Production and Partial Purification of Pectinases Produced by Three Novel *Cladosporium* Species Isolated from Egypt

Ahmed Mohamed Moharram

Assiut University

Abdel-Naser Ahmed Zohri

Assiut University

Abd El-Latif Hesham (✉ hesham_egypt5@agr.bsu.edu.eg)

Beni-Suef University

Mohamed Al-Amin Maher

Assiut University

Osama Abdel-Hafeez Al-Bedak

Assiut University Mycological Centre, Assiut University

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Abstract

Based on phenotypic characters and sequencing of the internal transcribed spacers (ITS), 28S Large Subunit (LSU) and partial actin (ACT), three novel species of *Cladosporium* were described in this study as *C. parasphaerospermum*, *C. chlamydosporum*, and *C. compactisporum*. Activities of pectinases produced by the three strains were 5700, 3720, and 3480 U mg⁻¹ enzymes, at the optimum pH (6.0, 7.0, and 8.0) and temperature (35, 30, and 35 °C), respectively. The ions potassium and manganese had a strong activating impact on the activity of *C. compactisporum* pectinase (124.4 and 172%, respectively). When tested under optimal conditions, EDTA and the other metal ions inhibited the activity of the pectinases in varied degrees. The strongest inhibitory effects of *C. parasphaerospermum*, *C. chlamydosporum*, and *C. compactisporum* pectinases were with Ni⁺², Zn⁺², and EDTA, respectively. *Cladosporium* species have a long history of biotechnological uses, and here we add another: pectinase makers. We offer three unique suppliers of low-temperature active fungal pectinases for biotechnological applications such as the management of Pectinacious wastes in the juice industry.

Introduction

Cladosporium is one of the largest and most complex genera of hyphomycetes, which currently includes more than 728 names. Until recently, all types of unrelated dematiaceous hyphomycetes characterized by amero-to-phragmosporous conidia formed in acropetal chains had been referred to *Cladosporium*¹. Species of *Cladosporium* are well adapted to be spread easily in large numbers over long distances, therefore they are cosmopolitan and widely present in all various types of plants and other debris, mostly isolated from air, soil, seeds, grains, food, paint, textiles and other organic matter¹⁻⁹. Other species of this genus are plant pathogenic causing leaf spots and other lesions¹⁰, or they occur as hyperparasites on other fungi¹¹.

Cladosporium species are also known to be common endophytes¹²⁻¹⁴ as well as phylloplane fungi¹⁵⁻¹⁸. Some *Cladosporium* species including *C. cladosporioides*, *C. chlorocephalum* and *C. uredinicola* were recorded as entomopathogens of Aphids and whiteflies^{19,20}. The Genus *Cladosporium* is considered a rich source of diverse and bioactive natural compounds²¹. Some species were reported to produce anticancer compounds such as L-asparaginase²², paclitaxel²³, as well as enzymes like cellulases²⁴, and pectinases²⁵⁻²⁷.

Pectinases are enzymes that target pectin and depolymerize it by hydrolysis, transelimination, and deesterification processes, which hydrolyze the ester connection between pectin's carboxyl and methyl groups. These enzymes degrade pectin, a complex polysaccharide present in the cell walls of higher plants that serves as a cementing substance for the cellulose network. Pectinases account for 10% of all industrial enzymes manufactured globally, and their market is growing by the day²⁸⁻³⁰. As a result, the current study attempted to identify some unique *Cladosporium* species isolated from air and fruits in

some Egyptian governorates, as well as to produce and partially purify pectinases in submerged fermentation.

Results

Molecular studies

The ITS of *Cladosporium* strain AUMC 10865 was 99.64% (554/556) similar to *C. cladosporioides* (KJ767065), *C. parahalotolerans* (MK262909) and *C. halotolerans* (MK258720). ACT sequence was 96.21% (127/132) similar to *C. halotolerans* (MF084398 and MF084399) and *C. omanense* (MH716046). LSU sequence was 98.96% (1140/1152) similar to *C. delicatulum* (JQ732984 and JQ732983). ITS of *Cladosporium* strain AUMC 11340 was 99.27% (549/553) similar to *C. floccosum* (MK460809). ACT sequence was 99.13% (228/230) to *C. herbarum* (EF679510). Comparing the ACT sequence to the type strains, it was 98.26% (226/230) similar to *C. macrocarpum* (EF679529), 97.83% (225/230) to *C. herbarum* (EF679516), and 95.15% (216/227) to *C. versiforme* (KT600613). LSU was 98.74% (1184/1199) similar to *C. herbarum* (MH047193), and *C. allicinum* (GU214408).

ITS sequence of *Cladosporium* strain AUMC 11366 was 98.9% (548/554) similar to *C. cladosporioides* (MT367253, MF077224 and MF319920). By comparing to ITS sequences of the type species, it was 98.55% (542/550) similar to *C. verrucocladosporioides* (MH863939), 98.53% (536/544) to *C. vicinum* (MF473311) and 98.88% (530/536) to *C. kenpeggii* (KY646222). ACT of *Cladosporium* strain AUMC 11366 was 100% similar to *C. cladosporioides* (KY886457, MZ734607 and HM148501). By comparing with ACT sequences of the type materials, it was 97.38% similar to *C. proteacearum* (MZ344213), 98.15% to *C. devikae* (MZ344212), and 95.65% similar to *C. cladosporioides* (HM148490). LSU was 98.77% (1206/1221) similar to *C. delicatulum* (JQ732985) and *C. uredinicola* (EU019264).

This analysis included 55 sequences. The full parsimony dataset consisted of 808 characters with 437 constant characters (no gaps, no N), 114 variable characters which were parsimony-uninformative (26.1% of constant characters), and 33 characters were counted as parsimony informative (7.6% of constant). Tamura 3-parameter (T92 + G) was the best nucleotide substitution model. The most parsimonious tree, resulted from the maximum parsimony analysis with the highest log likelihood (-5359.90), a tree length of 802 steps, consistency index of 0.520478, retention index of 0.807270, and a composite index of 0.524071 for all sites and parsimony-informative sites, is shown in Fig. 1. Nexus file of the sequence alignments for all data sets (ITS + ACT) were uploaded to TreeBASE <http://purl.org/phylo/treebase/phyloids/study/TB2:S23783> (study no. 29214).

Taxonomy

Cladosporium parasphaerospermum sp. nov., Moharram AM, Zohri AA, Maher MA and Al-Bedak OA

MycoBank: MB839546

Etymology

Name refers to globose to subglobose conidia near to that of *C. sphaerospermum*.

Holotype: Egypt, Beni Suef, Air, Maher MA, AUMC 10865. Ex-type culture: EMCCN: 2062

Macroscopic and microscopic characteristics

Colonies on PDA reaching 18–20 mm diameter after 7 d at 28 °C, raised at the center, radially sulcate, radially furrowed under the colony, olive, olive green at the center (2F6). Margin curled, paler than the colony center (3F8). Sporulation profuse. Exudates absent. Colonies on SNA reaching 17–19 mm in diameter after 7 d at 28 °C, flat, slightly raised at the center, circular, olive, olive green (3F4-6). Margin entire. Sporulation abundant. Exudates absent. On OA colonies attaining 16–19 mm in diameter after 7 d at 28 °C, circular, flat, somewhat lanuginose, dark olive, dark olive green (1F4-6). Margin entire. Sporulation abundant. Exudates absent. Mycelium abundantly formed, branched, 3–5 µm wide, septate, pale brown to brown, smooth. Conidiophores macronematous, micronematous, abundantly formed, arising terminally or laterally, more or less straight to flexuous, cylindrical, pale brown to brown, smooth, septate, commonly (–35) 75–100 × 3–5 µm (av. 87.5 × 4) µm (n = 50), not constricted at septa. Ramoconidia integrated, terminal, intercalary, cylindrical, smooth, thick-walled, 0–1 septate, 8–18 × 3–5 µm (av. 13 × 4) µm (n = 50), with 1–3 loci per cell. Loci usually confined to small lateral shoulders, protuberant, conspicuous, short cylindrical, 1–2 µm wide, up to 1–2 µm high. Conidia brown to dark brown, smooth, thick-walled, globose, subglobose, lemon-shaped, 0–septate, 4–6 × 3–5 µm (av. 5 × 4) µm (n = 50). Chlamydospores not formed (Fig. 2).

Cladosporium chlamydosporigenum sp. nov., Moharram AM, Zohri AA, Maher MA and Al-Bedak OA

MycoBank: MB839547

Etymology

refers to the formation of chlamydospores in culture.

Holotype: Egypt, Sohag, Grapevine fruits, Maher MA, AUMC 11340. Ex-type culture: EMCCN: 2332.

Macroscopic and microscopic characteristics

Colonies on PDA reaching 15–17 mm diameter after 7 d at 28 °C, raised at the center, wrinkled, irregular, olive, olive green (3F6-7). Margin undulate, narrow, paler than the center (3E5-6). Sporulation abundant. Exudates absent. On SNA colonies attaining 9–11 mm in diameter after 7 d at 28 °C, flat, filamentous, olive, olive green (3E2). Margin filiform, narrow, (3E6-7). µm (av. 250 × 5) µm (n = 50), not constricted at septa. Ramoconidia integrated, terminal, intercalary, cylindrical, 11–22 × 6–8 µm (av. 16.5 × 7) µm (n = 50), verruculose to finely roughened, 0–2 septa with 1–3 loci per cell, Loci usually confined to small lateral shoulders, protuberant, conspicuous, short cylindrical, 1–2 µm wide, up to 1 µm high. Conidial chains unbranched or branched, conidia pale brown, straight, subglobose, obovoid to ellipsoid, verruculose to finely roughened, 0–septate, 4–11 × 5–7 µm (av. 7.5 × 6) µm (n = 50). Chlamydospores

produced in hyphae, intercalary, aggregated, brown to dark brown, thick-walled, globose, subglobose, 15–25 × µm (Fig. 3).

Cladosporium compactisporum sp. nov., Moharram AM, Zohri AA, Maher MA and Al-Bedak OA

MycoBank: MB839548

Etymology

refers to the compact conidial chains.

Holotype: Egypt, Qena, Air, Maher MA, AUMC 11366. Ex-type culture: EMCCN: 2358.

Macroscopic and microscopic characteristics. Colonies on PDA attaining 25–28 mm after 7 d at 28 °C, raised, umbonate, circular, olive to olive green (3E3-3F4). Margin entire, narrow, about 3.0 mm in width, paler than the colony center (3E1-3). Sporulation profuse. Exudates absent. Colonies on SNA attaining 17–20 mm diameter after 7 d at 28 °C, raised, umbonate, olive to olive green (3F6-7). Margin entire, about 3.0 mm in width, paler than the colony center (3E2-3). Sporulation abundant. Exudates absent. Colonies on OA attaining 19–23 mm in diameter after 7 d at 28 °C, raised, umbonate, lanuginose, olive grey (2E1-2). Margin undulate, narrow, dark olive grey (2F2). Sporulation abundant. Exudates lacking. Mycelium abundantly formed, branched, 3–5 µm wide, septate, swollen, pale brown to brown, smooth.

Conidiophores macronematous and micronematous, abundantly formed, arising terminally or laterally, more or less straight to flexuous, nodulose, geniculate at the upper part, cylindrical, pale brown to brown, smooth, septate, branched, 100–300 µm × 3.0–6.0 µm (av. 200 × 4.5) µm (n = 50). Ramoconidia integrated, terminal, intercalary, cylindrical, 7–22 × 3–4 µm (av. 14.5 × 3.5) µm (n = 50), smooth, 0–1 septa with 1–3 loci per cell. Loci usually confined to small lateral shoulders, protuberant, conspicuous, short cylindrical, 1 µm wide, up to 1–2 µm high. Conidia formed in compact and branched chains, pale brown, subglobose, obovoid to ellipsoid, smooth, 0-septate, 4–6 × 3–4 µm (av. 5 × 3.5) µm (n = 50).

Chlamydospores not formed (Fig. 4).

Yield and Activity of Pectinases at pH and temperature

The three fungi generated pectinases with a rather high output in submerged fermentation. Per liter of fermentation media, *C. parasphaerospermum* produced 3.0 g pectinase powder, *C. chlamydosporum* produced 3.65 g, and *C. compactisporum* produced 2.85 g. The pectinases appeared to be active; the greatest activity for the microbial pectinases was 1840, 893, and 930 U ml⁻¹ min⁻¹ at pH 6.0, 7.0, and 8.0, respectively. *C. parasphaerospermum*, *C. chlamydosporum*, and *C. compactisporum* had specific activity of 4221 ± 138 Ug⁻¹, 1698 ± 63 Ug⁻¹, and 2120 ± 15 Ug⁻¹ pectinase, respectively (Fig. 5). The optimum temperature was 35, 30, and 35 °C, at pH 6.0, 7.0, and 8.0 for the three microbial pectinases produced by *C. parasphaerospermum*, *C. chlamydosporum*, and *C. compactisporum*, respectively. The specific activity values increased to 5758 ± 159 Ug⁻¹, 3716 ± 3.8 Ug⁻¹, and 3482 ± 50 Ug⁻¹ pectinase, respectively (Fig. 6).

Effect of some ions and EDTA on pectinases activity

The ions potassium and manganese exhibited a high activating influence (124.4% and 172%, respectively) on the activity of *Cladosporium compactisporum* pectinase. When evaluated under the optimal circumstances, EDTA and the metal ions showed varying inhibitory effects on the activity of the pectinases generated. In the case of *C. parasphaerospermum*, *C. chlamydosporigenum* and *C. compactisporum* pectinases, the highest inhibitory effects were with Ni, Zn, and EDTA, respectively (Table 1).

Discussion

Identification of novel species is at the heart of biodiversity research, and in recent years biodiversity efforts have been favouring DNA based method to morphology-based ones^{31–35}. Understanding of the microbial composition aids awareness of host-microbe interactions and their environmental function, revealing a complex and delicate balance that can be easily upset^{36–38}. Due to the complexity of fungal genomes and the lack of verified databases documenting appropriate biodiversity, such metagenomics studies in fungi have been limited. Identification of novel species is crucial in biodiversity research, which has lately adopted DNA-based methodologies. In this work, *Cladosporium parasphaerospermum*, *C. chlamydosporigenum*, and *C. compactisporum* were identified as new *Cladosporium* species based on phenotypic features of their cultures and sequencing of the ITS, ACT, and LSU.

Morphologically, *C. parasphaerospermum* AUMC 10865 has conidiophores that are 75–100 µm shorter than *C. cladosporioides* (350 µm). It may also be recognized from *C. cladosporioides*¹, *C. halotolerans*³⁹, and *C. parahalotolerans*³⁹ by its smaller ramoconidia (8–18 µm), which measure 15–50 µm, 15–37 µm, and 24–37 µm, respectively. It may also be recognized from *C. omanense*⁴⁰ by its slower developing colonies on PDA (18–20 mm diam/7 days) compared to *C. omanense*'s 83 mm/14 days. *Cladosporium omanense* hyphae are in a polysaccharide-like substance and produce brief cross connections between hyphae, which *C. parasphaerospermum* lacks. *Cladosporium chlamydosporigenum* AUMC 11340 has smaller conidia (4–11 µm) than *C. floccosum*³⁹ and *C. versiforme*⁴¹, which have terminal (6–15 µm and 4–18 µm) or intercalary (10–21 µm and 6–21 µm) conidia, respectively. It also has smaller ramoconidia (11–22 µm) with 0–2 septa than *C. versiforme*, whereas *C. versiforme* generates 45 µm ramoconidia with up to 5 septa. *C. herbarum*¹ has several nodulose swellings in quick succession, but *C. allicinum*¹ has head-like swellings with additional intercalary swellings, which *C. chlamydosporigenum* lacks. *C. chlamydosporigenum* may also be distinguished from *C. floccosum*, *C. herbarum*, *C. allicinum*, and *C. macrocarpum* by the presence of chlamydospores.

Cladosporium compactisporum AUMC 11366 contains smaller ramoconidia (7–22 µm) with 1–3 loci compared to *C. cladosporioides* (15–50 µm) with up to 4 loci packed at the apex, and *C. vicinum*³⁹ which measures 20–60 (–70) µm with up to 7 loci packed at the apex plus 1–2 loci. *Cladosporium compactisporum* differs from *C. verrucocladosporioides*¹ and *C. delicatulum*³⁹ by having nodulose and smooth conidiophores, as well as smaller ramoconidia (7–22 µm) compared to non-nodulose conidiophores and larger ramoconidia of *C. verrucocladosporioides*. *Cladosporium uredinicola*¹

possesses branching, aggregated hyphae that create thick hyphal nets, but *C. compactisporum* does not. The three new strains, *Cladosporium parasphaerospermum*, *C. chlamydosporum*, and *C. compactisporum* were used in this study to produce pectinases with high specific activities at low temperatures (10 °C) in SmF. Because of their biodegradability, non-toxicity, high selectivity, and high yields, microbial enzymes are superior to chemical synthesis (Bruno et al. 2019). The global enzyme market was valued at \$9.9 billion in 2019 and is expected to grow at a 7.1% annual rate from 2020 to 2027 ⁴². Pectinase has lengthy been used in commercial food processing to degrade pectin and aid in various processing steps such as liquefaction, clarification, and juice extraction ⁴³. Pectinases are among the most widely used enzymes, accounting for 40% of all food enzymes ^{44,45}. Several *Cladosporium* species have been shown to produce active pectinases as well ^{9,25,26,46–48}. Due to minor changes in methodology, it is difficult to compare the values of enzyme activity between different researches. As a result, comparisons should be made with caution.

The three novel strains could create active pectinases at pH 6.0, 7.0, and 8.0, respectively, while the optimum temperatures for *C. parasphaerospermum*, *C. chlamydosporum*, and *C. compactisporum* were 35, 30, and 35 °C, respectively. The majority of commercial enzymes, including pectinase, are now mesophilic or thermophilic. In the food sector, and particularly in the fruit processing sector, there has been an increasing desire to replace high-temperature procedures with low-temperature processes. Specific economic and environmental benefits, such as energy savings, retention of biologically inert and aromatic fragrance components, contamination mitigation, and eradication of any residual enzyme activity, which is deactivation of enzyme when temperature is raised, are driving this shift in trend ^{42,43,49–51}.

Conclusion

In the current study, three novel *Cladosporium* species were introduced and described as *C. parasphaerospermum*, *C. chlamydosporum*, and *C. compactisporum*. The three novel species appeared to produce mesophilic pectinases that had high activity at pH 6.0 and 35 °C, pH 7.0 and 30 °C, and pH 8.0 and 35 °C, respectively, of which *C. parasphaerospermum* was the most active. We may now add the generation of active pectinases to the vast list of *Cladosporium* species that have been documented. We also describe three novel species that can be exploited as active microbial pectinase producers in biotechnological solutions. Although further research is needed, these unique species might be employed to degrade troublesome and resistant pertinacious wastes as well as clear fruit juices.

Materials And Methods

Isolation and Maintenance of *Cladosporium* Strains

Three *Cladosporium* isolates involved in the current study, of which two were isolated from air of Beni Suef and Qena cities and one from grapevine fruits in Sohag city, Egypt. Exposure method ⁵² was

employed for isolation of *Cladosporium* from air and direct plating technique⁵³ for isolation from grapevine fruits. Czapek's Dox agar was used as an isolation medium. The isolation medium composed of (g/L): Sucrose, 30, Na₂NO₃, 2, K₂HPO₄, 1, KCl, 0.5, MgSO₄.7H₂O, 0.5, FeSO₄, 0.01, ZnSO₄, 0.01, CuSO₄, 0.005, Rose Bengal, 0.05, chloramphenicol, 0.25, agar, 15 and the final pH 7.3. The interesting isolates obtained were preserved as frozen and lyophilized cultures in the culture collection of Assiut University Mycological Centre (AUMC) and in the Egyptian Microbial Culture Collection Network (EMCCN) as AUMC 10865 = EMCCN 2062 (air, Beni Suef), AUMC 11340 = EMCCN 2332 (grapevine fruits, Sohag) and AUMC 11366 = EMCCN 2358 (air, Qena). The novel species were registered at the MycoBank with their description with accession numbers of MB839546, MB839547 and MB839548, respectively.

Morphological studies of the *Cladosporium* strains

Spore suspension (prepared in a 30 % glycerol, 0.2 % agar and 0.05 % Tween 80 solution) of the three *Cladosporium* was inoculated in a three-point pattern using inoculum size of 1.0 µl/spot on potato dextrose agar (PDA,^{54 46 54 54 54 54 54 33 54} [54] (Smith & Onions, 1994) (54) [54] [54] [33] [33] (Smith and Onions 1994) (Smith and Onions 1994) [33], synthetic nutrient agar (SNA) and Oat meal agar (OA) as described by^{54,55}. Morphological characteristics and growth rates on SNA were examined in lacto-phenol cotton blue. Colony colors were identified according to⁵⁶.

Molecular identification of the *Cladosporium* strains

DNA extraction

For DNA isolation, a small portion from fungal growth of 7-day-old colonies of *Cladosporium* isolates AUMC 10865, AUMC 11340 and AUMC 11366 grown on PDA at 25 °C were collected and transferred individually to 2 ml-Eppendorf tube. The DNA extraction was performed following CTAB method described in Moubasher, et al.⁵⁷.

PCR and sequencing of ITS, ACT and LSU

The universal primers ITS1 and ITS4⁵⁸ were used for amplification of the internal transcribed spacer (ITS) region, LROR and LR7 primers⁵⁹ for amplification of the large subunit (LSU) and ACT512F and ACT783R for amplification of ACT gene⁶⁰. PCR was done following Al-Bedak and Moubasher⁶¹.

Alignments and phylogenetic analyses

Sequences of *Cladosporium* species (ITS, ACT, LSU) in this study were compared to sequences of the type and ex-type species in GenBank (Table 2). The ITS and ACT sequences of the strains in this study and those downloaded from GenBank were concatenated using Sequence Matrix version 1.8⁶². The produced concatenated sequences were aligned together using MAFFT (version 6.861b) with the default options⁶³, and *Cercospora beticola* CBS 116456 was used as the outgroup. Alignment gaps and parsimony uninformative characters were treated by BMGE⁶⁴. Maximum-likelihood (ML) and Maximum

parsimony (MP) phylogenetic analyses were performed using PhyML 3.0⁶⁵. The robustness of the most parsimonious trees was evaluated by 1000 replications⁶⁶. The best optimal model of nucleotide substitution for the ML analyses was determined using Smart Model Selection (SMS) version 1.8.1⁶⁷. The phylogenetic tree was drawn and visualized using MEGA X 10.2.6⁶⁸. The resulting tree was edited using Microsoft Power Point (2016) and saved as TIF file⁹.

Production of Pectinases in Submerged Fermentation (SmF)

Previously, the three *Cladosporium* species AUMC (10865, 11340 and 11366) were discovered to be capable of producing large quantities of pectinases in SmF at 10 °C reaching 8.0 U/mg, 1.78 U/mg and 7.52 U/mg, respectively²⁷. For pectinases production by the three strains, *Cladosporium* species were employed in Erlenmeyer flasks (500 ml) to produce pectinases in SmF at 10 °C using sucrose-free Czapek's (initial pH 7.0) supplemented with 1.0 % pectin (as only carbon source). *Cladosporium* species were inoculated with 1.5×10^8 spore/ml spore suspensions obtained from 7-day-old cultures. The incubation period lasted 7 days at 10°C and 150 rpm. Following the incubation time, cell-free supernatants were recovered by centrifuging at 10,000 rpm for 10 min. At 4 °C, total protein was isolated using 70 % saturation of ammonium sulphate. A freeze dryer (VirTis, model #6KBTES-55, NY, USA) was used to separate and lyophilize the precipitated total protein. Lyophilized protein was dissolved in citrate buffer (pH 5.0) and dialyzed twice for 2 hours at room temperature (cutoffs: 12-14 KD) against the same buffer, eliminating the buffer each time, before being refrigerated overnight at 4 °C to remove small molecules. The dialyzed protein was then lyophilized, weighed, and used in enzyme characterization experiments as a partly pure fungal pectinases enzymes.

Impact of pH, Temperature and Some Ions and Inhibitors on Pectinases Activities

A 0.01 g enzyme powder and 0.01 g citrus peel pectin (each dissolved in 1.0 ml of 50 mmol buffer solution) were included in this test in a water bath. The impact of pH (3.0–10.0) and temperature (30–60 °C) on pectinases activity were investigated. After the reaction time (20 min), the reaction was terminated by introducing 2.0 ml of 3,5-dinitrosalicylic acid (DNS) according to Miller⁶⁹ and Jayani, et al.⁷⁰, and the pectinase activity was determined as the amount of the enzyme that releases $1 \mu\text{mol ml}^{-1} \text{ min}^{-1}$ galacturonic acid under standard assay conditions. The buffers used were citrate buffer (pH 3.0–6.0), phosphate buffer (pH 7.0–8.0), and borate buffer (pH 9.0–10.0). Also, some ions (Na^+ , K^+ , Ca^{+2} , Co^{+2} , Ni^{+2} , Cu^{+2} , Fe^{+2} , Mg^{+2} , Mn^{+2} , and Zn^{+2}) were evaluated by introducing them at 5 mmol/ml concentrations as NaCl, KCl, CaCl_2 , CoCl_2 , NiSO_4 , CuSO_4 , FeSO_4 , MgSO_4 , MnSO_4 , and ZnSO_4 . 5 mmol/ml ethylenediaminetetraacetic acid was used to evaluate an enzyme inhibitor (EDTA). Under standard conditions, the activity of the microbial pectinases in the absence of metal ions or EDTA was evaluated to define 100 % activity. All experiments were conducted in three repetitions.

Accessibility of data: Pure cultures of the type materials of the novel species are deposited in the culture collection of Assiut University Mycological Centre as AUMC 10865, AUMC 11340 and AUMC 11366, and

in the Egyptian Microbial Culture Collection Network as EMCCN 2062, EMCCN 2332 and EMCCN 2358. Nexus file of the sequence alignments for all data sets were uploaded to TreeBASE <http://purl.org/phylo/treebase/phyloids/study/TB2:S23783> (study no. 29214).

Declarations

Author Contributions: A.M.M.: Supervision of the study, writing, revising, A-N.A.Z.: Supervision of the study, writing, revising, M.A.M.: Fungal isolation, enzymatic production, A.E.H.: Editing, revising, O.AA.-B.: Molecular work, data analysis, writing, revising. All authors contributed to data analysis, drafting, or revising the manuscript. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement: “The datasets generated and/or analysed during the current study are available in the GenBank repository, [<https://www.ncbi.nlm.nih.gov/genbank/>]”.

Conflicts of Interest: The authors declare that there are no potential conflict of interest regarding the publication of this paper.

Data Availability: All datasets generated or analyzed during this study are included in the manuscript.

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Tables

Table 1. Effect of metal ions and EDTA (5 mmol mL⁻¹) on pectinase activity produced by *C. parasphaerospermum*, *C. chlamydosporum*, and *C. compactisporum* (mean $\pm$ SD, n = 3).

The results are expressed as the activity in the tested inhibitory conditions compared to the pectinase activity in the control without inhibitors (in bold).

Metal ions and inhibitors	<i>C. parasphaerospermum</i>	<i>C. chlamydosporum</i>	<i>C. compactisporum</i>
	Specific activity U g ⁻¹	Specific activity U g ⁻¹	Specific activity U g ⁻¹
Control	5758±159	3716±3.8	3482±50
Na ⁺	4027±22	3600±40	2025±18
K ⁺	3272±24	3086±26	4287±85
Fe ⁺²	3068±71	759±55	1865±15
Cu ⁺²	3406±49	2851±38	1336±14
Ca ⁺²	3875±52	2734±15	3379±67
Mg ⁺²	2231±45	1682±15	3215±33
Zn ⁺²	4185±30	913±15	2182±26
Ni ⁺²	2106±19	2954±31	3432±30
Co ⁺²	3473±106	1553±31	2390±36
Mn ⁺²	2955±26	1553±29	5992±51
EDTA	2383±15	2165±100	1253±22

Table 2. List of specimens and GenBank accession numbers of sequences used in this study. Bold accession numbers were generated from this study.

<i>Cladosporium</i> species	Strain	Locality	ITS	ACT	LSU
<i>C. parasphaerospermum</i>	AUMC 10865	Egypt	MN826828	OL514008	MW205008
<i>C. chlamydosporigenum</i>	AUMC 11340	Egypt	MN826919	OL514009	MW205067
<i>C. compactisporum</i>	AUMC 11366	Egypt	MN826822	OL514010	MW205086
<i>C. aerium</i>	DTO:323-B4	China	MF472897	MF473747	-
	DTO:323-G7	China	MF472899	MF473749	-
	DTO:323-G6	China	MF472898	MF473748	-
<i>C. allicinum</i>	CBS 121624	Belgium	MH863126	EF679502	MH874678
	DTO 111-A5	Denmark	KP701924	KP702047	-
	DTO 249-G3	Netherlands	KP701975	KP702097	-
<i>C. antarcticum</i>	CBS 690.92	Antarctica	NR_121332	EF679484	-
<i>C. cladosporioides</i>	CBS 112388	Germany	NR_119839	HM148490	-
	CPC 15167	Slovenia	HM148052	HM148539	-
	GZYQ-08-01	China	MK852271	MK852272	-
<i>C. colombiae</i>	CBS 274.80B	Colombia	MH861262	FJ936166	-
<i>C. cycadicola</i>	CBS 137970	Australia	NR_156279	KJ869227	NG_058881
<i>C. delicatulum</i>	DTO 145-C4	Germany	KP701940	KP702062	-
	CPC 14372	Denmark	HM148089	HM148578	-
	CPC 14363	Denmark	HM148088	HM148577	-
<i>C. domesticum</i>	CPC 22307	USA	NR_156348	MF473805	-
	DTO 308-B1	USA	MF472966	MF473816	-
	CPC:22413	USA	MF472961	MF473811	-
<i>C. exasperatum</i>	CBS 125986	Australia	MH863865	HM148579	MH875326
<i>C. floccosum</i>	DTO 323-H6	China	MF472979	MF473829	-
	CPC 22968	USA	MF472978	MF473828	-
	CPC 22399	USA	MF472977	MF473827	-
<i>C. halotolerans</i>	CBS 119416	USA	NR_119605	EF101397	-

<i>Cladosporium</i> species	Strain	Locality	ITS	ACT	LSU
	DTO 257-F4	Netherlands	KP701989	KP702111	-
<i>C. herbaroides</i>	CBS 121626	Israel	NR_119655	EF679509	-
<i>C. herbarum</i>	CBS 289.49	Switzerland	MH856530	EF679558	MH868058
	CPC 12183	Netherlands	EF679368	EF679521	-
<i>C. iridis</i>	CBS 107.20	Netherlands	MH854682	EF679522	MH866199
	CBS 138.40	Netherlands	NR_111271	EF679523	-
<i>C. kenpeggii</i>	CPC 19248	Australia	KY646222	KY646225	-
<i>C. macrocarpum</i>	CPC 12759	USA	EF679380	EF679534	-
	CPC 14305	Denmark	MF473141	MF473990	-
<i>C. ossifragi</i>	CBS 842.91	Norway	MH862342	EF679535	-
<i>C. parahalotolerans</i>	DTO 307-H4	Mexico	MF473161	MF474009	-
	DTO 324-B7	China	MF473169	MF474017	-
<i>C. perangustum</i>	CBS 167.54	Netherlands	HM148124	HM148613	-
	CBS 126365	USA	MH863940	HM148612	MH875401
<i>C. sphaerospermum</i>	CBS 193.54	Slovenia	NR_111222	EU570269	-
	DTO 255-H7	Netherlands	KP701988	KP702110	-
<i>C. spinulosum</i>	CBS 119907	Slovenia	NR_119660	EF679542	-
<i>C. subcinereum</i>	UTHSC DI-13-257	USA	NR_148193	LN834617	-
<i>C. subinflatum</i>	CBS 121630	Slovenia	MH863129	EF679543	MH874681
<i>C. tenuissimum</i>	CBS 125995	USA	MH864840	HM148687	MH876286
<i>C. tuberosum</i>	UTHSC DI-13-217	Spain	LN834417	LN834601	MH878168
<i>C. uredinicola</i>	CPC 5390	USA	AY251071	HM148712	EU019264
<i>C. variabile</i>	CBS 121635	USA	MH863131	EF679557	MH874683
<i>C. verrucocladosporioides</i>	CBS 126363	South Korea	MH863939	HM148717	-
<i>C. versiforme</i>	CBS 140491	Netherlands	NR_152297	KT600613	-

<i>Cladosporium</i> species	Strain	Locality	ITS	ACT	LSU
<i>C. vicinum</i>	CPC 22316	USA	MF473311	MF474161	-
	CPC 15457	New Zealand	HM148060	HM148547	-
<i>C. wyomingense</i>	CPC 22310	USA	MF473315	MF474165	-
<i>Cercospora beticola</i>	CBS 116456	USA	NR_121315	AY840458	NG_068999

Abbreviations used: AUMC = Assiut University Mycological Centre, Assiut University, Assiut, Egypt, CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, CPC = Culture Collection of Pedro Crous, housed at CBS, Utrecht, The Netherlands, DTO = Culture Collection of Jos Houbraken, UTHSC = The University of Tennessee Health Science Center, Monroe Avenue, Memphis, Tennessee, USA.

Figures

Figure 1

Maximum likelihood tree based on concatenated ITS and ACT sequences of *Cladosporium parasphaerospermum*, *C. chlamydosporigenum* and *C. compactisporum* in this study (in bold blue color) with the most similar sequences belonging to *Cladosporium* in GenBank. Bootstrap support values (1000 replications) for ML/MP $\geq 50\%$ are indicated near the respective nodes. The tree was rooted to *Cercospora beticola* CBS 116456 as outgroup (in bold red color).

Figure 2

Cladosporium parasphaerospermum (AUMC 10865). A-C. 7-day-old colonies on PDA, SNA and OA at 25 °C. D-F. Macronematous conidiophores and conidial chains. G. Globose to subglobose conidia. Scale bar = 20 μm (D), 10 μm (E-G).

Figure 3

Cladosporium chlamydosporigenum (AUMC 11340). A-C.7-day-old colonies on PDA, SNA and OA at 25 °C. D. Mycelium with abundant chlamydospores. E-H. Macronematous conidiophores and conidial chains. I. Aggregated chlamydospores. Scale bar = 20 µm.

Figure 4

Cladosporium compactisporum (AUMC 11366). A-C. 7-day-old colonies on PDA, SNA and OA at 25 °C. D. Geniculate conidiophore. E. Swollen conidiophore. F. Compact chains of conidia. Scale bar = 20 µm.

Figure 5

Effect of pH on the activity of pectinases produced by *Cladosporium* strains.

Figure 6

Effect of temperature on the activity of pectinases produced by *Cladosporium* strains