natural environmental conditions should be pursued.

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