International Scholars Journals

African Journal of Virology Research ISSN 3421-7347 Vol. 3 (3), pp. 001-010, March, 2009. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Antifungal activity and bioactive compounds produced by *Bacillus mojavensis* and *Bacillus subtilis*

Mounia YOUCEF-ALI[1]*, Noreddine KACEM CHAOUCHE[1], Laid DEHIMAT[1], Insaf BATAICHE[1], Mounira KARA ALI[1], Hélène CAWOY[2] and Philippe THONART[2]

[1]Laboratory of Mycology, Biotechnology and Microbial activity, FSNV, University Constantine-1 Constantine, 25000 Algeria. *E-mail: mouniayoucefali@yahoo.fr

[2]Walloon Centre for Industrial Biology, Agro-Bio Tech, University of Liege Gembloux, 5030 Belgium.

Accepted 20 January, 2007

The soil bacteria, CWBI-B1567 and CWBI-B1568, isolated from arid regions and identified as *Bacillus subtilis* (accession number KC341751) and *Bacillus mojavensis* (accession number KC341749) respectively, were screened and evaluated for their antifungal activity against *Candida albicans*, one of the most important human fungal pathogens. *In vitro* assay with the antagonists and their cell-free culture supernatants on agar plates showed that the two *Bacillus* strains effectively inhibited growth of the yeast. The capacity of these *Bacillus* strains to produce the cell-wall degrading enzymes was further studied. *B. subtilis* and *B. mojavensis* are able to produce cellulase and protease, but not chitinase. Bioactive molecules were produced by *B. subtilis* CWBI-B1567 and *B. mojavensis* CWBI-B1568 in a liquid culture medium optimised for lipopeptide production. The antifungal activity was equally demonstrated by testing the resulting supernatants and lipopeptide-enriched extracts. The electro-spray mass spectrometry coupled to liquid chromatography (ESI-LC-MS) analysis showed that both the *B. subtilis* and the *B. mojavensis* produced surfactin and iturin. However the fengycin group was produced only by *B. mojavensis*. Through this study, we have demonstrated that *B. subtilis* and *B. mojavensis* have a strong antifungal activity especially against *C. albicans*. This growth inhibition is probably due to the production of cell-wall degrading enzymes and different families of lipopeptide including iturins, fengycins and surfactins.

Key words: *Bacillus subtilis* CWBI-B1567, *Bacillus mojavensis* CWBI-B1568, antifungal activity, *Candida albicans*, lipopeptides, electro-spray mass spectrometry coupled to liquid chromatography (ESI-LC-MS), arid regions.

INTRODUCTION

Candida albicans is a dimorphic fungus (Molero et al., 1998); it is both, a commensal on human mucosal surfaces and one of the most important human fungal pathogens which become the fourth leading cause of nosocomial infections. The diseases caused by *C. albicans* ranges from relatively benign infections of skin to oral and vaginal thrush, deep-seated mycoses and life-threatening sepsis. *Candida* infections constitute today a problem of growing clinical importance. The incidence of infections has increased dramatically over the past three decades, and this trend, will inevitably continue into the 21st century (Odds, 1988; Pfaller et al., 1998; Edmond et al., 1999), especially, when some microorganisms continue to develop resistance against antibiotics treatment.

Bioactive natural products constitute a library of compounds with a large and privileged structural diversity,

showing a variety of biological activities. In fact, many of them have been used for pharmaceutical applications (Salas and Mendez, 2007). Inside microorganisms, several species of *Bacillus* genus are able to produce biological active substances that are capable of disintegrating the fungal cell walls (Tserkovniak et al., 2009). In the midst of these substances, several lipopeptide compounds, serve as a class of microbial surfactants with increasing scientific, pharmaceutical and biotechnological interest.

Lipopeptides from *Bacillus* are classified in three families of cyclic compounds: surfactin, iturin and fengycin. These lipopeptides, made of amino acids and a fatty acid, are amphiphilic membrane-active biosurfactants and peptide antibiotics with potent antifungal activities. Each family contains variants with the same peptide length but with different residues at specific positions. Moreover, each variant can have several homologues of different length and isomer of the fatty acid chain, leading to a remarkable structural heterogeneity (Ongena and Jacques, 2008; Moyne et al., 2004).

In recent years, there has been a considerable interest in using *Bacillus* strains producing lipopeptide antibiotics like iturin A and surfactin as biocontrol agents due to their antagonistic and repressive activities against pathogens (Bais et al., 2004). These amphiphilic cyclic biosurfactants have many advantages: low toxicity, high biodegradability and environmentally friendly characteristics (Kim et al., 2004). In literature, several approaches for lipopeptide identification have been proposed but their biological activity needs to be determined after isolation procedures (Price et al., 2007).

In fact, the list of novel microorganisms, especially *Bacillus*, and products found in microbiologically unexplored ecosystems around the world suggest that a careful exploration of other habitats such as arid regions might continue to be useful. The Algerian arid soil presents an ecosystem with numerous opportunities as a source of novel microorganisms producing new compounds.

Bacillus strains have been investigated previously for agricultural applications and have proven to be an effective biocontrol agent against important phytopathogens (Monteiro et al., 2005). In this paper, the antifungal activity of *Bacillus subtilis* CWBI-B1567 and *Bacillus mojavensis* CWBI-B1568 against *Candida albicans* was investigated. These two strains were isolated from soil of different palm trees situated in South-East of Algeria.

MATERIALS AND METHODS

Isolation of bacterial strains

Soil samples were collected from soil of different palm trees of arid regions, situated in South-East of Algeria. 1 g of each sample was suspended in 9 ml sterile physiological water and shaken vigorously for 2 min. The soil suspension was serially diluted in sterile physiological salt (0.85% NaCl), (from 10^{-1} to 10^{-6}); 0.1 ml of all dilutions were plated on YPD medium (Glucose, 20 g; Peptone, 10 g; Yeast extract, 10 g; Agar, 15 g; distilled water, 1 L) supplemented with a commercial antifungal to inhibit fungi growth. Petri dishes were incubated at 30°C (Gerhardt, 1994). The bacterial strains were maintained on YPD medium at 4°C before experimental use, and stored at -80°C in cryotubes according to the manufacturer recommendations (Microbank, Prolab Diagnostic, Richmond Hill, Canada) for long-term storage.

Screening of antagonists strains by *in vitro* antagonism experiments

The antifungal activity of the isolates against *C. albicans* was determined according to the plate diffusion method (Barakate et al., 2002; Errakhi et al., 2007). The yeast strain was isolated in Laboratory of Parasitology and Mycology (CHU Constantine, Algeria) from a patient's mouth suffering from candidiasis. It was maintained on Yeast Malt Agar (YMA: Glucose, 20 g; Peptone, 10 g; Yeast extract 10 g; Agar, 15 g; distilled water 1L) containing gentamicin and chloramphenicol.

Isolates were grown on YPD medium for 24 h, discs (6 mm in diameter) were cut and placed on YMA which was seeded by spreading of the yeast suspension. Plates were first kept in a refrigerator (4°C) for at least 2 h to allow the diffusion of any antibiotics produced, then incubated at 30°C. Inhibition zones were determined after 24 and 48 h of incubation. The same method was used to test the antagonistic effect of cell-free culture supernatant. In this case, 80 µl aliquots of filter (0.2 µm) sterilized supernatant samples were dispensed in wells (performed with a sterile cork borer, of 6mm diameter) made in the gelified medium previously spread by the yeast suspension (Touré et al., 2004).

Among the strains which developed antifungal activity, two potential bacterial strains (coded as CWBI-B1567 and CWBI-1568) were selected for further study and screened for lipopeptide and cell-wall degrading enzymes production.

Characterisation of the selected bacteria

To identify the strains CWBI-B1567 and CWBI-B1568, Gram staining, catalase test, morphology and standard biochemical tests, as listed in Bergey's Manual of Determinative Bacteriology, and their ability to utilise various carbon sources (elements) in the API 50CH (Biomerieux) were assayed (Logan and Berkeley, 1984). The two selected antagonistic bacterial strains were also identified by sequencing of their 16S rDNA.

Sporulation test

Microscopy

The two strains were grown in a liquid culture medium (YPD: Glucose, 20g; Peptone, 10g; Yeast extract, 10g; distilled water, 1 L) at 30°C for 3 days in agitated flasks at 200 rpm. The cells were examined under optic Zeiss Axioskop 2 MOT microscope.

Thermal treatment

The strains CWBI-B1567 and CWBI-B1568 were inoculated into

Table 1. Primers used for PCR and sequencing of 16S rDNA of bacterial strain.

Technique	Primer	Sequence	Source
PCR	16SPO	5'AAGAGTTTGATCCTGGCTCAG-3'	Ventura et al., 2001
	16SP6	5'-CTACGGCTACCTTGTTACGA-3'	Ventura et al., 2001
Sequencing*	F1	5'-CTGGCTCAGGAYGAACG-3'	(Sigma-Proligo)
	F2	5'-GAGGCAGCAGTRGGGAAT-3'	(Sigma-Proligo)
	F3	5'-ACACCARTGGCGAAGGC-3'	(Sigma-Proligo)
	F4	5'-GCACAAGCGGYGGAGCAT-3'	(Sigma-Proligo)

*Synthetic primers used for sequencing were deduced from the alignment of 16S rDNA genes collected from EMBL (European Molecular Biology Laboratory) databases and were supplied by Sigma-Proligo.

nutrient broth and incubated with shaking for 24 h at 30°C. The cultured samples were diluted into a minimal medium salt base, and the cell suspensions were incubated for 12 min at 80°C. Samples of treated and untreated cultures were diluted and plated on Nutrient Agar for CFU counting.

Identification by sequencing 16S rDNA gene

The 16S rDNA gene sequence was determined by direct sequencing. Total DNA was isolated by using the Wizard® genomic DNA purification kit (Promega, Madison, USA). Primers used for PCR and DNA sequencing are presented in Table 1. PCR amplification was performed with the primer pair SPO/SP6 targeted against regions of 16S rDNA (Ventura et al., 2001).

Amplification of DNA was performed in a Mastercycler thermal cycler (Eppendorf). PCR conditions included a hot start at 96°C (5 min), 25 cycles consisting of hybridisation at 55°C (30 s), elongation at 72°C (2 min), denaturation at 95°C (30 sand a final extension at 72°C (10 min). PCR products were resolved by electrophoresis in 1% (w/v) agarose gel and visualised by ethidium bromide (1 µl/10 ml) staining. 16S rDNA PCR amplicons were purified following the microcon YM-100 kit (Bedford, MA, USA) and sequenced using the Big Dye Terminator V3.0 kit as specified by the supplier with the primers described in Table 1. Phylogenetic analysis was realised by the alignment of sequences of the 16S rDNA with genes collected in an international database (GenBank). The results were then expressed as percentage of homology between the submitted sequence and the sequences contained in the database.

Determination of the enzymatic activities

Enzymatic activities (cellulase, protease and chitinase) were assessed in a qualitative way through halo formation on solid media. To obtain active cell populations, the strains were streaked on solid YPD medium 24 h before transfer to enzymatic test medium.

Cellulase activity

Bacterial strains were first grown on carboxymethyl cellulose liquid medium (CMC: Peptone, 1.5 g; Yeast extract, 1.5 g, CMC, 35 g; Distilled water, 1 L) at 30°C for 48 h, and cultures were centrifuged at 10,000 g for 20 min. Bacterial cultures and their supernatants were tested against a commercial cellulase on CMC agar containing (g/l): KH_2PO_4, 1.0; $MgSO_4.7H_2O$, 0.5; NaCl, 0.5; $FeSO_4.7H_2O$, 0.01; $MnSO_4.H_2O$, 0.01, NH_4NO_3, 0.3; CMC, 10.0; agar, 12.0. The pH was adjusted to 7.0 with 1 M NaOH. The CMC agar plates were incubated at 37°C for five days to allow for the secretion of cellulase. At the end of the incubation, the agar medium was flooded with an aqueous solution of Congo red (1% w/v) for 15 min. The Congo red solution was then poured off, and the plates were further treated by flooding with 1 M NaCl for 15 min. The formation of a clear zone of hydrolysis indicated cellulose degradation (Ariffin et al., 2006).

Protease activity

For protease activity, the fresh colonies were used to inoculate liquid Luria cultures (Peptone, 10 g; Yeast extract, 5 g; NaCl, 5 g; glucose, 1 g; distilled water, 1 L). After overnight incubation at 30°C, these cultures were filtered (0.2 µm acetate cellulose membrane) in order to obtain cell free supernatants which were spotted (15 µl on a circle of Whatmann paper, 5 mm diameter) on protease test medium plates. Plates were observed after 24 h of incubations at 30°C. The detection of enzymatic activity was based on the formation of clear halos. Papain 1 mg/ml (powder at 35 000 USP) was used as a positive control.

Chitinase activity

The bacterial strains were grown on solid medium, containing colloidal chitin, at 30°C for 2-7 days. The formation of a clear zone of hydrolysis indicated positive chitinase activity.

Production and purification of lipopeptides

The strains CWBI-B1567 and CWBI-B1568 were grown at 30°C for 72 h in agitated flasks at 200 rpm in 350 ml of a liquid culture medium optimised for lipopeptide production (named Opt medium) as described by Jacques et al. (1999). Cultures were centrifuged at 17,400 *g* for 20 min. The supernatant was filtered through a 0.2 µm sterile filter membrane for testing by the plate diffusion method. For safety, these tests were performed against *Yarrowia lipolytica*.

In order to obtain lipopeptide-enriched extracts, purification of the resulting supernatants was realised by solid-phase extraction on a 300mg C18 column (Touré et al., 2004); this is sufficient to fraction 10-20 ml of classic supernatant (agitated culture of 3 days in rich medium).

Table 2. Concentrations of the solvents used and their times.

Time (min)	Solvent A (H_2O 0.1 FA)%	Solvent B (ACN 0.1 FA)%
0	55	45
10	5	95
18	5	95
19	55	45
24	55	45

ACN, Acetonitrile; FA, formic acid.

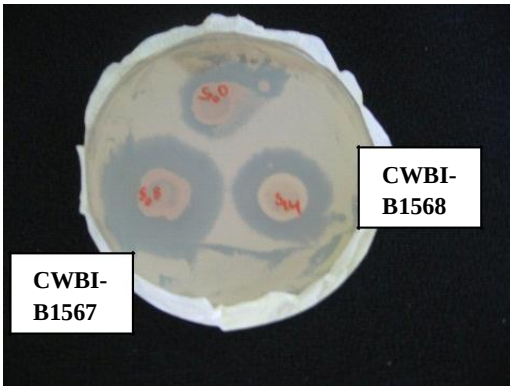

Figure 1. *In vitro* growth inhibition of *Candida albicans* caused by strain CWBI-B1567 and CWBI-B1568 on yeast malt agar (YMA) medium. The antagonism was scored after incubation of the plates for two days at 30°C.

10 ml of crude supernatant was loaded on an ISOLUTE C-18 CE type cartridge (International Sorbent Technology) previously activated with 10 ml of methanol and equilibrated with 5 ml of Milli-Q water. After sample loading, the column was washed with 3 ml of Milli-Q water. Hydrophobic compounds were eluted with 1 ml of 100% MeOH. This methanolic supernatant extract was also tested.

Characterisation and identification of lipopeptides using ESI/LC/MS

Lipopeptide-enriched extracts prepared as described above were analysed using a reverse phase HPLC (HPLC Waters Alliance 2695/ diode array detector) coupled with a single quad mass spectrometer (Waters SQD mass analyser) on a X-terra MS (Waters) 150*2.1 mm, 3.5 µm C18 column. A diode array detector was integrated into the HPLC system. The flow rate and temperature of the column were set at 0.5 ml/min and 40°C, respectively. The elution gradient mode was used to separate the various homologous compounds (Table 2). The ionisation type was electrospray. Desolvation and source temperatures were 250 and 130°C, respectively. Nitrogen flow was set at 500 l/h. Elution solvents were acetonitrile and Milli-Q water, with 0.1% formic acid. Specific cone voltages of 70, 85 and 130 V were used for surfactins, iturins and fengycins, respectively. The positive ion mode was used for analysis of all three families because a higher signal/background ration was obtained compared to negative ion recording.

RESULTS

Isolation and screening of antagonistic strains by *in vitro* antagonism experiments

Over 42 bacterial isolates were obtained from soil of arid regions situated in South-East of Algeria. In order to assess whether the bacterial strains isolated may be useful sources for natural bioactive compounds, the antifungal activity of the isolates and their supernatants was determined. Two strains showed a strong antagonistic effect when faced with *C. albicans* by developing clear zones of lyses of 23 and 19 mm by strain CWBI-B1567 and CWBI-B1568 respectively (Figure 1).

Identification of bacterial strains

Upon microscopic examination, catalase activity and the Gram stain showed that the two strains were catalase and Gram positive. The two sporulation tests and identification made by API 50CH revealed that these strain belongs to the genus: *Bacillus*.

However, nucleotide sequences of 16S rDNA of the two *Bacillus* sp. were determined to confirm biochemical identification. The obtained 16S rDNA sequences were compared with available 16S rDNA sequences in the GenBank database.

Obtained sequence identifications were very reliable (>99% similarity with the database). This comparison showed that the strain CWBI-B1567 was *B. subtilis* (accession number KC341751) while; the strain CWBI-B1568 was *B. mojavensis* (accession number KC341749).

Determination of the enzymatic activities developed by the antagonistic *Bacillus* strains

The capacity of the *Bacillus* strains to produce cell-wall degrading enzymes was qualitatively studied. The results show that both *B. subtilis* and *B. mojavensis* produced protease and cellulase (Figures 2 and 3) but not chitinase (Table 3).

Figure 2. Detection of protease activity developed by *B. Subtilis* CWBI-B1567 and *B. mojavensis* CWBI-B1568 on protease medium.

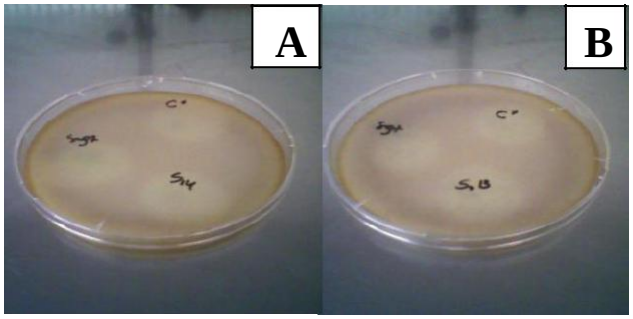

Figure 3. Detection of the cellulase activity on CMC medium, bacterial cultures and their supernatants were tested against commercial cellulase (C^+). **A,** *B. mojavensis* CWBI-B1568; **B,** *B. subtilis* CWBI-B1567.

Purification and identification of lipopeptides produced by the selected *Bacillus* strains

Implication of lipopeptides in the observed antagonism was at first studied by testing filter-sterilised crude supernatants obtained from culture in the Opt medium. A total of 80 µl of this solution was sufficient to clearly inhibit the development of the test yeast suggesting the involvement of secreted antifungal metabolites. A similar level of antagonistic activity was observed by testing lipopeptide-enriched extracts.

Members of the different families were detected in the methanolic extract obtained from the culture supernatant. They were identified by liquid chromatography/ electrospray ionization mass spectrometry (LC/ESI-MS) on the basis of their retention times (Figure 4) compared to purified molecules used as standards and on the basis of their fragmentation profiles (Figure 5). A wide variety of homologous compounds were detected within each group; both the *B. subtilis* and the *B. mojavensis* produced surfactin and iturin. However, the fengycin group was produced only by *B. mojavensis* (Table 4).

DISCUSSION

Bacillus species have the interesting property of producing secondary metabolites with a wide spectrum of antibiotic activity and very diverse structures. Therefore, they have been very valuable for medical applications. *Bacillus* strains produce gene-encoded antibiotics as well as a variety of small antibiotic peptides (<2000 Da) synthesized non-ribosomally (Moyne et al., 2004). They include lipopeptide antibiotics such as iturin, surfactin, and fengycin.

In this article, we have described the use of *Bacillus* strains CWBI-B1567 and CWBI-B1568 isolated from arid soil as well as their secreted secondary metabolites to act as antifungal products against *C. albicans*. These strains showed a powerful antifungal activity; both the anta-gonistic strains and their cell-free culture supernatants significantly inhibited growth of the yeast (inhibition zone ≥ 19 mm). This strong activity expressed by a large zone of inhibition on agar plates indicated, as mentioned by Barakate et al. (2002), that these two isolates produce water soluble antimicrobial metabolites. It is well known that several species of *Bacillus* are capable of producing biologically active molecules, including antifungal substances (Yun-Feng et al., 2012; Caldeira et al., 2011; Zhenzhen et al., 2010).

Based on the physiological, biochemical characteristics and 16S rDNA sequence analysis, strains CWBI-B1567 and CWBI-1568 were identified as *B. subtilis* and *B. mojavensis* respectively. *B. mojavensis* Roberts, Nakamura, and Cohan was distinguished from the *B. subtilis* complex on the basis of whole-cell fatty acid composition, divergence in DNA sequence, and resistance to genetic transformation (Roberts et al., 1994). The type of this bacterium is an isolate from the Mojave Desert, California. This group of species in addition to the desert origins has additional physiological traits that serve as characteristics of the species. All strains of this species are antagonistic to fungiand are all endophytic. These traits suggest that this species has outstanding traits as an endophytic biocontrol agent.

Antifungal power of *B. subtilis* and *B. mojavensis* was further exemplified by testing filter-sterilised crude supernatant obtained from their culture in the Opt medium. The electro-spray mass spectrometry coupled to liquid chromatography (ESI-LC.MS) analysis showed that *B. mojavensis CWBI-B1568* produced the three lipopeptides families. However, *B. subtilis CWBI-B1567* produce only two types of lipopeptides (surfactin and iturin). These antifungal substances have been widely reported in *Bacillus* cultures such as *B. amyloliquefaciens* B94 (Yu et al., 2002) and *B. subtilis* JA (Chen et al., 2008;

Table 3. The cell-wall degrading enzymes produced by *B. subtilis* and *B. mojavensis* on enzymatic test mediums.

Strain	Protease	Cellulase	Chitinase
B. subtilis	+++ (34 mm)	+++ (30 mm)	-
B. mojavensis	++ (20 mm)	+++ (30 mm)	-

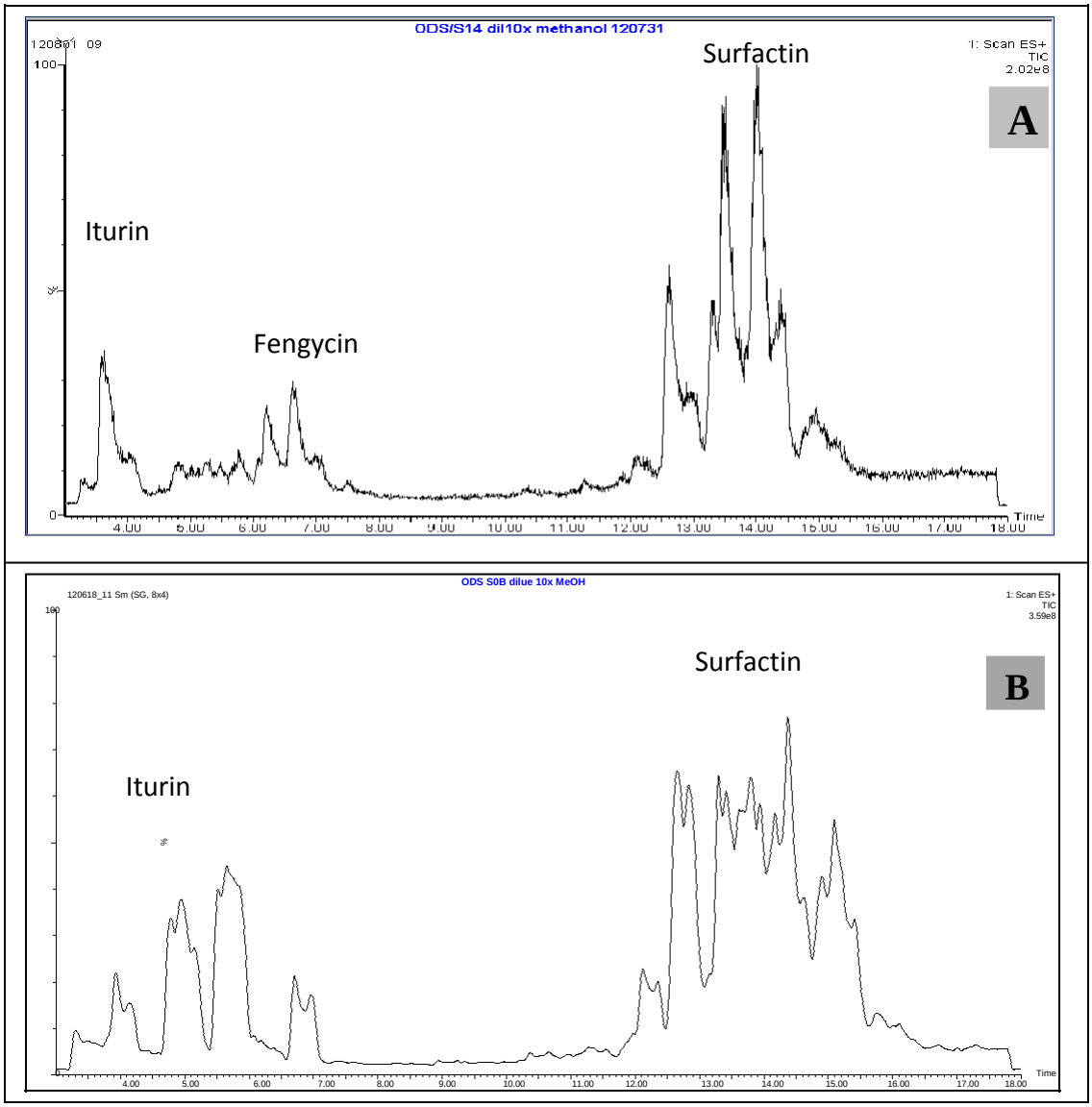

Figure 4. Chromatograms corresponding to lipopeptides analyzed by ESI/LC/MS technique. **A,** lipopeptides families produced by *B. mojavensis* CWBI-B1568; **B,** lipopeptides produced by *B. subtilis* CWBI-B1567.

Fernandes et al., 2007). The two tested *Bacillus* strains produced cellulase and protease but the chitinase activity was not observed in either *Bacillus* strain.

The antifungal activity detected in this work can be explained by the capacity of the *Bacillus* isolates to produce the cyclic lipopeptides and the cell-wall degrading enzymes (Glick et al., 1999; Dunne et al., 1993). Lipopeptides, including surfactin, iturin and fengycin, were described previously as a major class of *Bacillus* peptide antibiotics, which have a great potential

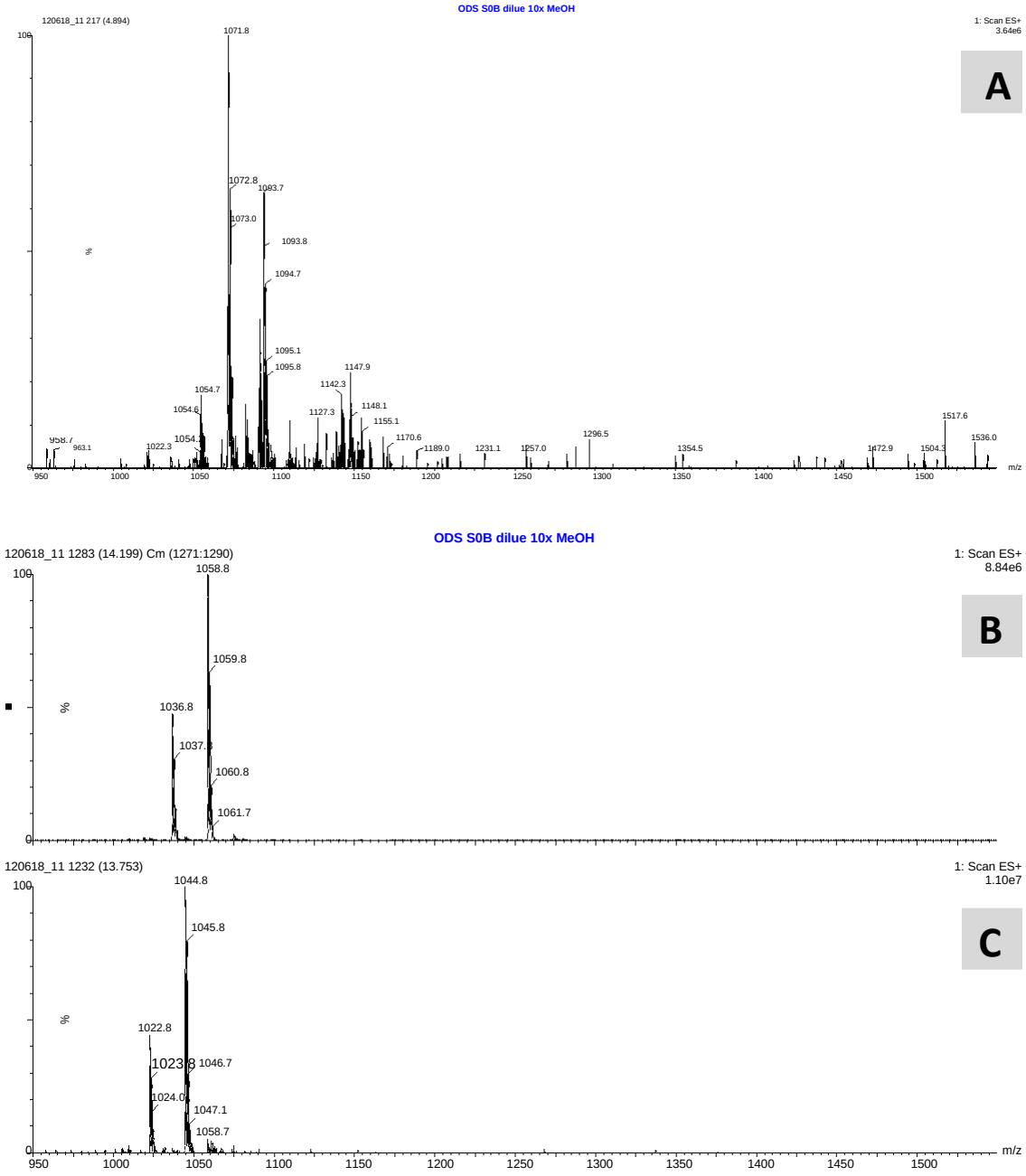

Figure 5. Mass spectra of some detected lipopeptides in *B. subtilis* CWBI-B1567 supernatant, analyzed by ESI-LC.MS. A, Iturin C_{16}, m/z= 1071.8; B, Surfactin C_{15}, m/z=1058.8; C, Surfactin C_{14}, m/z=1044.8.

for biotechnological and biopharmaceutical applications (Peypoux et al., 1999). Iturins and fengycins display a strong antifungal activity, however, surfactins are not fungitoxic by themselves but retain some synergistic effect on the antifungal activity of Iturin A (Maget-Dana et al., 1992). Results found by Ongena et al. (2005), strongly suggest that lipopeptides are responsible for most of the antifungal activity developed *in vitro* by *B. subtilis* toward multiple plant pathogens.

Through this study, we have demonstrated that *B. subtilis* and *B. mojavensis* have a strong antifungal activity especially against *C. albicans*. Both the *B. subtilis* and the *B. mojavensis* have a potential of lipopeptides and cell-wall degrading enzymes production. Thus, these bioactive components might provide an alternative resource for the pharmaceutical control of *C. albicans*. Further studies are needed to determine the ability of these *Bacillus* strains to control diseases caused by this

Table 4. Lipopeptide homologues produced by *B. subtilis* and *B. mojavensis* after growth for 72h in Opt medium.

***Bacillus* isolate**	**Lipopeptides**	**Retention times (min)**
	Fengycins	
	/	/
	Iturins	
	C_{14}	3,305
	C_{15}	3,925
	C_{16}	4,937
	C_{17}	5,653
	C_{15}+C_{17}	6,081
Bacillus subtilis	C_{15}	6,22
CWBI-B1567	C_{18}	6,753
	Surfactins	
	C_{12}	12,121
	C_{13}	12,645
	C_{14}	13,291
	C_{15}	14,373
	C_{16} +C_{15}	14,609
	C_{15} +C_{16}	14,906
	C_{16}	15,098
	Fengycins	6.22
	Iturins	
	C_{17}	3,602
Bacillus mojavensis CWBI-B1568	Surfactins	
	C_{12}	12,112
	C_{13}	12.61
	C_{14}	13,483
	C_{15}	14,015

human pathogenic yeast.

ACKNOWLEDGEMENTS

This work was financially supported by University Constantine 1, we thank CWBI for his reception into the laboratories of bioindustry. H. C's Ph.D. thesis is also partially supported by a grant from the Fonds pour la formation à la Recherche dans l'Industrie et dans l'Agriculture (F.R.I.A.).

REFERENCES

Ariffin H, Abdullah N, Umi Kalsom MS, Shirai Y, Hassan MA (2006). Production and characterisation of cellulase by *Bacillus pumilus* EB3. Int. J. Eng. Technol. 3:47-53.

Bais HP, Fall R, Vivanco JM (2004). Biocontrol of *Bacillus subtilis* against infection of *Arabidopsis* roots by *Pseudomonas syringae* is facilitated by biofilm formation and surfactin production. Plant Physiol. 134:307-319.

Barakate M, Ouhdouch Y, Oufdou KH, Beaulieu C (2002). Characterization of rhizospheric soil *Streptomyces* from Moroccan habits and their antimicrobial activities. World J. Microbiol. Biotechnol. 18:49-54.

Caldeira AT, Santos Arteiro JM, Coelho AV, Roseiro JC (2011). Combined use of LC-ESI-MS and antifungal tests for rapid identification of bioactive lipopeptides produced by *Bacillus amyloliquefaciens* CCMI 1051. Process Biochem. 46:1738-1746.

Chen H, Yuan C, Cai K, Zheng Z, Yu Z (2008). Purification and identification of iturin A from *Bacillus subtilis* JA by electrospray ionization mass spectrometry. Acta. Microbiologica Sinica 48:116-120.

Dunne C, Crowley JJ, Loccoz YM, Dowling DN, De Bruijn F, Gara FO (1993). Biological control of *Pythium ultimum* by *Stenotrophomonas maltophilia* W81 is mediated by an extracellular proteolytic activity. Microbiology 143:3921- 3931.

Edmond MB, Wallace SE, McClish DK, Pfaller MA, Jones RN, Wenzel RP (1999). Nosocomial bloodstream infections in United States hospitals: a three-year analysis. Clin. Infect. Dis. 29:239-244.

Errakhi R, Bouteau F, Lebrihi A, Barakate M (2007). Evidences of biological control capacities of *Streptomyces* spp. against *Sclerotium rolfsii* responsible for damping-off disease in sugar beet (*Beta* 1608 Afr. J. Microbiol. Res. *vulgaris* L).World. J. Microbiol. Biotechnol. 23:1503-1509.

Fernandes PAV, De Arruda IR, Dos Santos AFAB, De Araujo AA, Souto Maior AM, Ximenes EZ (2007). Antimicrobial activity of surfactants produced by *Bacillus subtilis* R14 against multidrug-resistant bacteria. Braz. J. Microbiol. 38:704-709.

Gerhardt P (1994). Methods for General and Molecular Bacteriology, Washington, DC, American Society for Microbiology.

Glick BR, Patten CL, Holguin G, Penrose DM (1999). Biochemical and genetic mechanisms used by plant growth promoting bacteria. J. Theor. Biol. 190:63-68.

Jacques P, Hbid C, Destain J, Razafindralambo H, Paquot M, De Pauw E, Thonart P (1999). Optimisation of biosurfactant lipopeptide production from *Bacillus subtilis* S499 by Plackett-Burman design. Appl. Biochem. Biotechnol. 77:223- 233.

Kim PI, Bai H, Bai D, Chae H, Chung S, Kim Y, Park R, Chi YT (2004). Purification and characterization of a lipopeptide produced by *Bacillus thuringiensis* CMB26. J. Appl. Microbiol. 97:942-949.

Logan NA, Berkeley RCW (1984). Identification of *Bacillus* strains using the API system. J. Gen. Microbiol. 130:1871-82.

Maget-Dana R, Thimon L, Peypoux F, Ptack M (1992). Surfactin/Iturin A interactions may explain the synergistic effect of surfactin on the biological properties of iturin A Biochimie 74:1047-1051.

Molero G, Díez-Orejas R, Navarro-García F, Monteoliva L, Pla J, Gil C, Sánchez-Pérez M, Nombela C (1998). *Candida albicans*: genetics, dimorphism and pathogenicity. Int. Microbiol. 1:95-106.

Monteiro L, Mariano RLR, Souto-Maior AM (2005). Antagonism of *Bacillus* ssp. Against *Xantomonas campestris* pv. *Campestris*. Braz. Arch. Biol. Technol. 48:23-29.

Moyne AL, Cleveland TE, Tuzun S (2004). Molecular characterization and analysis of the operon encoding the antifungal lipopeptide bacillomycin D. FEMS. Microbiol. Lett. 234:43-9.

Odds FC (1988). Candida and Candidosis, London, Baillière Tindall.

Ongena M, Jacques P (2008). Involvement of fengycin-type lipopeptides in the multifaceted biocontrol potential of *Bacillus subtilis*. Trends. Microbiol. 16:115-125.

Ongena M, Jacques P, Toure Y, Destain J, Jabrane A, Thonart P (2005). *Bacillus* lipopeptides: versatile weapons for plant disease biocontrol. Appl. Microbiol. Biotechnol. 69:29-38.

Peypoux F, Bonmatin JM, Wallach J (1999). Recent trends in the biochemistry of surfactin. Appl. Microbiol. Biotechnol. 51:553-563.

Pfaller MA, Jones RN, Messer SA, Edmond MB, Wenzel RP (1998). National surveillance of nosocomial blood stream infection due to *Candida albicans*:frequency of occurrence and antifungal susceptibility in the SCOPE Program. Diagn. Microbiol. Infect. Dis. 31:327-332.

Price NPJ, Rooney AP, Swezey JL, Perry E, Cohan FM (2007). Mass spectrometric analysis of lipopeptides from *Bacillus* strains isolated from diverse geographical locations. FEMS. Microbio. Lett. 271:83-9.

Roberts MS, Nakamura LK, Cohan FM (1994). *Bacillus mojavensis* sp. nov., distinguishable from *Bacillus subtilis* by sexual isolation, divergence in DNA sequence, and differences in fatty acid composition. Int. J. Syst. Bacteriol. 44:256-264.

Salas JA, Mendez C (2007). Engineering the glycosylation of natural products in actinomycetes.Trends. Microbiol. 15:219-232.

Touré Y, Ongena M, Jacques P, Guiro A, Thonart P (2004). Role of lipopeptides produced by *Bacillus subtilis* GA1 in the reduction of grey mould disease caused by *Botrytis cinerea* on apple. J. Appl. Microbiol. 96:1151-1160.

Tserkovniak LS, Roi AO, Kurdysh IK (2009). Synthesis of amino acids of *Bacillus subtilis* IMV V-7023 in the medium with glycerophosphates. Mikrobiol. Z. 71:218-232.

Ventura M, Elli M, Reniero R, Zink R (2001). Molecular microbial analysis of *Bifidibacterium* isolated from different environments by the species-specific amplified ribosomal DNA restriction analysis (ARDRA). FEMS. Microbiol. Ecol. 36:113-121.

Yu GY, Sinclair JB, Hartman GL, Bertagnolli BL (2002). Production of iturin A by *Bacillus amyloliquefaciens* suppressing *Rhizoctonia solani*. Soil Biol. Biochem. 34:955-963.

Yun-Feng YE, Qi-Qin LI, Gang FU, Gao-Qing Y, Jian-Hua M, Wei L (2012). Identification of antifungal substance (Iturin A2) produced by *Bacillus subtilis* B47 and its effect on southern corn leaf blight. J. Integr. Agric. 1:90-99.

Zhenzhen Z, Qiushuo W, Kaimei W, Kemp B, Changhong L, Yucheng G (2010). Study of the antifungal activity of *Bacillus vallismortis* ZZ185 *in vitro* and identification of its antifungal components. Biores. Technol. 101:292-297.