Morphological characterization of soil *Penicillium* sp. strains - potential producers of statins

E. Seydametova, R. Bt. Hj. Kambol, N. Bt. Zainol

Universiti Malaysia Pahang, Kuantan 26300, Malaysia
Corresponding author: e_seydametova@yahoo.com

ABSTRACT

At present time, search for fungal species of *Penicillium* genus – potential producers of biotechnologically valuable metabolites, has gained considerable momentum. The aim of present study was to examine the fungal isolates from soils of Pahang state, proposed to belong to *Penicillium* genus. Twenty fungal cultures were examined for characters of morphology. The variables used in this study were macromorphological characters: colony diameter, obverse and reverse colony colour, and the presence or absence of exudates. Moreover, microscopic characteristics of the fungal isolates were examined using an optical microscope. The colony appearance of each fungal isolate was characterized on different agar media: CZ, CYA, YES, MEA. The macro- and micromorphological characters of isolated cultures appeared to be distinctive. Based on colony macromorphology, as well as the structure of conidiophores, it was revealed that fungal isolates belong to *Penicillium* genus. Results of this research have demonstrated that some soils in Pahang state can be considered as a valuable natural source of filamentous fungi from *Penicillium* genus.

Key words: filamentous fungi, isolation, identification

INTRODUCTION

The 20th century witnessed the discovery, isolation and chemical characterization of a vast diversity of natural products from different species of filamentous fungi. However, members of the *Penicillium* genus have been of special interest to research community because of their biotechnological potential. Among the most prominent fungal metabolites useful for pharmaceutical purposes, the β-lactams such as penicillin from *Penicillium* species, have been subject to a wealth of scientific publications and reviews. The advent of penicillin not only led to the development of a new field of antibiotic research but also created an entirely new industry (10).

Although the early emphasis on secondary metabolite discovery was mainly devoted to antibiotics, later in the 1970s and 1980s it was realized that filamentous fungi from *Penicillium* genus are able to produce a number of compounds that possess other biological activities. At that time, researchers entered into a new era in which fungal metabolites were applied as cholesterol-lowering drugs, the statins (5).

The cholesterol biosynthetic pathway, starting from acetyl-CoA, involves more than 25 enzymes, but the rate-limiting step is the conversion of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) to mevalonate by HMG-CoA reductase. Statins can inhibit HMG-CoA reductase activity. The mechanism involved in the control of endogenous cholesterol levels makes these molecules suitable for therapeutic use. In recent years, these compounds have been reported as potential therapeutic agents for the treatment of many dyslipidaemias in the patients with various diseases (1, 2, 4, 6, 7).

Natural statins are secondary metabolites that can be produced by various filamentous fungi including some species of *Penicillium*. Currently, large-scale processes have been developed only for a few of the cholesterol-lowering drugs described in the literature (3).

For other molecules research is still ongoing and therefore greatly susceptible to future development.

It must be noted that industrial biotechnology field has utilized only a very minor portion of nature's microbial arsenal for the discovery of statins. Cholesterol-lowering activity of secondary metabolites from *Penicillium* species has spurred efforts towards the isolation of these fungi from different natural sources. Soil as a reservoir for a wide variety of filamentous fungi has been recognized for a long time. The majority of soil fungi are represented by rapidly growing and nutritionally nonexacting fungi of *Penicillium* genus. The aim of this study was to examine the soil fungal isolates from Pahang state, proposed to belong to *Penicillium* genus.

MATERIALS AND METHODS

Soil sampling

Samples of soil were collected from eleven different sites within three areas in Pahang state of Malaysia: Kuantan (3 sites), Balok (4 sites), and Cherating (4 sites). Soil samples were taken at a depth of approximately 10-30 cm in sterilized polyethylene bags using sterilized spatula and stored at 4 °C until examination.

Fungi isolation

The fungi were originally isolated by plating collected soil samples on PDA. Individual colonies of filamentous fungi were picked up and purified by streaking on agar medium. The colonies formed on PDA were transferred onto fresh agar medium. The fungal isolates were kept on agar medium at 4 °C and recultured every 4 weeks.

Morphological identification of fungal isolates

The fungal isolates were identified on the basis of macroscopic analysis in different identification media: CZ, CYA, YES, MEA. Fungal cultures were all incubated following the recommendations of Pitt (1979). The macroscopic variables analyzed included colony diameter, obverse and reverse colony colour, and the presence or absence of exudates. Microscopic characteristics of the fungal isolates were examined using an optical microscope (Primo Star Carl Zeiss Microimaging GmbH, Germany). These techniques allowed the fungal cultures to be identified at the genus level.

RESULTS AND DISCUSSIONS

According to the aim of the present study, soil samples collected from various localities of Pahang state were examined to isolate filamentous fungi of *Penicillium* genus. In contrast to bacteria and yeasts, the identification of most groups of filamentous fungi including *Penicillium* species continues to be based on their morphology. The gross appearance of colonies developed on agar media recommended for this particular fungal genus (CZ, CYA, YES, MEA) is considerable importance in identification. In addition, within the main sections of *Penicillium* genus the crucial taxonomic character is the structure of bodies on which the spores form. These two characteristics are valuable at the generic level (8).

In current research work a total of 20 fungal cultures were isolated. The fungal isolates were purified and identified at the genus level by standard procedures.

Morphological features showed variability between examined fungal isolates (Table 1). The obtained data indicate that colony diameters of fungal isolates vary in significant ranges on different agar media: 4-20 mm (CZ), 19-44 mm (CYA), 25-50 mm (YES), and 14-60 mm (MEA). In addition, examined fungal cultures have different obverse and reverse colony colours. In general, almost all fungal isolates have white to cream obverse and pale reverse colony colour on CZ. Colonies on CYA white to cream, yellow, glaucous, and

Table 1. Some morphological characteristics of examined fungal isolates on different agar media

Fungal isolate	CZ			CYA			YES			MEA		
	CD, mm	COC	CRC	CD, mm	COC	CRC	CD, mm	COC	CRC	CD, mm	COC	CRC
FI 1	5	wh/cr	p	21	d/gr	p	26	wh/cr	p	19	d/gr	y
FI 2	7	wh/cr	p	20	d/gr	p	25	gl	y	14	d/gr	y
FI 3	8	wh/cr	p	41	d/gr, y	p	35	wh/cr	p	42	wh/cr, y, d/gr	y, l/br
FI 4	12	wh/cr	p	44	gl	p	50	wh/cr, gr	y	60	wh/cr, y	y, or
FI 5	14	wh/cr	p	40	gl, y	p	43	wh/cr	p	38	wh/cr, y	or, l/br
FI 6	15	wh/cr	p	40	gr, y	l/br	35	wh/cr	p	46	wh/cr, y, gr	y
FI 7	10	wh/cr	p	40	wh/cr, y	p	40	wh/cr	p	56	wh/cr, y	y, or
FI 8	10	wh/cr	p	42	d/gr, y	p	41	wh/cr	p	50	y, gr	y
FI 9	15	wh/cr	p	40	wh/cr, y	p	49	wh/cr, gl	p	50	wh/cr, y, gr	y
FI 10	11	wh/cr	p	42	d/gr, y	p	42	wh/cr, gl	p	50	d/gr	y, or
FI 11	12	wh/cr	p	42	wh/cr, y, gl	p	40	wh/cr, gl	l/br	55	wh/cr, y	y, or
FI 12	12	wh/cr	p	43	wh/cr, y, d/gr	y	48	wh/cr	y	40	y, d/gr	y, l/br

Table 1. (continued)

Fungal isolate	CZ			CYA			YES			MEA		
	CD, mm	COC	CRC	CD, mm	COC	CRC	CD, mm	COC	CRC	CD, mm	COC	CRC
FI 13	20	wh/cr, y	y	37	d/gr, y	d/br	37	wh/cr, gl	or	50	wh/cr, y	or, l/br
FI 14	11	wh/cr	p	38	wh/cr, y, d/gr	p	46	wh/cr	y, l/br	46	wh/cr, y	y, or
FI 15	10	wh/cr	p	42	wh/cr, y	p	45	wh/cr	p	50	wh/cr, y, gl	y
FI 16	7	wh/cr	p	38	wh/cr, y	p	40	wh/cr	p	58	wh/cr, y	y
FI 17	12	wh/cr	p	32	wh/cr, y	or	37	wh/cr	p	50	wh/cr, y, gl	y, br
FI 18	11	wh/cr	p	42	wh/cr, y	or	48	wh/cr, y	p	50	wh/cr, y, gl	y
FI 19	4	wh/cr	p	19	gl	p	34	d/gr, gl	or	20	d/gr	y
FI 20	10	wh/cr	p	44	wh/cr, y, d/gr	or	38	wh/cr, y	y	35	wh/cr, y, d/gr	y, br

[a]Abbreviations: CD = colony diameter, COC = colony obverse colour, CRC = colony reverse colour, wh/cr = white to cream, y = yellow, gl = glaucous, gr = green, d/gr = dark green, p = pale, or = orange, l/br = light brown, br = brown, d/br = dark brown

dark green colour on obverse whereas in reverse - pale, yellow, orange, light brown, and dark brown. On YES medium both obverse and reverse colony colours of examined cultures vary from white and cream to dark green and from pale to light brown, respectively. For the most part of examined fungal isolates on MEA medium obverse colony colours are white to cream, yellow, and dark green; colony reverse is yellow, orange or light brown. The obtained results were in accordance with what had been observed by Pitt (1979). Pitt (1979) described the colonies of *Penicillium* species as rapid growing, flat, and filamentous. The colonies are initially white and become dark green, blue green, gray green, olive gray, yellow or pinkish in time. Moreover, various pigments are more or less typical for the *Penicillium* species. The colony reverse is usually pale to yellowish or brownish.

Further observation of macromorphological variables of studied fungal isolates showed that almost all examined fungal cultures do not produce exudates on CZ and YES. Presence of uncoloured, yellow, brown, and dark brown exudates was observed only on CYA and MEA. According to literature data, some *Penicillium* species indeed are able to produce distinct exudates droplets, for instance, yellow in *P. chrysogenum* and dark brown in P. *venetum* (9).

In addition to colony macromorphology, micromorphological characteristics such as conidiophores branching and its elements are very significant in identification of *Penicillium* fungi. Figure 1 shows the light micrographs of some fungal cultures examined in current study.

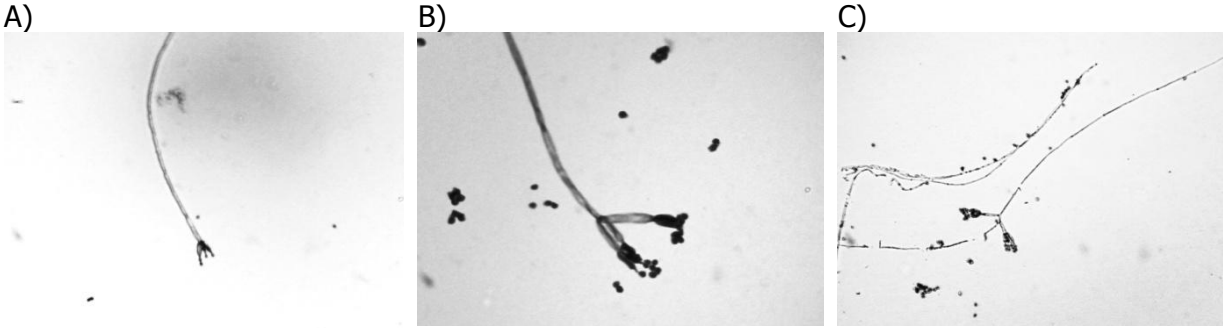

Fig. 1. Light micrographs of some fungal isolates: (A) *Penicillium* sp. FI14; (B) *Penicillium* sp. FI7; (C) *Penicillium* sp. FI5. Image is magnified 400×

From Figure 1 it can be seen that among the examined fungal isolates there are cultures both with simple (Fig.1(A)) and branched (Fig.1(B), (C)) conidiophores with metulae (Fig.1(B), (C)), phialides, and conidia. As Pitt (1979) mentioned, for *Penicillium* species simple or branched conidiophores, metulae, phialides, and conidia are observed. The appearance of the spore head is like that of a brush; and the spore head is called a penicillus, which is Latin for a brush. Thus, based on microscopic investigations it was proved that examined fungal isolates are characterized by conidiophores and microscopic elements typical for *Penicillium* genus (8).

CONCLUSION

Twenty fungal cultures were isolated from soil samples. Based on colony morphology and microscopic analysis, it was revealed that fungal isolates belong to *Penicillium* genus. Thus,

this study demonstrates that some soils in Pahang state can be considered as a valuable natural source of filamentous fungi from *Penicillium* genus. Isolated fungal cultures can be recommended for further studies in terms of determination of their ability for statins production.

ACKNOWLEDGEMENTS

This research was financially supported by the Research and Management Center of the Universiti Malaysia Pahang (UMP).

REFERENCES

1. Buemi, M., Senatore, M., Corica, F., Aloisi, C., Romeo, A., Cavallaro, E., Floccari, F., Tramontana, D., Frisina, N. (2002). Statins and progressive renal disease. *Medicinal Research Reviews*, **22**:76-84.

2. Chong, P.H., Seeger, J.D., Franklin, C. (2001). Clinically relevant differences between the statins: implications for therapeutic selection. *American Journal of Medicine*, **111**: 390-400.

3. Demain, A.L. (2006). From natural products discovery to commercialization: a success story. *J Ind Microbiol Biotechnol*, **33**: 486-95.

4. Eckert, G.P., Wood, W.G., Muller, W.E. (2005). Statins: drugs for Alzheimer's disease? *Journal of Neural Transmission*, **112**:1057-71.

5. Endo, A. (2004). The origin of the statins. *International Congress Series,* **1262**: 3-8.

6. Furberg, C.D. (1999). Natural statins and stroke risk. *Circulation*, **99**: 185-88.

7. Maron, D.J., Fazio, S., Linton, M.F. (2000). Curent perspective on statins. *Circulation*, **101**: 207-13.

8. Pitt, J.I. (1979). The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. Academic Press, London.

9. Raper, K. B., Thom Ch. (1949). A manual of the *Penicillia*. Williams & Wilkins Co.: Baltimore, Maryland.

10. Wainwright, M. (1990). Miracle cure: the story of penicillin and the golden age of antibiotics. Blackwell Publishing, Oxford.