

SCREENING FOR ANTIMICROBIAL ACTIVITY FROM UV-TOLERANT ACTINOBACTERIA AND SPORE-FORMING BACTERIA STRAINS ISOLATED FROM THE ALGERIAN SAHARA DESERT SOILS AFTER UVC EXPOSITION

Okba SELAMA^{***}, Khelil SIFI^{*}, Anis Sofiane DJABROUHO^{*}, Abdelghani ANAD^{*},
Ahmed ABDERRAHMANI^{*}, Elizabeth M.H. WELLINGTON^{**}, Hocine HACENE^{*}

^{*} Microbiology Group, Laboratory of Cellular and Molecular Biology, Faculty of Biological Sciences, USTHB- BP 32, EL ALIA, Bab Ezzouar, Algiers, Algeria

^{**} Environmental Microbiology, School of Life Sciences, University of Warwick, United Kingdom

Correspondence author: Okba Selama, Microbiology Group, Laboratory of Cellular and Molecular Biology, Faculty of Biological Sciences, University of Science and Technology Houari Boumediene (USTHB), BP 32, El Alia 16111 Bab Ezzouar, Algiers, Algeria, phone: +213 555190579, e-mail: okba.selama@gmail.com

Abstract. Extreme environments harbor such unusual bacterial groups that distinguish from normal ones with noteworthy bioactive molecules. The aim of this study was the isolation of UV-tolerant Actinobacteria and spore-forming bacteria strains with antimicrobial activity from the Algerian Sahara desert. Three soils were used in this study, two of which were from the Algerian Sahara desert (Melghir and Taghit) and one from an agriculture field within the university field (Algiers). Suspension/dilution on agar media followed by 15 min of UVC exposure were applied as protocol in this work. From all the irradiated plates, 46 colonies were best retrieved from both the two Algerian Sahara desert soils while less (24 colonies) from the university soil. In total, 29 distinct colonies were selected and analyzed for their macro- and micro-morphology and antimicrobial activities from which 10 strains were distinguished as UV-tolerant Actinobacteria and spore-forming bacteria with at least an antimicrobial activity against one test-microorganism. These isolates were also subject to enzymatic screening for amylase, caseinase, and lecithinase using culture dependent methods and for the presence of genes coding for putative antifungal compounds using a polymerase chain reaction (PCR) based method. In this study, we report the presence of a potential strain GT11 from the genus *Bacillus* with a particular activity against the pathogen fungus *Fusarium oxysporum* f. sp. *albedinis*. In addition to their UV-tolerant character, the presence of genes coding for putative antifungal compounds, combined with the antimicrobial activity against a broad range of indicator strains and their enzymatic potential, this would make these bacteria strains suitable for sustainable biotechnology applications.

Keywords: UVC; *Fusarium oxysporum* f. sp. *albedinis*; *Staphylococcus aureus* MRSA; Fengycin.

INTRODUCTION

Urgent discovering for new antibiotics are worldwide required to tackle human, animal and plant pathogens in particular these which have developed resistance against existing antibiotics and or where biological solution is mandatory.

Among the well-known pathogens to human and in many animal hosts, methicillin resistant *Staphylococcus aureus* (MRSA) has been known as one of the main pathogens associated with the development of antimicrobial resistance. The advent of AMR in *S. aureus* has been well registered in Algeria and worldwide and this throughout different molecular mechanisms [7].

As an example of the devastating phytopathogens in plant kingdom the fungus *Fusarium oxysporum* f. sp. *albedinis* that causes dangerous disease called Bayoud in date palm (*Phoenix dactylifera* L.). Knowing that dates are one of the most important crops growing in Algeria, Arab world and some neighboring countries disease caused by this pathogen is devastating to the local population and to national economies [13, 14, 36].

Biological solution is highly required for these and many other pathogens throughout the search for antimicrobial agents and or highly adapted and efficient antimicrobial producers. One particular research worth opportunity is the search for such solution in non or less explored environments like deserts. These microorganisms isolated from such ecosystems have the capability to survive the harsh

conditions like high pH, desiccation, high temperature, soil salinity, and intense solar radiation [16, 38].

The Sahara is the largest hot and dry desert in Africa. It extends on 9 millions km² from Mauritania to Egypt. This immense arid region can be considered as a paradigm of extreme environment for life because in these ecosystems the main limiting factor is water combined with highly and drastic contrasted temperatures. During the day, at the surface of the desert, the water is almost absent due to high temperature and high level of sunlight irradiation, and, except to some rare rainfall events, the only other source comes from dew. Under this limiting situation, there is a direct link between light intensity and water availability [25].

Algeria stands among the countries which are covered mostly by the Sahara, nearly 80 percent of the total surface of the country, yet we have been less interested to its bacterial biodiversity comparing to other environments. The few investigations show a poor microbial registered diversity: only (134/194861) bacterial registered sequences as observed using keywords (Algeria desert/ desert) and filtering for bacteria on NCBI Nucleotide database or better, 404 sequences, when using keyword (Algeria sahara) (<https://www.ncbi.nlm.nih.gov/nucleotide>: accessed in August 2020) [23, 24, 38, 39]. Interestingly, from these studies it has been noticed rare and remarkable worthy microorganisms, mainly some Actinobacteria and aerobic spore-forming bacteria strains which flourish under such extreme conditions and play a key role in the ecosystem, we might cite few newly isolates

Melghirimyces algeriensis [2], *Melghiribacillus thermohalophilus* [3], *Virgibacillus ainsalahensis* [5], *V. natechei* [6], *Thermoactinomyces khenchelensis* [29].

The ultraviolet are invisible wavelengths to the eye yet remarkable by their effects. We now define the ultraviolet range as roughly the 10-400 nm wavelength region. UV radiation can be divided into three sub-regions: UVA (400–315 nm), UVB (315–280 nm) and UVC (280–100 nm), we can also add the 10-180 nm range which is known as the vacuum ultraviolet because the radiation can be transmitted only by a vacuum [15, 20].

Similar to human, animals and plants, microorganisms are very susceptible to UV radiation, due to their small size, short generation time and the absence of shield or pigmentations in many cases [44]. The biological effects of UVA are habitually attributed to enhanced production of reactive oxygen species, which results in oxidative damage to DNA, lipids and proteins [37]. UVB and UVC radiation cause direct DNA damage by inducing the formation of DNA lesions (photoproducts), most remarkably pyrimidine dimers, which block RNA transcription and DNA replication [32]. However, some microorganisms overcome these extreme effects and withstand daily exposure of UV particularly some strains of bacteria found in desert. These bacteria have developed different degree of resistance to UV radiation [25, 30, 37].

Many studies have been interested either to the isolation of microorganisms from these extremes environments for studying adaption mechanisms to different extremes parameters of temperature, salinity, desiccation, and UV tolerance or for the potential of their biomolecules worldwide and locally [10, 12, 16]. However, to our knowledge this is the first time we investigated the potential of production of antimicrobial agents from UVC tolerate bacteria strains isolated from Algerian Sahara. We reported in this paper about the isolation of some selected bacteria strains from the unique and extreme ecological niche of the Algerian Sahara after high UVC exposure and their screening for antibiotic production against methicillin resistant *Staphylococcus aureus* (MRSA) and *Fusarium oxysporum* f. sp. *albedinis*, along with molecular profiling of genes for bioactive compounds. We believe that this ecosystem has not been studied for detailed for UV tolerant bacteria, so this seems to be the first comprehensive report on culturable

Actinobacteria and spore forming UV tolerant bacteria diversity of the Algerian Sahara.

MATERIALS AND METHODS

Isolation and morpho-cultural study

For enumeration and isolation of UV-tolerant culturable bacteria three soils were used: two soils from the Sahara desert: Taghit (T) (West) the second from Melghir (M) (East), and the third from the university field of Algiers (University of Sciences and Technology Houari Boumediene: USTHB, Bab-ezzouar) (U) (North Center of Algeria, an agricultural field), this last soil was used as the control soil sample.

Nearly 1 kg of ten composite samples of 100 g distanced 20 cm from 1-5 cm depth of each of the soils were collected on March 2018. The same soils have been previously characterized in different studies (Table 1).

Approximately one gram sample of each composite samples soil was placed in each of two tubes containing 9 mL sterile saline solution (0.9 %, and 10 % NaCl w/v). Tubes were vortexed for 5 min then soil particles were allowed to sediment for 5 min. A volume of 0.1 mL of the liquid phase was spread onto the surface of each of the five different culture media used in this study: nutrient agar (NA) (with and without 10% NaCl w/v), Widdel and Bak agar (BW) (with and without 10% NaCl w/v) [45], and International *Streptomyces* Project 2 (ISP2) (with 10% NaCl w/v) [28]. In this experiment, twelve plates of each medium were irradiated and three others were kept as non-irradiated as control plates. Irradiation was performed for 15 min using UVC laminar flow cabinet lamp (distance 45 Cm, TUV30W G30T8 25PK, Philips).

Plates were kept at 30 °C and the growth of microorganisms was evaluated daily for three weeks. Total counts of colonies were reported, and distinguished ones with Actinobacteria and spore-forming bacteria morphology were purified and stored.

The selected bacteria were tested for antimicrobial activities against: methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 43300; two Gram negative bacteria were used *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 25853; and against *Fusarium oxysporum* f. sp. *albedinis* a pathogen fungus of date palms provided by (Laboratoire de Recherche sur les Zones Arides, USTHB). Bacterial

Table 1. Characterization Soil samples from previous studies

Sample	Soil type	Moisture (%)	Organic matter (%)	pH	Conductivity (mS/cm)	Na	Cl	K	Ca	Reference
Melghir (M) g/L	Saline Alkaline soil/ Clay-Loamy soil	1.8	0.35	8.22	68.935	96.3	199.3	2.7	12.15	Amarouche-Yala 2009 [4]
Taghit (T) mg/L	Sand	0.26	0.5	8.1	0.545	18	13.5	8.8	79.2	Bahri et al. 2012 [9]
University campus (U) mg/Kg	Fine-loamy	3.5	4.6	6.95	0.46	10.50	12.50	18.6	25.9	Benhabylès et al. 2020 [11]

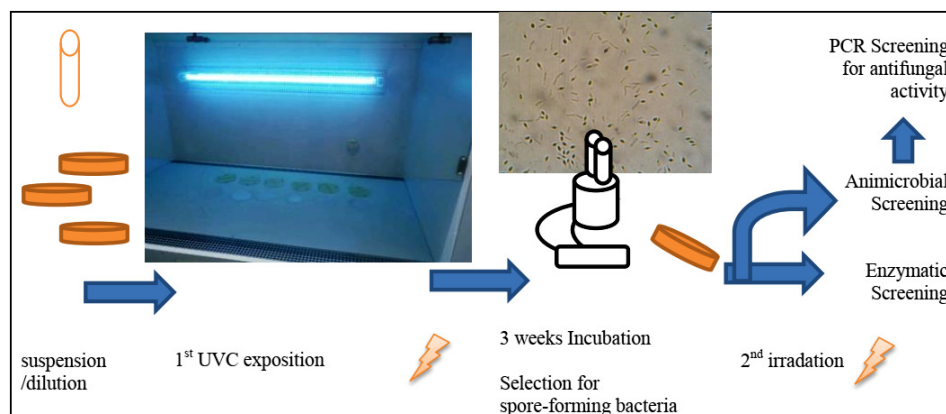


Figure 1. Isolation and selection procedure applied in this study for active UV-tolerant Actinobacteria and spore-forming bacteria

target strains were cultured on nutrient agar at 35 °C and the fungus at 25 °C on Sabouraud agar.

Hydrolysis of casein is tested on nutrient agar supplemented with 10% of skimmed milk by inoculation of 10 μL of bacterial suspension and incubated at 30 °C/ 48 h; a positive reaction is reflected by appearance of a transparent halo around the colony. Amyolytic activity was detected on Tryptic Soja Agar (TSA 1/10) supplemented with 1 % starch; after a spot inoculating, dishes are incubated and revelation is done by flooding dishes with an iodine solution (lugol). Starch hydrolysis results in a clear halo around the colony, unlike blue-starched areas. Revelation of lecithinase was carried out on a nutrient agar supplemented with an emulsion of egg yolk and distilled water (2 mL/20 mL). Then, the opacification of agar around the colony reflects the presence of a lecithinase while the appearance of a white opaque halo means the presence of lipase [28].

Fig. 1 shows isolation and selection procedures used in this study.

Molecular identification and screening

Bacterial strains of interest were identified using 16S rRNA gene sequencing. PCR was performed for direct amplification of 16S rRNA gene. Universal primers designated 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1429r (5'-TACGGYTACCTTGTACGACTT-3') were used [27]. The PCR mixture formed with 15 μL master mix (Sigma, UK), 1 μL each of forward and reverse primers (10 μM each) (Sigma, UK), 1 μL of Bovine Serum Albumin (10 mg/mL) (Promega, Madison, WI, USA), and 7 μL sterile distilled water in a final volume of 25 μL . PCR was performed with Mastercycler pro (Eppendorf). Agarose gels (1% w/v) were photo-

graphed after staining with ethidium bromide at 0.5 $\mu\text{g mL}^{-1}$ with a minivisionary imaging system. Sizes of the fragments were estimated using the Fermentas 1 kb Plus DNA ladder (Fermentas, UK).

The nucleotide sequencing for the 16S rRNA gene for prominent bacteria strains were carried out by GATC Biotech (UK). The isolates were identified using the EzTaxon-e server (<http://eztaxon-e.ezbiocloud.net/> accessed in August 2020) on the basis of 16S rRNA sequence data [26].

From the selected strains, these with antifungal activity were subjected to molecular screening for genes coding for antimicrobial compounds using two primer sets (Table 2). The sets of primers were designed by Tapi et al. [1, 43] amplified adenylation and thiolation nucleic acid domains genes of the NRPS (Non-Ribosomal Peptide Synthetase) system implicated in the biosynthesis of active lipopeptides of fengycins and bacillomycins.

The PCR conditions for the screening of NRPS system biosynthesis genes were determined experimentally for the sets of primers Abl1-F/Tbl1-R and Af2-F/Tf1-R. To find the correct annealing temperatures for each pair of primers, a PCR using an annealing temperature gradient was carried out in an Eppendorf AG (Hamburg, Germany) Mastercycler gradient. The PCR thermal cycle program included an initial denaturation at 94 °C for 3 min, followed by 30 cycles, with a denaturation step at 94 °C for 1 min, an annealing step of 30 s, at 45 °C with Abl1-F/Tbl1-R, and 40 s with Af2-F/Tf1-R followed by extension step during 45 s at 72 °C for both primers. Final extension was performed at 72 °C for 10 min. The PCR mixture consisted of 1.5 μL of each primer (20 μM), 12.5 μL (1.2 μM) of Master Mix (Fermentas GmbH, St-Leon-Rot, Germany), 8 μL of H_2O , and 1.5 μL of DNA template (10-30 ng μL^{-1}) in a final volume of 25 μL .

Table 2. List and characteristics of primers used in this study

Primer names	Sequence of primers	HyC	Expected fragments size (bp)	Non-ribosomal identified lipopeptides	References
Af2-F/	GAATAYMTCGGMCGTMTKGA	34	443, 452, 455	Fengycins	Tapi et al. 2010 [43]
Tf1-R	GCTTTWADKGAATSBCCGCC	72			
Abl1-F	GATSAWCARGTGA AAAATYCG	16	428, 431, 434	Bacillomycins	Abderrahmani et al. 2011 [1]
Tbl1-R	ATCGAATSKCCGCCRARATCRAA	32			

HyC: a Coefficient of hybridization calculated as described by Tapi et al. [43]

A volume of 10 µL of the amplified products after PCR was analyzed by electrophoresis in 1% (w/v) agarose gels stained with ethidium bromide (0.5 µg mL⁻¹). The gels were visualized with an Ultraviolet Illuminator and Digital Recorder (Geldoc Bio-Rad, Hercules, USA). PCR was scored positive when a band of the appropriate size was visualized on the agarose gel.

RESULTS

Enumeration and isolation

Regarding cultural diversity different culturable bacterial communities have been noticed for different soils on the used media. Overall and without irradiation, colonies of microorganisms were most pronounced on (NA) and (NA with 10% NaCl) for the university soil, best on BW and BW with 10% NaCl from Melghir soil (for both over 300 > colonies) and most noticed on BW for Taghit (77 colonies). After 15 min of irradiation drastic effect on microbial abundance and diversity for different soil and for all used media was observed. From all irradiated plates 46 colonies were best retrieved from both 2 soils Melghir and Taghit while 24 colonies from the university soil (Table 3).

From all the irradiated plates of the three soils 117 colonies were developed from which 29 distinct colonies were selected and analyzed for their

morphology (macroscopic, microscopic) and antimicrobial activities. Only 10 strains were distinguished as UV-tolerant Actinobacteria and spore-forming bacteria with an antimicrobial activity against at least one test-microorganism. These strains were the subject of testing for the following experimentations with 6 isolates from Taghit (on: 4 BW, 2 NA), and 4 from Melghir (all on NA) (Table 4).

Both UV-tolerant and antimicrobial activities were tested twice to confirm these properties. All strains had a positive antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (MRSA). In addition, 5 of them had an activity against *Escherichia coli* ATCC 25922. The strain GM3 had an antimicrobial activity on three tested bacteria and the strain GT11 had an antimicrobial activity against *Fusarium oxysporum* f. sp. *albedinis* (Fig. 2).

Most of the strains had caseinase activity and 4 had either amylase or lipase and two strains (GM2, GT1) produced all the three enzymes.

From the morphology description we could distinguish that 8 of the strains were *Bacillus* related and 2 strains belong to Actinobacteria phylum.

The molecular screening for potential genes of antifungal compounds on the only active strain GT11 revealed a band of expected length of 450 (bp) for *fengycin*. Another band is generated and this due to the nature of the primers (degenerate primers) (Fig. 3).

Table 3. Microbial abundance with and without irradiation for different soils and on different isolation media

Media \ Site	Bab-ezzouar (U)	Taghit (T)	Melghir (MG)
Non-irradiated			
ISP 2 10%	9	0	0
NA	>300	21	17
NA 10%	>300	4	39
BW	25	77	>300
BW 10%	0	0	>300
Irradiated			
ISP 2 10%	4	0	0
NA	17	2	0
NA 10%	7	5	17
BW	0	39	0
BW 10%	0	0	29
Total	25	46	46

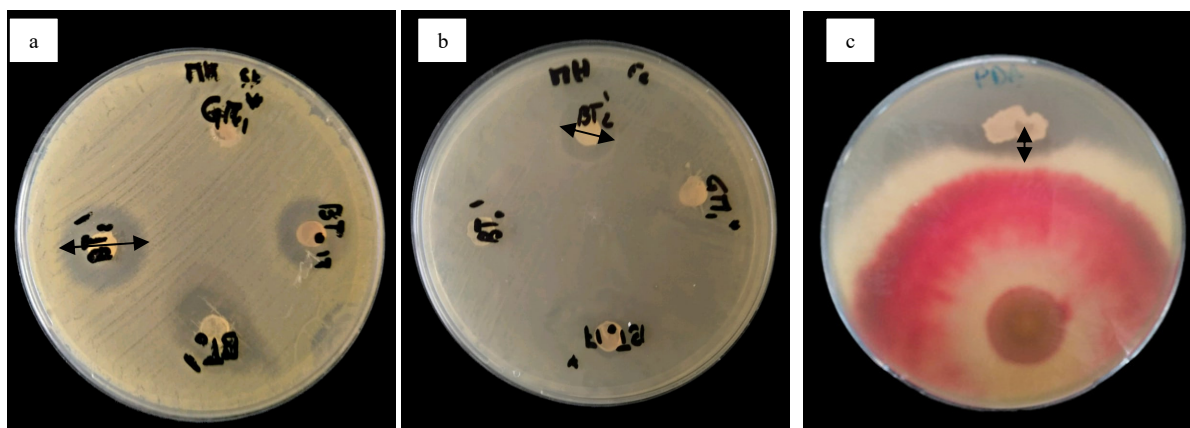


Figure 2. Antimicrobial activities of some strains among the selected strains (a) antibacterial activity against *Staphylococcus aureus* (b) against *Escherichia coli*. (c) antifungal activity against *Fusarium oxysporum* f. sp. *Albedinis*

Table 4. Isolation site, applied media, antimicrobial activity, enzymatic activity, and morphology description of the ten selected strains

Tests Strain	Soil origin	Isolation media	Antimicrobial activities			Enzymatic activities			Microscopy	Size (mm)	Macroscopic studies						
			<i>Ec</i>	<i>Pa</i>	<i>Sa</i>	<i>Fos</i>	A	C			L	Form	Elevation	Margin	Surface	Opacity	Consistency
GM2	M	NA	+	-	+	-	++	+	+	Isolated rods	2	Circular	Convex	Undulate	Rough	Opaque	dry
GM3	M	NA	+	+	+	-	++	+	-	Isolated rods	1	Punctiform	Plane	Entire	Smooth	Opaque	Creamy
GM4	M	NA	-	-	+	-	-	+	+	Isolated rods	2	Irregular	Raised	Undulate	Rough	Opaque	dry
GM5	M	NA	-	-	+	-	++	+	-	Isolated rods	2	Circular	Flat	Entire	Rough	Opaque	dry
BT0'17	T	BW	+	-	++	-	++	+	-	Filamentous	1	Circular	Flat	Entire	Rough	Opaque	dry
BM2	T	BW	+	-	+	-	-	-	+	Isolated rods	1	Circular	Flat	Entire	Smooth	Translucent	viscid
BT'2	T	BW	+	-	+	-	++	+	-	Filamentous	1.5	Circular	Flat	Entire	Rough	Opaque	dry
BT'0	T	BW	-	-	+	-	-	-	+	Isolated rods	2	Irregular	Raised	Undulate	Smooth	Translucent	Creamy
GT1	T	NA	-	-	+	-	+	+++	+	Isolated rods	2.5	Irregular	Flat	Undulate	Smooth	Opaque	Creamy
GT11	T	NA	-	-	+	+	-	+++	+	Isolated rods	2.5	Circular	Flat	Entire	Smooth	Opaque	Creamy

M: Melghir ; T: Taghit

Ec: *Escherichia coli* ATCC 25922

Pa: *Pseudomonas aeruginosa* ATCC 25853

Sa: methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 43300

Fos : *Fusarium oxysporum* f. sp. *albiedinis*

A: Amylase

C: Caseinase

L: Lecithinase

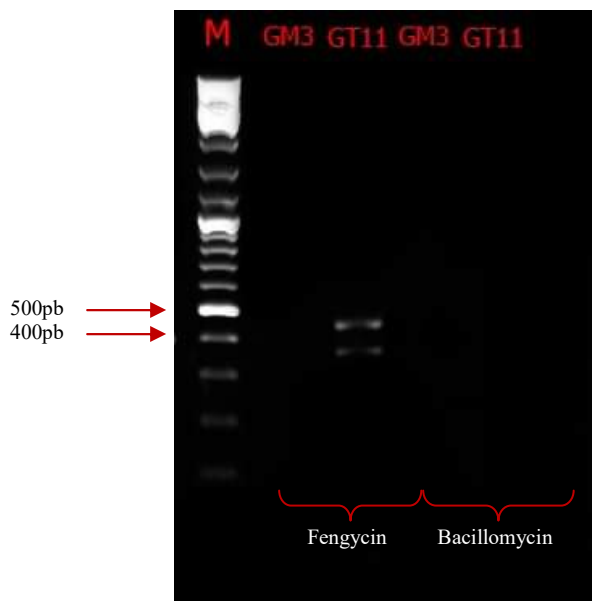


Figure 3. Agarose 1% gel electrophoresis of PCR products from genomic DNA of two strains (GT11: active strain, GM3: control strain) of the present study with selective fragments amplification range 400–500 bp using primers: Af2-F/Tf1-R and Abl1-F/Tb11-R (M: GeneRuler Express DNA Ladder)

Molecular Identification

Molecular identification of the active strain (GT11) against pathogenic fungus (*Fos* : *Fusarium oxysporum* f. sp. *albedinis*) shows that the strain was related to *Bacillus velezensis* (at 99.86% of similarity). The 16S RNA gene sequence of the strain was registered in NCBI Genbank with accession number (MN822797). Phylogenetic relationship GT11 is represented in Fig. 4.

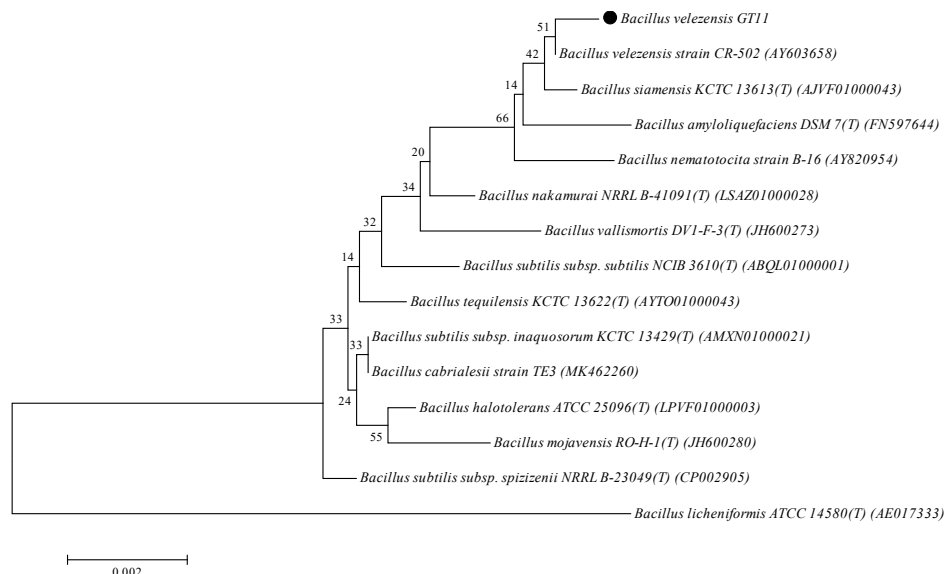


Figure 4. Molecular phylogeny of the bacteria strain *Bacillus velezensis* GT11 and the most related type strains species using partial 16S rRNA sequences. Alignment was performed using Muscle [17]. The evolutionary history was inferred using the Neighbor-Joining method [35]. The optimal tree with the sum of branch length = 0.03255945 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches [19]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method [41] and are in the units of the number of base substitutions per site. The analysis involved 15 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 1388 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 [42]. *Bacillus licheniformis* ATCC 14580^(T) was added as an out group for this tree.

DISCUSSION

The need for new active biomolecules from unexploited environments such as deserts is an urgent priority due to the severe consequences and the devastating nature of pathogens antibiotic resistance. The Sahara desert of Algeria is one of those environments which have not been yet explored fully for antimicrobial producing organisms. In this perspective, we have been following this fascinating ecosystem, in particularly, soils nearly over 3 decades, many of which are located in remote and protected areas. We have been in particularly interested into two types of soils the saline alkaline soils called Sabkha-Chott and sandy soil with or without association to palm trees, oasis. Published data have shown that this extreme environment may harbor exceptional and novel bacteria strains which can probably produce new antimicrobial drugs [22-24].

In addition to media composition and growth cultural conditions in particular high salinity, we have been interested in this study to isolate UVC tolerant microorganisms. Although the UVC region is not environmentally relevant, it is useful for investigating the UV sensitivity of microorganisms that are highly tolerant or insensitive to high doses of UVB, and to allow understanding of the molecular basis for the variability in UV sensitivity [37, 40].

Sahara diversity and abundance of bacteria can be low ranging from 10 to 10⁴ UFC/g of soil where the physicochemical parameters mainly of the soil are controlling factors [23]. Applying additional selection factors during isolation process reduce microbial abundance and diversity several folds. In our study,

this was observed with drastically decrease when using UVC comparing to control plates. Regarding general diversity, isolates were more of Gram positive Actinobacteria and spore-forming bacteria, one Gram negative bacterium was distinguished yet none of the pigmented colonies were present. Cultural biodiversity and microbial isolation in general and for the UVC bacteria tolerant depend deeply on isolation site and the culture media of isolation. More colonies were developed on both Saharan soils (46 each) than from the university soil after irradiation. This is totally in accordance with natural exposition of these soils to various extreme factors such as very low air humidity, a nearly complete absence of rains, and most important to high-flux solar radiation [18, 25]. In a similar study performed by Paulino-Lima et al. (2013), five sites from Atacama desert were investigated on three media LB, TGY and Marine agar. Results have shown for example that no growth was observed in LB plates from site 1, neither in TGY plates from site 3, after up to 7 days of incubation at 28 °C. Marine Agar was the best culture medium for microbial enumeration of samples from sites 1, 3, and 5, whereas LB medium was the best for sites 2 and 4. Samples from sites 2 and 4 showed the highest colony-forming units per gram of soil as showed in all types of culture media. The sample from site 3 exhibited the lowest colony forming units per gram of soil on average, although the lowest score was observed for site 1 plated on TGY. A total of 39 distinguished colonies with different pigmented morphotypes were obtained after visual inspection of all irradiated plates [31].

To protect themselves and resist radiation particularly to UV, microorganisms have to follow either or both ways of shield up and cell damage reparation. From the ten selected strains five showed creamy-viscid morphology which may suggest the production of external coat of exopolysaccharides that are worth investigate for both fundamental and applied biotechnology. Recently, a particular resistance mechanism to radiation was revealed by Guesmi et al. (2019) in strains from the *Bacillus* group. The mechanism implicates the production of radio-protective exopolysaccharides [21, 31].

In the other hand, the intrinsic mechanisms mainly engaging several molecular and enzymatic processes for the synthesis and reparations at different levels of DNA, proteins and lipids are so fascinating with a potential source of valuable biomolecules and enzymes [34, 44]. Interestingly, the so called extremozymes in particular which show a higher level of stability and activity over a wider range of conditions. The screened enzymes found in this study (proteases, amylases, and lipases) would be economically valuable since they were screened from strains thriving in such particular ecosystem and are likely to exhibit rare properties required for biotechnology industries [8, 38, 46].

The different patterns of activity against the targeted microorganisms observed in this study may indicate a variety of the produced active biomolecules

against in particular *Staphylococcus aureus* (MRSA). One prominent strain GT11 producing both antifungal and antibacterial active biomolecules was phylogenetically related to *Bacillus velezensis*. Genomic analysis has revealed that *B. velezensis* possesses strain-specific clusters of genes related to the biosynthesis of secondary metabolites, which play significant roles in both pathogen suppression and plant growth promotion. More specifically, *B. velezensis* exhibits a high genetic capacity for synthesizing cyclic lipopeptides known for their antimicrobial activity (i.e., surfactin, bacillomycin-D, fengycin), and siderophore as bacillibactin. Secondary metabolites produced by *B. velezensis* can also trigger induced systemic resistance in plants, a process by which plants defend themselves against recurrent attacks by virulent microorganisms [33]. In this study, we have been able to highlight the presence of *fengycin* gene but not *bacillomycin* one suggesting that the antifungal activity may be due to this molecule yet a biochemistry studies in particular using mass spectrometry is further needed to confirm the chemical nature of the active biomolecules.

To our knowledge, this is the first time to insight on UV-tolerant Actinobacteria and spore-forming bacteria strains biodiversity of the Algerian Sahara desert after UVC exposition. In this study, we have highlighted the interesting presence of diverse strains with potential antimicrobial biomolecules and enzymes. Future work will concentrate on more cloning and sequencing for whole clusters and genomes, chemical characteristics, identification by application of mass spectroscopy, and other enzymatic and biochemical techniques that would be more suitable for better determination of the nature of the elaborated compounds produced by the strains identified in this study in particular of *Bacillus velezensis* GT11.

Finally, these Sahara desert microorganisms should be investigated and reinvestigated in local and a global effort to understand not only their physiology, adaptation and biotechnological potential but also for their distribution locally and worldwide. Recently, we reported a terrible ecological accident arrived to one of the sites of our study that of Melghir with oil pipeline leak (<http://www.aps.dz/en/economy/tag/Oil%20pipeline> accessed: Friday, 04 September 2020 19:23). This is still under investigation and we do not have clues of the damages and the changes of the microbial component within the ecosystem. More studies are needed to confirm the position of our bacteria and their adaptation to this new situation.

Acknowledgments. The authors would like also to thank the Algerian Ministry of Higher Education and Scientific Research, The Islamic Development Bank for grant 600031378, and the University of Warwick for supporting this work. The authors would also like to thank the anonymous reviewers for the analysis and the enrichment of this paper. And to all people who have contributed to the successfulness of this research work.

REFERENCES

- [1] Abderrahmani, A., Tapi, A., Nateche, F., Chollet, M., Leclère, V., Wathelet, B., Hacene, H., Jacques, P., (2011): Bioinformatics and molecular approaches to detect NRPS genes involved in the biosynthesis of kurstakin from *Bacillus thuringiensis*. Applied Microbiology and Biotechnology, 92(3): 571-581.
- [2] Addou, A.N., Schumann, P., Spröer, C., Hacene, H., Cayol, J.L., Fardeau, M.L., (2012): *Melghirimyces algeriensis* gen. nov., sp. nov., a member of the family *Thermoactinomycetaceae*, isolated from a salt lake. Int J Syst Evol Microbiol, 62(Pt 7): 1491-1498.
- [3] Addou, N.A., Schumann, P., Spröer, C., Ben Hania, W., Hacene, H., Fauque, G., Cayol, J.-L., Fardeau, M.-L., (2015): *Melghiribacillus thermohalophilus* gen. nov., sp. nov., a novel filamentous, endospore-forming, thermophilic and halophilic bacterium. International Journal of Systematic and Evolutionary Microbiology, 65(Pt_4): 1172-1179.
- [4] Amarouche-Yala, S., (2009): Etude de la microflore du Chott Melghir (W. D'El Oued) : Isolement, identification et évaluation du degré de la pollution Magister Thesis, University of Sciences and Technology Houari Boumediene, Algeria.
- [5] Amziane, M., Darenfed-Bouanane, A., Abderrahmani, A., Selama, O., Jouadi, L., Cayol, J.L., Nateche, F., Fardeau, M.L., (2017): *Virgibacillus ainsalahensis* sp. nov., a moderately halophilic bacterium isolated from sediment of a saline lake in south of Algeria. Curr Microbiol, 74(2): 219-223.
- [6] Amziane, M., Metiaz, F., Darenfed-Bouanane, A., Djenane, Z., Selama, O., Abderrahmani, A., Cayol, J.L., Fardeau, M.L., (2013): *Virgibacillus natechei* sp. nov., a moderately halophilic bacterium isolated from sediment of a saline lake in southwest of Algeria. Curr Microbiol, 66(5): 462-6.
- [7] Antri, K., Akkou, M., Bouchiat, C., Bes, M., Martins-Simoes, P., Dauwalder, O., Tristan, A., Meugnier, H., Rasigade, J.-P., Etienne, J., (2018): High levels of *Staphylococcus aureus* and MRSA carriage in healthy population of Algiers revealed by additional enrichment and multisite screening. European Journal of Clinical Microbiology & Infectious Diseases, 37(8): 1521-1529.
- [8] Azua-Bustos, A., González-Silva, C., (2014): Biotechnological applications derived from microorganisms of the Atacama Desert. BioMed Research International, 2014: 909312.
- [9] Bahri, F., Saïbi, H., (2012): Characterization, classification, bacteriological, and evaluation of groundwater from 24 wells in six departments of Algeria. Arabian Journal of Geosciences, 5(6): 1449-1458.
- [10] Belov, A.A., Cheptsov, V.S., Vorobyova, E.A., (2018): Soil bacterial communities of Sahara and Gibson deserts: Physiological and taxonomical characteristics. AIMS microbiology, 4(4): 685-710.
- [11] Benhabylès, L., Djebbar, R., Miard, F., Nandillon, R., Morabito, D., Bourgerie, S., (2020): Biochar and compost effects on the remediative capacities of *Oxalis pes-caprae* L. growing on mining technosol polluted by Pb and As. Environmental Science and Pollution Research, 27(24): 30133-30144.
- [12] Boubetra, D., Sabaou, N., Zitouni, A., Bijani, C., Lebrihi, A., Mathieu, F., (2013): Taxonomy and chemical characterization of new antibiotics produced by *Saccharothrix* SA198 isolated from a Saharan soil. Microbiological Research, 168(4): 223-230.
- [13] Boucenna-Mouzali, B., Gaceb-Terrak, R., Rahmania, F., (2018): GC-MS analysis of cell wall-bound phenolic compounds and lignin quantification in date palm cultivars that are resistant or susceptible to *Fusarium oxysporum* f. sp. *albedinis*. Arabian Journal for Science and Engineering, 43(1): 63-71.
- [14] Bouguedoura, N., Bennaceur, M., Benkhalifa, A., (2010): Le palmier dattier en Algérie: situation, contraintes et apports de la recherche. pp. 16-22. In Aberlenc-Bertossi, F., (ed.): Biotechnologies du palmier dattier, IRD Éditions, Paris.
- [15] Brill, T.B., (1980): Light: Its interaction with art and antiquities. Springer, New York, 287 p.
- [16] Cherif, A., Tsiamis, G., Compant, S., Borin, S., (2015): BIODESERT: Exploring and exploiting the microbial resource of hot and cold deserts. BioMed Research International, 2015: 289457.
- [17] Edgar, R.C., (2004): MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research, 32(5): 1792-1797.
- [18] Fagliarone, C., Mosca, C., Ubaldi, I., Verseux, C., Baqué, M., Wilmotte, A., Billi, D., (2017): Avoidance of protein oxidation correlates with the desiccation and radiation resistance of hot and cold desert strains of the *Cyanobacterium chroococcidiopsis*. Extremophiles, 21(6): 981-991.
- [19] Felsenstein, J., (1985): Confidence limits on phylogenies: An approach using the bootstrap. Evolution, 39(4): 783-791.
- [20] Grupen, C., Rodgers, M., (2016): What about non-ionising radiation? pp. 133-143. In Grupen, C., Rodgers, M., (eds.): Radioactivity and radiation: What they are, what they do, and how to harness them, Springer International Publishing, Cham.
- [21] Guesmi, S., Chouchane, H., Neifar, M., Hosni, F., Cherif, A., Sghaier, H., (2019): Radiation-inducible radioprotective exopolysaccharides of *Bacillus siamensis* CV5 from irradiated roots of *Cistanche violacea* to decrease free radical damage produced by ionizing radiation. International Journal of Radiation Biology, 95(11): 1552-1563.
- [22] Hacene, H., (1986): Détermination des actinomycètes producteurs d'antibiotiques isolés du sol de trois palmeraies du Sud-Ouest algérien. Doctorat Thesis, University of Sciences and Technology Houari Boumediene, Algeria.
- [23] Hacène, H., Rafa, F., Chebhouni, N., Boutaiba, S., Bhatnagar, T., Baratti, J.C., Ollivier, B., (2004): Biodiversity of prokaryotic microflora in El Golea salt lake, Algerian Sahara. Journal of Arid Environments, 58(3): 273-284.
- [24] Hadj-Rabia-Boukhalifa, Y., Eveno, Y., Karama, S., Selama, O., Lauga, B., Duran, R., Hacène, H., Eparvier, V., (2017): Isolation, purification and chemical characterization of a new angucyclinone compound produced by a new halotolerant *Nocardioopsis* sp. HR-4 strain. World Journal of Microbiology and Biotechnology, 33(6): 126.
- [25] Heulin, T., De Luca, G., Barakat, M., Gommeaux, M., de Groot, A., Blanchard, L., Ortet, P., Achouak, W., (2017): Bacterial adaptation to hot and dry deserts. pp. 75-98. In Stan-Lotter, H., Fendrihan, S., (eds.): Adaption of microbial life to environmental extremes: Novel research results and application, Springer International Publishing, Cham.
- [26] Kim, O.-S., Cho, Y.-J., Lee, K., Yoon, S.-H., Kim, M., Na, H., Park, S.-C., Jeon, Y.S., Lee, J.-H., Yi, H.,

- (2012): Introducing EzTaxon-e: A prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *International Journal of Systematic and Evolutionary Microbiology*, 62(3): 716-721.
- [27] Lane, D.J., Pace, B., Olsen, G.J., Stahl, D.A., Sogin, M.L., Pace, N.R., (1985): Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. *Proceedings of the National Academy of Sciences*, 82(20): 6955-6959.
- [28] Marchal, N., Bourdon, J.-L., Richard, C., (1982): Les milieux de culture pour l'isolement et l'identification biochimique des bactéries. Doin, France, 482 p.
- [29] Mokrane, S., Bouras, N., Meklat, A., Lahoum, A., Zitouni, A., Verheecke, C., Mathieu, F., Schumann, P., Spröer, C., Sabaou, N., Klenk, H.P., (2016): *Thermoactinomyces khenchelensis* sp. nov., a filamentous bacterium isolated from soil sediment of a terrestrial hot spring. *Antonie Van Leeuwenhoek*, 109(2): 311-7.
- [30] Normand, P., Daffonchio, D., Gtari, M., (2014): The family *Geodermatophilaceae*. pp. 361-379. In Rosenberg, E., DeLong, E.F., Lory, S., Stackebrandt, E., Thompson, F., (eds.): *The prokaryotes: Actinobacteria*, Springer Berlin Heidelberg, Berlin, Heidelberg.
- [31] Paulino-Lima, I.G., Azua-Bustos, A., Vicuña, R., González-Silva, C., Salas, L., Teixeira, L., Rosado, A., da Costa Leitao, A.A., Lage, C., (2013): Isolation of UVC-tolerant bacteria from the hyperarid Atacama Desert, Chile. *Microbial Ecology*, 65(2): 325-335.
- [32] Pfeifer, G.P., (1997): Formation and processing of UV photoproducts: effects of DNA sequence and chromatin environment. *Photochem Photobiol*, 65(2): 270-283.
- [33] Rabbee, M.F., Ali, M.S., Choi, J., Hwang, B.S., Jeong, S.C., Baek, K.-H., (2019): *Bacillus velezensis*: A valuable member of bioactive molecules within plant microbiomes. *Molecules (Basel, Switzerland)*, 24(6): 1046.
- [34] Rainey, F.A., Oren, A., (2006): *Extremophiles*. Elsevier, Academic Press, London, 821 p.
- [35] Saitou, N., Nei, M., (1987): The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 4(4): 406-425.
- [36] Saleh, A.A., El_Komy, M.H., Eranthodi, A., Hamoud, A.S., Molan, Y.Y., (2015): Variation in a molecular marker for resistance of Saudi date palm germplasm to *Fusarium oxysporum* f. sp. *albedinis* the causal agent of Bayoud disease. *European Journal of Plant Pathology*, 143(3): 507-514.
- [37] Santos, A.L., Oliveira, V., Baptista, I., Henriques, I., Gomes, N.C.M., Almeida, A., Correia, A., Cunha, Â., (2013): Wavelength dependence of biological damage induced by UV radiation on bacteria. *Archives of Microbiology*, 195(1): 63-74.
- [38] Selama, O., Amos, G.C., Djenane, Z., Borsetto, C., Laidi, R.F., Porter, D., Nateche, F., Wellington, E.M., Hacene, H., (2014): Screening for genes coding for putative antitumor compounds, antimicrobial and enzymatic activities from haloalkalitolerant and haloalkaliphilic bacteria strains of Algerian Sahara soils. *BioMed Research International*, 2014: 317524.
- [39] Selama, O., James, P., Nateche, F., Wellington, E.M., Hacene, H., (2013): The world bacterial biogeography and biodiversity through databases: a case study of NCBI Nucleotide Database and GBIF Database. *BioMed Research International*, 2013: 240175.
- [40] Sundin, G., Jacobs, J., (1999): Ultraviolet radiation (UVR) sensitivity analysis and UVR survival strategies of a bacterial community from the phyllosphere of field-grown peanut (*Arachis hypogaea* L.). *Microbial Ecology*, 38(1): 27-38.
- [41] Tamura, K., Nei, M., Kumar, S., (2004): Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences of the United States of America*, 101(30): 11030-11035.
- [42] Tamura, K., Stecher, G., Peterson, D., Filipiński, A., Kumar, S., (2013): MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, 30(12): 2725-2729.
- [43] Tapi, A., Chollet-Imbert, M., Scherens, B., Jacques, P., (2010): New approach for the detection of non-ribosomal peptide synthetase genes in *Bacillus* strains by polymerase chain reaction. *Applied Microbiology and Biotechnology*, 85(5): 1521-1531.
- [44] Weihs, P., Schmalwieser, A.W., Schaubberger, G., (2013): UV Effects on Living Organisms. pp. 609-688. In Laws, E.A., (ed.): *Environmental toxicology: Selected entries from the encyclopedia of sustainability science and technology*, Springer New York, New York, NY.
- [45] Widdel, F., Bak, F., (1992): Gram-negative mesophilic sulfate-reducing bacteria. pp. 3352-3378. In Balows, A., Trüper, H.G., Dworkin, M., Harder, W., Schleifer, K.-H., (eds.): *The prokaryotes: A handbook on the biology of bacteria: ecophysiology, isolation, identification, applications*, Springer New York, New York, NY.
- [46] Zolfaghar, M., Amoozegar, M.A., Khajeh, K., Babavalian, H., Tebyanian, H., (2019): Isolation and screening of extracellular anticancer enzymes from halophilic and halotolerant bacteria from different saline environments in Iran. *Molecular Biology Reports*, 46(3): 3275-3286.

Received: June 26, 2020

Accepted: October 5, 2020

Published Online: October 16, 2020

Analele Universității din Oradea, Fascicula Biologie

<http://www.bioresearch.ro/revistaen.html>

Print-ISSN: 1224-5119

e-ISSN: 1844-7589

CD-ISSN: 1842-6433

University of Oradea Publishing House