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EXTRACELLULAR ENZYMATIC ACTIVITIES OF 32 FUNGAL SPECIES

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SUMMARY

Qualitative determinations of extracellular enzyme production (amylase, cellulase, caseinase, phosphatase, lipase, pectinase and urease) by 32 fungal species were investigated. The enzymes detected in the agar cultures of the fungi studied vary widely.

Amylase, urease and phosphatase were detected in cultures of most fungal species. Cellulase was detected particularly in cultures of ***Aspergillus flavus, A. fumigatus, Cladosporium cladosporioides. Penicilium brevicompactum, Rhizopus stolonifer, Scopulariopsis flava*** *and* ***Trichothecium roseum. Penicillium digitatum*** *and* ***P. duclauxi*** *showed of being high producers of caseinase. Lypolytic activity was detected in cultures with synthetic fats Tweens 20, 40, 60 and 80 as substrates for lipase.*

Lipolytic activity for the four synthetic fats showed ***A. flavus, Aureobasidium pullulans, C. cladosporioides, Gliocladium, roseum, Mycotypha, microspora Microsporum gypseum, P. brevicompactum and Scopulariopsis brumptii.*** *Pectinase was detected in cultures of isolates of* ***A. flavus, C. cladosporioides, Fusarium oxysporum, P. brevicompactum and Ulocladium atrum.***

RESUMEN:

[*Actividad enzimática extracelular investigada en 32 especies fúngicas*]

Se investigó en 32 especies fúngicas, la producción de enzimas extracelulares y sus determinaciones cualitativas (amilasa, celulosa, caseinasa, fosfatasa, lipasa, pectinasa y ureasa). La detección de las enzimas en los cultivos fúngicos en agar varió ampliamente.

En la mayoría de los cultivos fúngicos se detectaron: : amilasas, ureasas, y fosfatasas. Las celulasas se pesquizaron particularmente en cultivo de ***Aspergillus flavus, A. fumigatus, Cladosporium cladosporioides, Penicillium brevicompactum, Rhizopus stolonifer, Scopulariopsis flava y Trichothecium roseum. Penicillium digitatum y P.duclauxi*** *fueron productores de caseinasa en un alto grado. La actividad lipolítica se detectó en cultivos con grasas sintéticas Tweens 20, 40, 60, y 80 como sustrato para lipasa. Indicaron actividad lipolítica para estos sustratos* ***A. flavus, Aureobasidium pullulans, C. cladosporioides, Gliocladium roseum, Mycothypha microspora, Microsporum gypseum, P. brevicompactum y Scopulariopsis brumptii.*** *Productores de pectinasas fueron las cepas de* ***A. flavus, C. cladosporioides y Fusarium oxysporum, P. brevicompactum y Ulocladium atrum.***

Several excellent general and specific researches have appeared during the last 20 years, investigating the extracellular enzymes produced by fungi. Some factors which influence the synthesis of enzymes by fungi and other micro-organisms were reported by Davies (1963). The detection of extracellular enzymes produced by different species or genera of fungi is important in the study of spoilage of foods and biodeterioration of stored cereals and for taxonomical purposes too.

In this paper, the results of a screening on 32 species of filamentous fungi to detect their ability to release extracellular enzymes into the cultural media are reported.

MATERIALS AND METHODS.

Fungal species used in this study were isolated from the following habitat: 14 from the air sampling at Pavia (Italy) (determined by the agar plate method), 4 from horse dung, 10 from animal hair, 3 from soil and 1 from water (Table 1). All were cultured at 25° C on potato dextrose agar (PDA) until sufficient growth had occurred. Amylolitic, cellulolytic, lipolytic, pectinolytic and phosphatase activity were tested by the methods of Hankin and Anagnostakis (1975); caseinolysis was demonstrated on skimmed milk agar (Ahearn et al., 1968). Urease production was tested on Christensen's urea agar (Seeliger, 1956).

Qualitative estimates of enzymes production were made. When an enzyme was detected in a fungal culture, a plus (+) sign was used to indicate this; lack of enzyme detection was indicated by a minus (–) sign. Intensity of enzymatic activity was indicated by (++), (+++) signes. Urease production was based on colour intensity.

RESULTS AND DISCUSSION

Results of the extracellular enzymatic activities invstigated in 32 fungal species into the cultural media are given in Table 1 and illustrated in figures 1, 2, 3, 4, 5. The enzymes detected in the fungi studied vary widely.

The lowest number of enzymes were detected in cultures of **M. gypseum** and **P. inaequalis** (only lipolytic activity), **M. spinosus** (lipolytic activity and urease production), **Ch. globosum** (cellulolytic activity and urease production), **H. elegans, Myc. microspora** (lipolytic activity, urease and phosphatase production) and **Rh. stolonifer** (amilolytic, cellulolytic activity and phosphatase production).

Urease was detected in cultures of 29 species of fungi. **Phosphatase** was not detected in cultures of nine fungal species, i. e. **A. pullulans, B. piluliferum** and some species of the genus **Scopulariopsis.**

Cellulase was detected in cultures of 21 fungal species, particularly of species of the genera **Aspergillus, Penicillium, Scopulariopsis** and **C. cladosporioides, Rh. stolonifer** and **T. roseum.**

Pectinase was detected only in cultures of 11 species of fungi and particularly in the species of **A. flavus, C. cladosporioides, F. oxysporum, P. brevicompactum** and **U. atrum.**

Amylase was detected in cultures of 21 fungal species; most of these were species of the genera **Aspergillus, Penicillium** and **Scopulariopsis.**

Caseinase was detected only in cultures of 17 species of fungi; **P. digitatum** and **P. duclauxi** showed of being high producers of this enzyme.

Data of lipolytic activity detected in cultures of all fungal species with synthetic fats Tweens 20, 40, 60 and 80 as substrates (Polyoxyethylene sorbitane monolaurate, monopalmitate, monostearate and monooleate respectively), were the following:

1) Ch. globosum, Rh. stolonifer and U. atrum were the only fungi that did not produce lipase;
2) the fungal species showing to possess lipolytic activity for the four synthetic fats were **A. flavus, A. pullulans, C. cladosporioides, G. roseum, Myc. microspora, M. gypseum, P. brevicompactum** and **S. brumptii;**
3) strong lipolytic activity for monolaurate and monopalmitate showed **A. pullulans, C. cladosporioides, M. gypseum** and **S. brevicaulis;**
4) **H. elegans** and **P. inaequalis** showed lipolytic activity for monopalmitate.

Comparing the enzymatic activities of fungal species in relation to their different isolation, the following conclusions may be drawn:

1) the principal fungi which showed cellulolytic and pectinolytic activities were strains of fungi isolated from the air. These molds, principally **Penicillium, Aspegillus** spp., **E. purpurascens** and **Rh. stolonifer** have been reported to be of widespread occurrence on agricultural products and some is well known attack seeds in storage and associated with the spoiled of fruits (Adisa, 1985). Occurrence of mycotoxins in food-stuffs by **A. flavus** and **A. ochraceus** has also been reported.

The easy growth of these fungi in wood materials, fruits, textiles, paper and stored cereals may perhaps be explained to their capability to produce these extracellular enzymes. Cellulolytic enzymes may play a role in invasion of plants by pathogens, intra- and intercellular penetration of host tissues (Walker et al., 1955; Wood, 1960) and the development of wilts (Beckman, 1956, Blackhurst and Wood, 1963; Husain and Kelman, 1958). Cellulases also may be essential to the maintenance of the pathogen during periods when it is living saprophytically (Mandels and Reese, 1965).

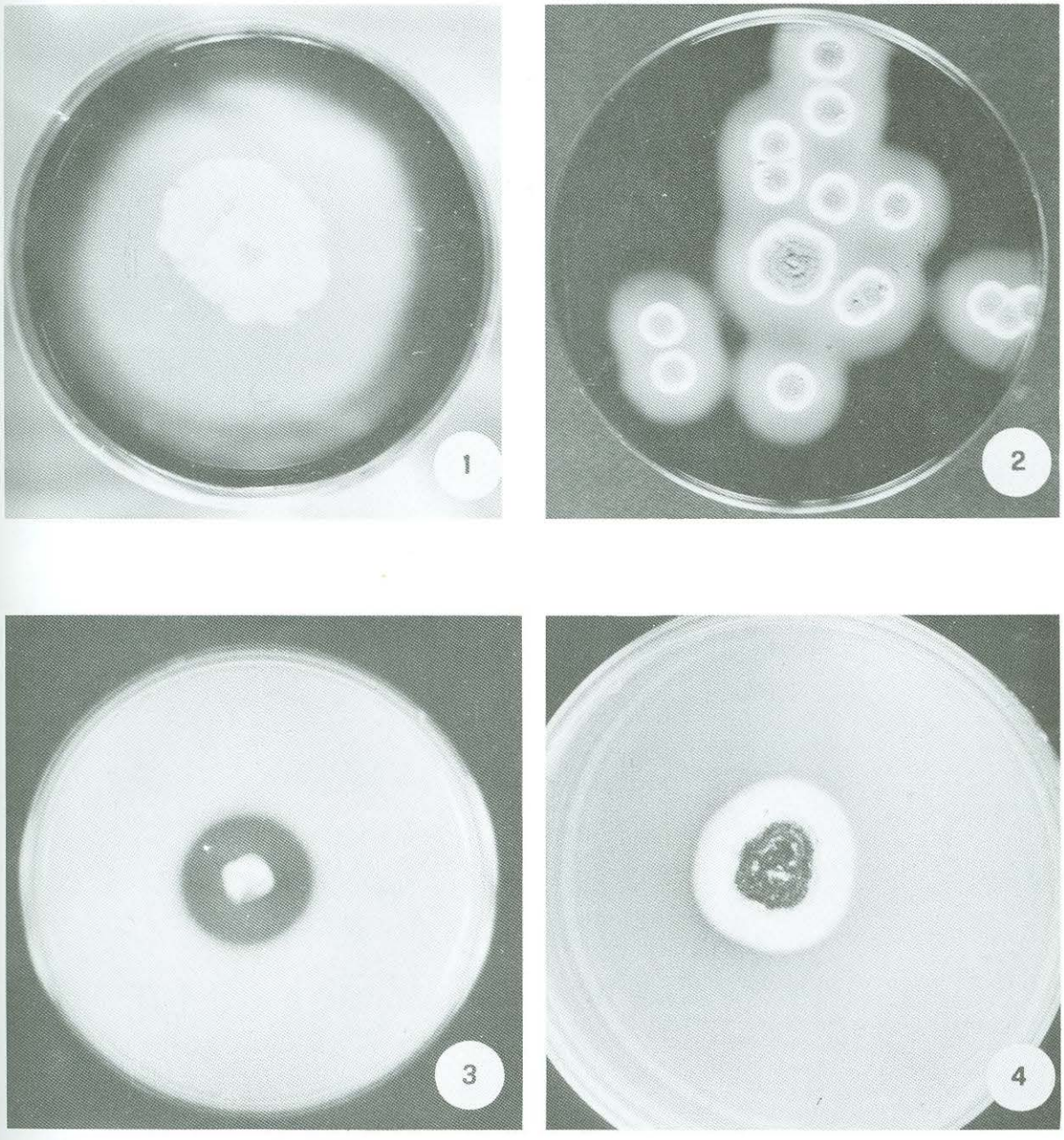
2) The principal fungi which showed lipolytic activities were fungal strains isolated from air (**A. pullulans, Myc. microspora** and **C. cladosporioides**) but particularly strains isolated from animal dungs (**H. elegans, Pet. guttulata, Pod. inaequalis**), from the fur of animals (**Scopulariopsis** spp.) and from the soil **M. gypseum.**

These results agree with Cochrane's (1958) statement that most fungi have capability to produce lipase and with Sierra's (1957) statement that the Tweens may be useful in detecting the specificity of lipases on fungi.

The fungi, **M. gypseum** and **Scopulariopsis** spp. are "non pathogenic keratinophilic fungi", occasionally causative agents of skin and of the nails diseases.

These fungi have also been recovered "in vivo" from the skin and the fur of animals which show no signs of disease. The ability of these fungi to survive on skin might be related to the skin surface lipids, particularly to the mono-, di- and tri-glycerides fraction, evaluated to be 31,7 o/o of a skin surface lipids fraction of adult human male scalp (Lewis and Hayward, 1971).

Glycerides of the lauric, palmitic and stearic acids might be sufficient nutrients available to allow the growth of this keratinophilic fungal group. The keratinolytic ability would appear to be suited to survival as a soil saprophyte.



Examples of extracellular enzymatic activities of some fungal species.

Fig. 1 and 2 – Amylase, a clear halo around the colony of **A. pullulans** and **P. brevicompactum**.
Fig. 3 – Caseinase, **P. digitatum**.
Fig. 4 – Pectinase, **G. roseum**.

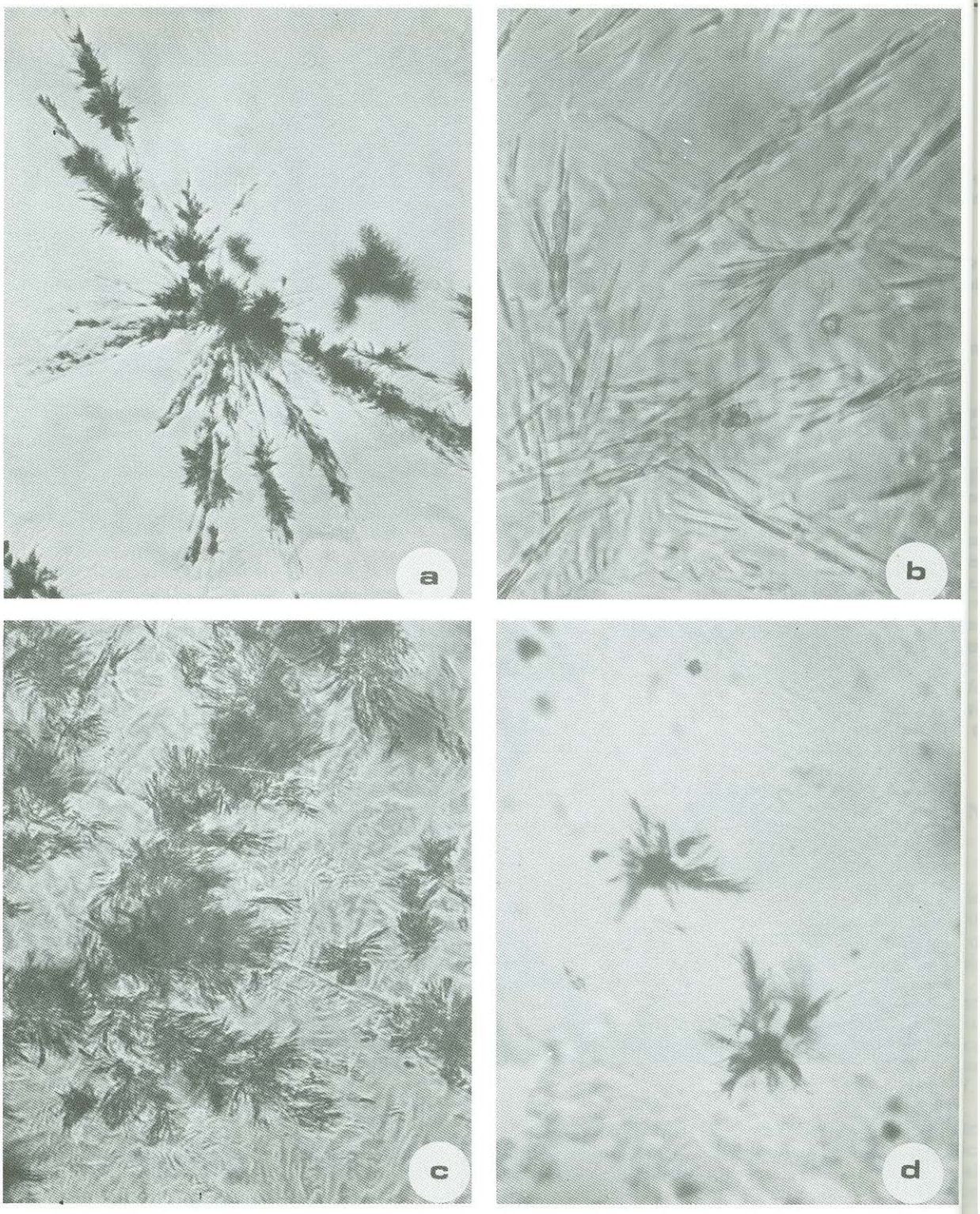


Fig. 5 – Lipase: characteristic crystals formed in haloes in the immediate vicinity of the colonies on plate with different Tweens.
a) calcium laurate crystals on plate with Tween 20, **A. pullulans** X100; b) Ca palmitate crystals Tween 40, **S. candida** X250; c) Ca stearate crystals Tween 60, **M. microspora** X400; d) Ca oleate crystals Tween 80, **M. gypseum** X100.

TABLE 1 – Qualitative determinations of extracellular enzymes production by 32 fungi

	ORGANISMS	AMYLASE	CASEINASE	CELLULASE	PHOSPHATASE	LIPASA Tween 20	LIPASA Tween 40	LIPASA Tween 60	LIPASA Tween 80	PECTINASE	UREASE
A	**Aspergillus flavus** Link: Fries	+	+	+++	+++	+	+	+	+	+++	+++
A	**A. fumigatus** Fresen.	+	–	++++	+++	+	+	–	–	++	±
A	**A. niger** V. Tieghem	+	–	+++	+++	+±	–	–	–	++	±
S	**A. ochraceus** Wilhelm	+	±	–	+++	+±	+	–	+	++	±
A	**Aureobasidium pullulans** (de Bary) Arn.	+	–	±	–	+++	+++	+	±	++	++
D	**Botryotrichum piluliferum** Sacc. & Marchal	–	+	±	–	+	+	–	–	–	+++
A	**Chaetomium globosum** Kunze ex Steud	–	±	++	–	–	–	–	–	–	+++
A	**Cladosporium cladosporioides** (Fresen.) de Vries	–	–	+++	+++	++++	+++	+	+	+++	+++
A	**Epicoccum purpurascens** Ehrenberg ex Schlecht.	+	–	++	++	–	+	+	+	++	+++
A	**Fusarium oxysporum** Schelecht.	+	±	–	+++	++	++	–	–	+++	+++
S	**Gliocladium roseum** (Link) Bainier	–	–	+++	++	+	+	+	+	++	+++
D	**Helicostylum elegans** Corda	–	–	–	++++	–	+++	–	–	–	+++
A	**Mycothypha. microspora** Fenner	–	–	–	++	++++	+	+++	+	–	++
S	**Microsporum gypseum** (Bodin) Guiart & Grigorakis	–	–	–	–	++	+++	++	+	–	–
H	**Mucor circinelloides** v. Tieghem	–	–	–	+	±	–	–	+	+	+
A	**M. spinosus** v. Tieghem	–	–	–	–	++	–	–	–	–	+
S	**Penicillium brevicompactum** Dierckx	+	+	+++	+	+±	+	+±	+	+++	++
A	**P. digitatum** (Pers: Fr) Sacc.	+	+++	++	++	+	+	–	+	–	±
W	**P. duclauxi** Delacr.	+	+++	++	++	–	+	–	–	–	++
A	**Paecilomyces lilacinus** (Thom) Samson	+	++	–	+++	–	+	+	+	–	+++
D	**Petriella guttulata** Barron & Cain	+	+	++	+++	+	+	+	+	–	++
D	**Podospora inaequalis** (Cain) Cain	–	–	–	–	–	+++	–	–	–	–
A	**Rhizopus stolonifer** (Ehrenb. ex Link) Lind	+	–	++++	++++	–	–	–	–	–	–
H	**Scopulariopsis** sp	+	++	–	–	+	+	–	+	–	++
H	**S. brevicaulis** (Sacc.) Bain.	+	++	++	±	+±	+++	–	–	–	++
H	**S. brumptii** Salvanet - Duval	–	±	++	±	++	+	+	+	–	+
H	**S. candida** (Gueg.) Vuill.	+	+	++	±	+	+	±	–	–	++
H	**S. flava** (Sopp) Morton & Smith	+	±	++	–	+	+	–	±	–	++
H	**S. koningii** (Oud.) Vuill.	+	±	+++	–	+	±	–	+	–	++
H	**Trichoderma viride** Pers. ex Gray	+	–	–	++++	+	+	–	–	–	++
A	**Trichothecium roseum** Link: Fies	+	–	+++	±	–	+	–	+	–	±
H	**Ulocladium atrum** Preuss	+	±	++	+	–	–	–	–	+++	±

(A = air; H = dung; S = soil; W = water)

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