

Isolation and Identification of Indigenous *Penicillium chrysogenum* Series

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ABSTRACT

The present study describes the isolation and identification of native isolates of *Penicillium chrysogenum*. Several fungal isolates obtained from different samples comprising of fruits, vegetables, bread and grains from market around Faisalabad were identified as *Penicillium* species by their morphological features. Slide culture method was adopted for the identification of fungal isolates. Only two isolates, one from spoiled mango and other from maize were found closely related to *Penicillium chrysogenum*. Most of the cultural characteristics of *Penicillium chrysogenum* isolates were observed on Sabouraud's glucose agar medium and Czapek yeast autolysate agar medium. Being the local isolates, these were provisionally named as *Penicillium chrysogenum* series University of Agriculture, Faisalabad Rafi 1 (UAF R-1) and *Penicillium chrysogenum* series University of Agriculture, Faisalabad Rafi 2 (UAF R-2).

Key Words: *Penicillium chrysogenum*; Characterization

INTRODUCTION

Penicillin is one of the oldest discoveries among the naturally occurring antibiotics. More than 30 different derivatives are being prepared from 6-aminopenicillanic acid and playing a vital role in the treatment of mixed infections (Akhtar, 1999). Penicillin is most actively produced by representatives of the species *Penicillium* (*P.*) *chrysogenum*. Several strains of *P. chrysogenum* are being used for penicillin production at laboratory scale or on commercial level in various countries of the World (Eriksen *et al.*, 1994; Justen *et al.*, 1998). Research is still continuing in an effort to economize its production by fermentation. Special attention is being paid to develop genetically modified strains for maximum penicillin productivity, the utility of cheap raw materials as substrate and to employ simple methods of cultivation for better penicillin production.

The present project was aimed at to isolate *P. chrysogenum* from local sources and providing basic data related to the morphological and cultural characteristics of the local isolates in comparison to the already defined characteristics of the existing standard isolates. Moreover, the present study may further provide an opportunity to evaluate the isolated series of *P. chrysogenum* for the penicillin production potential.

MATERIALS AND METHODS

Sample collection. Spoiled fruits (mangoes, apples, citrus), vegetables, bread and grains (wheat, maize) were collected for the isolation of *Penicillium* species as described by Alexopoulos (1961) and Malik (1996).

Primary isolation. Sabouraud's glucose agar was used for the primary isolation of mold. The fruiting bodies of mold

from the samples were grasped and rubbed with a pair of sharp forceps on the Sabouraud's glucose agar plates to spread spores as described by Cappuccino and Sherman (1999). The plates were incubated in an inverted position at 25-28°C for seven days in a moist incubator.

Secondary isolation. The fungal isolates from Sabouraud's glucose agar were shifted separately for further isolation by slide culture method as recommended by Awan and Rahman (2002). Each slide was examined under the low and high power objective of microscope for arrangement of hyphae, conidiophore, sterigmata and conidia.

Purification of culture. Different colonies of *P. chrysogenum* appeared on Sabouraud's glucose agar plates were also transferred to Czapek yeast autolysate (CYA) agar plates under sterile conditions for the purification and further characterization of culture as recommended by Singh *et al.* (1991). The CYA agar medium was composed of (g/L): NaNO₃, 3.0, K₂HPO₄, 1.0; KCl, 0.5; MgSO₄.7H₂O, 0.5; FeSO₄.7H₂O, 0.01; yeast extract, 5.0; sucrose, 30.0; agar, 15.0 and trace metal solution, 1.0 mL. The trace metal solution was composed of ZnSO₄.7H₂O, 1.0 g; CuSO₄.5H₂O, 0.5 g in 100 mL distilled water. The inoculated plates were incubated at 25-28°C for 7 days under dark conditions.

RESULTS AND DISCUSSION

Isolation and identification of *Penicillium* spp. Spoiled fruits and vegetables along with bread and grains were found to contain a variety of mycoflora. Most of the fungal isolates belonged to *Penicillium*, *Aspergillus*, *Mucor* and other unidentified species of fungi. The relative occurrence of different fungal genera isolated through spoiled food sampling is given in Table I. In a similar work, Alexopoulos (1961) showed that various species of *Penicillium* were

frequently found on citrus and other fruits, on jellies and on other foodstuffs that had become contaminated with their spores. *Penicillium* species affect food and fruits quality, reported in the work of Malik (1996).

Table I. Cultural characteristics of mold upon the primary isolation on Sabouraud's glucose agar medium at 25-28°C after seven days of incubation

Source	No. of isolates	Colony characteristics
Mango	1	Bluish-green in colour
	2	Yellowish-green in colour
Citrus	1	Blackish-brown in colour
	2	Greyish-blue green in colour
	3	Creamish-yellow in colour
Apple	1	Bluish-green in colour
	2	White to yellow in colour
	3	Whitish-cream in colour
Wheat	1	White in colour
	2	White to yellow in colour
Maize	1	Dark green in colour
	2	Greyish-white in colour
Bread	1	Gray to brown in colour
	2	White in colour

Sabouraud's glucose agar medium was used for the primary isolation of mold, had also been attempted by Cruickshank *et al.* (1975), Malik (1996) and Cappuccino and Sherman (1999). Maximum of two to three types of colonies were visible from each sample on Sabouraud's glucose agar plates.

As it was difficult to process a complete range of mold isolates, the efforts were made to take up only those isolates which were having morphological and cultural characteristics similar to *Penicillium* species. A total of four isolates were selected for further processing and identification as detailed in Table II.

The colonies of Isolate I on Sabouraud's glucose agar were 4 to 5 cm wide at the 7th day of incubation. The colonies were velvety and sulcate, with blue-green in colour. The reverse side of the colonies was yellow. Microscopically, penicilli were terverticillate and the

conidia were spherical to elliptical in shape. Conidia were smooth and had a green colour reflection in the mass. The colonial and microscopic features of Isolate I were found similar to that of *P. chrysogenum* (Table II).

The colonies of Isolate II on Sabouraud's glucose agar were 2.5 to 3.0 cm in diameter at the 7th day of incubation. The colonies were white at the 3rd day of incubation later turned, gray-blue green in colour. The surface of colonies was velvety and sulcate. The reverse side of the colonies was yellowish-cream in colour. Microscopic examination showed that the penicilli were biverticillate. The phialides were ampulliform with spherical conidia. Conidia were smooth and had a greenish-yellow colour in the mass. The morphological and cultural characters of Isolate II were found similar to that of *P. citrinum* (Table II).

The colonies of Isolate III had limited growth on Sabouraud's glucose agar, achieving a diameter of 1.0 to 1.5 cm by the 7th day of incubation. The colonies were velvety and sulcate with blue-green in colour. The reverse side of the colonies was creamish-yellow in colour. Microscopically, the penicilli were monoverticillate. The phialides were ampulliform and the conidia were elliptical in shape. The conidia were smooth and had a greenish colour in the mass. The morphological and cultural characters of Isolate III were found similar to that of *P. capsulatum* (Table II).

The colonies of Isolate IV grew quickly on Sabouraud's glucose agar and were 4.0 to 4.5 cm in diameter on the 7th day of incubation. The colonies were velvety and sulcate having dark green colour. The reverse side of the colonies was yellow in colour. Microscopic examination revealed that the penicilli were biverticillate. The conidia were elliptical in shape with smooth surface and had a dark green colour in the mass. The morphological and cultural characters of Isolate IV were found similar to that of *P. chrysogenum* (Table II). The colonial and microscopic morphology of two isolates, one from the spoiled mango and other from maize were found closely related to that of *P. chrysogenum*. Being the local isolates, these were provisionally named as *P. chrysogenum* series UAF R-1 and *P. chrysogenum* series UAF R-2.

Table II. List of various isolates of *Penicillium* spp. on the basis of colonial and morphological characters on Sabouraud's glucose agar medium at 25-28°C after seven days of incubation

Colonial and microscopic morphology	Isolate I (source:mango)	Isolate II (source:citrus)	Isolate III (source:apple)	Isolate IV (source:maize)
Colony diameter	4.5 - 5 cm	2.5 - 3.0 cm	1.0 - 1.5 cm	4.0 - 4.5cm
Texture	Sulcate, velutinous	Radially sulcate, floccosse in center & velutinous at margins	Sulcate, centrally raised, velutinous	Sulcate, velutinous
Obverse	Bluish green	Greyish-blue green	Bluish green	Dark green
Reverse	Yellow	Yellowish-cream	Creamish-yellow	Yellow
Stipe	Short, smooth	Long, smooth	Short, smooth	Short, smooth
Penicilli	Terverticillate	Biverticillate	Monoverticillate	Biverticillate
Phialides	Ampulliform	Ampulliform	Ampulliform	Ampulliform
Collula	Short	Short	Short	Short
Conidia	Spherical to ellipsoidal, smooth, greenish	Spherical, smooth, greenish-yellow	Ellipsoidal, smooth, greenish	Ellipsoidal smooth, dark green

Cultural and morphological characters of *P. chrysogenum* series UAF R-1 on CYA agar medium.

Colonies on Czapek yeast autolysate (CYA) agar medium were 4.5 to 5.0 cm in diameter after seven days of incubation at 25-28°C under complete darkness. The colonies were bluish-green to dark green in colour with velvety and sulcate surface, having numerous spores and a white border of 2 to 3 mm wide. The reverse side of the colony and medium was usually pale yellow. Diameter of colonies became shorter (1.0-1.5 cm) on CYA agar medium after one week of incubation at 37°C (Table III, Plate 1).

Conidiophores were formed from the substrate in a thick layer. The stipes were short and smooth. The penicilli (brushes) were terverticillate and asymmetrical. The brushes had two branches, with four metulae; there were 4 to 5 phialides (sterigmata) on each verticil. The phialides were ampulliform, which bore chains of conidia. The conidial chains were normally in distinct columns. The individual conidia were spherical to elliptical in shape with smooth surface and green in the mass (Table IV, Plate 2).

Table III. Comparative cultural characters of *P. chrysogenum* series on CYA agar medium at 25-28°C after 7 days of incubation

Cultural characters	<i>P. chrysogenum</i> series UAF R-1	<i>P. chrysogenum</i> series UAF R-2
Colony diameter	4.5 – 5.0cm	5.5 – 6.0cm
Texture	Sulcate, velutinous and a white border 2-3mm wide	Sulcate, velutinous and a white border 2-3mm wide
Obverse	Bluish-green to dark green	Greyish-green to dark green
Reverse	Pale-yellow	Creamish-yellow
Colony diameter at 37°C	1.0 – 1.5 cm	1.5 – 2.0cm

Table IV. Comparative morphological characters of *P. chrysogenum* series on CYA agar medium at 25-28°C after 7 days of incubation

Morphological characters	<i>P. chrysogenum</i> series UAF R-1	<i>P. chrysogenum</i> Series UAF R-2
Stipes	Short, smooth	Short, smooth
Penicilli	Terverticillate	Biverticillate
Phialides	Ampulliform	Ampulliform
Collula	Short	Medium – sized
Conidia	Spherical to ellipsoidal smooth, greenish	Ellipsoidal to spherical, smooth, dark green

Most of the colonial and microscopic features of *P. chrysogenum* series UAF R-1 were related to those as described by Florey *et al.* (1949) and Sing *et al.* (1991) and still differed in other cultural characteristics. It differed in that it had a bigger size colony (diameter 4.5 to 5.0 cm) surrounded by a white border of 2-3 mm wide. It was also noticeable that the growth appeared on CYA agar medium at 37°C of incubation with colony diameter of 1.0 to 1.5 cm

after seven days as compared to the already described series of *P. chrysogenum* which do not grow at 37°C.

Cultural and morphological characters of *P. chrysogenum* series UAF R-2 on CYA agar medium.

Colonies on CYA agar medium were 5.5 to 6.0 cm in diameter after seven days of incubation at 25-28°C in complete darkness. The colonies were grayish-green to dark green in colour. The colonies were velvety and sulcate with a white border about 2 to 3 mm wide. There were numerous spores. The reverse side of the colony and medium was creamish-yellow in colour. Colonies had limited growth on CYA agar medium at 37°C and were 1.5 to 2.0 cm in diameter between the 6th and 7th day of incubation (Table III, Plate 3).

Conidiophores were formed on the substrate. The stipes were short and smooth. The penicilli (brushes) were biverticillate and asymmetrical. The brushes generally had two metulae. There were 4 to 5 phialides (sterigmata) each in verticils. The phialides were ampulliform, which bore chains of conidia. The chains of conidia were arranged in columns. The conidia were elliptical or round-ovoid in shape with smooth surface and dark green in the mass (Table IV, Plate 4).

Most of the morphological and cultural characteristics of *P. chrysogenum* series UAF R-2 were in line with those of Bilai (1963b). But *P. chrysogenum* series UAF R-2 was different in certain cultural characteristics. It was found that it had a colony diameter of 5.5 to 6.0 cm and a white border of 2-3 mm wide in contrast to the already reported series having a diameter of 4.5 to 5.0 cm. It also differed in that it had growth on the CYA agar medium at 37°C and the colony diameter was 1.5 to 2.0 cm after seven days of incubation.

It can be concluded from the present study that the local series isolated from spoiled mango and maize showed morphological and cultural resemblance to that of standard series of *P. chrysogenum* with the exception of colony size and growth obtained at 37°C, may be represented as *P. chrysogenum* series UAF R-1 and *P. chrysogenum* series UAF R-2. Further studies may be conducted to test the ability of indigenous series for the possible penicillin production potential.

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