Plant Archives Vol. 20, Special Issue (AIAAS-2020), 2020 pp. 367-370 *ISSN 0972- 5210*

PHYSIOLOGICAL STUDIES OF SOME MUCORACEOUS STRAINS OF WESTERN UTTAR PRADESH

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Abstract

Members of the order Mucorales come within the realm of "Mucoraceous Fungi". Mucoraceous fungi have been found quite suitable for producing food, bioproducts including fuels like ethanol, fatty acids, lactic acid and fumaric acid at industrial scale and several extracellular enzymes. Utilization of Mucorales for all these biotechnological processes would necessarily involve their ample growth on suitable culture media. These fungi, commonly known as 'sugar fungi', grow profusely on media having simple organic compounds but exhibit considerable variability in their specific requirements for nutrients, pH, temperature etc. In the present study influence of these factors on growth of mucoraceous fungi isolated from soils of western Uttar Pradesh has been recorded. All test fungi exhibited best growth on fructose, maltose and glucose as carbon nutrient source while none of the strains exhibited best growth on media containing sucrose. The optimum set of conditions for each mucoraceous strain has also been worked out.

Keywords: Mucoraceous fungi, sugar fungi, soil, carbon and nitrogen, pH, temperature.

Introduction

The "Fungi" constitute an independent kingdom of highly diverse eukaryotes with immense ecological and economic impact. Zygomycota is one of the four phyla of Fungi recognized in the Dictionary of the Fungi (9th Edition: Kirk *et al.*, 2001). Mucoraceous fungi, constitute order Mucorales under the phylum Zygomycota. Mucoraceous fungi have been found quite suitable for producing food, bioproducts including fuels (Satari and Karimi, 2018; Molaverdi *et al*., 2019) and several industrially important acids utilizing inexpensive resources (Li and Yong, 2020). Utilization of Mucorales for all the biotechnological process would necessarily involve their growth on suitable culture media. The Mucorales exhibit wide diversity in their growth requirements (Pawlowska *et al*., 2019) although, many of these are quite selective with regards to their nutrients. Apart from this, their growth is influenced by several factors like pH and temperature. For instance, *Rhizomucor pusillus* is thermophilic but *Mucor hiemalis* is psychrotolerant.

Therefore, the present study was conducted to study the different physiological aspects like carbon and nitrogen nutrition, C:N ratio, temperature and pH of selected mucoraceous fungi.

Materials and Methods

Collection of soil samples

Soil samples were collected from soils, covering six districts of western U.P., namely Muzaffarnagar (29°28′N 77°42′E), Meerut (28.99°N 77.70°E), Shamli (29.45°N 77.32°E), Baghpat (28.95°N 77.22°E), Hapur (28.73°N 77.77°E) and Ghaziabad (28.67°N 77.42°E). A total of thirty soil samples, five soil samples from each of the six districts, were taken from apparently non-polluted agricultural lands. The surface layer of the soil was removed with the help of a trowel to remove extraneous litter/organic matter. Soil samples were then collected aseptically and brought to the laboratory for the isolation and further experiments.

Isolation of fungi from soils

All the five soil samples collected from the each district were mixed thoroughly to obtain one composite sample, thus obtaining six composite soil samples. Suspensions of 1:100, 1:1000, 1:10000 and 1:100000 dilutions were prepared using serial dilution method. Culturing was carried out on Potato Dextrose Agar medium (Thom and Raper, 1949) in order to isolate as many desired strains from each composite soil sample. The Petri dishes (in triplicate) containing medium (20 ml of cooled and sterilized PDA with 0.03 g of Rose Bengal) and the inocula (one ml aliquot) were incubated at 25±1°C for 3-8 days. A complete record of fungal species and their numbers in each case was maintained. The identification of the fungal species was done on the basis of their morphology and cultural characteristics following Gilman (1957), Tandon (1968), Ellis (1971, 1976), Subramanian (1971), Hesseltine and Ellis (1973), Domsch *et al.* (1980) and Nagamani *et al*. (2006). The total number of colonies and the number of colonies of individual fungal species growing were recorded.

Effect of different carbon and nitrogen nutrient sources

Axenic suspension cultures of selected strains were studied to ascertain the effects of different carbon and nitrogen

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nutrient sources on the growth of these fungal strains. Czapek‟s Dox broth was used as the basal medium. For the study of carbon nutrition, Czapek‟s Dox broth (without the carbon source) was used. To the basal medium sucrose, glucose, fructose and maltose were added as a sole sugar sources at a time, so as to supply 12.63 g of carbon per litre. For the study of nitrogen nutrition, Czapek‟s Dox broth (without the nitrogen source) was used. To the basal medium sodium nitrate, ammonium nitrate, potassium nitrate and urea were added as sole nitrogen sources at a time, so as to supply 0.49 g of nitrogen per litre.

Effect of different carbon and nitrogen sources on selected mucoraceous fungi was observed in terms of dry weight of biomass produced. 50 ml of a given medium were poured in each of sterilized 150 ml flasks. Material for inoculation was taken from the margin of actively growing young colonies (4 mm in diameter) in all the cases. The pH was adjusted to 6.0 in all cases. The experiments were run in triplicate and all the flasks were incubated for 10 days at 25+1°C. At the end of 10th day, fungal mats were collected on Whatman's filter paper, subsequently dried in an electric oven at 70°C for two consecutive days and kept in desiccators to cool at room temperature. The growth records were maintained by weighing the dried fungal mats on a digital balance.

Effect of different carbon and nitrogen ratios on the growth of selected strains

To evaluate the effect of different carbon and nitrogen nutrient ratios, three variants of the Czapek's Dox broth were prepared, with varying carbon and nitrogen nutrient concentrations. Standard Czapek's Dox broth was considered as 'N' (Normal). The medium containing half of the standard $NaNO_3$ concentration *i.e.* 1.5 g was taken as N/2 while that containing 0.75 g $NaNO_3$ was taken as N/4. Growth of the strains under test was assessed in these types of media of different C:N ratios following method described above.

Effect of variations in temperature

Each of the flasks containing 20 ml of Czapek's Dox broth was inoculated with 4 mm disc from the axenic culture of test fungi. Out of 12 flasks for one test fungi, 3 flasks were incubated at 20°C, 3 at 25°C, 3 at 30°C and 3 at 35°C for 5-7 days. After incubation, the contents of the flasks were filtered through pre-dried, pre-weighed and sterilized filter papers followed by drying of the mycelia mats on the filter paper at 70°C for 48 hrs. The weight of the (Whatman filter paper + dry mycelium) – weight of the filter paper, provided an idea of mycelial growth. Similar procedure was repeated for each subset containing different fungal strains.

Effect of variations in pH

To assess the effect of variations in pH on the growth of selected mucoraceous fungal taxa, four types of flasks containing Czapek's Dox broth of different pH (pH 6.0, pH 6.5, pH 7.0 and pH 7.5) were prepared. 50 ml of medium of given pH were added to each of a set of 150 ml capacity flasks. Every flask of a given subset was inoculated with 4 mm disc of a given strain (in triplicate). The flasks were incubated at 25±1°C for 5-7 days, the mycelium was harvested and its dry weight determined as described earlier. The media of pH 6.5, 7.0 and 7.5 were also utilized similarly.

Results and Discussion

A total of 29 taxa were isolated during the present study, out of which 10 taxa (including three genera *i.e. Cunninghamella*, *Mucor* and *Rhizopus*) belongs to order Mucorales. These includes-(i) *Cunninghamella elegans* Lendner; (ii) *Mucor hiemalis* Wehmer; (iii) *Mucor mucedo* Fresenius; (iv) *Mucor plumbeus* Bonorden; (v) *Mucor racemosus* Fresenius; (vi) *Rhizopus arrhizus* Fischer strain 1; (vii) *Rhizopus arrhizus* Fischer strain 2; (viii) *Rhizopus microsporus* Tieghem (ix) *Rhizopus stolonifer* Ehrenberg strain 1 and (x) *Rhizopus stolonifer* Ehrenberg strain 2. Moreover, *Mucor hiemalis, Mucor racemosus* and *Rhizopus arrhizus* remained persistent throughout the whole area of study and were recorded from each of the six districts while *Cunninghamella elegans* turned out to be the least frequent taxon, recorded from Ghaziabad district only.

Effect of different carbon and nitrogen sources on the growth of mucoraceous fungi

Effect of different combinations of carbon sources and nitrogen sources on the growth of ten strains of fungi was investigated. The results are represented in the table 1. The comparative growth of selected strains with different combinations of carbon and nitrogen sources is as under-

a) *Cunninghamella elegans*: Maltose+Urea >Maltose+KNO_3> Sucrose+Urea>Glucose+KNO_3

b) *Mucor hiemalis*: Fructose+NH_4NO_3>Fructose+$NaNO_3$> Fructose+KNO_3>Sucrose+$NaNO_3$

c) *Mucor mucedo*: Fructose+NH_4NO_3 >Maltose+KNO_3 >Sucrose+$NaNO_3$>Sucrose+KNO_3

d) *Mucor plumbeus*: Maltose+KNO_3 > Fructose+Urea > Sucrose+NH_4NO_3 > Sucrose+Urea

e) *Mucor racemosus*: Maltose+$NaNO_3$ > Maltose+Urea > Glucose+Urea > Glucose+NH_4NO_3

f) *Rhizopus arrhizus* strain 1: Glucose+$NaNO_3$ >Fructose+Urea>Sucrose+NH_4NO_3>Fructose+KNO_3

g) *Rhizopus arrhizus* strain 2: Maltose+Urea >Glucose+Urea>Sucrose+NH_4NO_3 >Glucose+KNO_3

h) *Rhizopus microsporus*: Maltose+NH_4NO_3 >Maltose+Urea>Sucrose+$NaNO_3$ >Glucose+$NaNO_3$

i) *Rhizopus stolonifer* strain 1: Glucose+$NaNO_3$ >Maltose+KNO_3>Maltose+$NaNO_3$ >Glucose+Urea

j) *Rhizopus stolonifer* strain 2: Glucose+$NaNO_3$ >Glucose+KNO_3>Sucrose+$NaNO_3$ >Glucose+NH_4NO_3

Effect of different carbon:nitrogen ratios on the growth of isolated mucoraceous fungi

Table 2 represents the effect of different C:N ratios on the growth of 10 isolated mucoraceous strains under study. In general, there was a decrease in the mycomass with decrease in nitrogen ratio *i.e.* increase in C:N ratio in the medium except in the case of *Mucor hiemalis*. The mycomass of different species in N medium were as under-

M. racemosus > *M. plumbeus* > *R. stolonifer* strain 1 > *M. hiemalis* > *R. arrhizus* strain 1 = *R. arrhizus* strain 2 > *R. microsporus* > *C. elegans* > *R. stolonifer* strain 2

In general, the species of *Mucor* exhibited better growth than the species of *Rhizopus* and *Cunninghamella* on all the three media.

Effect of temperature variations on the growth of isolated mucoraceous fungi

The effect of temperature variations on the growth of mucoraceous fungi under investigation are presented in table 3. *Cunninghamella elegans*, *M. hiemalis*, *R. microsporus* and *Rhizopus arrhizus* exhibited their best growth at 35°C. Below 30°C, the growth of all the fungal strains except *M. hiemalis* decreased with decrease in temperature. In the case of *M. hiemalis* more growth was exhibited at 20°C than at 25°C. Maximum growth at 35°C was exhibited by *M. hiemalis* followed by *R. arrhizus* strain 2, *C. elegans* and *R. microsporus*. At 30°C, maximum growth was exhibited by *M. mucedo* followed by *M. racemosus*, *M. plumbeus*, *R. arrhizus* and *R. stolonifer*. The best overall growth performance was of *M. mucedo* at 30°C while the least growth was exhibited by *C. elegans* at 20°C.

Effect of pH on the growth of isolated mucoraceous fungi

Cunninghamella elegans and *Rhizopus arrhizus* strains exhibited their maximum growth at pH 7.0. The remaining 8 fungal strains under test exhibited maximum growth at pH 7.5. None of the strains exhibited their best growth at pH 6.0 or 6.5. Of course, all the strains could grow on the media of pH 6.0 to 7.5. The growth of *Cunninghamella elegans*, *Mucor racemosus*, *M. plumbeus*, *Rhizopus arrhizus* strain 2 was very meager on the medium of pH 6.0. The maximum growth was recorded for *Mucor mucedo*, closely followed by *Rhizopus arrhizus* strain 1 at pH 7.5. The results are presented in table 4.

Conclusion

The main objective of this study was to work out the optimum conditions (carbon and nitrogen sources, pH and temprature) for the cultivation of the fungal strains under test. The optimum set of conditions for each mucoraceous strain has been worked out as under:

a) *Cunninghamella elegans*: Maltose+Urea, pH 7, Temp. 35°C

b) *Mucor hiemalis*: Fructose+NH4NO3, pH 7.5, Temp. 35°C

c) *Mucor mucedo*: Fructose+NH4NO3, pH 7.5, Temp. 30°C

d) *Mucor plumbeus*: Maltose+KNO3 , pH 7.5, Temp. 30°C

e) *Mucor racemosus*: Maltose+NaNO3, pH 7.5, Temp. 30°C

f) *Rhizopus arrhizus* strain 1: Glucose+NaNO3, pH 7.5, Temp. 30°C

g) *Rhizopus arrhizus* strain 2: Maltose+Urea, pH 7, Temp. 35°C

h) *Rhizopus microsporus*: Maltose+NH4NO3, pH 7.5, Temp. 35°C

i) *Rhizopus stolonifer* 1: Glucose+NaNO3, pH 7.5, Temp. 30°C

j) *Rhizopus stolonifer* 2: Glucose+NaNO3, pH 7.5, Temp. 30°C

References

Domsch KH, Gams W and Anderson T (1980). Compendium of Soil Fungi. Vols. I & II. Academic Press, London, N. Y.

Gilman JC (1957). A Manual of Soil Fungi. Iowa State University Press, U. S. A.

Hesseltine CW and Ellis JJ (1973). Mucorales. In: "*The Fungi"* (eds. Ainsworth, G.C., Sparrow F.K. and A.S. Sussman), Vol. 4b, Academic Press, New York, pp. 187-217.

Kirk PM, Cannon PF, David JC and Stalpers JA (2001). Ainsworth & Bisby's Dictionary of the Fungi *Ninth Edition*. CAB International, Wallingford, Oxon, UK.

Li X and Yong Q (2020). Production of fumaric acid from lignocellulose by *Rhizopus oryzae*. *Chemistry and Industry of Forest Products*, **40:** 1-7.

Molaverdi M, Karimi K, Mirmohamadsadeghi S and Galbe M (2019). High titer ethanol production from rice straw via solid-state simultaneous saccharification and fermentation by *Mucor indicus* at low enzyme loading. *Energy Conversion and Management*, **182:** 520-529.

Nagamani A, Kunwar IK and Manoharachary C (2006). Handbook of Soil Fungi. I.K. International Pvt. Ltd. New Delhi, Mumbai, Banglore.

Pawłowska J, Okrasińska A, Kisło K, Aleksandrzak-Piekarczyk T, Szatraj K, Dolatabadi S and Muszewska A (2019). Carbon assimilation profiles of mucoralean fungi show their metabolic versatility. *Scientific reports*, **9:** 1-8.

Raper KB and Thom C (1949). A Manual of Penicillia. Williams and Wilkins Co., Baltimore, Md.

Satari B and Karimi K (2018). Mucoralean fungi for sustainable production of bioethanol and biologically active molecules. *Applied Microbiology and biotechnology*, **102:** 1097-1117.

Subramanian CV (1971). Hyphomycetes. Indian Council of Agricultural Research, New Delhi.

Tandon RN (1968). Mucorales of India. Indian Council of Agricultural Research, India.

Table 1: Effect of different carbon and nitrogen sources on the growth of mucoraceous fungi

Carbon source	Nitrogen source	Fungal strain									
		Cunninghamella elegans	*Mucor hiemalis*	*Mucor mucedo*	*Mucor plumbeus*	*Mucor racemosus*	*Rhizopus arrhizus* strain 1	*Rhizopus arrhizus* strain 2	*Rhizopus microsporus*	*Rhizopus stolonifer* strain 1	*Rhizopus stolonifer* strain 2
Sucrose	$NaNO_3$	0.211	0.321	0.268	0.196	0.111	0.286	0.177	0.316	0.123	0.268
	KNO_3	0.188	0.1.6	0.241	0.276	0.222	0.201	0.147	0.123	0.130	0.172
	NH_4NO_3	0.197	0.233	0.198	0.308	0.145	0.305	0.281	0.276	0.183	0.144
	Urea	0.307	0.159	0.207	0.277	0.127	0.196	0.231	0.167	0.216	0.217
Glucose	$NaNO_3$	0.276	0.285	0.107	0.185	0.217	0.409	0.147	0.307	0.396	0.489
	KNO_3	0.296	0.093	0.096	0.182	0.231	0.222	0.276	0.265	0.187	0.370
	NH_4NO_3	0.188	0.263	0.150	0.279	0.281	0.170	0.243	0.158	0.187	0.230
	Urea	0.277	0.140	0.079	0.143	0.282	0.166	0.300	0.177	0.287	0.135
Fructose	$NaNO_3$	0.126	0.476	0.157	0.238	0.212	0.088	0.176	0.187	0.230	0.240
	KNO_3	0.251	0.412	0.235	0.269	0.079	0.287	0.161	0.185	0.075	0.079
	NH_4NO_3	0.099	0.513	0.389	0.149	0.263	0.180	0.091	0.143	0.175	0.123
	Urea	0.279	0.287	0.157	0.358	0.187	0.356	0.199	0.112	0.281	0.159
Maltose	$NaNO_3$	0.228	0.310	0.083	0.109	0.314	0.105	0.135	0.119	0.361	0.096
	KNO_3	0.327	0.211	0.361	0.381	0.212	0.210	0.142	0.128	0.381	0.158
	NH_4NO_3	0.226	0.089	0.217	0.101	0.141	0.329	0.169	0.399	0.284	0.134
	Urea	0.354	0.121	0.110	0.127	0.295	0.219	0.339	0.392	0.171	0.124

Table 2: Effect of different C:N ratios on the growth of selected fungi

Fungal Strain	Mycelial dry wt. (g)		
	N	N/2	N/4
Cunninghamella elegans	0.233	0.213	0.145
Mucor hiemalis	0.300	0.343	0.280
Mucor mucedo	0.278	0.134	0.122
Mucor plumbeus	0.385	0.158	0.211
Mucor racemosus	0.634	0.457	0.323
***Rhizopus arrhizus* strain 1**	0.245	0.187	0.122
***Rhizopus arrhizus* strain 2**	0.245	0.232	0.158
Rhizopus microsporus	0.242	0.222	0.101
***Rhizopus stolonifer* strain 1**	0.345	0.246	0.193
***Rhizopus stolonifer* strain 2**	0.134	0.074	0.014

Table 3: Effect of temperature variations on the growth of fungi

Fungal Strain	Mycelial dry wt. (g)			
	20°C	25°C	30°C	35°C
Cunninghamella elegans	0.044	0.233	0.436	0.665
Mucor hiemalis	0.533	0.300	0.654	0.754
Mucor mucedo	0.221	0.278	0.965	0.578
Mucor plumbeus	0.244	0.385	0.786	0.687
Mucor racemosus	0.356	0.634	0.866	0.506
***Rhizopus arrhizus* strain 1**	0.143	0.245	0.767	0.677
***Rhizopus arrhizus* strain 2**	0.134	0.245	0.345	0.675
Rhizopus microsporus	0.223	0.242	0.234	0.434
***Rhizopus stolonifer* strain 1**	0.255	0.345	0.774	0.574
***Rhizopus stolonifer* strain 2**	0.124	0.134	0.467	0.367

Table 4: Effect of pH variation on the growth of fungi

Fungal Strain	Mycelial dry wt. (g)			
	pH 6.0	pH 6.5	pH 7.0	pH 7.5
Cunninghamella elegans	0.041	0.233	0.287	0.256
Mucor hiemalis	0.354	0.300	0.433	0.568
Mucor mucedo	0.241	0.278	0.565	0.875
Mucor plumbeus	0.069	0.385	0.266	0.433
Mucor racemosus	0.089	0.634	0.264	0.764
***Rhizopus arrhizus* strain 1**	0.204	0.245	0.398	0.865
***Rhizopus arrhizus* strain 2**	0.043	0.245	0.543	0.356
Rhizopus microsporus	0.092	0.242	0.432	0.454
***Rhizopus stolonifer* strain 1**	0.197	0.345	0.399	0.645
***Rhizopus stolonifer* strain 2**	0.102	0.134	0.195	0.445