Metabolic characterization of toxigenic fungal strains isolated from post-harvest crops

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Tripathi C.K.M. & Agarwal S.C. 1998. Metabolic characterisation of toxigenic fungal strains isolated from post - harvest crops. *Geophytology* **26**(2): 39-42.

Fungal strains isolated from different post-harvest crops have been characterized with respect to their consistent physiological and biochemical properties related with growth parameters. All these strains (fifty eight in number) are toxic to mice in various degrees. Their metabolic characteristics have been compared with authentic reference strains and attempts have been made to confer these isloates a taxonomic status. The isolated strains belong to twelve different species of the genus *Penicillium*. Of these *P. crustosum*, *P. cyclopium*, *P. brevicompactum* and *P. commune* were of dominant occurrence while, *P. digitatum* and *P. granulatum* were scarcely isolated. A possibility of correlating toxicity with selected metabolic characteristics has been indicated.

Key-words - Fungi, post-harvest crops.

INTRODUCTION

FUNGI are the most common of all the contaminants found associated with various places and stuffs of human uses. The economic losses caused by fungal deterioration are substantial as they produce diverse arrary of chemical compounds in the occupied ecological microniches (Pitt 1979). Penicilli and Aspergilli are the most frequently isolated fungi from post-harvest crops (Mislivec & Tuite 1970). Various metabolites of the genus *Penicillium* bear concern with cereal industry, food technology, pharmaceutical industry and chemotherapy (Frisvad & Filtenborg 1989). Fungal isloates from food were found to be more efficient mycotoxin producers than the strains isolated from other sources (Frisvad & Filtenborg 1989). Strain characterization with respect to the culture's consistent physiological and biochemical properties related with growth products qualitatively and quantitatively responds well to the various enquiries by biotechnologists for assessment and extrapolation of the metabolic potential of the microorganisms. It has become apparent that classification based almost exclusively on macro- and micro-phenotypic characters may not always result in well defind taxa (Bridge *et al.* 1989). Very minor changes in culture conditions lead to substantial variations in colony characteristics of *Penicillium* strains (Okuda 1994) and situation is particularly complicated in fasciculate penicillia. We have examined some of the fungal strains isolated from post-harvest crops viz., wheat, rice, gram, maize and few millets belonging to different geographical regions of India. Based on morphological observations these strains were identified and their toxicity was evaluated in mice. Certain toxigenic strains of the genus *Penicillium* were chosen for metabolic characterization and identification. Authentic reference strains were used as positive controls in the study. Purpose of the study was to investigate the metabolic profile of the cultures to evaluate and explore its industrial uses.

MATERIALS AND METHODS

The previously isolated and preserved cultures of the genus *Penicillium* from post-harvest crops were revived and confirmed by preliminary microscopic observations.

Biochemical Studies

Growth of these fungi was studied on ammonium oxalate, citric acid, tannic acid, sodium nitrate, creatine, Tween B0; hydrolysis of cellulose,

Table 1. Characteristics of the reference and isolted strains.

Sl. No	Isolated strain Nos. Pl-	Reference strain Nos. and names	Biochemical Characters										
			Ao	Ca	Ta	Sn	Cr	TBO	Ce	Pe	Ge	Cn	Mt
1.	2267, 2292, 2327 2342, 2347, 2572, 2583, 2596	NRRL 3474 *P. cyclopium*	-	-	-	-	+	+	-	+	-	+	Pen
2.	2304, 2309, 2417, 2494, 2577, 2631, 2634	NRRL 859 *P. veridicatum*	+	+	+	+	+	+	-	+	+	+	My
3.	2314, 2412, 2620, 2635	NRRL 3712 *P. veridicatum*	-	+	-	+	+	+	-	+	+	+	Oc
4.	2234, 2260, 2419, 2617	NRRL 2096 *P. funiculosum*	-	+	+	-	+	-	+	+	+	-	ND
5.	241, 2395, 2425, 2471, 2616	NRRL 807 *P. chrysogenum*	-	+	+	-	+	+	-	-	+	-	ND
6.	2559, 2560, 2603	NRRL 783 *P. citrinum*	+	-	+	+	+	+	-	+	+	+	Ci
7.	2429, 2556, 2558, 2565, 2567, 2569, 2563, 2578, 2581	NRRL 868 *P. crustosum*	-	+	+	+	+	+	+	-	+	+	ND
8.	2408, 2444, 2562, 2575, 2576, 2590, 2602	NRRL 890 *P. commune*	-	-	+	+	-	-	+	-	-	+	ND
9.	2456, 2593, 2599	NRRL 976 *P. expansum*	+	+	+	-	+	+	-	+	-	+	Pa
10.	2503, 2512	NRRL 786 *P. digitatum*	-	-	-	+	-	-	+	-	+	+	ND
11.	2520, 2536	NRRL 2036 *P. granulatum*	+	+	+	-	-	+	+	+	+	+	Pa
12.	2561, 2585, 2595, 2568	NRRL 965 *P. verrucosum*	-	+	-	-	+	-	+	+	+	+	Oc

Ao - Ammonium oxlate, Ca - Citric acid, Ta - Tannic acid, Sn - Sodium nitrate, Cr - Creatine, TBO - Tween BO, Ce - Cellulose, Pe - Pectin, Ge - Gelatin, Cn - Casein, Mt - Mycotoxin, Pen - Penicillic acid, My - Mycophenolic acid, Oc - Ochratoxin A, Ci - Citrinin, Pa- Patulin, ND - Not detected.

pectin, gelatin and casein following the method of Bridge (1985).

Determination of Mycotoxin Production

Mycotoxin production was determined in YES medium (Ciegler *et al.* 1973). Cultures were grown for ten days on shaker (160 rpm) at 28°C. The extracellular and intracellular mycotoxins were elicited by TLC against authentic mycotoxin samples obtained from Sigma. Production of penicillic acid, mycophenolic acid, ochratoxin A, citrinin and patulin was detected by the method described by Ciegler *et al.* 1973. For comparing phenotypic and biochemical characteristics reference strains of the genus *Penicillium* were obtained from Northern Regional Research Laboratory, Illinois, USA.

RESULTS AND DISCUSSION

Observations for colony morphology on different media and microscopic characters were made and matched with the standard reference strains.

Metabolic characteristics of the investigated reference and isolated strains have been listed in Table 1. Reference strains have been used as positive controls helping to group the isolated strains. It was observed that *P. crustosum* was the most commonly occurring fungi as nine out of fifty eight isolates belonged to this species, followed by *P. cyclopium* (eight strains); *P. chrysogenum* (five strains), and *P. veridicatum, P. funiculosum, P. verrucosum* (four strains each).

It seems obvious that a set of biochemical characteristics of a particular group of strains indicate about more or less similar metabolic potentials. Frisvad (1981) has reported that only few species could produce more than two fundamentally different mycotoxins. However, sharing of certain physiological and chemotaxonomic properties among ochratoxin-citrinin producing strains was observed. In the present study, it was observed that pectin and casein hydrolysis were always associated with the production of mycotoxins. Strain lacking these hydrolytic activities failed to produce detected mycotoxins or other toxic metabolites in substantial amount as they show feeble toxicity to mice. It has been demonstrated in various studies that particular physiological attribute of any strain could be indicative of certain biological activities. Frisvad (1981) demonstrated that preservative resistant isolates of *P. cyclopium* appeared to be more active biochemically than the preservative sensitive isolates and of the lipase producing strains 72% were penicillic acid producers and of the nonproducers of tricaproinase only 33% produced penicillic acid. These studies indicate that diversified metabolic potential of a strain may have certain inter-relationship. Jimenez *et al.* (1986) studied various Pencillia and observed that toxin producing strains were toxic to Brine shrimp larvae and were antibacterial too. Their studies concluded that these strains produced some other active principles also along with mycotoxins with common biochemical pathways. In fact it is characteristic for secondary metabolites that the end products, in a biosynthetic route, are of much restricted distribution than their precursors (Frisvad & Feltenborg 1989) which can be utilized for the synthesis of other bioactive principles. From regulation point of view also, secondary metabolism is under less precise genetic control as several genes are involved in contrary to single gene in case of primary metabolites (Bu'Lock 1980). Thus presence of a particular metabolite may be a useful character but not much can be inferred from its absence (Bridge *et al.* 1989).

It is clear from table 1 that metabolic potential of the investigated strains exhibit enough variation. Mycotoxin producing cultures were found to be more active metabolically as they are positive for many biochemical characters. Jimenez *et al.* (1986) has reported that the metabolic profiles of certain strains of *Penicillium* exhibit multiple biological activities. *P. citrinum* and *P. crustosum* have been found to cause extrinsic bronchial asthma (Wel *et al.* 1993), other strain of *Penicillium* degrade phenol and hydroxyl compounds (Hofrichter *et al.* 1992-1993, Lin *et al.* 1996).

The present evaluation of biochemical diversity indicated that the post-harvest crops, which are very rich source of fungi, could be of enormous interest in pharmacology.

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