ISSN: 0976-3031

Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 7, Issue, 9, pp. 13481-13484, September, 2016

International Journal of Recent Scientific Research

Research Article

PREVALENCE AND EXTRACELLULAR ENZYME PRODUCTION OF ACREMONIUM SPECIES ISOLATED FROM FUNGAL KERATITIS CASES

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ARTICLE INFO

Article History:
Received 17th June, 2016
Received in revised form 21st July, 2016
Accepted 05th August, 2016
Published online 28th September, 2016

Key Words:
Acremonium spp., Prevalence, DNase, Lipase, Protease, Phospholipase,

ABSTRACT

Objective: To determine the prevalence and to screen the different types of hydrolytic enzymes produced by *Acremonium species* isolated from corneal scrapings of fungal keratitis cases. **Methods:** The isolation and enzymatic production of *Acremonium* species were tested on solid media enriched with the suitable substances. **Results:** Out of the 1,491 corneal scrapings, seven isolates were confirmed as genus *Acremonium* and it was noted that four isolates of *Acremonium* spp. showed positive for all the extracellular enzymes screened. **Conclusion:** The incidence rate of *Acremonium* species is increasing and species level identification would facilitate in the appropriate therapy and management.

INTRODUCTION

Fungal keratitis is a strange but potentially sight-threatening ocular infection. The greater part of fungal keratitis cases happens after a corneal injury, typically as a result of contact with a fungus contaminated plant material or any other predisposing factors. Species of *Fusarium*, *Aspergillus* and *Curvularia* are the prime causes of filamentous fungal keratitis, but many other species of *Acremonium*, *Scedosporium* have also been implicated. In recent years the number and the diversity of the infections caused by *Acremonium* species have increased (Fincher *et al*, 1991), and various species have been involved (Hoog and Guarro, 1995).

Acremonium is a filamentous fungus that comprises approximately 150 species, most of them being saprobes in soil and pathogens of plants, insects, and other fungi. Some species are considered as opportunistic environmental pathogens that lead to a superficial infection (Hoog *et al*, 2000; Fincher *et al*, 1991; Summerbell, 2003). The typical morphological features of *Acremonium* include slow-growing colonies, hyaline, septate mycelial elements with branched or intertwined hyphae. The species of *Acremonium* are morphologically alike to each other and at best can only be distinguished on the basis of slight differences, making their identification difficult. Thus, in most of the clinical cases, the etiological agent is reported only as an *Acremonium* spp. Several fungal virulence factors are associated with the production/secretion of extracellular enzymes including proteases, collagenases, phospholipases, esterases etc., Presence of these enzymes in corneal ulcers cases indicate that a correlation exists between the production of enzymes and inflammation of the cornea. But still, the exact cause and nature of the enzymatic harm are not yet studied. The aim of the present study was to determine the incidence and initial screening of extracellular enzymes, such as protease, DNase, lipase and phospholipase in the clinical isolates of *Acremonium* species from fungal keratitis.

MATERIALS AND METHODS

Collection and processing of samples

Corneal scrapings were carried out from fungal keratitis patients from a tertiary eye care hospital in Coimbatore, Tamil

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Nadu, India by the ophthalmologist using standard procedures. The material obtained by corneal scraping was processed based on the standard methods and the positive fungal growth were identified based on their morphological (microscopic and macroscopic) characteristics and were confirmed by standard mycological procedures.

Screening of Aspergillus species for the production of extracellular enzymes

Screening for Lipase production

At the center of the tributyrin agar plates, 50µl of the conidial suspension was placed and were incubated at 37°C for 7 days. A clear zone of lysis beneath and around the colony indicated a positive lipase activity (Griebeler *et al,* 2011).

Screening for DNase production

The DNase agar grew test isolates were flooded with 1N hydrochloric acid and were observed for lysis of DNA. The development of a clear zone around the colony confirmed the production of DNase by the test isolates (Sanchez *et al*, 2010).

Screening for Protease production

The assay for protease production amongst the *Aspergillus* isolates was done using Skim Milk Agar (SMA) and the zone of lysis by protease was studied as described for other extracellular enzymes of the study (Sharma *et al*, 2006).

Screening for Phospholipase production

Extracellular phospholipase production of the *Aspergillus* isolates was tested on egg yolk agar plates as described by Price *et al*. (1982).The phospholipase enzyme production was noted by the development of dense white precipitation zone around the colony. The degree of enzymatic activity was observed by measuring the diameter of the precipitation zone (mm) around the colony.

Determination of various extracellular enzyme activities indices of the fungal isolates

The enzyme activity index (EAI) was calculated by measuring the diameter of growth and the diameter of the zone (Blanco *et al*, 2002).

$$Enzyme\ activity\ index = \frac{Diameter\ of\ Zone}{Diameter\ of\ growt}$$

The enzyme screenings were carried out in triplicate and mean EAI values were calculated. Statistical tools like percentage analysis for finding the percentage of the fungal isolates having the specific enzyme activity were employed for the interpretation of the results.

RESULT AND DISCUSSION

Of the total 1,491 corneal scrapings, seven isolates were determined to belong to the genus *Acremonium* based on morphological (microscopic and macroscopic) characteristics. The characteristics included the production of flat to very thin cottony, whitish or yellowish colonies of moderate to slow growth with thin hyphal structures. Out of this 7 isolate, 4 was positive for KOH wet mount. All the 4 KOH positives correlated with culture results and rest three was positive for cultures alone. The other genus includes *Fusarium*, *Aspergillus*, *Scedosporium*, *Cylindrocarpon*, *Trichoderma*, *Rhizopus*, *Penicillium*, *Curvularia*, *Bipolaris*, *Alternaria*, *Exserohilum*, *Cladosporium*, *Aureobasidium*, unidentified hyaline and dematiaceous fungi were also isolated.

Acremonium spp. are becoming increasingly recognized as opportunistic fungal pathogens in causing keratitis. Fungal keratitis is an important ophthalmic problem in all parts of the world, as it leads to corneal blindness and sometimes loss of the vision. Its incidence is reported to vary from 7%- 40% in various parts of India (Bharathi *et al*, 2006; Shokohi *et al*, 2006). In the present study the prevalence rate of *Acremonium* spp. was 1.6% (table 1) which is more or less similar to the reports of Anusuya devi *et al*. (2013) 5.26%, 1.85% (Payal *et al*, 2013), 0.9% (Nhung *et al*, 2012), 4.7% (Rautaraya *et al*, 2011) and 1.5% (Ibrahim *et al*, 2009). Fincher *et al*. (1991) reported 15 cases of keratitis due to *Acremonium*; however, several more have been reported. Based on the previous reports the prevalence rate of *Acremonium* spp. was considerably less when compared to other genus but the rate of incidence is increasing worldwide.

Acremonium spp. related keratitis follows traumatic events with the inoculation of contaminated material and surgical abrogation are also reported to be associated with fungal keratitis which promotes this kind of ocular opportunistic infection. The patients with culture positives belonged to the adult age group (> 25 years and < 60 years) with an overall predominance of males over the females. This is probably because ocular trauma was the main risk factor and ocular trauma was found to be significantly more common in men than in women. In this study, among the seven cases reported with acremonium keratitis, 5 were exposed to the traumatic agents like vegetative matters, foreign body etc. with initial symptoms of redness and pain. Increased use of antifungals and immunosuppressive drugs all disturb host immunity and predispose to opportunistic fungal infections. Cases of acremonium endophthalmitis have also been reported, although rarely (Rao and Aquavella, 1979; Green *et al*, 1965; Cunha *et al*, 1970). The ability of *Acremonium* species to colonize soft contact lenses has been clearly confirmed (Yamamoto *et al*, 1979; Simmons *et al*, 1986; Liotet and Warnet, 1981), and this ability can predispose to ocular infection. For this reason, disinfection of lenses after exposure to potentially high concentrations of environmental fungi is prudent (Fincher *et al*, 1991).

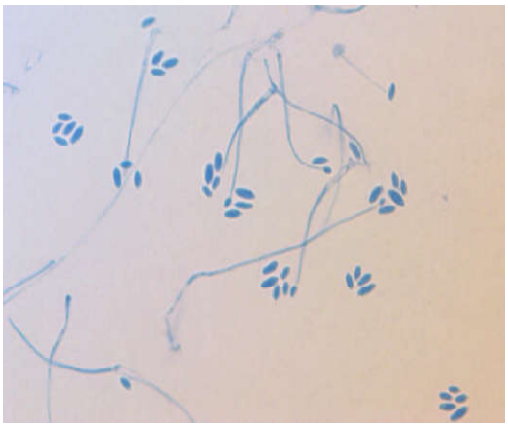

Figure 1 Morphological characteristic of *Acremonium* species

keratinase, lipase etc. are identified to play a vital role in several infections caused by fungi. In this part of the study, four enzymes like protease, phospholipase, lipase and DNase were screened in order to evaluate the overall spectrum of extracellular enzyme activity among the *Acremonium* spp. isolated from corneal scrapings (table 2). Four isolates showed positive enzyme activity for all the four extracellular enzymes screened.

These enzymes play a role in nutrition, tissue damage, fungal dissemination within the human body, iron acquisition and overcoming the host immune system which strongly affects fungal pathogenicity (Ibrahim *et al*, 1995).

Table 1 Prevalence of *Acremonium* spp. from the corneal scrapings of fungal keratitis cases

Isolates of *Acremonium* spp.	Age	Sex	Traumatic agent	Duration of symptoms	Initial symptoms	Prior therapy	Nature of drug	Surgical intervention
AC1	34	M	Iron nail	45D	Redness	Yes	Natamycin, Voriconazole, Itraconazole	TKP
AC2	41	M	Cow's tail	4D	Pain, redness, DOV	-	Natamycin, Itraconazole, Econazole, Voriconazole	-
AC3	59	F	Vigorous eye rubbing	8D	Pain, redness	Yes (AF)	Natamycin, Voriconazole, Itraconazole	TKP
AC4	25	M	Stick	5D	Irritation, pain, DOV	-	Natamycin, Econazole, Itraconazole	-
AC5	60	M	Sand	1W	Redness, pain, watering	-	Natamycin, Voriconazole, Itraconaozle, Ketoconazole	TKP
AC6	48	F	Foreign body	2D	Pain, redness	Yes (AB, AF)	Natamycin, Voriconazole, Itraconazole	-
AC7	34	M	-	3D	-	-	Natamycin, Voriconazole, Itraconazole	-

Note: AC-*Acremonium*; M-Male; F-Female; D-Days; W-Week; DOV-Defective vision: AB-Antibacterial: AF-Antifungal: TKP-Therapeutic Keratoplasty

Table 2 Various extracellular enzymatic activity of *Acremonium* spp.

Isolates of *Acremonium* spp.	Enzyme Activity Index (EAI)			
	DNase	Lipase	Protease	Phospholipase
AC1	**1.13**	**1.1**	**1.21**	**1.16**
AC2	**1.14**	**1.14**	**1.14**	**1.13**
AC3	**1.12**	**1.1**	-	**1.11**
AC4	**1.13**	**1.1**	**1.16**	**1.13**
AC5	**1.09**	**1.15**	**1.12**	**1.16**
AC6	-	**1.12**	**1.14**	**1.17**
AC7	**1.14**	**1.1**	-	**1.11**

Therapy was administered including natamycin, itraconazole, voriconazole, ketoconazole and econazole. After the topical treatment, four cases showed no evidence of infection but 3 cases were managed by means of the therapeutic keratoplasty. Topical therapy with keratoplasty or therapy with parenteral amphotericin B seems to be an adequate treatment (Fincher *et al*, 1991). Simonsz (1983) reported that therapy adopted with fluconazole, amphotericin B, griseofulvin, and itraconazole was unsatisfactory and keratoplasty combined with pharmacological therapy was reported to be the only curative treatment (Rodriguez *et al*, 2000). Creti *et al*, 2006 strongly recommend that, in the event of a compatible clinical picture, laboratory testing for mycetes even in the absence of traumatic events, and the use of the new generation azoles, in order avoid a keratoplasty intervention and disease progression to severe endophthalmitis. Virulence factors are products or attributes of a fungus that increases its capacity to attack the host. Many virulence factors are obvious and important, such as growth ability at 37ºC, physiological pH, extracellular enzymes which is essential for the pathogenicity of a fungus (Hogan *et al*, 1996). Certain extracellular enzymes such as protease,

CONCLUSION

In summary, several species of *Acremonium* are emerging as causative agents of fungal keratitis in humans. These fungi share morphological similarities with *Fusarium* spp. which can be confused in identification. So, molecular methods can be employed in the precise identification and which helps in the management of *Acremonium* fungal keratitis.

Acknowledgement

The support of Prof. Dr. M. Karunanithi, Chairman and Secretary, Vivekanandha Educational Institutions, Dr. B. T. Sureshkumar, Principal, Dr. A. Malarvizhi, Head, Department of Microbiology, Vivekanandha College of Arts and Sciences for Women, Namakkal, and Dr. C. S. Shobana, Associate Professor, PG & Research Department of Microbiology, PSG College of Arts & Science, Coimbatore are gratefully acknowledged.

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How to cite this article:

Punitha, T *et al*, 2016., Prevalence and Extracellular Enzyme Production of Acremonium Species Isolated from fungal Keratitis Cases. *Int J Recent Sci Res.* 7(9), pp. 13481-13484.