ISSN: 1675-7785
eISSN: 2682-8626

Available online at
https://scilett-fsg.uitm.edu.my/

Science Letters 18(1) 2024, 10 – 20.

Science Letters

Diversity of Chlorpyrifos-Degrading Fungi Isolated from Chlorpyrifos-Contaminated Agricultural Soil

Nur Fatin Nabihah Hasan, Wan Nurhayati Wan Hanafi, Muhd Fauzi Safian, Zaidah Zainal Ariffin*

Faculty of Applied Sciences, Universiti Teknologi MARA (UiTM), 40450 Shah Alam, Selangor, Malaysia

ARTICLE INFO

Article history:
Received 12 July 2023
Revised 14 September 2023
Accepted 3 October 2023
Online first
Published 22 January 2024

Keywords:
Chlorpyrifos,
Agricultural soil
Degradation
Pesticides
Bioremediation

DOI:
10.24191/sl.v18i1.23128

ABSTRACT

Chlorpyrifos is among the most extensively used organophosphorus pesticides in agriculture to control crop pests, reduce damage to insects, control mosquitoes, and reduce household pests. The extensive usage of chlorpyrifos has resulted in widespread contamination of the natural environment, affecting non-target organisms and causing potential environmental jeopardy. As for the toxicity of chlorpyrifos in the environment, the exploration of chlorpyrifos-contaminated soil fungus to remediate the contaminated area is of immense importance. This study isolated and identified fungi from chlorpyrifos-contaminated soil (6° 32' 30.5"N, 100° 15' 39.3") in pursuing our search to find a chlorpyrifos bioremediation agent. Five chlorpyrifos-surviving fungi were successfully isolated from the chlorpyrifos-contaminated soil and identified as *Fusarium oxysporus, Teleromyces verruculosus, Albifimbria terrestris, Aspergillus terreus* and *Purpureocillium lilacinum.* Their identification had been proven by molecular analysis based on 18S rRNA sequence homology and morphological characteristics. The identified strains had the potential to clean up chlorpyrifos pesticide-contaminated environment.

INTRODUCTION

Chlorpyrifos [O, O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphonothioate] is a globally widely used insecticide of chlorinated organophosphorus. It is a degradable compound. Thus, it has been used globally since its introduction in 1965 in the United States despite its high toxicity. Chlorpyrifos is a colourless to white crystalline solid [1] and is marketed as liquid, granular, pellets, wettable powder, dustable powder, and an emulsifiable concentrate.

It is promptly soluble in most organic solvents but has a very low water solubility (2 mg/L) [2]. Chlorpyrifos has a mild mercaptan odour, comparable to the smell of sulfur compounds in rotten eggs, garlic, and onions. Chlorpyrifos residues have been detected in freshwater and soil habitats, and due to their acute toxicity and persistence, they may cause serious human health problems [3]. According to the previous study, long environmental half-lives of chlorpyrifos can last up to four years, depending on the

* Corresponding author. *E-mail address*: drzaidah@uitm.edu.my

application rate, ecosystems, and pertinent habitats [4]. Microbes can degrade chlorpyrifos metabolically [5,6] or cometabolically [7]. The first indicates mineralization as a direct source of carbon and energy, while the second is the by-product metabolism in which the microbe transforms the compound with no advantages.

Numerous genera of bacteria can break down chlorpyrifos, including *Bacillus cereus* CP6, *Klebsiella pneumoniae* CP19 [8], and *Enterobacter* sp. SWLC2 [9], *Shewanella* BT05 [10], *Pseudomonas nitroreducens* AR-3 [11], *Pseudomonas putida* MB285 (12), *Ochrobactrum* sp. JAS2 (13), *Spingobacterium* sp. JAS3 (14) and *Sphingomonas* sp. DSP2 (15). *Flavobacterium* sp. ATCC 27551, *Arthrobacter* sp. [16], and *Enterobacter* sp. [17] were reported to mineralize chlorpyrifos. A study has shown that the *Alcaligenes faecalis* strain could degrade chlorpyrifos and utilize it as the sole source of carbon and phosphorus [18]. Various types of bacteria, including *Sphingomonas, Stenotrophomonas*, *Bacillus* sp., *Brevundimonas* sp. and *Pseudomonas* sp., were capable of 100% degrading 100 mg/L of chlorpyrifos which were obtained from polluted water samples in China and enriched in mineral salts medium [19]. In 4 days, *Paracoccus* sp. isolated from an activated sludge sample and cultivated in TYC medium completely degrades 50 mg/L of chlorpyrifos and 3,5,6-trichloro-2-pyridinol [20]. Fungi are known to have the ability to form extended mycelial networks. Their catabolic enzymes' low specificity and independence in using organic chemicals as a growth substrate make fungi suitable for bioremediation. However, there is limited study regarding the degradation of chlorpyrifos by fungi.

Aspergillus sp. [21], *Trichoderma viride* [22], and Fusarium [23] were used in earlier studies of chlorpyrifos biodegradation with varying degrees of success. Besides that, *Aspergillus terreus*, *Phanerochaete chrysosporium*, and *Verticillium* sp. DSP has also been investigated regarding their ability to degrade chlorpyrifos [24,25,26]. In addition, 83.9% of chlorpyrifos was used as a source of C and N by *Acremonium* sp. [27]. This study aimed to identify the chlorpyrifos-degrading fungi from the agricultural soil using the enrichment culture medium. Besides, the diversity of soil fungi also was determined.

EXPERIMENTAL

Soil Sample

Soil samples were collected as the inoculum from Ladang Harum Manis Bukit Reban Jalan Kaki Bukit, Perlis (6° 32' 30.5''N, 100° 15' 39.3'') that had been contaminated by chlorpyrifos for years in Perlis, Malaysia. Soil samples were obtained from 0 to 15 cm depth and air dried at room temperature, where all the debris was removed using a 2 mm mesh screen. All the samples were kept at 4°C for further studies.

Morphological Identification of Isolated Fungi

The fungi isolates were identified by assessing their macroscopic and microscopic characteristics. Macroscopic is based on the characteristics that are visible by the naked eye, such as the presence and absence of mycelium, pigment production, length, and growth, as well as the colour of the fungi. On the other hand, for microscopic observation, an adhesive tape was gently pressed onto the surface of the fungal colony to attach mycelia to the adhesive tape. The tape was then placed onto a glass slide containing a drop of methylene blue. The observation was performed using a CX12 light microscope under a total magnification of 1000.

DNA Extraction

The isolates were grown on Potato Dextrose Agar (PDA) plates for five days, and their morphology was investigated by light microscope. Colony morphology was observed on PDA plates incubated at 27°C at 1,2,3,4 and 5 days. The fungal isolates were used for genomic DNA extraction. The mycelia were scraped and collected, and genomic DNA was extracted according to the Fungi/Yeast Genomic DNA Isolation Kit protocol from Norgen Biotek Corporation.

https://doi.org/10.24191/sl.v18i1.23128

Molecular identification of isolated fungi

The primer pairs ITS1 (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') were used to amplify the DNA sample. The 25 uL reaction mixture included 50 ng of DNA template, 1 U of Taq polymerase, 10 mM dNTPs, and 10 μM of each primer. Amplifications were carried out using a thermocycler under the following conditions: initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute. The final extension was carried out at 72°C for 15 minutes. The PCR products were sent to the third party for sequencing, and the similarity between biological sequences was determined using the Basic Local Alignment Search Tool (BLAST). Based on results from the GenBank BLAST search and considering the average weighted infraspecific ITS variability in the kingdom fungi, which was calculated to be 2.51% with a standard deviation of 4.57%, it is reasonable to start with 80% query coverage and 97-100% sequence similarity for assigning a species name.

Phylogenetic tree

The phylogenetic analysis was performed with ITS gene sequences using the neighbour-joining method in MEGA 4 software (version 4.0) with 1000 bootstrap replicates. The resulting tree was trimmed to save space, and the closest relatives were retained.

RESULTS AND DISCUSSION

A total of five potential degraders have been successfully isolated from the soil and were grown on Potato Dextrose Agar (PDA). All the fungi were observed under macroscopic and microscopic observation and identified molecularly.

Molecular Identification of Isolated Fungi

The molecular identification method was performed using the Fungi/Yeast Genomic DNA Isolation Kit. The genomic DNA of selected chlorpyrifos-degrading fungi was first extracted, and the presence of the genomic DNA was confirmed by running the gel electrophoresis. The results of the extracted DNA on 1% agarose gel were visualized under UV light. The genomic DNA of isolated fungi was subjected to amplification by using the ITS1/ITS4 primers. The 18S rRNA genes of the ITS region were amplified by PCR to determine the efficacy and reliability of this technique. (Fig. 1). The bands that appeared on agarose gel were identified as *Fusarium oxysporum* strain SHBV2, *Talaromyces verruculosus* isolate ATT281, *Albifimbria terrestris* strain SCAU180, *Aspergillus terreus* strain LPSC 1180 and *Purpureocillium lilacinum* isolate HT003 after sequence alignment performed in GenBank database by using BLAST.

Fusarium oxysporum strain SHBV2

Fusarium oxysporum was observed to produce a white cottonish colony with the aerial mycelia becoming tinged in purple on Potato Dextrose Agar (PDA). Macroscopic and microscopic images shown in Fig. 2 were taken after five days of incubation. Large and complex conidia (macroconidia) and more simple conidia (microconidia) were observed under the microscope. The microconidia were usually non-septate and slightly curved. They can be seen accumulating around the conidiophores. Besides, macroconidia were large and were cumulative over the conidiophores. They can be seen having three to five septa. This description matched the morphological traits that had previously been published for *F. oxysporum* [28]. Besides, similar macroscopic and microscopic images of *F. oxysporum* were also reported [29]. *F. oxysporum* can be found in every type of soil and can be considered a normal constituent of fungal species in the rhizosphere of plants. Based on a study [30], all *F. oxysporum* strains are saprophytic and can withstand organic matter in soil and the rhizosphere of various plant species. Certain strains of *F. oxysporum* can penetrate the roots and invade the vascular system, but some cannot.

https://doi.org/10.24191/sl.v18i1.23128

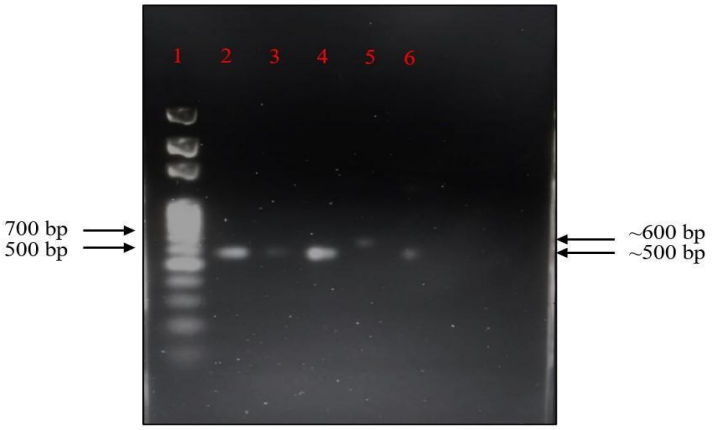
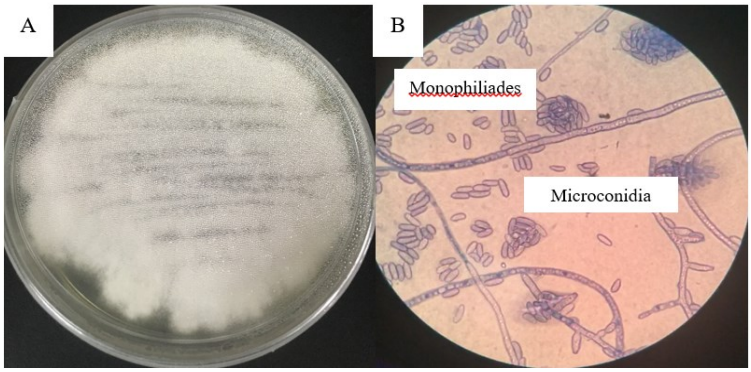


Fig. 1. Amplification of PCR of the ITS products obtained with ITS1/ITS4 primers. PCR products from several fungi are in 1% agarose gel and stained with Gel-Red. 1- 100bp ladder; 2- *Fusarium oxysporum* strain SHBV2; 3- *Teleromyces verruculosus* isolate ATT281; 4- : *Albifimbria terretris* strain SCAU180; 5- *Aspergillus terreus* strain LPSC 1180; 6- *Purpureocillium lilacinum* isolate HT003.

Talaromyces verruculosus isolate ATT281

Talaromyces verruculosus was observed as a white to greenish colony on Potato Dextrose Agar (PDA) (Fig. 3). It was considered a fast-growing fungus as the plate was fully covered after four days of incubation. Macroscopic and microscopic observations of this isolate after five days of incubation are shown in Figure 3. Globose to sub-globose in the shape of conidia was observed. The metulae and phialides were branched from the conidiophores. *Teleromyces verruculosus* is also known as *Penicillium verruculosum* [31]. Diverse strains of *T. verruculosus* were isolated primarily from the soils. For decades, *T. verruculosus* has been studied as a promising fungus for cellulase production and anti-microbial extrolites [32,33].

Fig. 2. *Fusarium oxysporum* strain SHBV2; 1000x magnification (A: Macroscopic view; B: Microscopic view)



https://doi.org/10.24191/sl.v18i1.23128

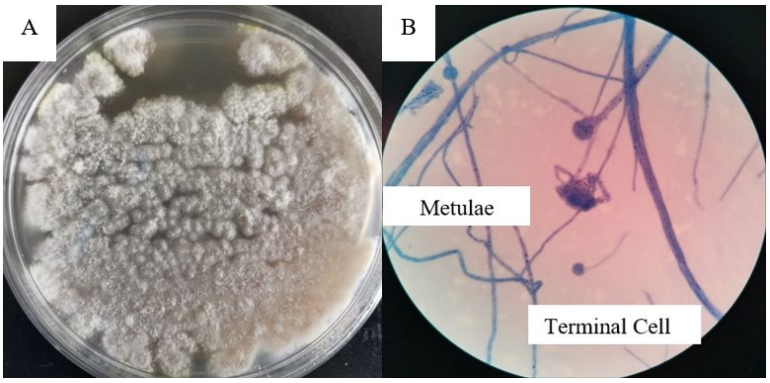


Fig. 3. *Teleromyces verruculosus* isolate ATT281; 1000x magnification (A: Macroscopic view; B: Microscopic view)

Albifimbria terrestris strain SCAU180

A white cottonish colony of *Albifimbria terrestris* was seen on Potato Dextrose Agar (PDA) (Fig. 4). *A. terrestris* was a fast-growing isolate as it was fully covered on PDA after six days of incubation. Microscopic observation of this isolate showed the metulae and philiade attached to the conidiophore. The conidia were globose in shape. However, the impacts and functions of these isolates still need to be researched.

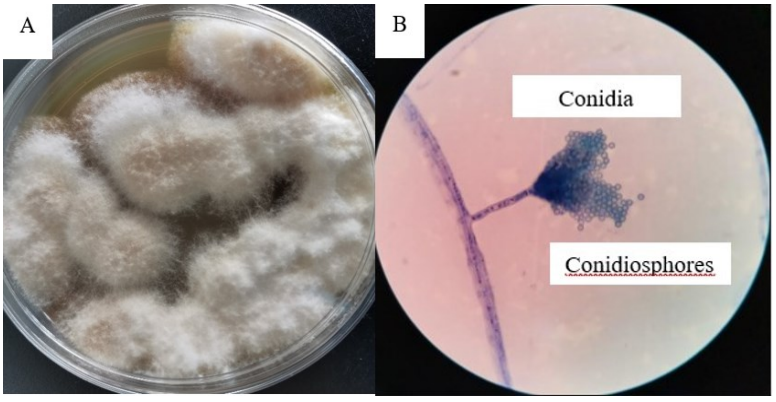


Fig. 4. *Albifimbria terretris* strain SCAU180; 1000x magnification (A: Macroscopic view; B: Microscopic view)

Aspergillus terreus strain LPSC 1180

Macroscopic observation of *Aspergillus terreus* showed bright orange-brownish colonies and appeared as various shades of cinnamon brown on Potato Dextrose Agar (PDA). Fig. 5 shows the colony formation after five days of incubation and is also considered a fast-growing isolate. Microscopically, the conidiophores attached to the foot cell were smooth, and the conidial head was compact. As shown in Figure 5, the metulae were as long as the philiade. *A. terreus* which belongs to the genus *Aspergillus*, is a natural soil saprophyte recovered from desert fields, grasslands, compost heaps, and stored contaminants of barley, peanuts, and corn [34]. *A. terreus* is a source of numerous bioactive substances with anticancer properties [35]. Lovastatin, an antihypercholesterolemic drug, was one of the secondary metabolites produced by *A. terreus* [36]. This species can cause infections, such as onychomycosis and invasive aspergillosis (IA), in severely immunocompromised hosts. Thus, *A. terreus* is clinically significant.

https://doi.org/10.24191/sl.v18i1.23128

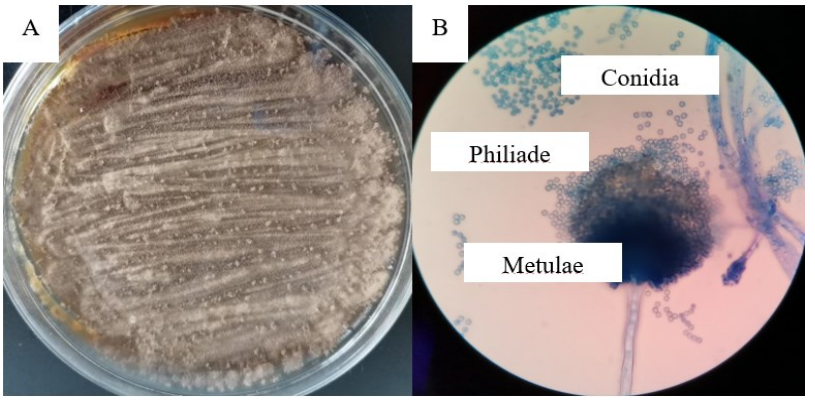


Fig. 5. *Aspergillus terreus* strain LPSC 1180; 1000x magnification. (A: Macroscopic view; B: Microscopic view)

Purpureocillium lilacinum **isolate HT003**

Fig. 6 shows the image of the isolate captured after seven days of incubation on Potato Dextrose Agar (PDA). Thus, this verified that this isolate was a fast-growing fungus [37] and is known as *Purpureocillium lilacinum*. *P. lilacinum* was observed to have an irregular shape and a slightly white cottonish texture. No color changes in the isolate after 14 days of incubation. Figure 5 also shows the microscopic image of the incubated colony after three days of incubation. It was observed that a large vesicle was covered by metulae and philiade was attached to the metulae on the conidiophore. Conidia was seen in globose-ellipsoidal to fusiform in shape. *P. lilacinum* (known initially as *Paecilomyces lilacinus*) is a member of the Ascomycota phylum [38]. *P. lilacinum* is a saprobic species that can be isolated from soil, insect and insect larvae, nematodes, decaying vegetation, humans, animals, and air. Based on previous studies [39,40,41], this species can cause contamination of creams and lotions, sodium bicarbonate solutions, and colonizing materials. *P. lilacinum* forms a biofilm, together with *Aspergillus*, *Fusarium*, and *Acremonium,* as it can grow on wet and moist surfaces of water distribution systems [42].

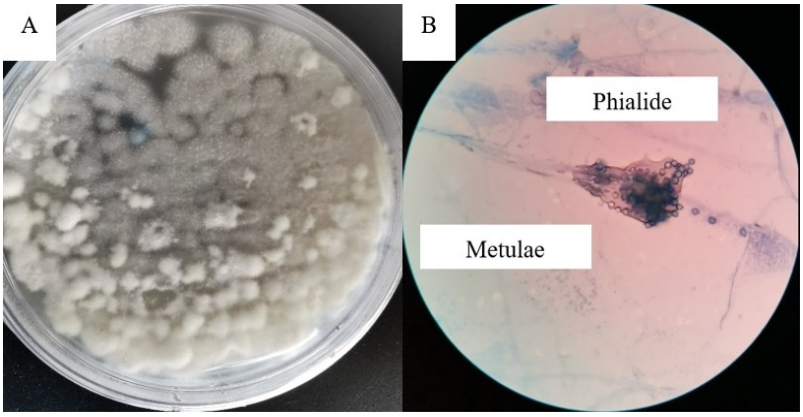


Fig. 6. *Purpureocillium lilacinum* isolate HT003; 1000x magnification. (A: Macroscopic view; B: Microscopic View)

https://doi.org/10.24191/sl.v18i1.23128

Phylogenetic tree of isolated fungi

The phylogenetic tree was constructed to study the evolutionary relationship or histories among the fungal isolates. The branching pattern in a phylogenetic tree determines how species and other groups evolved from a series of the same ancestor. The phylogram in Fig. 7 was constructed using MEGA 5.10 software. The root of the tree represents the ancestral lineage, and the tips of the branches represent the descendants of the ancestors. The horizontal line bar represents evolutionary lineage change over time. The clade is commonly known as a monophyletic group where the group shares the same ancestor and all the descendants of the ancestor. A clade was formed between *Talaromyces verruculosus* isolate ATT281 and *Purpureocillium lilacinum* isolate HT003, showing that these species shared the same ancestor and *Aspergillus terreus* strain LPSC 1180 is also closely related. Moreover, a clade formation of *Fusarium oxysporum* strain SHBV2 and *Albifimbia terrestris* strain SCAU180 showed they also share a common ancestor. All five isolated fungi diverged from a common ancestor a long time ago. A neighbor joining tree method was constructed and shown in Figure 7.

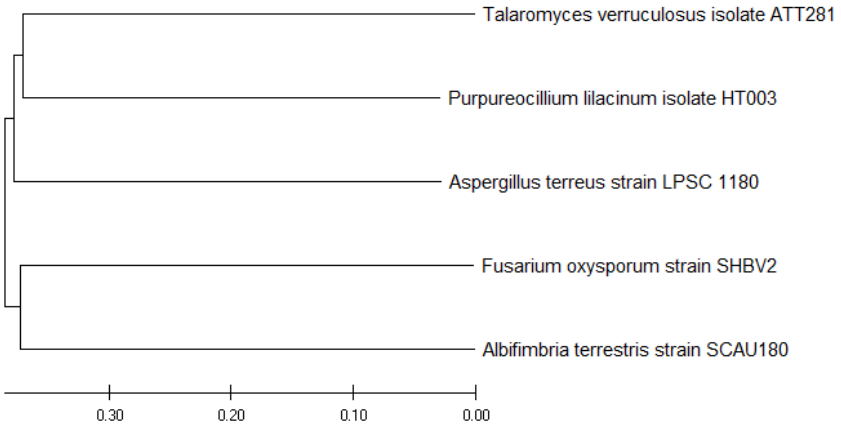


Fig.7. Phylogenetic tree of the isolated and identified soil fungi.

CONCLUSION

Five fungi have been successfully isolated and identified in this study which were *Fusarium oxysporus*, *Teleromyces verruculosus*, *Alfimbria terrestris*, *Aspergillus terreus* and *Purpureocillium lilacinum*. These fungi can be used as microbial inoculants with their properties to degrade pesticides to reduce contamination. In this study, all those isolated fungi were identified as CP-degrading fungi, and further research should be done to determine the ability of the degrader before successful bioremediation of the organisms can be implemented.

ACKNOWLEDGMENTS

The authors would like to acknowledge the support of the Faculty of Applied Sciences, Universiti Teknologi MARA, Shah Alam, Selangor, Malaysia, who provided this research's facilities and financial support.

https://doi.org/10.24191/sl.v18i1.23128

AUTHOR'S CONTRIBUTION

The article was written and revised by Nur Fatin Nabihah Hassan and Zaidah Zainal Ariffin, who also did the research. Zaidah Zainal Ariffin, Muhd Fauzi Safian and Wan Nurhayati Wan Hanafi conceptualized the main idea for the research, provided the theoretical foundation, designed the study, oversaw its progress, anchored the review, approved corrections, and approved the publication submission.

CONFLICT OF INTEREST STATEMENT

The authors affirm that there are no competing interests in the publication of this paper.

REFERENCES

[1] Tomlin C, British Crop Protection Council. (2006) The pesticide manual: a world compendium. British Crop Protection Council, Alton, Hampshire, 186-187

[2] Maya K, Singh RS, Upadhyay SN, Dubey SK (2011) Kinetic analysis reveals bacterial efficacy for biodegradation of chlorpyrifos and its hydrolyzing metabolite TCP. Process Biochem 46:2130–2136. doi: 10.1016/J.PROCBIO.2011.08.012

[3] Tony Hadibarata, Risky Ayu Kristanti, Muhammad Bilal, Murat Yilmaz, Palanivel Sathishkumar (2023) Biodegradation mechanism of chlorpyrifos by halophilic bacterium *Hortaea* sp. B15, Chemosphere, 312(1), 137260, doi:10.1016/j.chemosphere.2022.137260.

[4] Lu M-X, Jiang WW, Wang J-L, Jian Q, Shen Y, Liu X-J, et al. (2014) Persistence and Dissipation of Chlorpyrifos in Brassica Chinensis, Lettuce, Celery, Asparagus Lettuce, Eggplant, and Pepper in a Greenhouse. PLoS ONE 9(6): e100556. https://doi.org/10.1371/journal.pone.0100556

[5] Singh BK, Walker A, Morgan JAW, Wright DJ (2004) Biodegradation of Chlorpyrifos by *Enterobacter* Strain B-14 and Its Use in Bioremediation of Contaminated Soils. Appl Environ Microbiol 70:4855–4863. doi: 10.1128/AEM.70.8.4855-4863.2004

[6] John, E. M., Rebello, S., & Jisha, M. S. (2014). Chlorpyrifos degradation using bacterial consortium obtained from soil. Int J Environ Eng–IJEE, 1(4), 91-94.

[7] Anwar S, Liaquat F, Khan QM, Khalid ZM, Iqbal S (2009) Biodegradation of chlorpyrifos and its hydrolysis product 3,5,6-trichloro-2-pyridinol by *Bacillus pumilus* strain C2A1. J Hazard Mater 168:400–405. doi: 10.1016/J.JHAZMAT.2009.02.059

[8] Mohamed S. Elshikh, Khaloud Mohammed Alarjani, Dina S. Huessien, Hamzah A.M. Elnahas, Antonyrajan Roshini Esther (2022) Enhanced Biodegradation of Chlorpyrifos by *Bacillus cereus* CP6 and *Klebsiella pneumoniae* CP19 from municipal wastewater, Environ Res, 205, 112438, doi: 10.1016/j.envres.2021.112438.

[9] Jha, Sunil & Chishti, Zia & Arshad, Khaliq-ur-Rehman. (2022). *Enterobacter* sp. SWLC2 for biodegradation of chlorpyrifos in the aqueous medium: Modeling of the process using artificial neural network approaches. Computers and Electronics in Agriculture. 193. 106680. doi: 10.1016/j.compag.2021.106680.

[10] Muthusamy, Govarthanan & Ameen, Fuad & Kamalakannan, Seralathan & Thangaswamy, Selvankumar & Almansob, Abobakr & Alwakeel, S.S. & Kim, Woong. (2020). Rapid biodegradation of chlorpyrifos by plant growth-promoting psychrophilic *Shewanella* sp. BT05: An eco-friendly approach to cleaning up pesticide-contaminated environment. Chemosphere. 247. doi: 10.1016/j.chemosphere.2020.125948.

https://doi.org/10.24191/sl.v18i1.23128

[11] Aswathi, A., Pandey, A., & Sukumaran, R. K. (2019). Rapid degradation of the organophosphate pesticide - Chlorpyrifos by a novel strain of *Pseudomonas nitroreducens* AR-3. Bioresource technology, 292, 122025. doi: 10.1016/j.biortech.2019.122025

[12] Liu, J., Tan, L., Wang, J., Wang, Z., Ni, H., & Li, L. (2016). Complete biodegradation of chlorpyrifos by engineered *Pseudomonas putida* cells expressing surface-immobilized laccases. Chemosphere, 157, 200–207. doi:10.1016/j.chemosphere.2016.05.031

[13] Abraham, J., & Silambarasan, S. (2016). Biodegradation of chlorpyrifos and its hydrolysis product 3,5,6-trichloro-2-pyridinol using a novel bacterium *Ochrobactrum* sp. JAS2: A proposal of its metabolic pathway. Pesticide biochemistry and physiology, 126, 13–21. doi: 10.1016/j.pestbp.2015.07.001

[14] Abraham, J. & S., S. (2013). Biodegradation of chlorpyrifos and its hydrolyzing metabolite 3,5,6-trichloro-2-pyridinol by *Sphingobacterium* sp. JAS3. Process Biochemistry, 48(10):1559–1564. doi: 10.1016/j.procbio.2013.06.034

[15] Li, X., He, J., & Li, S. (2007). Isolation of a chlorpyrifos-degrading bacterium, *Sphingomonas* sp. strain Dsp-2, and cloning of the mpd gene. Research in microbiology, 158(2), 143–149. doi:10.1016/j.resmic.2006.11.007

[16] Mallick K, Bharati K, Banerji A, Shakil NA, Sethunathan N (1999) Bacterial Degradation of Chlorpyrifos in Pure Cultures and in Soil. Bull Environ Contam Toxicol 62:48–54. doi: 10.1007/s001289900840

[17] Anwar S, Liaquat F, Khan QM, Khalid ZM, Iqbal S (2009) Biodegradation of chlorpyrifos and its hydrolysis product 3,5,6-trichloro-2-pyridinol by *Bacillus pumilus* strain C2A1. J Hazard Mater 168:400–405. doi: 10.1016/J.JHAZMAT.2009.02.059

[18] Yang L, Zhao Y, Zhang B, Yang C-H, Zhang X (2005) Isolation and characterization of a chlorpyrifos and 3,5,6-trichloro-2-pyridinol degrading bacterium. FEMS Microbiol Lett 251:67–73. doi:10.1016/j.femsle.2005.07.031

[19] Li X, Jiang J, Gu L, Ali SW, He J, Li S (2008) Diversity of chlorpyrifos-degrading bacteria isolated from chlorpyrifos-contaminated samples. Int Biodeterior Biodegradation 62:331–335. doi:10.1016/J.IBIOD.2008.03.001

[20] Xu G, Zheng W, Li Y, Wang S, Zhang J, Yan Y (2008) Biodegradation of chlorpyrifos and 3,5,6-trichloro-2-pyridinol by a newly isolated *Paracoccus* sp. strain TRP. Int Biodeterior Biodegradation 62:51–56. doi: 10.1016/J.IBIOD.2007.12.001

[21] Liu X, You M, Wei Y, Liao J, Ye L, Chen J (2003) Isolation of chlorpirifos-degrading *Aspergillus* sp. Y and measurement of degradation efficiency. Chengdu Institute of Biology, Chinese Academy of Sciences

[22] Mukherjee I, Gopal M (1996) Degradation of chlorpyrifos by two soil fungi *Aspergillus niger* and *Trichoderma viride*. Toxicol Environ Chem 57:145–151. doi: 10.1080/02772249609358383

[23] Wang J, LS Z, Qin K (2005) Degradation characteristics of three fungi to chlorpyrifos | Request PDF. https://www.researchgate.net/publication/285687171_Degradation_characteristics_of_three_fungi_to_chlorpyrifos. Accessed 30 January 2019

[24] Bumpus JA, Kakar SN, Coleman RD (1993) Fungal degradation of organophosphorus insecticides. Appl Biochem Biotechnol 39–40:715–26

[25] Omar SA (1998) Availability of phosphorus and sulfur of insecticide origin by fungi. Biodegradation 9:327–36

https://doi.org/10.24191/sl.v18i1.23128

[26] Yu YL, Fang H, Wang X, Wu XM, Shan M, Yu JQ (2006) Characterization of a fungal strain capable of degrading chlorpyrifos and its use in detoxification of the insecticide on vegetables. Biodegradation 17:487–494. doi: 10.1007/s10532-005-9020-z

[27] Kulshrestha G, Kumari A (2011) Fungal degradation of chlorpyrifos by *Acremonium* sp. strain (GFRC-1) isolated from a laboratory enriched red agricultural soil. Biol Fertil Soils 47:219–225. doi:10.1007/s00374-010-0505-5

[28] Khan, Muhammad & Khan, Sajid & Waheed, Ummara & Raheel, Dr & Khan, Zulqarnain & Alrefaei, Abdulwahed & Alkhamis, Hussein. (2021). Morphological and genetic characterization of *Fusarium oxysporum* and its management using weed extracts in cotton. Journal of King Saud University: Science. 33.

[29] Cutuli MT, Gibello A, Rodriguez-Bertos A, Blanco MM, Villarroel M, Giraldo A, Guarro J (2015) Skin and subcutaneous mycoses in tilapia (*Oreochromis niloticus*) caused by *Fusarium oxysporum* in coinfection with *Aeromonas hydrophila*. Med Mycol Case Rep 9:7–11. doi:10.1016/j.mmcr.2015.06.002

[30] Garrett SD. 1970. Pathogenic root-infection fungi. London, UK: Cambridge University Press, 294

[31] Samson, R.A., Yilmaz, N., Houbraken, J., Spierenburg, H., Seifert, K.A., Peterson, S.W., Varga, J., Frisvad, J.C.,2011. Phylogeny and nomenclature of the genus *Talaromyces* and taxa accommodated in *Penicillium* subgenus *Biverticillium*.Stud. Mycol. 70, 159–183

[32] Solov'eva, I.V., Okunev, O.N., Vel'kov, V.V., Koshelev, A.V., Bubnova, T.V., Kondrat'eva, E.G., Skomarovskii, A.A., Sinitsyn, A.P., 2005. The selection and properties of *Penicillium verruculosum* mutants with enhanced production of cellulases and xylanases. Mikrobiologiia 74, 172–178

[33] Szakács, G., Réczey, K., Hernádi, P., Dobozi, M., 1981. *Penicillium verruculosum* WA30 is a new source of cellulase. Eur. J. Appl. Microbiol. Biotechnol. 11, 120–124

[34] Balajee SA (2009) *Aspergillus terreus* complex. Med. Mycol. 47: S42–S46

[35] Nadumane, Varalakshmi & Venkatachalam, Prerana & Gajaraj, B. (2016). Aspergillus Applications in Cancer Research. doi:10.1016/B978-0-444-63505-1.00020-8.

[36] Ja´nos Varga J, Bea´ta B, To´th T, Sa´ndor S, Kocsube´1 K, Farkas BB, Gyo¨rgy G, Szaka´cs S, Jo´zsef J, Te´ren T, Kozakiewicz Z Evolutionary relationships among *Aspergillus terreus* isolates and their relatives. doi: 10.1007/s10482-005-3870-6

[37] Luangsa-Ard J, Houbraken J, van Doorn T, Hong SB, Borman AM, Hywel-Jones NL, Samson RA (2011) *Purpureocillium*, a new genus for the medically important *Paecilomyces lilacinus*. FEMS Microbiol Lett 321:141–149. doi: 10.1111/j.1574-6968.2011.02322.x

[38] Khan, M., & Tanaka, K. (2023). *Purpureocillium lilacinum* for plant growth promotion and biocontrol against root-knot nematodes infecting eggplant. PloS one, 18(3), e0283550. https://doi.org/10.1371/journal.pone.0283550

[39] Pettit TH, Olson RJ, Foos RY & Martin WJ (1980) Fungal endophthalmitis following intraocular lens implantation. A surgical epidemic. Arch Ophthalmol 98: 1025–1039

[40] Orth B, Frei R, Itin PH, Rinaldi MG, Speck B, Gratwohl A & Widmer AF (1996) Outbreak of invasive mycoses caused by *Paecilomyces lilacinus* from a contaminated skin lotion. Ann Internal Med 125:799–806

[41] Itin PH, Frei R, Lautenschlager S, Buechner SA, Surber C, Gratwohl A &Widmer AF (1998) Cutaneous manifestations of *Paecilomyces lilacinus* infection induced by a contaminated skin lotion in patients who are severely immunosuppressed. J Am Acad Dermatol 39: 401–409

https://doi.org/10.24191/sl.v18i1.23128

[42] Anaissie EJ, Stratton SL, Dignani MC, Lee C, Summerbell RC, Rex JH, Monson TP & Walsh TJ (2003) Pathogenic molds (including *Aspergillus* species) in hospital water distribution systems: a 3-year prospective study and clinical implications for patients with hematologic malignancies. Blood 101:2542–2546

https://doi.org/10.24191/sl.v18i1.23128