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Bioactive Betulin produced by marine *Paecilomyces* WE3-F

Khouloud Barakat*, Mohamed Saleh

Marine Microbiology Laboratory, Division of Marine Environment, National Institute of Oceanography and Fisheries, Alexandria, Egypt.

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ABSTRACT

Objective: Marine fungi play an important role in human and animal health, leading compounds to new drug discoveries and prospects for their bioactivity potential.
Materials and Methods: *Paecilomyces* WE3-F was isolated from marine sediment (Red Sea, Shalateen, Egypt). Fungal isolate was screened for their antagonistic activity against four Gram-positive (*Bacillus cereus, Lesteria monocytogenes, Micrococcus luteus and Staphylococcu aureus*) and four Gram-negative (*Aeromonas hydrophila, Flavobacteruim* sp, *Pseudomonas aeruginosa and Vibrio cholera*,) pathogenic bacteria. *Paecilomyces* WE3-F was identified using 18S rRNA technology. Seven factors were chosen to be screened for bioactivity using the Placket Burman experimental design: sucrose, yeast extract, Na NO_3, temperature, initial pH, inoculum size, and incubation period.
Results: Among conditional factors, acidic pH and 1.5 ml inoculum size favored the bioactive metabolites. Furthermore, a number of solvents have been experimented for the extraction of the bioactive metabolite(s). Dichloromethane (DCM) crude extract from the fermentation broth of a marine *Paecilomyces* WE3-F showed the highest activity with averages of 26 and 24 mm against G-ve and G+ve, respectively. Under optimal culture conditions, the maximum extractable compound concentration in a 10-L culture medium reached 83.4 mg/L. Based on data obtained by thin layer chromatogram (TLC), gas chromatography - mass spectrum (GC-MS) and Fourier Transform Infrared (FTIR) the major compound, betulin was structurally identified.
Conclusions: The isolated marine *Paecilomyces* WE3-F, therefore, showed the ability to produce a betulin yield after optimal operating conditions for antibacterial potential.

INTRODUCTION

Betulin is a pentacyclic triterpenoid, manly found at the bark of several birch species (Alakurtti *et al*., 2006). The presence of reactive functional groups in betulin makes it an available and convenient starting material for synthesizing new biologically active compounds (Santos *et al*. 2009). In fact, the antibiotics era began in 1929 with the penicillin discovery by Fleming, who considered filamentous fungi as an important source of new drugs for therapeutic use (Yu and Keller 2005). Nowadays, this micromycetes by-product inspired scientists to re-explore new metabolites for biofactories, which synthesize desired pharmatheutical products for a healthy life. The production of terpenoids by endophytic fungi (65 sesquiterpenes, 45 diterpenes, 5 meroterpenes and 12 other terpenes) and their biological activities were reported by Jorgeane de Souza *et al*., (2011). Among these filamentous fungi, the ascomycete *Paecilomyces* species deserve special attention according to its reputation as a source of secondary metabolites without mycotoxins production (Mioso *et al*. 2015). *P. variotii* is found worldwide; it is commonly found in air, soil, and plant litter and was frequently recovered from aquatic environments where it probably resides as dormant conidia or ascospores (Marante *et al*., 2012; Silva *et al*., 2013).

Four new polyketides were isolated from marine *Paecilomyces variotii*. These compounds showed inhibitory activity against pathogenic bacteria, including methicillin-resistant *Staphylococcus aureus* 3089 and multi-drug-resistant *Vibrio parahemolyticus* 7001 (Liu *et al*., 2011).

In the continuous search for additional bioactive metabolites, a naturally occurring anacardic acid analogue was isolated from the same fungal strain showing cytotoxicity against human and antimicrobial activity against marine pathogens *Escherichia coli* DC2 and *Streptococcus iniae* FP5228 (Liu *et al*., 2012).

* *Corresponding Author*
Khouloud Barakat, Marine Microbiology Lab, Marine Environment Dept., National Institute of Oceanography and Fisheries, Alexandria, Egypt. Email: kh2m2[at]yahoo.com

Hyung *et al*., (2013) suggested that the viriditoxin (10) extract from marine *P. variotii* is effective for preventing and treating infections caused by fish pathogenic microorganisms, particularly *Aeromonas hydrophila*, *A. sobria*, *Edwardsiella tarda*, *Clostridium botulinum*, *Mycobacterium marinum*, *Streptococcus iniae*, *Vibrio parahaemolyticus*, *V. cholerae*, *V. vulnificus* and *V. ichthyoenteri*. These extracts that have been attributed to naturally occurring antibiotic in aquaculture could improve the safety of fish consumption, since it generates a lower environmental impact because of its low toxicity and rapid degradation (Mioso *et al*., 2014).

In the present study, the Placket Burmen experimental design was used to optimize the submerged culture conditions for the simultaneous production of bioactive betulin by *Paecilomyces* WE3-F. Meanwhile, the chemical characterization of betulin was carried out using TLC, GC-MS and FTIR.

MATERIALS AND METHODS

Fungal Isolation

Marine fungi were originally isolated form Shalateen coast, Red Sea, Egypt. Briefly, sediment sample was collected in sterile plastic bags (APHA, 1998). The isolation process was carried out using the pour plate technique. One gm sediment in 30ml sterile sea water were applied on glucose peptone agar (GPA) and then incubated at 30^{o}C for 7 days at pH 7. Slants containing pure cultures were stored at 4°C until further use. The macroscopic examination of fungal hyphea was then observed.

Bacterial Pathogens

Four Gram-negative bacteria: *Aeromonas hydrophila*, *Pseudomonas aeruginosa,*, *Flavobacterium* sp., *Vibrio cholera* and four Gram-positive bacteria*: Bacillus cereus, Lesteria monocytogenes; Staphylococcus aureus* Micrococcus *luteus* were kindly provided by the Microbiology Lab, Marine Environment Department, National institute of oceanography and fishers, Egypt. *Stock bacterial cultures were maintained on nutrient agar slants at 4 ˚C with monthly transfer.*

Antibacterial Potential

Screening for the antibacterial potential was carried using the disk-diffusion assay method according to Kirby-Bauer (Bauer *et al*., 1966) to test the inhibition of marine fungi against the tested pathogens. GPA medium was used to support the secondary metabolite production. One ml of pre-cultured tested bacteria (OD_{550} = 1.0) was mixed well using 25 ml of sterile and molten Muller-Hinton agar. Sterile discs of 6 mm diameter were immersed in the culture supernatant and placed on the seeded Muller-Hinton agar plates. All plates were incubated at 30oC for 24-48h. The inhibition zone diameters were recorded.

Identification of the Potent Isolate

Phylogenetical identification of the potent isolate was carried out using the manufacturer protocol of FavorPrep Fungi/ Yeast Genomic DNA Extraction Mini Kit (FAFYG 000). Gel documentation system (Geldoc-it, UVP, and England) was applied for data analysis using the Total lab analysis software, ww.totallab.com, (Ver.1.0.1). NCBI (http://www.ncbi.nlm.nih.gov/) software analysis was used for sequence alignment. Phylogenetic tree was constructed via ClustalW (http://www.ebi.ac.uk/Tools/msa/clustalw2/) software analysis. Isolated fungus was initially grown for 7 days on GPA medium in Petri dish.

A disc (5 mm) was cut from the margins of old culture of the agar plate culture and then transferred into the seed medium. The seed culture was grown in a 250 ml flask containing 100 ml GPA medium at 30^{o}C on a rotary shaker incubator at 120 rev/min for 7 days for further studies.

Effective Culture Media

Four culture media namely; potato dextrose, glucose peptone, czapek-dox and yeast-glucose liquid media, have been used to trace the antibacterial activity response. After adjusting pH to 7, the culture media were sterilized. Each medium was inoculated with 1 ml (1x107 spores/ml) then incubated at 30oC under shaking condition for 7 days.

Plackett-Burman Experimental Design

The effect of medium components on the bioactive compounds produced by marine fungus was studied by applying the Plackett Burman Experimental Design (Plackett and Burman, 1946; Greasham and Inamine, 1986). In this experiment, seven factors (medium components and culturing conditions) were determined in eight combinations organized according to the Plackett Burman matrix shown in Table 1. For each variable, a high level (+1) and a low level (-1) were tested (Rajendran *et al*., 2007). The factors tested were: Sucrose, $NaNO_3$ and yeast extract concentrations. Also, the pH, temperature inoculum size and incubation period of the tested fungus were also tested. The assays were performed in duplicate.

Table 1: Plackett-Burman design for seven variables.

Trials (n)	Independent variables						
	1	2	3	4	5	6	7
1	+	+	+	-	+	-	-
2	+	+	-	+	-	-	+
3	+	-	+	-	-	+	+
4	-	+	-	-	+	+	+
5	+	-	-	+	+	+	-
6	-	-	+	+	+	-	+
7	-	+	+	+	-	+	-
8	-	-	-	-	-	-	-

The main effect of each factor was determined using this equation: $Exi = (\Sigma Mi+ - \Sigma Mi-)/N$)

Where Exi is the variable main effect; Mi+ and Mi- are the calculated results of the bioactivities (inhibition zone in mm). They are recorded by trial which contains positive and negative levels of independent variables (xi), respectively. N is the number of trials divided by 2. A main effect figure with a positive sign

indicates that the positive level of this variable is nearer to optimum bio activity; while a negative sign indicates that the negative level of this variable is closer to optimum bioactivity. Using Microsoft Excel, statistical t-values for equal unpaired samples were calculated for the determination of variable significance (Al-Sarrani and El-Naggar, 2006).

Extraction of the Active Compound(S)

Four solvent systems namely: chloroform, dichloromethane, diethyl ether and ethyl acetate were individually applied for the extraction purpose. The organic layer was separated and concentrated under vacuum using rotary evaporator (STUART, RE300DB) to obtain the oily crude substance. Ten liters of culture filtrate of marine fungus were extracted from the supernatant using 1:1 (v/v) supernatant: dichlormethane. The overall crude extract amounted to 834 mg. The crude extract was then tested using the disc diffusion assay method.

ANALYTICAL METHODS

Thin layer chromatography

The selected dichloromethane crude extract was fractionated using the silica gel thin layer chromatography sheet (60GF254 Merck). Then, the crude was applied as a spot and left to complete separation. The plates stood for solvent evaporation and the R_f of the result spot was recorded. The collected samples were evaporated, weighed and dissolved in dichlormethane to be applied on pathogens inoculated plates. The bioactivity of the collected samples was checked for the eight pathogens using the disc diffusion assay.

Gas Chromatography-Mass Spectrum analysis

Oily crude was dissolved in 10 ml of dichlormethane and subjected to chromatographic analysis. The active dichlormethane fraction that was subjected to Gas chromatograph-mass spectrometer coupling (GC/MS) measurements were performed on an Agilent Technologies 7890A GC System with a flame ionization detector, a 5975C inert XL MSD Triple-Axis Mass Detector and Agilent 19,091S-433 Trace Analysis column. GC-conditions: 1 µL of sample was injected with an evaporation temperature of 250 °C, 1.8 bar, 2.5 mL/min, split 20:1. He, the carrier gas temperature was gradient 50 °C/1 min, 40 °C/min gradient 300 °C/min, 300 °C/5 min. The components were identified by comparing their retention times to those of authentic samples, as well as by comparing their mass spectra with those of Wiley 275 Library (Wiley, 2006). Quantitative data were obtained by the peak normalization technique using integrated FID response.

Fourier Transform Infrared analysis

Dichloromethane crude was subjected to FTIR-spectroscope (Perkin Elmer, USA) in the mid IR region of 400–4000 cm^{-1} with 16 scan speed using KBr discs.

RESULTS AND DISCUSSION

Paecilomyces sp. isolated from a broad range of habitats drives the diversification of new high-value-added secondary metabolites that play an important role in a healthy life, leading new drug discoveries, developments of new trends, and prospects for the bioactivity potential of this ascomycete (Mioso *et al*., 2015)

MARINE ACTIVE ISOLATE

After 7 days of incubation, marine *Paecilomyces* WE3-F was selected according to its highest antibacterial activities, where, the maximum activity recorded was 25 mm against *V. cholera* as Gram positive and 27 mm against *S. aureus* as Gram negative; whereas, lower activity recorded for both *B. cereus* and *L. monocytogenes* at 10 mm (Table 2).

Table 2: Bioactivity pattern of *Paecilomyces* WE3-F.

	Gram Negative				Gram Positive			
Test organism	*A. hydrophila*	*Flavobacter* sp.	*Peudomonas* sp.	*V. cholera*	*B. cereus*	*L. monocytogenes*	*M. luteus*	*St. aureus*
Inhibition zone (mm)	23	15	23	25	10	10	20	27

Compared with our results, cell free culture from *Paecilomyces fumosoroseus* obtained after 21 days of incubation exhibited highest activity for pathogenic bacteria including: *Escherichia coli* (32 mm), *Bacillus subtilis* (18 mm) and *Salmonella typhi* (12 mm); whereas, it was lower for *Pseudomonas aeruginosa* (10 mm), and showed no activity for *Klebsiella pneumoniae* (Gulwani *et al*., 2015). Also, different *Pacelomyces* sp. cultivated on PDA for 5 days, exhibited antibacterial activities against *B. subtilis*, *S. aureus* with averages of inhibition zone 27 and 29 mm, respectively (Lee *et al*., 2005).

In our present study, the marine potent isolate was analyzed for its 18S rRNA gene, and the phylogenetic relationship showed that this strain is very close to the strain type, *Paecilomyces* WE3-F (Figure 1).

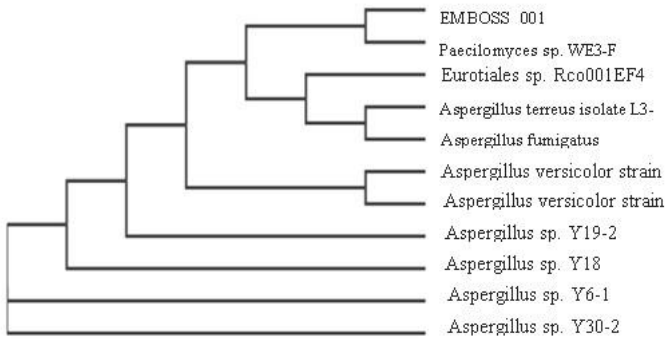

Fig. 1: Phylogenetic tree for *Paecilomyces* WE3-F based on specific gene sequence.

Table 3: Bioactivity of *Paecilomyces* WE3-F at different culture media for Gram -ve and Gram +ve bacteria.

Media	Inhibition zone (mm)									
	Gram -ve bacteria					Gram +ve bacteria				
	A. hydrophila	*Flavobacter sp.*	*Peudomonas* sp	*V. cholera*	AVG	*B. cereus*	*L. monocytogenes*	*M. luteus*	*St. aureus*	AVG
Potato Dextrose	6	11	6	10	8.25	0	7	10	7	6
Glucose Peptone	23	15	23	25	21.5	10	10	20	27	17.5
Czapek-dox	25	15	25	27	23	15	12	25	25	19.25
Yeast-Glucose	7	0	12	12	7.75	0	7	9	9	6.25

Table 4: Degree of positive or negative effect of independent variables on bioactivity of *Paecilomyces* WE3-F according to levels in the Plackett Burman experiments.

Trials (n=8)	Variables (Factors)													
	Inhibition zone (mm)													
	Sucrose		$NaNO_3$		Yeast		pH		Temperature(ºC)		Inoculum size		Incubation period	
	+	-	+	-	+	-	+	-	+	-	+	-	+	-
1	3.4		3.4		3.4		3.4		3.4		3.4		3.4	
2	5.4		5.4		5.4		5.4			5.4		5.4		5.4
3	10.5			10.5		10.5		10.5	10.5			10.5		10.5
4	17.2			17.2		17.2		17.2		17.2	17.2		17.2	
5		19.9	19.9		19.9			19.9		19.9	19.9			19.9
6		7.8	7.8		7.8			7.8	7.8			7.8	7.8	
7		4.1		4.1		10.5	4.1			4.1		4.1	4.1	
8		9.8		9.8		17.2	9.8		9.8		9.8			9.8
N	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
Mean	**9.1**	**10.4**	**9.1**	**10.4**	**9.1**	**10.4**	**5.7**	**13.9**	**7.9**	**11.6**	**12.6**	**7.0**	**8.1**	**11.4**
Main effect	**-1.3**		**-1.2**		**-1.2**		**-8.2**		**-3.8**		**5.6**		**-3.3**	
t-value	0.278		0.269		0.269		2.59		0.871		1.405		0.744	
Deg. of sign.	N.S		N.S		N.S		95% (-)		N.S		80% (+)		N.S	

*t-value significant at the 1% level = 3.70; 5% level = 2.45; 10% level = 1.94; 20% level =1.37. Standard t-values were obtained from statistical methods (Snedecor and Cochran, 1989).

EFFECTIVE MEDIA FOR BIOACTIVITY

Averages of inhibition zone of *Paecilomyces* WE3-F using four culture media was represented in Table 3 a & b. Using Czapek-dox medium, containing yeast and sucrose as main N and C sources, the highest activities were recorded at 23 mm and 19.24 mm against G-ve and G+ve, respectively; whereas, lower activities were represented using potato dextrose and yeast glucose medium. Two different liquid culture using *P. fumosoroseus* were tested for biocontrol efficacy using basal salts medium (Sandoval-Coronado *et al*., 2001). Another ten different culture media were used to investigate a mycelia growth of *P. fumosoroseus*, where, only two media were the favorable carbon and nitrogen source for maximal growth (Shim *et al*., 2003). Recently, the growth of *P. hepiali* in various agar media was studied, where, the addition of peptone improved mycelial growth and the most favorable carbon sources were mannose, fructose and glucose (Chioza, and Ohga, 2013).

Our study revealed that Czapek-dox medium containing in g/l: sucrose 20; $NaNO_3$ 2; yeast 2, were further used within an experimental design to elucidate the maximum activity of *Paecilomyces* WE3-F.

ELUCIDATION OF MEDIUM COMPONENTS FOR THE BIOACTIVITY

Optimization of the components of the selected Czapek-dox broth medium for the maximum activity using *Paecilomyces* WE3-F was determined. The nutrient medium factors and cultivation conditions were screened by applying the Plackett-Burmann design as described in the materials and methods section. Table 4 shows that trial 5 yielded the highest activity of *Paecilomyces* WE3-F at 19.9 mm which was obtained by incubating 1.5 ml (2.3×10^7 spores/ml) of the fungus for 5 days in 100 ml medium containing in g/l: sucrose 10, $NaNO_3$ 2.5 and yeast extract 3, at incubation temperature of 25°C and pH 5. These results revealed that the degree of significance of pH and inoculum size was at 95 and 80%, respectively. It also showed that the decrease of pH and the increase of inoculum size caused an increase in activities using marine *Paecilomyces* WE3-F (at the 5 and 20 % level of significance) (Figure 2). Using response surface methodology to optimize the extract process of mycelial polysaccharides from *P. hepiali*, as well, test antioxidant activities were studied by Yu *et al* (2011).

In addition, the best growth for *P. hepiali* in an optimized media was obtained at 25°C in 14 days with initial pH range from 6 to 8 (Chioza, and Ohga, 2013). Giraldo *et al*. (2012) observed that *P. variotii* is able to produce extracellular invertases when grown for 96 h, where, the optimal temperature and pH values were 60°C and 4·0–4·5, respectively. Statistical method involves changing one independent variable was carried out for medium optimization condition for *Inonotus obliquus* yield of betulin, where, optimal pH was at 6.0 and optimum temperature was at 25°C (Bai *et al*., 2012).

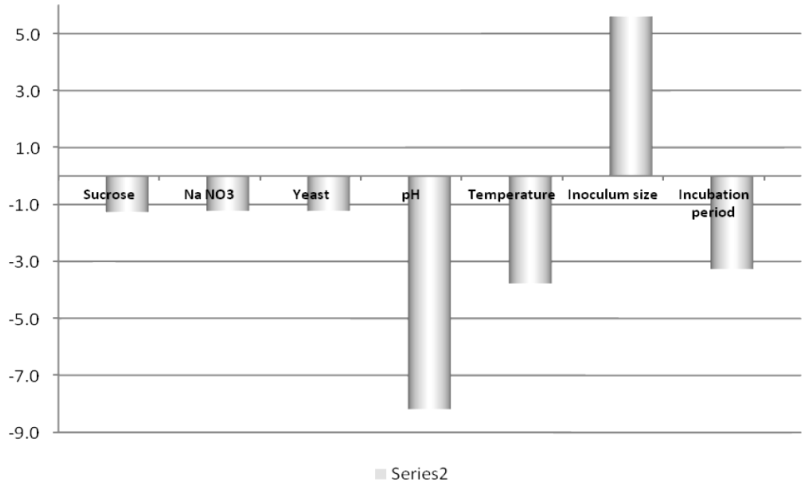

Fig. 2: Bioactivity main effect of the medium constituents after applying Plackett-Burman experimental design.

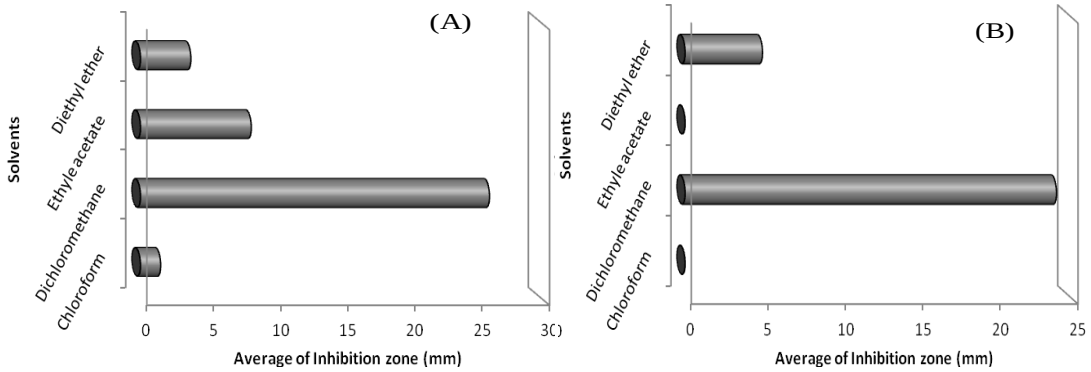

Fig. 3: Bioactivity of *Paecilomyces* WE3-F extracts against the Gram +ve (A) and Gram -ve bacteria using different solvents.

Fig. 4: Antibacterial activity of dichloromethane extract against Gram-positive and Gram-negative tested bacteria using disc-diffusion method.

DETERMINATION OF THE APPROPRIATE SOLVENT(S) FOR EXTRACTION OF THE ACTIVE AGENT

The results showed that dichloromethane solvent was selected for the extraction procedures where the yielded extract obtaining showed the highest average zone of inhibition; 26 and 24 mm against Gram –ve and +ve, respectively (Figure 3). However, the other solvent showed low or no antimicrobial activity against the eight pathogens (Figure 4). Compared with our results, the extracts from *P. lilacinus* were evaluated for antimicrobial activity by disc diffusion method showed activity for Gram-positive *S. aureus* and *L. monocytogenes* at average inhibition zone 20.76 mm, even while, Gram-negative bacteria were not affected by the tested compounds (Teles *et al.*, 2013a). The antimicrobial activity of the methanol crude extract from marine-derived *Paecilomyces* sp. was determined for MRSA, *Escherichia coli*, *A. hydrophila* and *C. albicans* at 18, 6, 9 and 7 mm, respectively (Liu *et al.*, 2011). Ethyl acetate extract of *P. variotii* FEL24 presented antimicrobial activities ranged from 19 to 35 mm (Silva *et al*, 2011). The active ethyl acetate extract of *P. lilacinus* showed antibacterial activity for some pathogenic bacteria by agar diffusion assay: 19, 19.3 and 16 mm against *Ps. syringae*, *R.*

solanacearum and *E. coli;* whereas, there was no activity against *X.vesicatoria, B. cereus B. subtilis* and *S. aureus* (Srivastava and Anandrao, 2015).

PHYSICOCHEMICAL CHARACTERIZATION OF THE CRUDE EXTRACT

Paecilomyces genus includes many species capable to produce secondary metabolites which belongs to different chemical groups with wide biological activity such as paecilosetin (Lang *et al*., 2005), paecilaminol (Ui *et al*., 2006), paecilodepsipeptide A (Isaka *et al*., 2007), paecilin A and B (Guo *et al*., 2007). Using silica gel TLC plate, dichloromethane crude extract was run as concentrated spots (R_f = 0.90) then scratched to estimate its bioactivity for the eight tested pathogens by disc diffusion assay. The extracts from *P. lilacinusto* assayed were prepared by dissolution on a suitable solvent and applied in readymade TLC plates that developed a yellow color in the plates (Teles and Takahashi, 2013b). The extract of *P. variotii* FEL32 strain submitted to TLC yielded two spots of R_f 0.48 and 0.70 (Silva *et al*, 2011). Spectroscopic analysis of the separated spot showed a major sharp peak observed at acquisition time 29 min with the highest count percentage $1x10^2$ on gas-chromatography. This major peak was identified as betulin with the aid of mass spectrum library (Figure 5A & B). More recently, a new strain of *P. variotii* was isolated from a marine habitat showed 28 structural groups of volatile organic compounds identified by GC-MS from liquid fermented biomass, where, active terpenoids were represented (Marante *et al*., 2012). Moreover, mass spectrum of viriditoxin obtained from marine *P. variotii* was studied by Silva *et al*. (2013).

FT-IR spectrum of betulin showed the absorption band frequency at 3448 cm^{-1} with the presence of O-H stretch. In the area of 3059 cm^{-1} appears derivates from C-H stretch for alkane in CH_2 group and at 1765 cm^{-1} appears a very intensive absorption characteristic ribbon of carbonyl group (C=O). The1598 cm^{-1} band can be assigned to C=C stretching and CH_2 bending vibrations of the methyl and of the CH_2 groups in the rings. The band at 1006 cm^{-1} can be assigned to C-O stretching vibrations in the CH_2OH group. A strong absorption band at 713 cm^{-1} indicated the presence of wagging vibrations in the terminal group aromatic C-H (Figure 6).

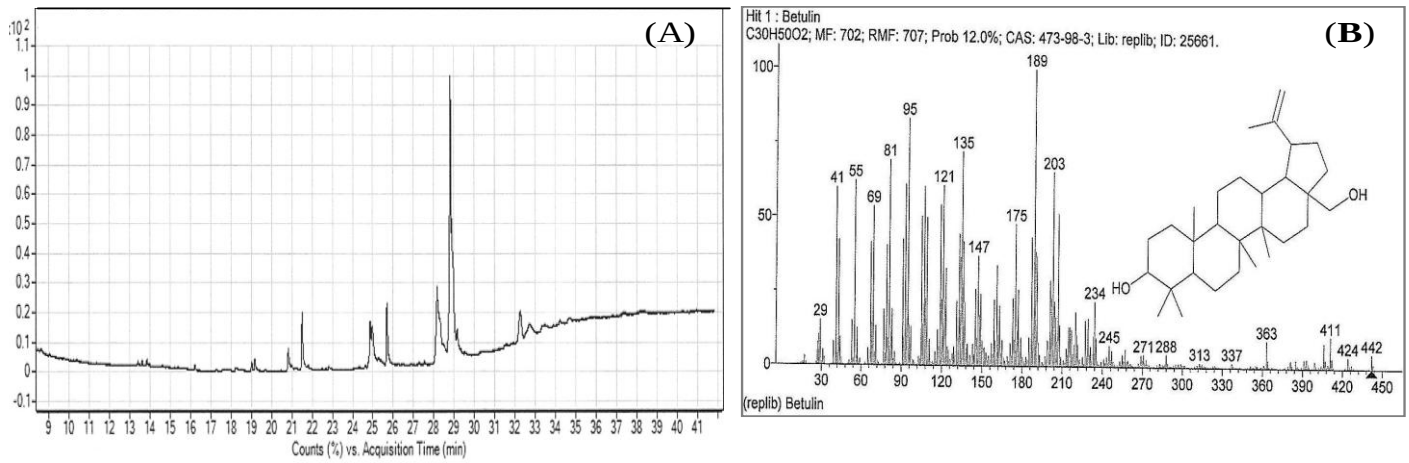

Fig. 5: Gas Chromatography-Mass Spectra showing the major peak in GC analysis (A) and identified compound "betulin" in Mass Spectral analysis (B).

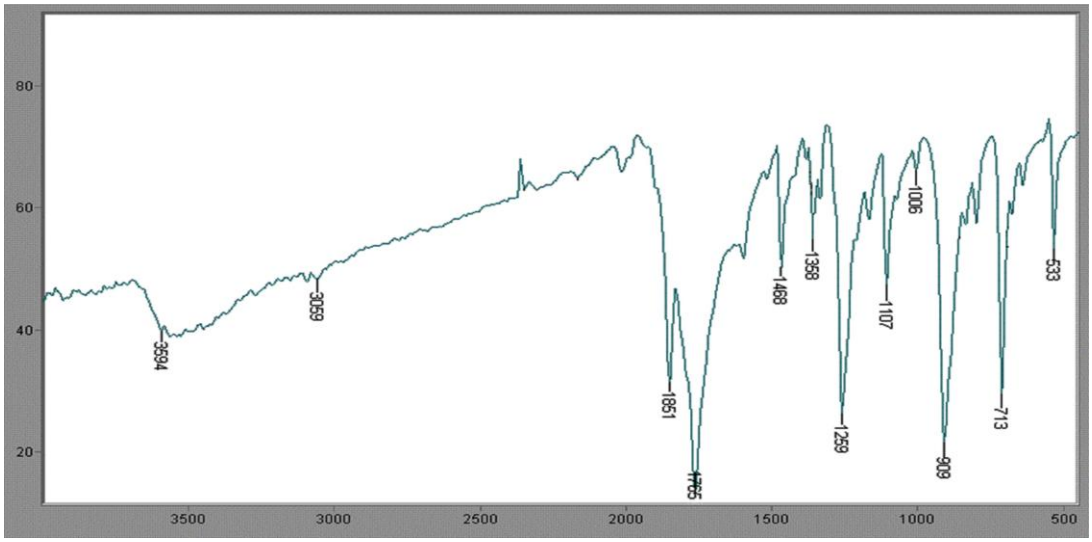

Fig. 6: FT-IR trace of betulin as a major product.

The FT-IR spectrum of bioactive exopolysaccharide from a native *Paecilomyces* sp. showed characteristic bands at different wave lengths (Naturforsch, 2011). In conclusion, work reports the isolation of a marine *Paecilomyces* WE3-F showing the ability to produce betulin with antibacterial potential.

Using Plackette Burman experimental design, it was possible to determine optimal operating conditions to obtain a high betulin yield. This metabolite could be used in further investigation as a potential candidate for analytical purposes and /or further biotechnological applications.

REFERENCES

Alakurtti S, Makela T, Koskimies S, Yli-Kauhaluoma J. Pharmacological properties of the ubiquitous natural product betulin. Eur J Pharm Sci, 2006; 29:1–13.

Al-Sarrani A, El-Naggar M. Application of Plackett-Burman factorial design to improve citrinin production in *Monascus ruber* batch cultures. Bot Stud, 2006; 47:167-174.

APHA (American Public Health Association). 1998. American Water Works Association, and Water Pollution Control Federation, Standard methods for the analysis of water and wastewater (20th ed.): Washington, D.C., American Public Health Association.

Bai Y-H, Feng Y-Q, Mao D-B, Xu Ch-P. Optimization for betulin production from mycelial culture of *Inonotus obliquus* by orthogonal design and evaluation of its antioxidant activity. J Taiwan Inst Chem Engineers, 2012; 43: 663–669.

Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol, 1966; 45: 493–496.

Chioza A, Ohga S. Mycelial Growth of *Paecilomyces hepiali* in Various Agar Media and Yield of Fruit Bodies in Rice Based Media. Advances Microbiol, 2013; 3: 529-536.

Giraldo MA, Silva TM, Salvato F, Terenzi HF, Jorge JA, Guimarães LHS. Thermostable invertases from *Paecylomyces variotii* produced under submerged and solid-state fermentation using agroindustrial residues. World J Microbiol Biotechnol, 2012; 28: 463–472.

Greasham R, Inamine E. 1986. Nutritional improvement of processes. In: Manual of Industrial Microbiology and Biotechnology. Demain AL & Solomon NA (eds). American Soc Microbiol, Washington D.C. 41-48.

Gulwani N, Shukla H, Sandhu SS. Screening the Antibacterial Potential of *Paecilomyces fumosoroseus* against Some Pathogenic Bacteria. American J Adv Drug Delivery, 2015; 3(1): 24-31.

Guo Z, She Z, Shao C, Wen L, Liu F, Zheng Z, Lin Y. 1H and 13C NMR signal assignments of paecilin A and B, two new chromone derivatives from mangrove endophytic fungus *Paecilomyces* sp. (tree 1-7). Magnetic Resonance Chem, 2007; 45:777-780.

Hyung JJ, Kang H, Joong JJ, Soo KY. 2013. *Paecilomyces variotii* extracts for preventing and treating infections caused by fish pathogenic microorganisms. KR Patent, 2013051523.

Isaka M, Palasan S, Lapanun S, Sriklung K. Paecilodepsipeptide A, an antimalarial and antitumor cyclohexadepsipeptideo from the insect pathogenic fungus *Paecilomyces cinnamomeus* BCC9616. J Nat Prod, 2007; 70:675-678.

Jorgeane de Souza J, Vieira IJC, Rodrigues-Filho E, Braz-Filho R. Terpenoids from Endophytic Fungi. Molecules, 2011; 16(12):10604-18.

Lang G, Blunt JW, Cummings NJ, Cole ALJ, Munro MHG. Paecilosetin, a new bioactive fungal metabolite from a New Zealand isolate of *Paecilomyces farinosus*. J Nat Prod, 2005; 68:810-811.

Lee S-Y, Nakajima I, Ihara F, Kinoshita H, Nihira T, Cultivation of entomopathogenic fungi for the search of antibacterial compounds. Mycopathologia, 2005; 160: 321–325.

Liu J, Li F, Kim EL, Li JL, Hong J, Bae KS, Chung HY, Kim HS, Jung JH. Antibacterial Polyketides from the Jellyfish-Derived Fungus *Paecilomyces variotii*. J Nat Prod, 2011; 74: 1826–1829.

Liu J, Li F, Lee Y-M, Li JL, Hong J-K, Yoon W-D, Kim E-K, Jung J-H. An anacardic acid analog from the jellyfish-derived fungus *Paecilomyces variotii*. J Nat Prod, 2012; 18: 8-12.

Marante TFJ, Mioso R, Barrera BJ, González GJE, Rodríguez SJJ, Bravo H., de Laguna I. Structural characterization and metabolite profiling of the facultative marine fungus *Paecilomyces variotii*. Ann Microbiol, 2012; 62: 1601–1607.

Mioso R, Marante FJT, Bravo de Laguna IH, Bessonart M. Química de productos naturales y acuicultura: un enfoque interdisciplinar. Química Nova, 2014; 37: 513–520.

Mioso R, Marante FJT, Bravo de Laguna IH. The Chemical Diversity of the Ascomycete Fungus *Paecilomyces variotii*. Appl Biochem Biotechnol, 2015; 177(4): 781-791.

Naturforsch ZC. Antibacterial activity of an oligosaccharide of native *Paecilomyces* sp. and its aminoglycosylated derivative. 2011; 66(3-4): 123-128.

Plackett RL, Burman JP. The design of optimum multifactorial experiments. Biometrika, 1946; 33:305-325.

Rajendran A, Sundaramurthy AB, Thangavelu V. Statistical evaluation of medium components using Plackett-Burman experimental design and kinetic modeling of lipase production by *Bacillus sphaericus*. Chem Biochem Eng, 2007; 21:181-188.

Sandoval-Coronado CF, Luna-Olvera HA, Arévalo-Niño K, Jackson MA, Poprawski TJ, Galán-Wong LJ. Drying and formulation of blastospores of *Paecilomyces fumosoroseus* (Hyphomycetes) produced in two different liquid media. World J Microbiol Biotechnol, 2001; 17(4): 423-428.

Santos RC, Salvador JAR, Marín S, Cascante M. Novel semisynthetic derivatives of betulin and betulinic acid with cytotoxic activity. Bioorg Med Chem, 2009; 17(17): 6241–6250.

Shim SM, Lee KR, Kim SH, Im KH, Kim JW, Lee UY, Shim JO, Lee MW, Lee TS. The Optimal Culture Conditions Affecting the Mycelial Growth and Fruiting Body Formation of *Paecilomyces fumosoroseus*. Mycobiol, 2003; 31(4): 214-220.

Silva MRO, Almeida AC, Arruda FVF, Gusmão N. 2011. Endophytic fungi from brazilian mangrove plant *Laguncularia racemosa* (L.) Gaertn. (Combretaceae): their antimicrobial potential Science against microbial pathogens: communicating current research and technological advances A. Méndez-Vilas (Ed.) 1260-1266.

Silva MRO, Kawai K, Hosoe T, Takaki CGM, Gusmão BN, Fukushima K. 2013. Viriditoxin, an antibacterial substance produced by mangrove endophytic fungus *Paecilomyces variotii*. In A. Méndez-Vilas (Ed.), Microbial pathogens and strategies for combating them: science, technology and education, 1406–1411. Badajoz: Formatex Res. Pub.

Snedecor GW, Cochran WG. Statistical Methods. 1989. Eighth Edition, Iowa State University Press.

Srivastava A, Anandrao RK. Antimicrobial Potential of Fungal Endophytes Isolated From Leaves of *Prosopis Juliflora* (Sw.) Dc., An Important Weed. Inter J Pharm Pharm Sc, 2015; 7(12): 128-136.

Teles APC, Atalibab GS, Takahashib JA. Modulation of *Paecilomyces lilacinus* antimicrobial metabolite production by co-culturing with *Salmonella typhimurium*. Nat Prod Res, 2013a; 27(17): 1598–1601.

Teles APC, Takahash JA. Paecilomide, a new acetylcholinesterase inhibitor from *Paecilomyces lilacinus*. Microbiolog Res, 2013b; 168(4): 204–210.

Ui H, Shiomi K, Suzuki H, Hatano H, Morimoto H, Yamaguchi Y, Masuma R, Sakamoto K, Kita K, Miyoshi H, Tomoda H, Tanaka H, Omura S. Paecilaminol, a new NADH-fumarate reductase inhibitor produced by *Paecilomyces* sp. FKI-0550. J Antibiotics, 2006; 59:591-596.

Wang H, Hong J, Yin J, Moon HR, Liu Y, Wei X, Oh DC, Jung JH. Dimeric Octaketide Spiroketals from the Jellyfish-Derived Fungus *Paecilomyces variotii* J08NF-1. , 2015; 78(11): 2832-2836.

Wiley J. Wiley Registry of Mass Spectral Data, eighth Edition "software". 2006; Wiley's Scientific, Technical, and Medical Databases.

Yu J-H and Keller N. Regulation of Secondary Metabolism in Filamentous Fungi. Ann Rev Phytopath, 2005; 43: 437-458.

Yu SJ, Zhang Y, Li CR, Zhang Q, Ma ZY, Fan MZ. Optimization of ultrasonic extraction of mycelial polysaccharides from *Paecilomyces hepiali* using response surface methodology and its antioxidant activity. African J Biotechnol, 2011; 10(75): 17241-17250.

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