

## ISOLATION OF MUCORALES FROM PROCESSED MAIZE (*ZEA MAYS* L.) AND SCREENING FOR PROTEASE ACTIVITY

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### SHORT COMMUNICATION

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#### ABSTRACT

Mucorales were isolated from maize flour, corn meal and cooked cornflakes using surface and depth plate methods. *Rhizopus oryzae*, *Circinella muscae*, *Mucor subtilissimus*, *Mucor hiemalis* f. *hiemalis*, *Syncephalastrum racemosum*, *Rhizopus microsporus* var. *chinensis* and *Absidia cylindrospora* showed protease activity.

**Key-words:** Zygomycetes, food, contamination, isolation

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Mucorales are ubiquitous, morphologically simple terrestrial fungi that belong to the Zygomycetes. These molds are widespread in nature, and have been already isolated from soil, decaying vegetables, fruits and seeds (22). Some species of Mucorales are well-known as food contaminants, thus providing an important source of information concerning the hygiene condition of food deposits, imperfection on the storage process, extreme contamination of grain feedstock and presence of mycotoxins in substrata (6,29).

Proteases account for approximately 60% of all enzyme sales (14). The major uses of free proteases occur in dry cleaning, detergents, meat processing, cheese making, silver recovery from photographic film, production of digestive and certain medical treatments of inflammation and virulent wounds (19). Several studies report the efficient kinds of protease biosynthesis by *Rhizopus microsporus* var. *rhizopodiformis* (3), *Mucor circinelloides* (30), *R. oryzae* (4) and *Syncephalastrum racemosum* (9), confirming the real potential of Mucorales in producing this enzyme.

The aims of this work were to isolate and identify Mucorales from maize flour, corn meal and cooked cornflakes and to characterize the species according to the presence or not of protease activity.

#### Isolation and identification of Mucorales

Processed products derived from maize, such as maize flour, corn meal and cooked cornflakes, and commercially available in Recife, Pernambuco, Brazil were used as substrata. Three different packages showing different packing dates were analyzed and discarded after the analysis.

Surface and depth plate methods were used for the isolation, following Silva *et al.* (27). In the former, 1:10, 1:100 and 1:1000 dilutions were performed. From each of these three dilutions, 0,1mL was transferred to different sets of Petri dishes containing dichloran rose bengal chloramphenicol agar medium (DRBC) (15) and Sabouraud rose bengal chloramphenicol agar medium (SRBC) (17). Dilutions were the same for the surface and depth plate methods, but in the last method 1mL from each resulting suspension was inoculated in empty sterilized Petri dishes. After that, 15mL of DRBC and SRBC media cooled at 45°C were added to each Petri dish and put into motion, with soft circular movements. Colony growth was observed during 72 hours at room temperature (28°C ± 2°C). The inoculations to each medium and each dilution were done in triplicate.

Identification of Mucorales was carried out according to Benny (5), Hesseltine & Fennel (10), Hesseltine & Hellis (11), Hesseltine & Hellis (12,13), Schipper (24), Schipper (25) and Schipper (26).

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### Protease activity

Fragments of grown cultures (7 days) were transferred to the center of Petri dishes containing casein media (17). The dishes in triplicate were kept at room temperature (TA 28°C ± 1°C) for 7 days. In order to confirm the presence of a true proteolytic activity, an acid solution of mercury chloride was added to each plate. Protease production was certificated by the formation of a transparent halo (17,23).

A total of 86 specimens representing 13 species of Mucorales were identified (Table 1). The highest number of CFU/g was observed in *Mycocladius corymbifer* followed by *R. oryzae* and *Mycocladius ramosus*. Only *R. oryzae* was isolated from three different products. Among these three substrats, corn meal showed the highest number of species (12) and also CFU/g (59) followed by maize flour (3 species and 10 CFU/g) and cooked cornflakes (2 species and 17 CFU/g), corresponding to 68.60%, 11.63% and 19.77%, respectively. The isolation of Mucorales from maize products has been reported by several authors. As also observed in this manuscript, *Rhizopus oryzae* (21), *Rhizopus* sp. and *Cunninghamella* sp. (2) were isolated from corn meal. *Absidia cylindrospora* (21) and was isolated from maize flour. *Rhizopus* sp., *Syncephalastrum* sp., *Absidia* sp. and *Mucor* sp. (7), were isolated from maize foods in general. According to Banwart (4) and Sinha & Sinha (28) *Mucor* sp. and *Rhizopus* sp. are common contaminants in places where products are stored, supporting the results described on this paper.

The presence of Mucorales in the studied products and the differences in the number of CFU/g among the three substrata are probably consequences of maize contamination in the field or during the harvest, processing, transporting, packaging, storage and also the low quality of grain feedstock (18,28). The contamination of stocked maize grains can be caused by high humidity and temperature levels, inappropriate sanitary conditions, high level of fungi inoculation and exposition of grains to insects and mites (8,19,28).

*Absidia cylindrospora*, *Circinella muscae*, *Mucor hiemalis* f. *hiemalis*, *M. subtilissimus*, *Rhizopus microsporus* var. *chinensis*, *R. oryzae* and *Syncephalastrum racemosum* showed positive protease activity. Their halo reached up to 9 cm (total diameter of Petri plate) after 7 days of incubation.

Protease activity in Mucoralean fungi has also been reported by several authors in the past years for *R. oryzae* (16), *R. chinensis* (31), *S. racemosum* (9), *Mucor hiemalis* f. *hiemalis*, *M. hiemalis* f. *luteus*, *M. subtilissimus* (2), in accordance with the results obtained in this research.

The results of the present study showed that several species of Mucorales are able to infect food products derived from maize. The great majority of the isolated Mucorales species are reported here for the first time on these substrata. The detection of protease activity indicates that those species of Mucorales may be able to produce this enzyme. However, specific studies should be carried out in order to verify the real potential of

**Table 1.** Number of Mucorales (CFU/g) isolated from corn meal, cooked cornflakes, and maize flour using surface and depth plate methods.

Species/substrate	Corn meal			Cooked cornflakes			Maize flour			Total
	I	II	III	I	II	III	I	II	III	
<i>Absidia cylindrospora</i> Hagem	0	0	3	0	0	0	0	2	5	10
<i>Mycocladius corymbifer</i> (Cohn) Váňová	17	1	1	0	0	0	0	1	0	20
<i>M. hyalospora</i> (Saito) J. H. Mirza	1	3	0	0	0	0	0	0	0	4
<i>M. ramosus</i> (Zopf) Váňová	7	0	11	0	0	0	0	0	0	18
<i>Circinella muscae</i> (Sorokin) Berl. & De Toni	0	0	1	0	0	0	0	0	0	1
<i>Mucor amphibiorum</i> Shipper	1	0	0	0	0	0	0	0	0	1
<i>M. subtilissimus</i> Oudem	0	2	2	0	0	0	0	0	0	4
<i>M. hiemalis</i> f. <i>hiemalis</i> Wehmer	1	0	2	0	0	0	0	0	0	3
<i>M. hiemalis</i> f. <i>luteus</i> (Linnem.) Schipper	1	0	0	0	0	0	0	0	0	1
<i>Syncephalastrum racemosum</i> Cohn ex J. Schröt	1	0	0	0	0	0	0	0	0	1
<i>Rhizopus microsporus</i> var. <i>chinensis</i> (Saito) Schipper & Stalpers	0	0	0	3	0	0	0	0	0	3
<i>R. microsporus</i> var. <i>rhizopodiformis</i> (Cohn) Schipper & Stalpers	1	0	0	0	0	0	0	0	0	1
<i>R. oryzae</i> Went. & Prinsen Geerl	0	0	3	3	0	11	0	0	2	19
Total	30	6	23	6	0	11	0	3	7	86
Percentage	68.60%			19.77%			11.63%			

I, II, III = different packages from each corn product (substrate).

enzyme production. This is the first report of proteolytic activity for *Absidia cylindrospora* and *Circinella muscae*.

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### RESUMO

#### Isolamento de Mucorales de milho processado (*Zea mays* L.) e seleção quanto à atividade proteásica

Mucorales foram isolados da farinha de milho, fubá e flocos de milho pré-cozidos pelos métodos de plaqueamento em superfície e em profundidade. *Rhizopus oryzae*, *Circinella muscae*, *Mucor subtilissimus*, *Mucor hiemalis* f. *hiemalis*, *Syncephalastrum racemosum*, *Rhizopus microsporus* var. *chinensis* e *Absidia cylindrospora* exibiram atividade proteásica.

**Palavras-chave:** Zygomycetes, alimento, contaminação, isolamento

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