Bull. Pharm. Sci., Assiut University, Vol. 45, Issue 1, 2022, pp. 359-369.

Bulletin of Pharmaceutical Sciences
Assiut University
Website: http://bpsa.journals.ekb.eg/
e-mail: bullpharm@aun.edu.eg

PRODUCTION OF CELL WALL-HYDROLYZING ENZYMES AND GC-MS EXTRACT ANALYSIS OF BYSSOCHLAMYS LAGUNCULARIAE, A NEW RECORD ISOLATED FROM EGYPT

Osama A. Al-Bedak[1], Mohamed A. Abdel-Sater[2], Hagar M.K. Galal[3], and Sedky H.A. Hassan[3,4*]

[1]*Assiut University Mycological Centre, Assiut University, Assiut 71511, Egypt*
[2]*Department of Botany and Microbiology, Faculty of Science, Assiut University, Assiut, Egypt*
[3]*Department of Botany and Microbiology, Faculty of Science, New Valley University, El-Kharga 72511, Egypt*
[4]*Department of Biology, College of Science, Sultan Qaboos University, Muscat 123, Oman*

In the present work, six strains of Byssochlamys laguncularіae were isolated from alkaline wastewater of textile factory polluted with azo dyes in Alexandria, Egypt. Out of the six strains, only one was identified using the sequencing of the internal transcribed spacer gene (ITS). ITS sequence was uploaded to GenBank (MT939911) and a pure culture was maintained in the culture collection of Assiut University Mycological Centre (AUMC) as B. laguncularіae AUMC 14498. The strain shared some morphological characteristics indicating species such as the existence of smooth chlamydospores, ascospore size, and good growth on Czapek's yeast autolysate agar (CYA) at 30 °C. However, it was different from the type species by providing larger conidia and positive acid production on creatine sucrose agar (CREA) at 30 °C. Extrolite analysis of this strain was performed using gas chromatography mass spectrometry (GC-MS). The extrolite analysis extract revealed that more than fifty chemical compounds were detected, which have an important medical application. All B. laguncularіae strains tested, when grown on oat spelt xylan could utilize oat spelt xylan to produce endoglucanase, exoglucanase and xylanase as well as maize starch and pectin, respectively for amylase and pectinase production. Three strains of B. laguncularіae achieved maximum specific activity for amylase ranged from 75.65 to 114.67 IU/mg, while one of these three was superior for endoglucanase (59.1 IU/mg), exoglucanase (102.84 IU/mg), pectinase (34.33 IU/mg), and xylanase (90.21 IU/mg) production.

Keywords: *Byssochlamys laguncularіae, B. laguncularіae enzymes, B. laguncularіae extrolite, : Byssochlamys isolation.*

INTRODUCTION

Byssochlamys is ascomycete's genus belonging to family Aspergillaceae and order Eurotiales. Its species produce ascospores which are heat-resistant, and survive considerable periods of heat above 85 °C[1&2]. Furthermore, *Byssochlamys* species can grow under very low oxygen tensions[3], and can produce pectinases. These three physiological characteristics make *Byssochlamys* species very important spoilage fungi in pasteurized and canned fruit[4]. Stolk and Samson[5] synonymized it with *B. nivea*. Houbraken *et al.*[6] demonstrated that *Byssochlamys* and its associated anamorph species can be separated into at least nine taxa by investigating the micro- and macroscopical characteristics of *Byssochlamys* and *Paecilomyces variotii*-like isolates. Recently, it was synonymized into three species namely *B. laguncularіae, B. spectabilis*, and *B. zollerniae* (Index Fungorum database).

Received in 24/8/2021 & Accepted in 10/11/2021

*Corresponding author: Sedky H.A. Hassan, E-mail: sedkyhassan@sci.nvu.edu.eg

The plant cell wall, composed of lignin, cellulose, hemicellulose, and pectin, known to be a green chemical raw material[7]. Fortunately, the availability of multiple components makes it sustainable, and its enzymatic hydrolysis usually involves the activity of a series of enzymes. As a result, the production of plant cell-wall hydrolytic enzymes is under intensive research iterest. So many investigations have been performed on the production of amylase, cellulase, pectinase and xylanase due to the sheer number of applications of these enzymes[8]. Cellulase (endoglucanase, exoglucanase, β-glucosidase, together) degrade the most abundant polymer present in the plant cell wall, cellulose to monomers, while amylase, pectinase and xylanase function on starch, pectin and xylan, respectively. Therefore, this study demonstrates the utilization of maize starch, pectin and oat spelt xylan for the production of plant cell wall hydrolytic enzymes (amylase, cellulases, pectinase and xylanase) under submerged fermentation by *B. laguncullariae* AUMC 14498 isolated from alkaline wastewater polluted with textile azo dyes.

MATERIALS AND METHODS

Fungal isolation

Pour plate technique was employed for fungi isolation from the wastewater polluted with textile azo dyes. Three ml of each water sample were pipetted into each 9.0 cm Petri dish containing 20 ml of 1 % glucose-Czapek's agar with the following composition (g/l): Glucose, 10; Na_2NO_3, 2; K_2HPO_4, 1; KCl, 0.5; $MgSO_4.7H_2O$, 0.5; $FeSO_4$, 0.01; $ZnSO_4$, 0.01; $CuSO_4$, 0.005; Rose Bengal, 0.05; chloramphenicol, 0.25; agar, 15 and the final pH 7.3[9]. The plates were incubated for 15 days at 25 °C. Pure cultures of the obtained fungal isolates were maintained on Czapek's agar slants[10], as well as on cotton balls as described by Al-Bedak *et al.*[11] in the culture collection of Assiut University Mycological Centre (AUMC) and the accession numbers were given.

Morphological studies

Morphological features and growth rates of *Byssochlamys* sp. AUMC 14498 were monitored on malt extract agar (MEA,[12] and creatine sucrose agar (CREA;[13] at 30 °C and 37 °C. Spore suspension 10^6 (prepared in a 30 % glycerol, 0.2 % agar and 0.05 % Tween 80 solution) was used for inoculation. Plates were inoculated in three-point pattern using a micropipette and inoculum size of 1 µl per spot. Microscopic features on MEA were examined in lacto-phenol cotton blue.

Molecular identification of the fungal strain

DNA extraction

A small portion of the *Byssochlamys* sp. AUMC 14498 mycelia (grown on MEA for 7 days at 30 °C) was collected and transferred to 2 ml-Eppendorf tube. DNA extraction was performed following the method of Moubasher *et al.*,[14].

PCR for rDNA and sequencing using ITS1 and ITS4 primers

The PCR reaction was carried out using the universal primers ITS1 and ITS4[15] and SolGent EF-Taq. In the PCR tubes 1µl of DNA template, 1 µl 2.5 mM dNTP mix, 0.2 unit of Taq polymerase, 5 µl of 10x complete buffer and 40 µl of sterile ddH_2O, 10 pmol of ITS1 and ITS4 were added. Amplification was conducted using the following PCR conditions: denaturation at 95 °C for 15 min (one cycle), denaturation at 95 °C for 20 sec (30 cycles) then annealing at 50 °C for 40 sec and extension at 72 °C for 1 min (30 cycles), with a final extension step of 72 °C for 5 min. Prior to sequencing, the PCR products were purified using the SolGent PCR Purification Kit-Ultra (SolGent, Daejeon, South Korea). The purified PCR products were confirmed on 1 % agarose gel by electrophoreses, then eluted and sequenced in the forward and reverse directions using the same primers (ITS1 and ITS4) and the incorporation of ddNTP in the reaction mixture[16].

Alignments and phylogenetic analyses

Sequences of the nearest closely related species were retrieved from GenBank including sequences of the available type specimens. Sequence of *Byssochlamys* sp. AUMC 14498 in the present study and those downloaded from GenBank were aligned using MAFFT[17]. Alignment gaps and parsimony uninformative characters were treated by BMGE[18]. Maximum-likelihood (ML) and Maximum parsimony (MP) phylogenetic analyses were performed using PhyML 3.0[19]. The robustness of the most parsimonious trees was evaluated by 1000 bootstrap replication[20]. The best optimal model of nucleotide substitution for the ML analyses

was determined using Smart Model Selection (SMS) version 1.8.1[21]. The phylogenetic tree was visualized using FigTree version 1.4.3[22]. The resulting tree was edited using Microsoft Power Point (2016) and saved as TIF file.

Enzymes production in submerged fermentation (SmF)

The fungal strains were cultivated each in a 250 ml Erlenmeyer conical flask containing 50 ml Czapek's broth medium supplemented with 1 % soluble starch (for amylase production) or 1 % pectin (for pectinase production) or 1 % oat spelts xylan (for cellulases and xylanase production). The flasks were inoculated each with 1 ml spore suspension containing 1.5 x 10^8 spore/ml of 7-day-old cultures. The flasks were then incubated for 7 days at 30 ^oC in shacking condition (150 rpm). The medium was filtered through Whatman No.1 filter paper after incubation, and the cell-free supernatant was obtained by centrifugation (10000 xg at 4 ^oC for 10 min) and used as enzyme source.

Enzymes assay and protein estimation

Amylase, endoglucanase, exoglucanase, pectinase and xylanase production were determined by mixing 0.1 ml filtered crude enzyme with 0.9 ml of 1 % starch, CMC, avicel, pectin and oat spelts xylan respectively (prepared in 50 mM Na-citrate buffer, pH 5.0). The reaction mixture was incubated at 50 °C for 20 min [23] and the process was stopped by introducing 2 ml of 3, 5-dinitrosalicylic acid (DNS) and boiling in a water bath for 10 min [24]. After cooling, the color absorbance was measured at 540 nm using UV-Visible spectrophotometer (T80+). The amount of reducing sugar liberated was quantified using standard curves of glucose (for amylase, cellulase), or galacturonic acid (for pectinase) or xylose (for xylanase). One unit of the enzyme is defined as the amount of enzyme that liberates 1 µmol of the reducing sugar (glucose or xylose) equivalent per minute under the standard assay conditions[25]. The enzyme activity was calculated according to the following formula[26].

$$\text{Enzyme activity} = \text{Absorbance x DF x } \left(\frac{1}{x}\right)\left(\frac{1}{y}\right)\left(\frac{1}{t}\right)\left(\frac{1}{slope}\right)$$

Where: DF= the dilution factor for enzyme; x= the volume of enzyme used; y= the volume of hydrolysate used for assay of reducing sugars; t = the time of hydrolysis; slope is determined from a standard curve. Soluble protein was estimated by Folin Lowry's method using bovine serum albumin as standard ranged from 1-10 mg/g[27].

Gas Chromatography-Mass Spectrometry (GC-MS)

For analysis of the culture extract of *Byssochlamys* sp. AUMC 14498 using GC-MS, it was grown on MEA at 30 °C for 15 days. Three discs (6 mm diameter) were taken from the fungal growth and sonicated in 0.5 ml ethyl acetate: dichloromethane: methanol (3:2:1) for 60 min according to the method described by[28]. The fungal crude extract was re-dissolved in 0.5 ml ethyl acetate: dichloromethane: methanol (3:2:1) and filtered through 0.2 µm filter syringe (Millipore) by which the filtrate submitted to the GC-MS (Trace 1300 Gas Chromatography and Single Quadrupole Mass Spectrometer ISQ 7000). The analysis parameters and conditions are summarized in Table (1).

Table 1: GC-MS analysis parameters and conditions used to detect the compounds in the fungal extract of *Byssochlamys* sp. AUMC 14498.

GC-MS analysis parameters and conditions	
Maximum temperature	280 °C
Prep-run timeout	10.00 min
Equilibration time	0.50 min
Ready delay	0.00 min
Cryogenics threshold	50.0 °C
Cryogenics timeout	60.0 min
Initial temperature	80.0 °C
Initial hold time	5.00 min
Number of ramps	4
Ramp 01 rate	10.0 °C/min
Ramp 01 final temperature	150.0 °C
Ramp 01 hold time	10.0 min
Ramp 02 rate	10.0 °C/min
Ramp 02 final temperature	200.0 °C
Ramp 02 hold time	10.0 min
Ramp 03 rate	5.0 °C/min
Ramp 03 final temperature	250.0 °C
Ramp 03 hold time	10.0 min
Ramp 04 rate	5.0 °C/min
Ramp 04 final temperature	280.0 °C
Ramp 04 hold time	12.0 min

RESULTS AND DISCUSSION

Results

Water sample involved in this study registered an alkaline pH of 8.85. Six strains related to genus *Byssochlamys* were isolated from the examined sample. All isolates obtained were morphologically identical, of which the strain AUMC 14498 was confirmed by sequencing of the internal transcribed spacer (ITS) gene as *B. laguncullariae*.

Description of *Byssochlamys laguncullariae* strain AUMC 14498

Byssochlamys laguncullariae AUMC 14498 grow fast on MEA, attaining 80-90 mm in diameter within 7 d at 30 °C and 37 °C, powdery, dirty white to pale buff. Conidiophores irregularly branched. Phialides ampulliform, pointed towards the apex, 30-45 µm. Conidia in chains, subglobose to ellipsoidal with truncate ends, commonly (3-) 5-7 (-10)×3-5 µm. Chlamydospores present, uncoloured, smooth-walled, 6-10×5-6 µm. Ascomata formed on agar media. Asci subglobose, 8-10×8-10 µm. Ascospores ellipsoidal, 4-5 × 3-4 µm (Figure 1). This strain had good growth and acid production on CREA at 30 °C and 37 °C.

Phylogenetic analysis

Phylogenetic analysis of ITS dataset was employed to determine the taxonomic status of our strain relative to other members of Aspergillaceae. The entire ITS dataset comprised 19 sequences. The maximum parsimony dataset consisted of 579 characters with 477 constant characters (no gaps, no N), 70 variable characters which were parsimony-uninformative (14.7% of constant characters), and 39 characters were counted as parsimony informative (8.2% of constant). GTR was the perfect model for substitution of nucleotides. The dataset for maximum parsimony (MP) yielded 7 parsimonious trees. The maximum likelihood (ML) analysis yielded one tree with a final ML optimization likelihood value of -1637.6579, tree size of 0.39130, tree length of 161 steps, a consistency index of 0.692982, a retention index of 0.832536, and a composite index of 0.576933, for all sites and parsimony-informative sites, to represent and discuss the phylogenetic relationships among taxa (Figure 2). Estimated base frequencies were: f(A)= 0.18177, f(C)= 0.31046, f(G)= 0.29324, f(T)= 0.21453; substitution rates AC= 1.92672, AG= 3.00084, AT= 2.38474, CG= 0.33974, CT= 4.74314, GT= 1.00000; gamma distribution shape parameter (α)= 1.101.

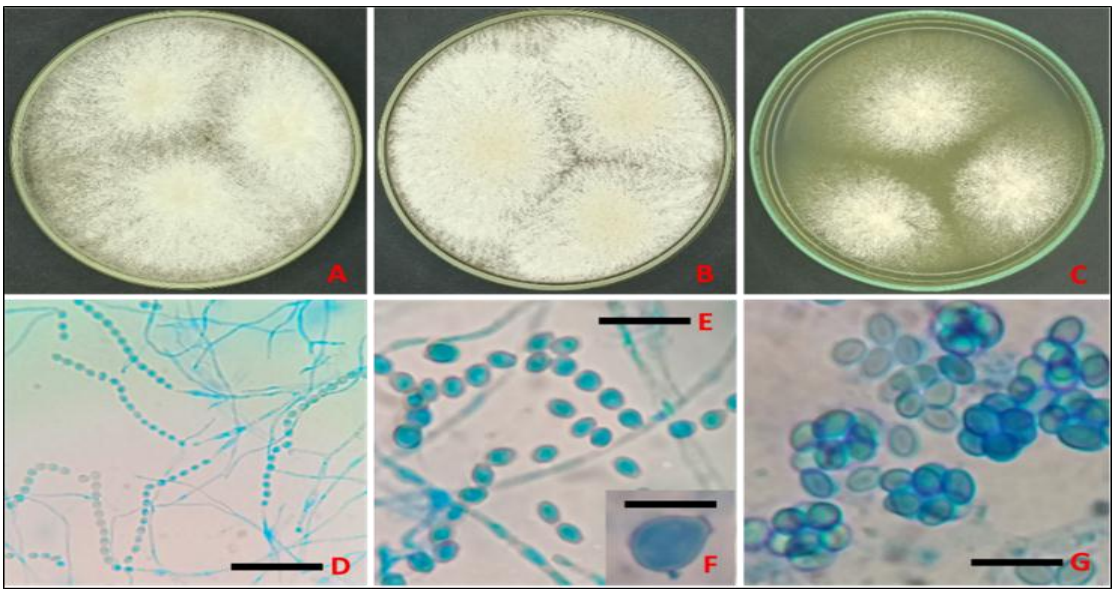

Fig. 1: Macroscopic and microscopic characteristics of *B. laguncullariae* strain AUMC 14498. A-C 7-day-old colonies on MEA at 30 °C, MEA at 37 °C and CREA at 30 °C. D-E conidiophores and phialides bearing subglobose to ellipsoidal conidia in chains. F 8-spored asci. Scale bars: D = 50 µm; E = 20 µm; F-G = 10 µm.

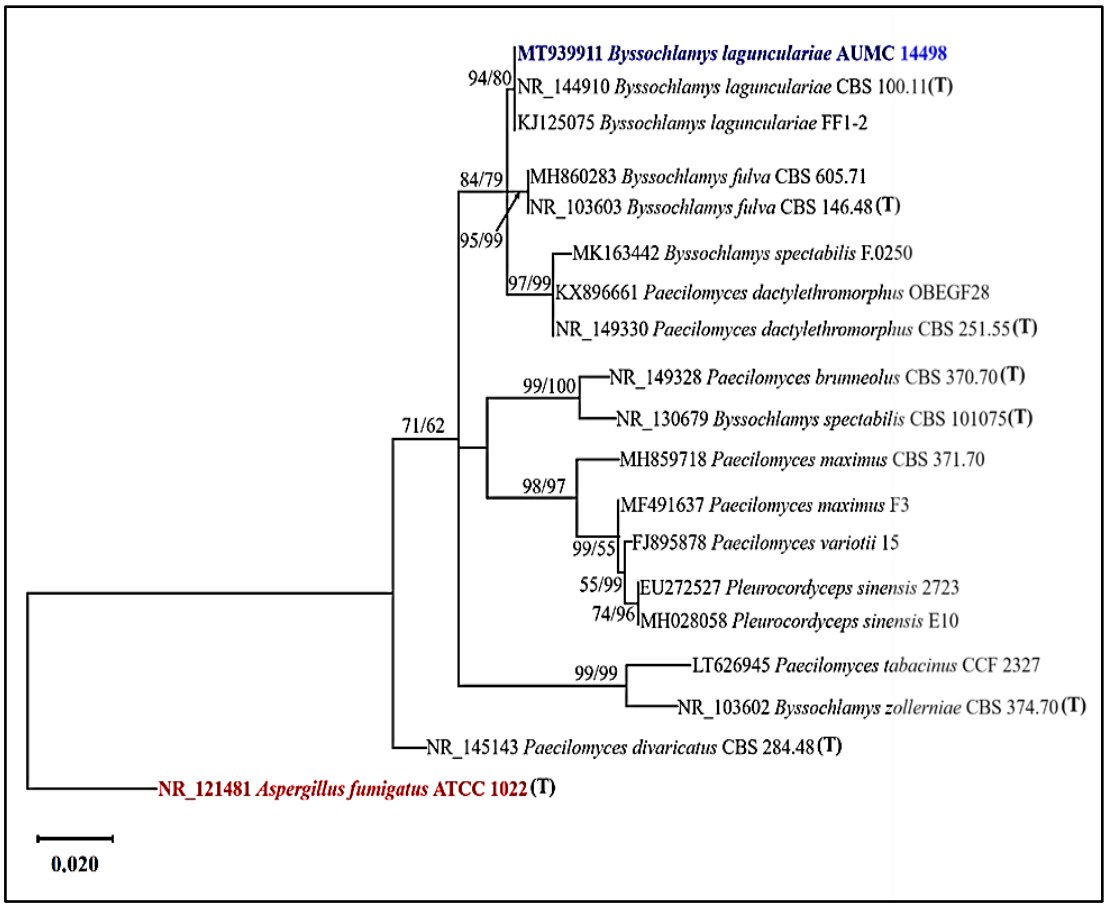

Fig. 2: Phylogenetic tree generated from MP analysis based on ITS sequence data of *Byssochlamys laguncolariae* AUMC 14498 associated to other related genes in the ITS gene sequences belonging to Aspergillaceae. Sequence of the species in this study are in blue color. Bootstrap support values (1000 replications) for ML/MP combination equal to or greater than 50% are indicated at the respective nodes. The tree is rooted to *Aspergillus fumigatus* ATCC 1022 as out group (in red color). Taxa derived from type materials are indicated with superscripts **(T)**.

The phylogenetic result depicts the relationships of our strain with other *Byssochlamys* species. The ML tree revealed strong bootstrap support for most of the terminal clades and the tree backbone. The strain AUMC 14498 located within *Byssochlamys laguncolariae* clade on the same branch as *Byssochlamys laguncolariae* CBS 100.11 and *Byssochlamys laguncolariae* FF1-2 endorsing strong bootstrap value (62% ML/64 % MP). The three species having 100% similarity of their ITS sequences, so the strain AUMC 14498 can be identified as *Byssochlamys laguncolariae*.

GC-MS analysis

GC-MS analysis of *B. laguncolariae* AUMC 14498 culture extract revealed that more than fifty chemical compounds were detected, of which the highest detectable compounds were 3-Trifluoroacetoxypentadecane; Ethyl iso-allocholate; Hexadecane, 1,1-bis (dodecyloxy)-; Phenol, 2,4-bis (1,1-dimethylethyl)-; 1-Hexadecanol, 2-methyl-; 7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione; Oxiraneoctanoic acid, 3-octyl-, cis-; Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester and Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester (Figure 3), which have an important medical application. The retention time, molecular formula and molecular weight of these highest detectable chemical compounds are proved in Table (2) and the structures of these compounds are showed in Figure (4).

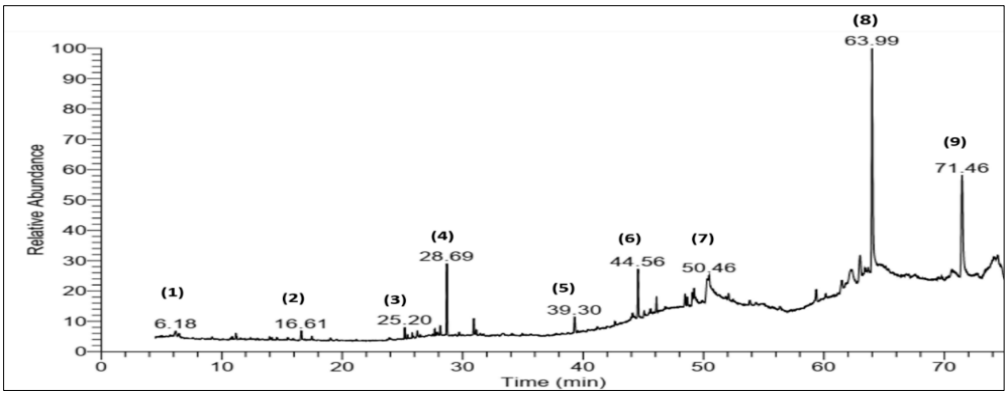

Fig. 3: Chromatogram of the culture extract of *B. laguncullariae* AUMC 14498 showing: **(**1) 3-Trifluoroacetoxypentadecane; (2) Ethyl iso-allocholate; (3) Hexadecane, 1,1-bis(dodecyloxy)-; (4) Phenol, 2,4-bis(1,1-dimethylethyl)-; (5) 1-Hexadecanol, 2-methyl-; (6) 7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione; (7) Oxiraneoctanoic acid, 3-octyl-, cis-; (8) Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester and (9) Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester detected by GC-MS.

Table 2: Retention time, molecular formula and molecular weight of the highest detectable compounds detected by GC-MS in the culture extract of *B. laguncullariae* AUMC 14498.

No.	Retention time (min)	Chemical compound	Molecular formula	Molecular weight
1	6.18	3-Trifluoroacetoxypentadecane	$C_{17}H_{31}F_3O_2$	324
2	16.61	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436
3	25.20	Hexadecane, 1,1-bis (dodecyloxy)-	$C_{40}H_{82}O_2$	594
4	28.69	Phenol, 2,4-bis (1,1-dimethylethyl)-	$C_{14}H_{22}O$	206
5	39.30	1-Hexadecanol, 2-methyl-	$C_{17}H_{36}O$	256
6	44.56	7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276
7	50.46	Oxiraneoctanoic acid, 3-octyl-, cis-	$C_{18}H_{34}O_3$	298
8	63.99	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$	330
9	71.46	Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester	$C_{39}H_{76}O_5$	624

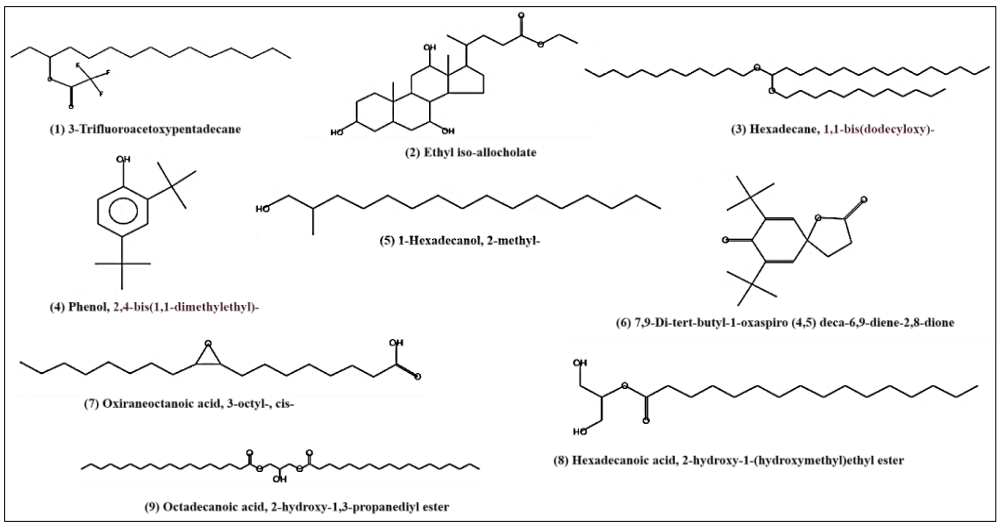

Fig. 4: Chemical structures of the highest detectable chemical compounds analyzed by GC-MS in the culture extract of *B. laguncullariae* AUMC 14498.

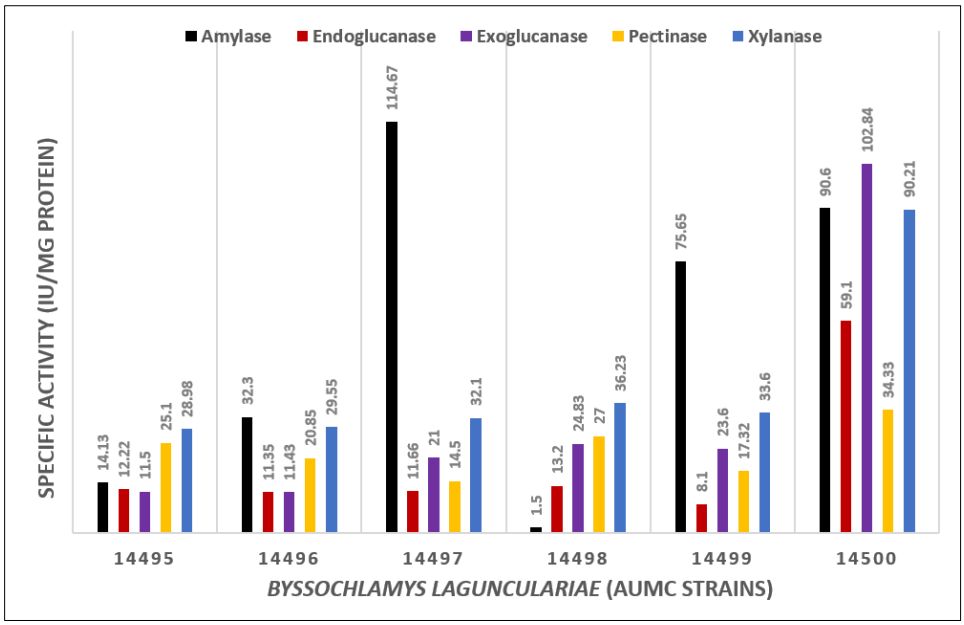

Fig. 5: Specific activity (IU/mg protein) for amylase, endoglucanase, exoglucanase, pectinase and xylanase produced by *B. laguncullariae* strains in submerged fermentation at 30 °C.

Enzymes production and activities

The current results showed that all the *B. laguncullariae* strains tested, when grown on oat spelt xylan, could produce endoglucanase, exoglucanase and xylanase as well as maize starch and pectin respectively for amylase and pectinase. Strains AUMC 14497, AUMC 14499 and AUMC 14500 could achieve maximum specific activity of 114.67 IU/mg, 75.65 IU/mg and 90.6 IU/mg respectively for amylase, while AUMC 14500 produced the highest specific activity (59.1 IU/mg, 102.84 IU/mg, 34.33 IU/mg and 90.21 IU/mg) for endoglucanase, exoglucanase, pectinase and xylanase, respectively (Figure 5).

Discussion

Byssochlamys is ascomycete's genus belonging to family Aspergillaceae. Its species produce ascospores which are heat-resistant, and survive considerable periods of heat above 85 °C [1, 2]. Furthermore, *Byssochlamys* species can grow under very low oxygen tensions[3], and can generate pectinases. These three physiological characteristics make *Byssochlamys* species very important spoilage fungi in pasteurized and canned fruit [4].

In the current study, *Byssochlamys laguncullariae* was isolated for the first time in Egypt. Morphologically, *B. laguncullariae* is similar to *B. nivea* and shares various characters such as fast growth rate on MEA at 30 °C and globose to ellipsoidal conidia with a flattened base, as well as presence of uncolored and smooth-walled chlamydospores. In addition, the conidia and ascospores of *B. laguncullariae* are generally smaller in size. Another difference is that *B. laguncullariae* grows well on CYA while *B. nivea* grows rather poorly. In the original description by[29], *B. laguncullariae* was described as a variety of *B. nivea*, distinguished by its smaller conidia and ascospores.

Stolk and Samson[5] synonymized it with *B. nivea*. Houbraken *et al.*[6] demonstrated that *Byssochlamys* and its associated anamorph species can be separated into at least nine taxa by investigating the micro- and macroscopical characteristics of *Byssochlamys* and *Paecilomyces variotii*-like isolates. Recently, it included three species namely *B. laguncullariae*, *B. spectabilis*, and *B. zollerniae* (Index Fungorum database). The strain *B. laguncullariae* AUMC 14498 obtained in this study shared some morphological characteristics with the type species such as presence of smooth chlamydospores, ascospores size (4-5 × 3-4 µm) and good growth on CYA at 30 °C after 7 days. However, it was different from the type species by having larger conidia (5-7 × 3-5 µm) and positive acid production on CREA at 30 °C.

The ex-type culture of *B. laguncullariae* was isolated from wood of *Laguncularia racemosa* (mangue) in Brazil and other strains identified as this species were isolated from soil, pasteurized strawberries and aloe juice[4&30&31],

and from citrus raw material of spoiled fruit jelly products in Japan[32].

Gas chromatography mass spectrometry (GC-MS) analysis of *Byssochlamys laguncullariae* AUMC 14498 culture extract revealed that more than fifty chemical compounds were detected, of which the highest detectable compounds were 3-Trifluoroacetoxypentadecane; Ethyl iso-allocholate; Hexadecane, 1,1-bis (dodecyloxy)-; Phenol, 2,4-bis (1,1-dimethylethyl)-; 1-Hexadecanol, 2-methyl-; 7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione; Oxiraneoctanoic acid, 3-octyl-, cis-; Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester and Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester. Such compounds are reported here for the first time in *Byssochlamys laguncullariae* and have never been documented in any species of *Byssochlamys* before. *Byssochlamys laguncullariae* (the type species) has been reported to produce byssochlamic acid and mycophenolic acid [4]. Viriditoxin and other compounds with characteristic UV-spectra were detected in *B. spectabilis* extrolites while no known extrolites were reported to be developed in *B. zollerniae* cultures [4].

The production of plant cell-wall digestive enzymes is now a focus of current research. Many such research have been done into the production of amylase, cellulase, pectinase and xylanase due to the huge number of application scenarios of these enzymes[26&33-45]. In the present work, six strains of *Byssochlamys laguncullariae* were estimated for their amylase, endoglucanase, exoglucanase, pectinase and xylanase production.

In this study, three strains of *Byssochlamys laguncullariae* could generate maximum specific activity for amylase. *Byssochlamys* species have been documented to generate pectinases[3] and *Byssochlamys fulva* CMI 40021 has been generated extracellular α-amylase and low levels of amyloglucosidase[46]. The current results have shown that all the *Byssochlamys laguncullariae* strains tested when grown on oat spelt xylan could generate endoglucanase, exoglucanase and xylanase. These three enzymes have never been recorded for any species with the genus.

Conclusions

Byssochlamys laguncullariae, a new record from alkaline fabric wastewater in Alexandria, Egypt has been isolated and deposited in AUMC as *Byssochlamys laguncullariae* AUMC 14498. Extrolite analysis of this strain was performed using gas chromatography mass spectrometry (GC-MS). The extrolite analysis of *B. laguncullariae* AUMC 14498 revealed that more than fifty chemical compounds were detected, which have an important medical application. Three strains of *B. laguncullariae* achieved maximum specific activity for amylase ranged from 75.65 to 114.67 IU/mg, while one of these three was superior for endoglucanase (59.1 IU/mg), exoglucanase (102.84 IU/mg), pectinase (34.33 IU/mg), and xylanase (90.21 IU/mg) production. The emerging trend in this research is the usage of *Byssochlamys laguncullariae* strains as a strong source of useful enzymes that have many industrial applications.

Acknowledgments

This paper was supported financially by the Academy of Scientific Research and Technology (ASRT), Egypt.

REFERENCES

1- L. R. Beuchat and S. L. Rice, "*Byssochlamys* spp. and their importance in processed fruits", In ***Advances in Food Research,*** Elsevier, 25, 237-288 (1979).
2. L. R. Beuchat, "*Food and beverage mycology*", Springer Science & Business Media, (1987).
3. M. H. Taniwaki, "Growth and mycotoxin production by fungi under modified atmospheres", (1997).
4. R. Samson, J. Houbraken, J. Varga and J. C. Frisvad, "Polyphasic taxonomy of the heat resistant ascomycete genus *Byssochlamys and its Paecilomyces* anamorphs", ***Persoonia,*** *22*, 14-27(2009).
5. A. C. Stolk and R. A. Samson, "Studies on Talaromyces and related genera I Hamigera gen. nov. and Byssochlamys", ***Persoonia-Molecular Phylogeny and Evolution of Fungi,*** *6*(3), 341-357, (1971).
6. J. Houbraken, R. A. Samson and J. C. Frisvad, "*Byssochlamys*: significance of heat resistance and mycotoxin production", In ***Advances in Food Mycology,*** Springer, pp 211-224 (2006).
7. R. Kumar; S. Singh and O. V. Singh, "Bioconversion of lignocellulosic biomass:

biochemical and molecular perspectives", ***J Ind Microbiol Biotechnol,*** *35* (5), 377-391, (2008).
8. S. Ghorai, S. P. Banik, D. Verma, S. Chowdhury, S. Mukherjee and S. Khowala, "Fungal biotechnology in food and feed processing", ***Food Res Inter,*** *42* (5-6), 577-587, (2009).
9. M. Ismail, A. Moubasher, R. Mohamed and O. Al-Bedak, "Extremophilic fungi and chemical analysis of hypersaline, alkaline lakes of Wadi-El-Natrun, Egypt", ***Inter J Tech Res Sci,*** 1 (10), 345-363, (**2017)**.
10. K. B. Raper and D. I. Fennell, "The genus *Aspergillus*", ***The genus Aspergillus***, (1965).
11. O. A. Al-Bedak, R. M. Sayed and S. H. Hassan, "A new low-cost method for long-term preservation of filamentous fungi", ***Biocatal Agric Biotech,*** *22*, 101417, (2019).
12. R. Samson, J. Houbraken, U. Thrane, J. Frisvad and B. Andersen, "Food and indoor fungi: CBS-KNAW fungal biodiversity centre", ***Utrecht,*** (2010).
13. J. C. Frisvad, "Physiological criteria and mycotoxin production as aids in identification of common asymmetric penicillia", ***Appl Environ Microbiol,*** *41* (3), 568-579, (1981).
14. A. Moubasher, M. Ismail, O. Al-Bedak and R. Mohamed, "*Ramophialophora chlamydospora*, a new species from an alkaline lake of Wadi-El-Natron, Egypt", ***Asian J Mycol,*** *2* (1), 110-117, (2019).
15. T. J. White, T. Bruns, S. Lee and J. Taylor, "Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics", ***PCR protocols: a guide to methods and applications,*** *18* (1), 315-322, (1990).
16. O. Al-Bedak and A. Moubasher, *"Aspergillus gaarensis*, a new addition to section Circumdati from soil of Lake El-Gaar in Wadi-El-Natron, Egypt", ***Stud in Fungi,*** *5* (1), 59-65, (2020).
17. K. Katoh and D. M. Standley, "MAFFT multiple sequence alignment software version 7: improvements in performance and usability", ***Mol Biol Evolut,*** *30* (4), 772-780, (2013).
18. A. Criscuolo and S. Gribaldo, "BMGE (Block Mapping and Gathering with Entropy): a new software for selection of phylogenetic informative regions from multiple sequence alignments", ***BMC Evolut Biol,*** *10* (1), 210, (2010).
19. S. Guindon, J.-F. Dufayard, V. Lefort, M. Anisimova, W. Hordijk and O. Gascuel, "New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0", ***Syst Biol,*** *59* (3), 307-321, (2010).
20. J. Felsenstein, "Confidence limits on phylogenies: an approach using the bootstrap", ***Evolution,*** *39* (4), 783-791, (1985).
21. V. Lefort, J.-E. Longueville and O. Gascuel, "SMS: smart model selection in PhyML", ***Mol Biol Evolut,*** *34* (9), 2422-2424, (2017).
22. A. Rambaut and A. Drummond, ***FigTree version*** 1.4. 0. (2012).
23. M. J. Bailey, P. Biely and K. Poutanen, "Interlaboratory testing of methods for assay of xylanase activity", ***J Biotech,*** *23* (3), 257-270, (1992).
24. G. L. Miller, "Use of dinitrosalicylic acid reagent for determination of reducing sugar", ***Anal Chem,*** *31* (3), 426-428, (1959).
25. T. Ghose and V. S. Bisaria, "Measurement of hemicellulase activities: Part I Xylanases", ***Pure Appl Chem,*** *59* (12), 1739-1751, (1987).
26. A. Moubasher, M. Ismail, A. Mohamed and O. Al-Bedak, "Xylanase and cellulase production under extreme conditions in submerged fermentation by some fungi isolated from hypersaline, alkaline lakes of Wadi-El-Natrun, Egypt", ***J Basic Appl Myco (Egypt),*** *7*, 19-32, (2016).
27. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, "Protein measurement with the Folin phenol reagent", ***J Biol Chem,*** *193*, 265-275, (1951).
28. J. Smedsgaard, "Micro-scale extraction procedure for standardized screening of fungal metabolite production in cultures", ***J Chromatog A,*** *760* (2), 264-270, (1997).
29. C. Ram, "Timber-attacking fungi from the State of Maranhao, Brazil. Some new species of *Paecilomyces* and its perfect stage *Byssochlamys* Westl. VIII", ***Nova Hedwigia,*** *16*, 305-314, (1968).
30. S. Rice, L. Beuchat and R. Worthington, "Patulin production by *Byssochlamys* spp.

in fruit juices", ***Appl Environ Microbiol,*** *34* (6), 791-796, (1977).
31. R. Kramer, N. Davis and U. Diener, "Byssotoxin A, a secondary metabolite of *Byssochlamys fulva*", ***Appl Environ Microbiol,*** *31* (2), 249-253, (1976).
32. S. UEDA, N. KAWARA, T. YAGUCHI and S. I. UDAGAWA, "Identification and heat-resistance characteristics of *Byssochlamys laguncullariae*, a mold isolated from citrus raw material of spoiled fruit jelly products", ***Japanese Journal of Mycology,*** *51* (2), 48-58, (2010).
33. D. N. Adhyaru, N. S. Bhatt and H. Modi, "Enhanced production of cellulase-free, thermo-alkali-solvent-stable xylanase from *Bacillus altitudinis* DHN8, its characterization and application in sorghum straw saccharification", ***Biocatal Agri Biotechol,*** *3* (2), 182-190, (2014).
34. M. Sanjivkumar, T. Silambarasan, A. Palavesam and G. Immanuel, "Biosynthesis, purification and characterization of β-1, 4-xylanase from a novel mangrove associated actinobacterium *Streptomyces olivaceus* (MSU3) and its applications", ***Prot Express Purif,*** *130*, 1-12, (2017).
35. B. E. Far, Y. Ahmadi, A. Y. Khosroshahi and A. Dilmaghani, "Microbial alpha-amylase production: progress, challenges and perspectives", ***Advan Pharm Bull,*** *10* (3), 350, (2020).
36. Y. Xue, J. Han, Y. Li, J. Liu, L. Gan and M. Long, "Promoting cellulase and hemicellulase production from *Trichoderma orientalis* EU7-22 by overexpression of transcription factors Xyr1 and Ace3", ***Bioresour Technol,*** *296*, 122355, (2020).
37. J.-X. Li, F. Zhang, D.-D. Jiang, J. Li, F.-L. Wang, Z. Zhang, W. Wang and X.-Q. Zhao, "Diversity of Cellulase-Producing Filamentous Fungi From Tibet and Transcriptomic Analysis of a Superior Cellulase Producer *Trichoderma harzianum* LZ117", ***Front Microbiol,*** *11*, 1617, (2020).
38. A. Moubasher, M. Ismail, R. Mohamed and O. Al-Bedak, "Production and purification of extreme xylanase from *Aspergillus flavus* AUMC 10331 in sub-merged fermentation", ***Eur J Biol Res,*** *9* (1), 20-28, (2019).
39. H. Jalis, A. Ahmad, S. Khan and M. Sohail, "Utilization of apple peels for the production of plant cell-wall degrading enzymes by *Aspergillus fumigatus* MS16", ***J Anim Plant Sci,*** *24* (2), 64-67, (2014).
40. M. Ismail, A. Moubasher, R. Mohamed and O. Al-Beddak, "Agro–industrial residues as alternative sources for cellulases and xylanases production and purification of xylanase produced by *Aspergillus flavus* AUMC 10331 isolated from extreme habitat", ***Curr Res Environ Appl Mycol,*** *8* (3), 313-322, (2018).
41. A. Moubasher, M. Ismail, R. Mohamed and O. Al-Bedak, "Synergistic production and purification of extreme xylanase produced by *Aspergillus flavus* AUMC 10331 and *A. oryzae* AUMC 10329 from rice husk in solid-state fermentation", ***J Multidiscip Sci,*** *1* (1), 1-7, (2019).
42. S. P. Edor, O. P. Edogbanya and J. R. Kutshik, "Cellulase activity of *Aspergillus niger* in the biodegradation of rice husk", (2018).
43. B. Nikhil, D. Adhyaru and P. Thakor, "Production of xylanase by *Aspergillus flavus* FPDN1 on pearl millet bran: Optimization of culture conditions and application in bioethanol production", ***Int J Res Chem Environ,*** *2* (3), 204-210, (2012).
44. A. A. Keera, F. E. Mouafi, T. Kahil, M. Fadel and A. Abedo, "Hyper production of glucoamylase by *Aspergillus oryzae* FK-923 under solid state fermentation", ***World Appl Sci J,*** *30* (11), 1447-1461, (2014).
45. S. Pan, N. Ding, J. Ren, Z. Gu, C. Li, Y. Hong, L. Cheng, T. P. Holler and Z. Li, "Maltooligosaccharide-forming amylase: Characteristics, preparation, and application", ***Biotechnol Adv,*** *35* (5), 619-632, **(2017).**
46. E. Doyle, A. Noone, C. Kelly, T. Quigley and W. Fogarty, "Mechanisms of action of the maltogenic α-amylase of *Byssochlamys fulva*", ***Enz Microbiol Technol,*** *22* (7), 612-616, (1998).

Bull. Pharm. Sci., Assiut University, Vol. 45, Issue 1, 2022, pp. 359-369.

نشرة العلوم الصيدلية
جامعة أسيوط

BULL. PHARM. SCI. Assiut Univ.

إنتاج الإنزيمات المحللة للجدار الخلوي والتحليل الكيميائي لمستخلص Byssochlamys laguncullariae والمسجل لأول مرة في مصر

أسامة عبد الحفيظ البيدق[١] – محمد أحمد عبد الساتر[٢] – هاجر محمد كمال جلال[٣] – صدقي حسن علي حسن* [٤،٣]

[١]مركز الفطريات بجامعة أسيوط ، جامعة أسيوط ، أسيوط ٧١٥١١ ، مصر

[٢]قسم النبات والميكروبيولوجي ، كلية العلوم ، جامعة أسيوط ، أسيوط ، مصر

[٣]قسم النبات والميكروبيولوجي ، كلية العلوم ، جامعة الوادي الجديد ، الخارجة ٧٢٥١١ ، مصر

[٤]قسم الأحياء ، كلية العلوم ، جامعة السلطان قابوس ، مسقط ١٢٣ ، عمان

في الدراسة الحالية ، تم عزل ست سلالات من *Byssochlamys laguncullariae* من مياه الصرف القلوية لمصنع النسيج الملوث بصبغات الآزو في الإسكندرية ، مصر. من بين السلالات الست ، تم تعريف واحدة فقط باستخدام (ITS). تم وضع تسلسل ITS إلى بنك الجينات و حفظه بالرقم (MT939911) GenBank وتم حفظ السلالة الفطرية بمركز الفطريات بجامعة أسيوط (AUMC) باسم ***B.*** *laguncullariae* AUMC 14498. وتتميز السلالة الفطرية بالعديد من الصفات المورفولوجية و الفسيولوجية. تم إجراء تحليل المستخلص لهذه السلالة باستخدام جهاز (GC-MS). وبينت التحاليل أنه تم اكتشاف أكثر من خمسين مركبًا كيميائيًا ، والتي لها تطبيقات طبية مهمة. تم اختبار جميع سلالات *B. laguncullariae* ، عند نموها على مادة الشوفان المحتوي علي الزيلان ، كما يمكن أن يستخدم الشوفان لإنتاج إندوجلوكاناز وإكسوجلوكاناز وزيلانيز بالإضافة إلى نشا الذرة والبكتين ، على التوالي لإنتاج أنزيمات الأميليز والبكتيناز. حققت ثلاث سلالات من *B. laguncullariae* أقصى قدر من النشاط النوعي للأميلاز تراوحت من ٧٥.٦٥ إلى ١١٤.٦٧ وحدة دولية / مجم ، بينما تفوق أحد هذه السلالات الثلاثة على انتاج إندوجلوكاناز ب (٥٩.١ وحدة دولية / مجم) ، وإكسوجلوكاناز (١٠٢.٨٤ وحدة دولية / مجم) ، وبكتيناز (٣٤.٣٣ وحدة دولية / مجم) ، وإنتاج الزيلانز(٩٠.٢١ وحدة دولية / مجم)