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Isolation of Polyvinyl Alcohol Degrading Fungi and Improvement of Degradation Activity

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IN THE PRESENT study, thirty-one isolates were collected from different locations in Tanta city, Gharbia Governorate, Egypt. These isolates were screened for their polyvinyl alcohol (PVA) - degrading ability as a sole carbon source in the culture medium. Among the thirty-one tested isolates, only Seventeen were found PVA degraders belonging to genera *Penicillium*, *Aspergillus*, *Trichoderma*, *Fusarium* and *Ulocladium*. The highest degradation percentages were recorded for *Penicillium polonicum* ON024147 (25.7%) and *Aspergillus niger* ON012795 (24.28%). The culture conditions were optimized for maximum PVA-degradation of 88% and 90% by *P. polonicum* and *A. niger*, respectively. The optimum static culture conditions for *P. polonicum* were adjusted at pH 5 and incubation at 30°C for 6 days in the presence of starch as a co-carbon source and KNO_3 as a nitrogen source with PVA conc. 10g/L. For *A. niger*, the optimum growth conditions were at pH=6, 30°C, static culture, for 6 days, cellulose as a co-carbon source, sodium nitrate as a nitrogen source and PVA conc.= 10g/L.

Keywords: *Aspergillus niger*, Degradation, Fungi, Hydrolysis, *Penicillium polonicum*, PVA**.**

Introduction

Polyvinyl alcohol (PVA) is a water-soluble polymer which has recently incorporated in the manufacture of biodegradable plastic materials (Solaro et al., 2000). PVA has excellent physical properties, such as film forming, emulsifying, viscosity, dispersing power, adhesive strength, flexibility and tensile strength. PVA is also resistant not only to water, but also to oil, grease, and solvent. Based on these properties PVA is broadly used in adhesives and films, paper coating, in forming oxygen impermeable films and as a finishing agent in the textile industry (Amange & Minge, 2012). Because of all these uses, huge every year amounts of PVA are released into water systems. So, waste water can contain an extensive amount of PVA as a result of the slow rate of degradation (Mollasalehi, 2013). Synthetic petrochemical-based plastics have been produced in ever growing volumes globally and since their first commercial introduction; they have been continually developed with regards to quality, colour, durability, and resistance. With some exceptions, such as polyurethanes, most plastics are very stable and are not readily degraded when they enter the ground as waste, taking decades to biodegrade and therefore are major pollutants of terrestrial and marine ecosystems. During the last thirty years, extensive research has been conducted to develop biodegradable plastics as more environmentally benign alternatives to traditional plastic polymers. The production of PVA all over the world has reached about 1,250kt in 2007. About 76% of the worldwide production capacity is located in Asian countries, among which China alone accounted for 45% of the world capacity in 2006, up from 34% in 2002. Global use of PVA was expected to continue increasing at an overall rate of about 2.5% annually between 2006 and 2011 (Kawai & Hu, 2009). The global polyvinyl alcohol (PVA) market is expected to reach USD 1.21 billion by 2025, reflecting a compound annual growth rate of 4.59%. Although PVA isn't toxic to organisms, it has strong surface activity which yields large amounts of foam and prevents recovery of oxygen in water. This will cause a critical threat

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to water-borne organisms and humans (Kawai & Hu, 2009).

There are physical and chemical environmental factors involved in degradation of plastic such as heat, moisture, light and chemical conditions (Krzan et al., 2006). These factors cause changes in polymer's properties like erosion, discoloration and cracking (Pospisil & Nespurek, 1997). PVA may be the only synthetic polyvinyl-type polymer possessing biodegradability (Shah et al., 2008). Microbial degradation is better than physical and chemical methods as the degradation pathway leads to complete degradation and mineralization of polymer (Mollasalehi, 2013).

Since the first report of degradation by *Fusarium lini* (Nord 1936), Suzuki et al. (1973) started extensive studies of PVA biodegradation where a diversity of microorganisms have been reported for the ability to degrade PVA. Most of these isolated strains are bacteria such as *Bacillus megaterium* (Mori et al., 1996a), *Alcaligenes faecalis* (Matsumara et al., 1994), *Pseudomonas* sp. (Mori et al., 1996b; Watanabe et al., 1976; Hatanaka et al., 1995); although *Pycnoporus cinnabarinus* (Larking et al., 1999), *Flammulina velutipes* (Tsujiyama et al., 2011), *Phanerochaete chrysosporum* (Mejia et al., 2003) and *Penicillium* sp. *(*Qian et al., 2004) were recorded as capable fungal species of degrading PVA. Moreover, *Aspergillus flavus, Aspergillus niger*, *Aureobasidium pullulans* and *Aspergillus oryzae* were reported to have the ability to grow on PVA films (Qian et al., 2004; Tsujiyama et al., 2011).

As the production and consumption of PVA are continuously increasing, the biodegradation of PVA is getting attention worldwide, leading to continuous isolation of new PVA-degraders. The present study aimed to screen the ability of some isolated fungi to degrade polyvinyl alcohol-based plastic wastes.

Materials and Methods

Collection of samples

Four samples were collected from four localities in Tanta, Gharbia Governorate in 2019 as following: Sewage water from Drinking Water and Sanitation Company, Waste water from Tanta Petroleum Pipe Company, Paper and plastic wastes from garbage dumps and petroleum-contaminated soil from Tanta Petroleum Pipe Company. The solid samples were stored in polyethylene bags at room temperature while the water samples were taken up in sterilized glass jars and preserved at 4ºC.

Isolation of fungi from collected samples

The fungi were isolated from the collected samples using the dilution plate method on Czapek's Dox Agar (Johnson et al., 1959), incubated at 28°C till observed growth then individual colonies were subcultured on new sterile plates. The purified fungi were stored on slants of Potato Dextrose Agar medium (Vanderzant & Splittstosser, 1992). Mycelial growth of the isolates was covered with sterilized glycerol (50%) at -20ºC.

Identification of the isolated fungi

The fungal colonies that appeared on the plates were purified and identified on the basis of morphological and microscopic features according to the following references: Raper & Thom (1949), Gilman (1959), Raper & Fennel (1965), Raper et al. (1968), Booth (1971), Ellis (1971), Moubasher (1993) and Klick (2002). The identification of the selected strains were confirmed by partial sequencing of their 18s rRNA, whereas their genetic material was extracted separately according to Sambrook et al. (1989), amplified by PCR machine (Qiagen, USA) according to Mullis et al. (1986), using universal primers (F: 5' TGAGCCTTGTAAGCGTCCAC 3' ; R: 5' TTCATGCCGTGCTTCTCCAG 3'), then PCR products were analyzed and partially sequenced at Sigma Aldrich, Egypt.

Qualitative screening of PVA-fungal degraders

Pure disc (5mm) of each fungus was transferred onto the plates with mineral salt agar medium containing PVA (2.5g/L). After incubation period of about 2 weeks, the plates were flooded by iodine-boric acid reagent (1 iodine : 4 boric acid), left for 15min in dark. The degrading ability of the tested fungi was screened by the formation of clear zone around the fungal growth. (Min et al., 2012) The composition of mineral salt medium was (g/L) as follows: PVA 2.5 (13,000-23,000 MW, 98% hydrolyzed, Sigma-Aldrich), K_2HPO_4 2.2, KH_2PO_4 0.8, NaCl 0.1, $CaCl_2.2H_2O$ 0.02, NH_4NO_3 2.5, $MgSO_4$ 0.7, $MnSO_4$ 0.001, $FeSO_4.7H_2O$ 0.01, agar 15 and chloramphenicol 0.3.

Quantitative estimation of PVA-degradation activity

One ml of 1×10^6 of spores of each isolated fungus was inoculated in sterile 250mL conical

flasks containing 50mL of mineral salt medium supplemented with PVA (2.5g/L), as the sole carbon source then the flasks were incubated on a rotary shaker (Human Lab, Korea) at 100rpm. After two weeks, PVA degrading activity was determined by using iodine-boric acid at 690nm (Labomed Inc., China). One ml of filtrate was drawn into a tube containing 3.5ml of boric acid (40g/L) and 0.75mL of KI-I_2 (25-12.7g/L), then the residual PVA concentration in the liquid medium were calculated comparing to a standard calibration curve; each separate estimation was performed in a triplicate and their mean value was recorded (Mollasalehi, 2013).

Optimization of culture conditions

To maximize the PVA degrading activity of the most potent fungi. Mineral salt liquid medium was prepared as a basic nutrition medium, dispensed as 100 ml medium for each 250mL conical flasks, sterilized, inoculated separately with the selected fungi. Then growth conditions were varied for more detailed study, separately in a successive order as follows; various pH values (5, 6, 7, 8, and 9); various temperatures (25, 30, 35, 40 and 45°C); agitation speeds (0, 50, 100 and 150rpm) whereas all cultures were incubated for 12 days; incubation periods (2, 4, 6, 8, 10 and 12 day); co-carbon sources (cellulose, carboxy methyl cellulose (CMC), glucose and starch) ; nitrogen sources (ammonium chloride, ammonium nitrate, ammonium sulphate, sodium nitrate, potassium nitrate, asparagine and peptone) and PVA concentrations (0.3, 0.6, 1.25, 2.5, 5, 10, 20 and 30g/L) were investigated after incubation for the optimum period. For each parameter, at the end of experiment, mycelia were collected, dried at 40°C till constant weight and recorded as the dry biomass value. Each separate estimation was performed in a triplicate and their mean value was recorded. Percentage of PVA degradation was calculated as previously mentioned.

Results

Table 1 shows the occurrence of fungi in the different localities of isolation, thirty-one different isolates were obtained, as the most predominant fungi was presented in the soil sample from Tanta Petroleum Pipe Company, bearing 14 isolates; followed by paper and plastics from Tanta public garbage dump that contaminated with 7 isolates. On the other hand, the lowest isolate number was recorded for sewage and waste water samples from Drinking Water and Sanitation Company; and Tanta Petroleum Pipe Company (5 isolates for each one).

The isolated fungi were morphologically identified and their frequencies were calculated and presented in Fig. 1. The most abundant fungus was *Aspergillus niger* (12.9%), followed by *Aspergillus flavus* (9.68%) and *Mucor spp.* (9.68%), then *Penicillium verrucosum*, *penicillium commune*, *penicillium chrysogenum, penicillium funiculosum* and *Fusarium solani* (6.45% for each). The lowest frequency was recorded for *Aspergillus ochraceus, Aspergillus candidus, Penicillium neoechinulatum, Penicillium polonicum, Trichoderma harzianum, Trichoderma viride, Trichoderma virens, Trichoderma hamatum, Ganoderma lucidum* and *Ulocladium botrytis* (3.23% for each).

The ability of identified fungi to degrade PVA was qualitatively screened and summarized in Table 2. Among the tested 31 fungi, positive results were recorded, only for 17 strains, namely: *A. flavus*, *A. niger*, *A. ochraceus*, *A. candidus*, *P. neoechinulatum*, *P. polonicum*, *P. tricolor*, *P. verrucosum*, *P. commune*, *P. chrysogenum*, *P. funiculosum*, *T. harzianum*, *T. viride*, *T. hamatum*, *T. virens*, *Ulocladium botrytis* and *Fusarium solani*. *Ganoderma lucidum* and *Mucor sp.* possessed no PVA degradation activity.

The PVA hydrolytic activity of the tested fungi was quantitatively estimated in order to select the potent PVA-hydrolyzing fungus. *P. polonicum* was the most active strain in this regard with a PVA-hydrolyzing activity approached 25.7%, followed by *A. niger* (24.28%), then *A. candidus* and *T. harzianum* (22.85% for each). The lowest activity was recorded for the fungus *A. ochraceus* (2.8%) (Fig. 2).

The most active fungal strains showing the maximum values of PVA degradation were subjected to molecular identification to confirm their identity; as the analysis of their partial sequences and similarities in the obtained phylogenetic tree revealed that the first target fungus was *Penicillium polonicum* ON024147 (more related with 95% to *Penicillium polonicum* MK140689.1); and the second fungus *Aspergillus niger* ON 012795 (more related with 95% to *Aspergillus niger* MT530261.1), as shown in Tables 3, 4; and Figs. 3, 4.

TABLE 1. List of identified fungal species isolated from different sources

Sample	Location (geographic coordinates + map link)	Fungal species
Sewage water	Drinking Water and Sanitation Company (30.7910436, 30.9694423 – https://maps.app.goo.gl/fMoqqyF3CqjVGUw6)	*Aspergillus flavus* Link, 1809 no.1
		Aspergillus niger Micheli,1729 no.1
		Penicillium verrucosum Dierchx,1901 no.1
		Fusarium solani Synder,1940 no.1
		Trichoderma harzianum Rifai,1969
Waste water	Tanta Petroleum Pipe Company (30.7911736, 30.9694296 – https://maps.app.goo.gl/v5x5TtRr2QK7a3z79)	*Aspergillus flavus* Link, 1809 no.2
		Aspergillus niger Micheli,1729 no.2
		Penicillium commune Thom,1910 no.1
		*Mucor sp***.** Fersen,1850 no.1
		Trichoderma viride Pers,1794
Paper and plastics	Tanta Public garbage dump (30.8017259, 30.9925674 – https://maps.app.goo.gl/b17ZDMvgNRssz8dj9)	*Aspergillus niger* Micheli,1729 no.3
		Penicillium chrysogenum Thom,1910 no.1
		Penicillium funiculosum Thom,1910 no.1
		Penicillium verrucosum Dierchx,1901 no.2
		Penicillium commune Thom,1910 no.2
		Fusarium solani Synder,1940 no.2
		Mucor sp. Fersen,1850 no.2
Petroleum-contaminated soil	Tanta Petroleum Pipe Company (30.7911736, 30.9694296 – https://maps.app.goo.gl/v5x5TtRr2QK7a3z79)	*Aspergillus flavus* Link, 1809 no.3
		Aspergillus niger Micheli,1729 no.4
		Aspergillus ochraceus Wilhelm,1877
		Aspergillus candidus Link, 1809
		Penicillium neoechinulatum Frisvad and Samson,2004
		Penicillium polonicum Zaleski,1927
		Penicillium chrysogenum Thom,1910 no.2
		Penicillium funiculosum Thom,1910 no.2
		Penicillium tricolor Samson and Mills,1994
		Mucor sp. Fersen,1850 no.3
		Trichoderma virens Mill, Giddens and Foster,1987
		Trichoderma hamatum Bonord,1906
		Ganoderma lucidum Karsten,1881
		Ulocladium botrytis Preuss,1851
Total		**31**

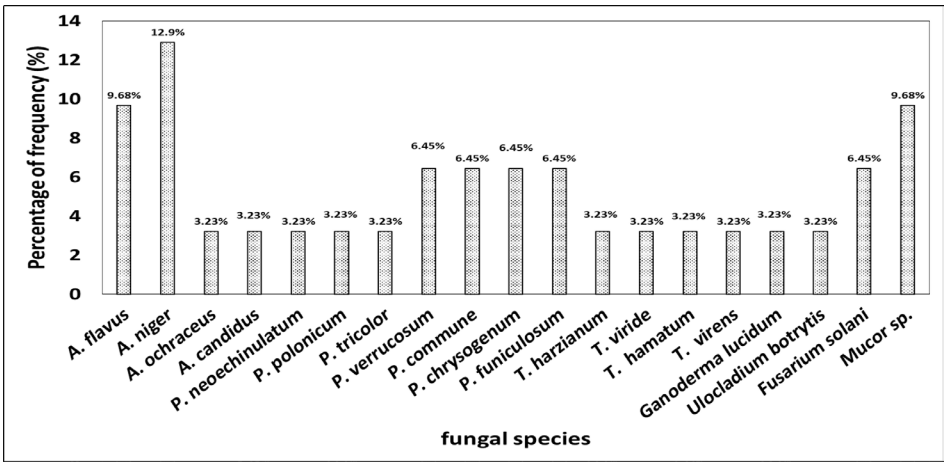

Fig. 1. Frequency of fungal species among the collected samples

TABLE2. Qualitative screening of PVA-degrading activity for the isolated fungi

Fungal species	PVA degradation	Fungal species	PVA degradation
Aspergillus flavus no.1	-	*Penicillium chrysogenum* no.1	+
A. flavus no.2	-	*P. chrysogenum* no.2	-
A. flavus no.3	+	*Penicillium funiculosum no.*1	-
Aspergillus niger no.1	-	*P. funiculosum* no.2	+
A. niger no.2	-	*Trichoderma harzianum*	+
A. niger no.3	-	*Trichoderma viride*	+
A. niger no.4	+	*Trichoderma virens*	+
Aspergillus ochraceus	+	*Trichoderma hamatum*	+
Aspergillus candidus	+	*Ganoderma lucidum*	-
Penicillium neoechinulatum	+	*Ulocladium botrytis*	+
Penicillium polonicum	+	*Fusarium solani* no.1	-
Penicillium tricolor	+	*F. solani* no.2	+
Penicillium verrucosum no.1	-	*Mucor sp.* no.1	-
P. verrucosum no.2	+	*Mucor sp.* no.2	-
Penicillium commune no.1	+	*Mucor sp.* no.3	-
P. commune no.2	-		

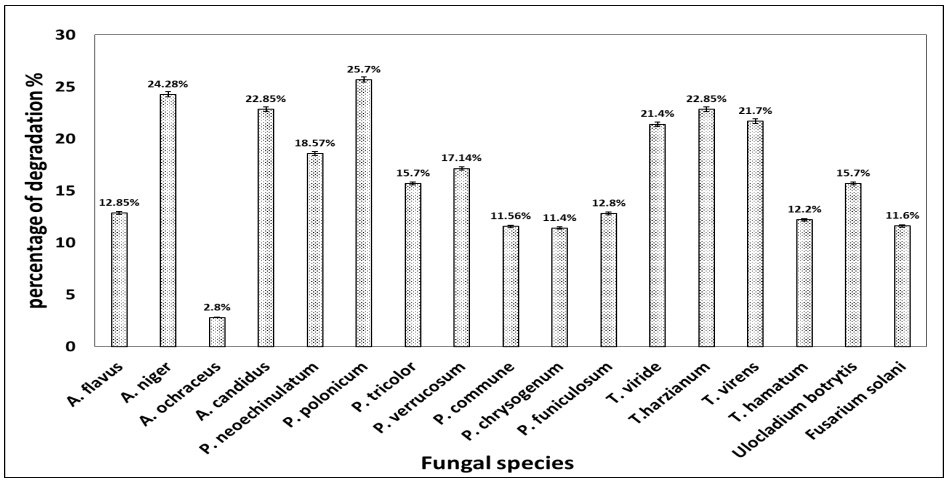

Fig. 2. Quantitative estimation of PVA degradation activity for the isolated fungi

TABLE 3. Partial sequencing of 18s rRNA of Penicillium polonicum

1	AACTCTGTCT GAAGATTGAA GTCTGAGTGA AAATATAAAT TATTAAAAAC TTTCAACAAC	60
61	GGATCTCTTG GTTCCGGCAT CGATGAAGAA CGCAGAGAAA TGCGATACGT AATGTGAATT	120
121	GCAAATTCAG TGAATCATCG AGTCTTTGAA CGCACATTGC GCCCCCTGGT ATTCCGGGGG	180
181	GCATGCCTGT CCGAGCGTCA TTGCTGCCCT CAAGCCCGGC TTGTGTGTTG GGCCCCGTCC	240
241	TCCGATTCCG GGGGACGGGC CCGAAAGGCA GCGGCGGCAC CGCGTCCGGT CCTCGAGCGT	300
301	ATGGGGCTTT GTCACCCGCT CTGTAGGCCC GGCCGGCGCT TGC	343

TABLE 4. Partial sequencing of 18s rRNA of *Aspergillus niger*

1	ATACCCTGTT GCTTCGGCGG GCCCGCCGCT TGTCGGCCGC CGGGGGGGCG CCTTTGCCCC	60
61	CCGGGCCCGT GCCCGCCGGA GACCCCAACA CGAACACTGT CTGAAAGCGT GCAGTCTGAG	120
121	TTGATTGAAT GCAATCAGTT AAAACTTTCA ACAATGGATC TCTTGGTTCCG GCATCGATG	180
181	AAGAACGCAG CGAAATGCGA TAACTAATGT GAATTGCAGA ATTCAGTGAA TCATCGAGTC	240
241	TTTGAACGCA CATTGCGCCC CCTGGTATTC CGGGGGGCAT GCCTGTCCGA GCGTCATTGC	300
301	TGCCCTCAAG CCCGGCTTGT GTGTTGGGTC GCCGTCCCCC TCTCCGGGGG GACGGGCCCG	360
361	AAAGGCAGCG GCGGCACCGC GTCCGATCCT CGAGCGTATG GGGCTTTGTC ACATGCTCTG	420
421	TAGGATTGGC CGGCGCCTGC CGACGTTTTC CAACCATTTT TTCCAG	466

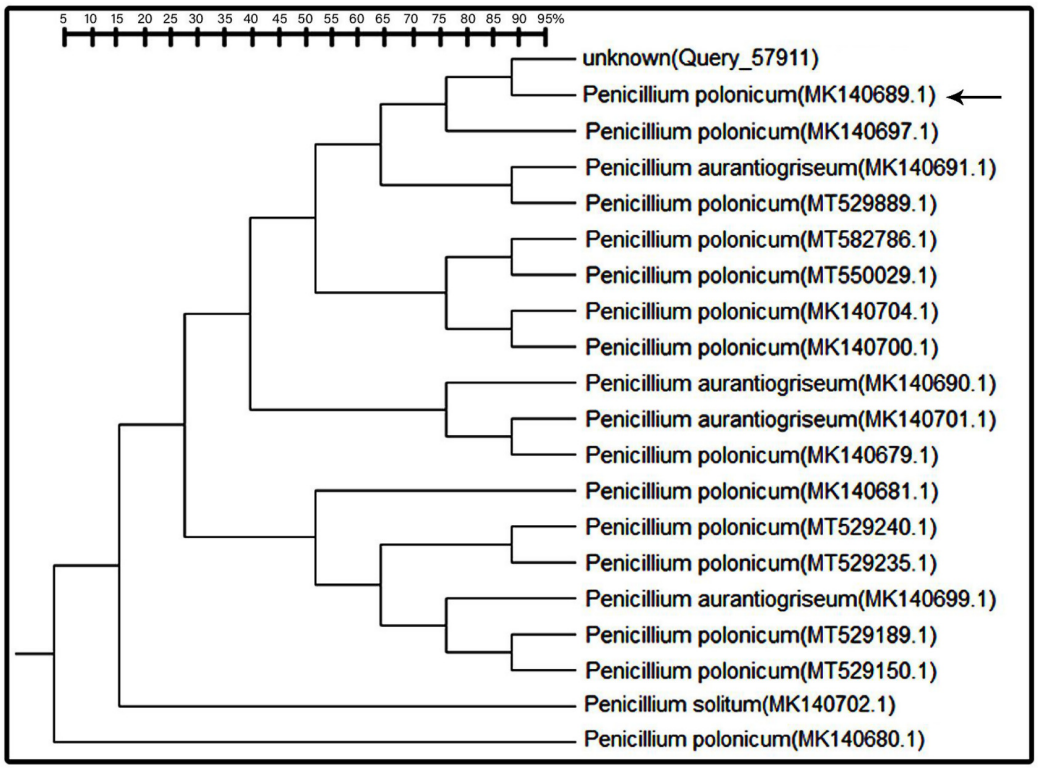

Fig. 3. Phylogentic tree for the identification probability of the selected *Penicillium polonicum* isolate

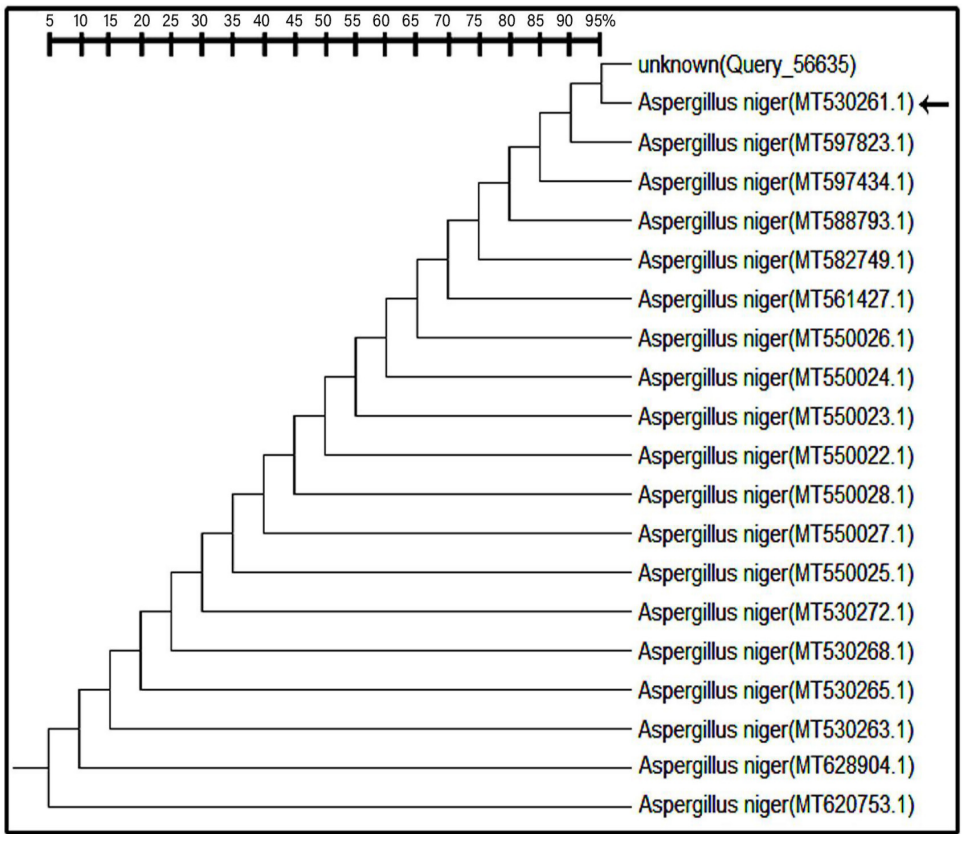

Fig. 4. Phylogentic tree for the identification probability of the selected *Aspergillus niger* isolate

For maximum PVA-hydrolyzing activity by the two selected potent fungal strains, the optimum growth conditions and nutrient requirements were examined in batches.

Figures 5, 6 illustrate the effect of different pH values on growth, fungal dry biomass (dry weight) and PVA degradation activity by the selected PVA-hydrolyzing strains. A maximum PVA degradation rate of 40% was achieved by *P. polonicum* at pH= 5, however, this rate was accomplished at pH= 6 for *A. niger*. Maximum values of mycelium dry weight were recorded at pH= 5 for *P. polonicum*, recording 5.6g/L and at pH= 6 for *A. niger* recording 3.5g/L. Maximum PVA degradation of 50.0% and the highest dry biomass 2.8g/L were observed when *P. polonicum* was incubated at 30ºC. Higher degradation of 58.0% and dry weight of 6.4g/L were determined in *A. niger* culture incubated at 30ºC (Figs. 7 and 8).

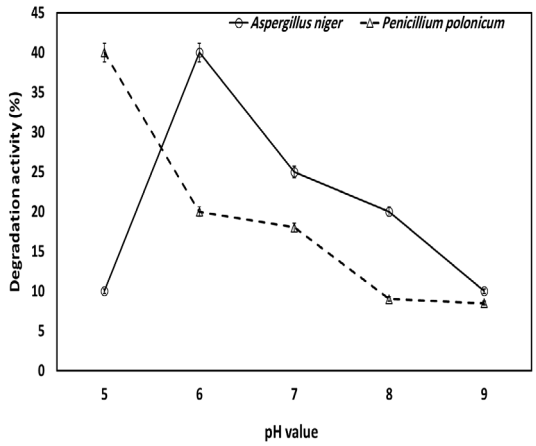

Fig. 5. Effect of different pH values on PVA degradation activity of *P. polonicum* and *A. niger*

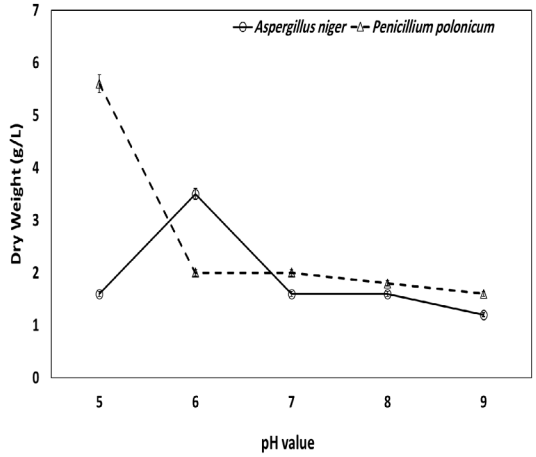

Fig. 6. Effect of different pH values on growth dry weight of *P. polonicum* and *A. niger*

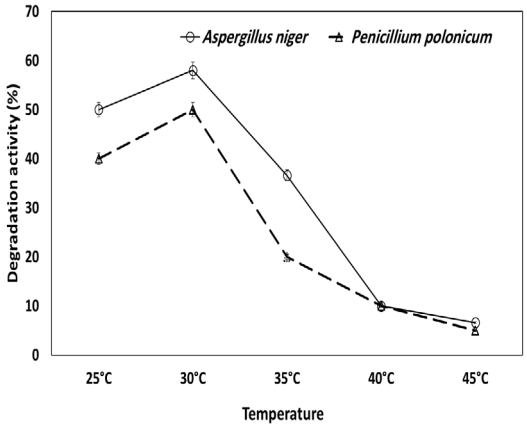

Fig. 7. Effect of different temperatures on PVA degradation activity of *P. polonicum* and *A. niger*

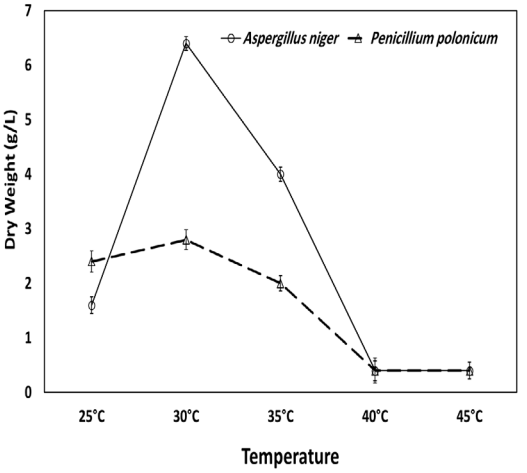

Fig. 8. Effect of different temperatures on growth dry weight of *P. polonicum* and *A. niger*

Both growth and PVA-degradation by the two examined strains were greatly affected by agitation (Figs. 9. 10). The two fungal candidates exhibited their maximum performance in stagnant cultures while their growth and PVA-hydrolyzing activity were decreased to the minimal values in highly agitated cultures at 150rpm.

In the study of the effect of different incubation periods on the growth dry weight and PVA degradation activity, the PVA degradation rate of 65.5% was achieved by *P. polonicum* after incubation for 6 days, however PVA degradation rate of 70.0% after 6 days was recorded for *A. niger*. Maximum value of mycelium dry weight (3.2g/L) recorded after 6 days of incubation for *P. polonicum* and recorded 2.8 g/l for *A. niger* (Figs. 11, 12).

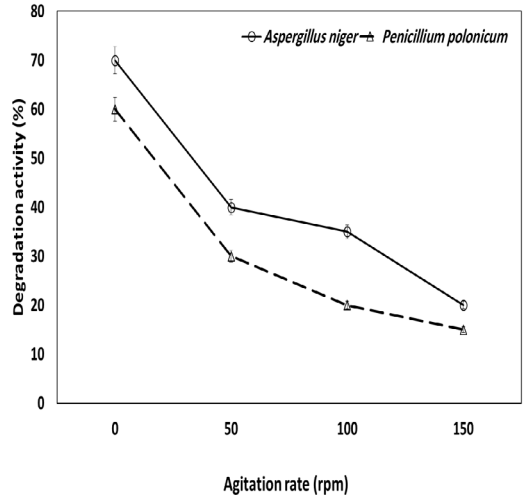

Fig. 9. Effect of agitation rate on PVA degradation activity of *P. polonicum* and *A. niger*

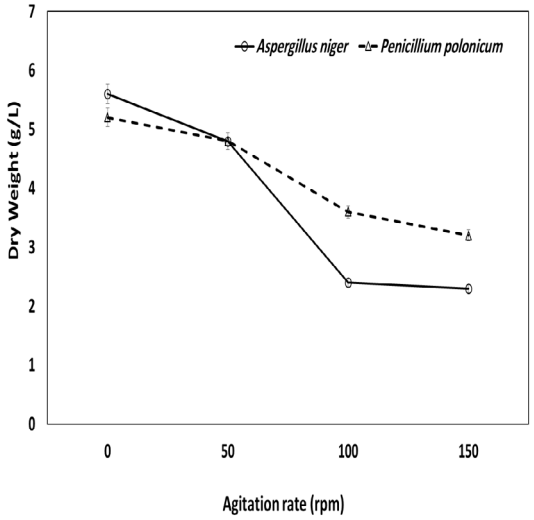

Fig. 10. Effect of agitation rate on the growth dry weight of *P. polonicum* and *A. niger*

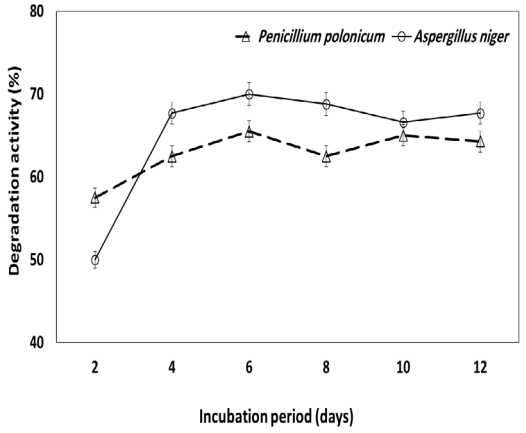

Fig. 11. Effect of different Incubation periods on PVA degradation activity of *P. polonicum* and *A. niger*

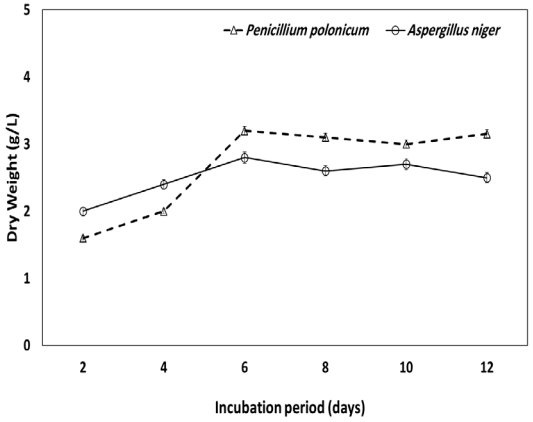

Fig. 12. Effect of different Incubation periods on the growth dry Weight of *P. polonicum* and *A. niger*

Figures 13, 14 compared the effect of different co-carbon sources on the PVA-degradation activity. Starch recorded the highest value of PVA degradation at 28.0% for *P. polonicum* with dry weight of 1.6g/L; *A. niger* revealed PVA-degrading activity at 30.65% and dry weight at 1.2g/L.

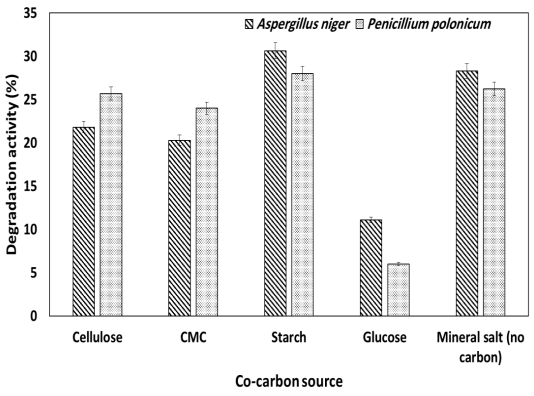

Fig. 13. Effect of co-carbon source PVA degradation activity of *P. polonicum* and *A. niger*

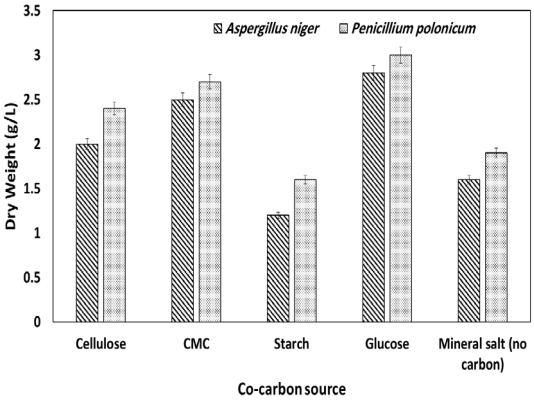

Fig.14. Effect of co-carbon source on the growth dry weight of *P. polonicum* and *A. niger*

Figures 15, 16 summarized the effect of nitrogen source on the PVA degradation activity and growth dry biomass of *P. polonicum* and *A. niger*. Potassium and Sodium Nitrates recorded PVA-degrading activity of 80.0% for both selected fungal strains; while dry biomass was 3.0g/L for *P. polonicum* and 3.5g/L for *A. niger***.**

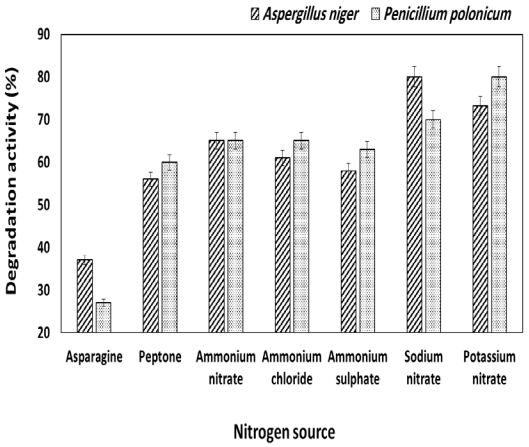

Fig. 15. Effect of different nitrogen sources on PVA degradation activity of *P. polonicum* and *A. niger*

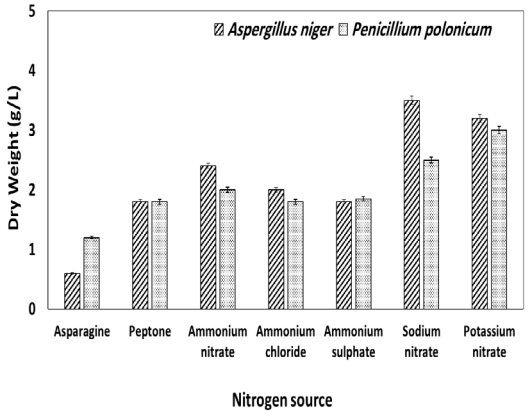

Fig. 16. Effect of different nitrogen sources on the growth dry weight of *P. polonicum* and *A. niger*

In the study of the effect of different concentrations of PVA on the growth dry weight and PVA degradation activity, Figs. 17 and 18 revealed that the highest efficiency of both fungi to degrade PVA was achieved at the initial PVA concentration of 10g/L, whereas *P. polonicum* recorded 88.0% and *A. niger* recorded 90%. And the highest value of dry weight was 5.6g/L for *P. polonicum* and 4.4g/L for *A. niger*.

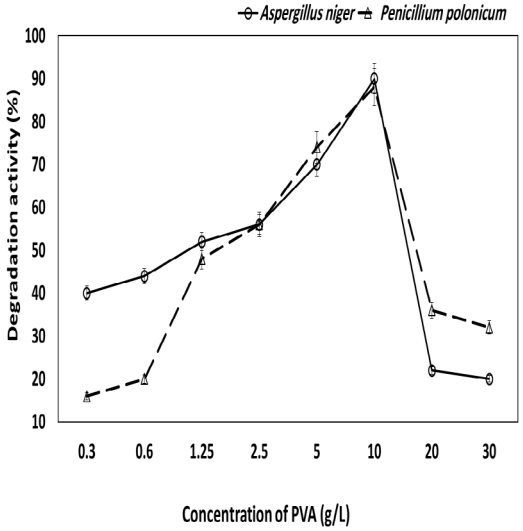

Fig. 17. Effect of different PVA concentrations on PVA degradation activity of *P. polonicum* and *A. niger*

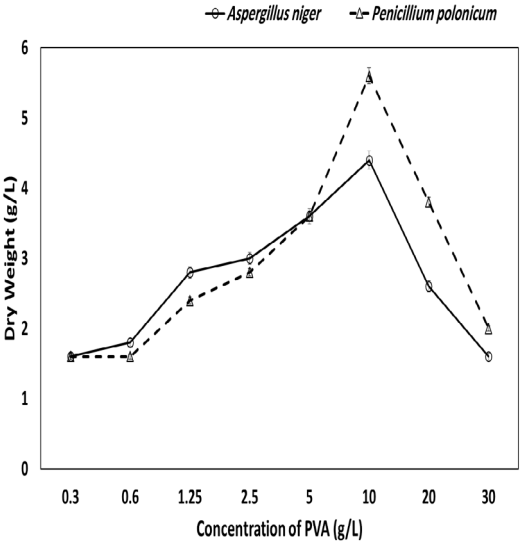

Fig. 18. Effect of different PVA concentrations on the growth dry weight of *P. polonicum* and *A. niger*

Discussion

In the study of the effect of pH of the medium on the PVA- degrading activity of *Penicillium polonicum* and *Aspergillus niger*, the results showed that pH had an observable effect on the degradation rate; as the acidic medium achieved optimum biodegrading ability from 25.7% for *P. polonicum* and 24.28% for *A. niger* to 40% for each. This indicated that the acidic conditions, (pH=5 and 6), resulted in the highest enzyme activity

which matched previous findings by Safari et al. (2005). Also, Qian et al. (2004) adjusted the culture medium at pH=6 in the study of biodegradation of PVA by *Penicillium sp*. Furthermore, Cajthaml et al. (2008) stated that fungi grow rapidly at acidic pH 5 and 5.5, supporting the present results. Also, Khalil et al. (2013) found that pH 5 was optimum for maximum biodegradation of polymers by fungi isolated from plastic garbage. Pawar (2015) found that fungi can degrade polycyclic aromatic hydrocarbons at acidic pH.

In this study, a range of incubation temperatures (25-45°C) was examined and it was found that 30°C was the most suitable temperature for degradation of PVA by the examined strains. At 30°C, the degradation activity increased to 50% and 58% for *P. polonicum* and *A. niger*, respectively. This was in agreement with Qian et al. (2004) who cultured *Penicillium sp.* in a study on biodegradation of PVA at the same temperature, Afifi & Bayoumi (2008) concluded that 30°C was the optimum for biodegradation of hydrocarbons by *Fusarium Oxysporum*. Moreover, Khalil et al. (2013) said that *Myceliophthora sp*. Produce plastic-degrading enzymes at the highest level at temperature 30°C; as well Ullah et al. (2018) recorded that the temperature at which the maximum degradation of PVA by *Stenotrophomonas sp*. was 30°C. Also, three bacterial strains which gave the highest degradation of crude oil at 30°C were recorded by Abdel-Aziz et al. (2018). The present data also was compatible with Deng et al. (2019) that recommended the temperature of 30°C for the biodegradation of PVA by *Eutypella sp*. fungus.

Although co-carbon sources gave high growth for the tested fungi, they were observed to decrease the efficiency of PVA-enzymatic degradation. This was in harmony with Ullah et al. (2019) who stated that the addition of another carbon source to the culture medium increased the growth of *Bacillus cereus* RA23 but decreased the removal of PVA and they brought it back to the dependence of the microorganism on the additive co-carbon source as an energy source and adapted for production of this substrate-specific enzymes, leading to decreasing production of PVA-degrading enzymes. Unlike that, nitrogen source enhanced the degradation activity and the fungal growth. The use of nitrate salts in the culture medium raised the degradation rate of PVA to 80% for both fungi. This is compatible with Kim et al. (2003); Chen et al. (2007); Ullah et al. (2019) and Kanjanasopa et al. (2020) who mentioned that inorganic nitrogen sources raised the degradation rate than organic nitrogen sources. Also, Afifi & Bayoumi (2008) recorded that nitrate salts had a significant effect on the biodegradation of hydrocarbons by *Fusarium solani*.

Finally, the performance of both fungi was negatively influenced by agitation rate. The obtained results revealed that static condition was more suitable for PVA degradation than any agitation speeds which can be considered an advantage of the process leading a reduction in costs due to energy saving. On the contrary, Deng et al. (2019) incubated the PVA- degrading fungus, *Eutypella sp*., on a shaker at 160 rpm. Maximum PVA-degradation activity of *P. polonicum* and *A. niger* reached 88% and 90% after studying the effect of different PVA concentrations; it was found that both fungi had ability to degrade PVA concentration reaching 10g/L after 6 days of incubation. These results agreed with Qian et al. (2004) showing the ability of *Penicillium sp.* to degrade 100% of 0.5% PVA after 12 days. Also, Yamatsu et al. (2006) recorded that *Sphingopyxis* sp. had a degradation rate 90% after 6 days. In this study, *P. polonicum* and *A. niger* had capacity to degrade high concentrations of PVA reaching 10g/L after 6 days of incubation. Kanjanasopa et al. (2020) got similar results when a mixed culture was used for degradation of 1% PVA after 5 days of incubation. In this regard, Shyam et al. (2021) used also PVA 1% during their study to degrade it by *Enterobacter cloacae*.

Conclusion

Data from the present work indicate that the selected fungal strains *Penicillium polonicum* (ON024147) and *Aspergillus niger* (ON012795) are potent PVA-degraders. The recommended growth conditions for maximum degradation activities are incubation at 30°C, culture pH 5 for *P. polonicum* and 6 for *A. niger*, using nitrate salts as a nitrogen source, in a stagnant culture. The degradation activities of *P. polonicum* and *A. niger* were maximized to 88% and 90% of 1% PVA after 6 days of incubation.

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عزل الفطريات المحللة لكحول عديد الفينيل و تحسين قدرتها التحليلية

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تم تجميع 31 عزلة من مواقع مختلفة بمدينة طنطا، محافظة الغربية، مصر. و تم إختبار قدرتها على تحليل الكحول عديد الفينيل كمصدر كربون منفرد أثناء زراعتها فى هذه الدراسة. بين ال 31 عزلة، وجدت فقط 17 عزلة محللة لعديد الفينيل تنتمى لأجناس البينيسيليوم، الأسبيرجيللاس، الترايكوديرما، الفيوزاريوم واليولوكلاديوم. وكانت أعلى نسبة تكسير مسجلة لفطر البينيسيليوم بولونيكام ON024147 (25.7%) والأسبيرجيللاس نايجر ON012795 (24.28%). كما تم تحسين ظروف الزراعة للوصول لأقصى كفاءة لتحلل عديد الفينيل، و التى سجلت 88% لفطر البينيسيليوم بولونيكام و 90% لفطر الأسبيرجيللاس نايجر. حيث كانت أفضل ظروف نمو لفطر البينيسيليوم بولونيكام فى مزرعة ساكنة تحت أس هيدروجينى = 5، درجة حرارة = 30°م، تحضين لمدة 6 أيام فى وجود النشا كمصدر كربون مساعد، نترات البوتاسيوم كمصدر نيتروجين، عديد الفينيل بتركيز 10 جم/لتر. بينما كانت أفضل ظروف نمو لفطر الأسبيرجيللاس نايجر فى مزرعة ساكنة تحت أس هيدروجينى = 6، درجة حرارة = 30°م، تحضين لمدة 6 أيام فى وجود السليلوز كمصدر كربون مساعد، نترات الصوديوم كمصدر نيتروجين، عديد الفينيل بتركيز 10 جم/لتر.