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Isolation and Characterization of Mesophilic and Thermophilic fungi from Agricultural fields

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ABSTRACT
Physiochemical parameters of samples and isolation of mesophilic and thermophilic fungi were undertaken from agricultural field. Thirty-three fungal forms were isolated from seventy-eight samples collected from different localities. The frequency of occurrence of isolated fungal forms was calculated. The physiochemical parameters of the collected sites were also evaluated with help of Eutech Oakton PCS Tester TM 35. The pH, moisture, EC, TDS and salinity were found to range from 5.2-8.5, 20-70 %, 235-428 µS, 412-915 ppm and 0.10-0.410 ppm respectively. Out of the isolated forms Aspergillus and Penicillium were more frequently encountered. Maximum growth of fungi wasevaluated on YpSs medium under the stationary condition.
Keywords: Mesophilic fungi, Thermophilic fungi, Physiochemical parameters, Agricultural field

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INTRODUCTION

Agriculture waste is one of the major environmental waste and pollutant, as well as it is one of the cheap natural resources which may be used for various activities. There is a huge amount of agro-waste produced every year in India as well as all over the world. Agro-waste can be utilised to produce industrially important enzymes through microbes. Important enzymes are xylanase (hemi-cellulase), cellulase, beta-glucosidase etc. Xylanase and beta-glucosidase have many biotechnological as well as industrial applications. Important biotechnological applications of these enzymes includes removal of non-reducing terminal glucosyl residues from saccharides; functions in glycolipid and exogenous glycoside metabolism in animals; cell wall lignifications; cell wall β-glucan turnover; phytohormone activation; release of aromatic compounds in plants; biomass conversion besides havingmany other agricultural and industrial applications.

Xylanase has very important role in pulping and bleaching of pulps[1] Xylanase also helps in pre-bleaching process [2] and bread making. Xylan contributes as dietary fibre thus is having impact on biochemical and physiological process in humans and otherorganisms [1]. With above applications in mind fungal strains were isolated and characterized from agricultural field having wastes for further testing of their xylanase activity.

MATERIAL AND METHODS

Different samples of agro-waste and soil were collected from Pantnagar, Pattarchata, Rudrapur, Kaladungi,Baelbadao, Bijnauragricultural fields. 78 different samples were collected aseptically from above region and brought aseptically to the laboratory. Samples were analysed for physio-chemical parameters like; pH, temperature, EC, TDS, and salinity by using Eutech Oakton PCS tester T35 analyser (Table 1). They were kept in deep freezer for further periodical studies.

Samples were used for microbial analysis, using different enriched media by direct and dilution plate methods [3].Petri plates were incubated at 30°C and 45 °C for isolation of fungal forms. Three replicates were taken for all the samples. Fungal forms isolated in pure form were transferred onto agar slants for further studies. Isolated fungi were identified using morphological, cultural characteristics, camera-lucida and photomicrograph by comparing with available literature. Isolated fungal forms were checked for

their growth on YpSs medium. Colony diameter method [4] was used to observe periodic growth at 45° C to check their nature.15 ml of solid medium was poured into Petri-plates and inoculated with single point inoculation.

RESULTS

Physiochemical parameters of the samples were analysed using multi-parameter device Eutech Oakton PCS tester T35 analyser and standard methods. The results have been presented in table 1. It is apparent from the tabulated data that the lowest pH 7.8was found from site 5 (Kaladungi) while the highest pH (8.5) was recorded from site 1. All the sites showed alkaline pH. The moisture content of site-3 (Haldi), site-5 and site -6 were the lowest, whereas the highest moisture content was recorded from site2 and site4. The highest salinity was recorded from site1 (Table 1) followed by site4 and 6, while the least was from site5. E.C and TDS was recorded maximally from site1 and site 7 i.e., 928 µS and 915 (ppm) respectively.

Seventy-eight different samples collected were analysed for their mycocommunities. The isolated forms with their frequency of occurrence from different sites have been presented in Table 2. Among the isolated forms the highest number of fungi belonged to the group Deutromycotina. Amongst the Zygomycotina*, Rhizopus* was more prevalent. *Mucor spp.* was isolated in maximum frequency from site-5 followed by 7 and 1, whereas they were isolated minimally from site-3 and 4. The highest frequency of *Rhizopus* was obtained at site-7, 6 and 1,while it was least on site-5.Amongst the Ascomycotina, species of *Chaetomium* were more prevalent whereas, frequency of *Thermomyces sp.* was the highest amongst the isolated forms in this group. *Chaetomium thermophile* was more prevalent from site-4, while *Chaetomium funicola* was prevalent from site-3. Amongst the isolated Ascomycotina, frequency of occurrence of *Chaetomium funicola* was the least.

Members of the Deutromycotina were dominant, of which the *Aspergillus* were the most prevalent. *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus*, *Penicillium notatum* and *Trichoderma viride* were more frequently isolated forms. Species of *Humicola* and *Aspergillus* were more prevalent at site-4, while the species of *Sporotrichum* and *Trichoderma* were prevalent at site-6. *Humicola sp.* was not isolated from site-7, while at site-3, *H. fuscoatra* was only isolated species. Among the isolated forms *Aspergillus niger, A. flavus , A. candidus , A. oryzae, A.terreus, Mucor spp., Rhizopus, Monellia, Penicillium and Trichoderma*were mesophilic while *Humicola insolens, H.grisea, H.fuscoatra, Sporotrichum thermophile , Chaetomium thermophile, C. globosum and C. funicola* were thermophilic as per their temperature requirements.

DISCUSSION

During the present study, Deuteromycotina was found as a dominant group with their fungal forms encountered more frequently. Amongst the Deuteromycotina, species of *Aspergillus* were the most frequent [5], whereas *Curvularia* showed the least occurrence*. Trichoderma viride, T.reesei, Humicola lanuginosa, Mucor spp. Rhizopus spp. Aspergillus niger, A.flavus, A.fumigatus, Monellia spp.*were the other frequently observed fungi. Isolation of these fungi in highfrequency indicted that these may be more active in degradation of agricultural waste so may have high enzymatic machinery for this purpose. It has already been reported that *Aspergillus niger, Trichoderma viride and Humicola spp.* showed high xylanase activity [6]. Agricultural waste comprises of hemi-cellulose (xylan), lignin and other components [6].Most of the fungus obtained showed the mesophilic nature and very few thermophilic fungi were also observed, indicating that environmental conditions of the agricultural waste in the field most of the months of the year are mesophilic while during summer months thermophilic forms were also encountered. Members of Deutromycotinai.e., *Aspergillus* [2], *Penicillium* [8] and *Trichoderma*[9] are mesophilic while *Humicola*, *Curvularia* and *Sporotrichum* [11] are thermophilic.Isolated members of Ascomycotina were mostly thermophilic while that of Zygomycotina were mesophilic. Site-1 showed the highest EC(928 µS) and pH i.e., 8.5 which showed the favourable condition for most of the fungus and amongst them *Aspergillus fumigatus* showed the highest frequency of occurrence followed by *Aspergillus niger* and *Penicillium notatum* while for other fungus such as *Aspergillus tamarii, Humicolafuscoatra, Sporotrichum thermophile and Trichodermahamatum*don't favour these parameter. Site-7 showed highest TDS (915ppm) least salinity (0.10 ppt) and pH 7.7 which favour the growth of *Rhizopus sps, Aspergillus nidulans and Aspergillus terreus.*

Table 1: Physiochemical parameters of different studied sites

Site	Form	Place	pH	Salinity (ppt)	Moisture %	EC (µS)	TDS (ppm)
S1	Soil	Site-1, Pantnagar University	8.5	0.41	60	928	541
S2	Soil	Site-2,Rudrapur	8.1	0.13	70	270	550
S3	Soil	Site-3,Haldi	7.9	0.14	20	300	619
S4	Soil & debris	Site-4,Aamdanda	7.9	0.17	70	369	645
S5	Debris	Site-5,Kaladungi	7.8	0.11	20	314	907
S6	Soil	Site-6,Baelpadao	7.9	0.16	20	364	622
S7	Soil	Site-7,Kaladungi	7.7	0.10	35	309	915

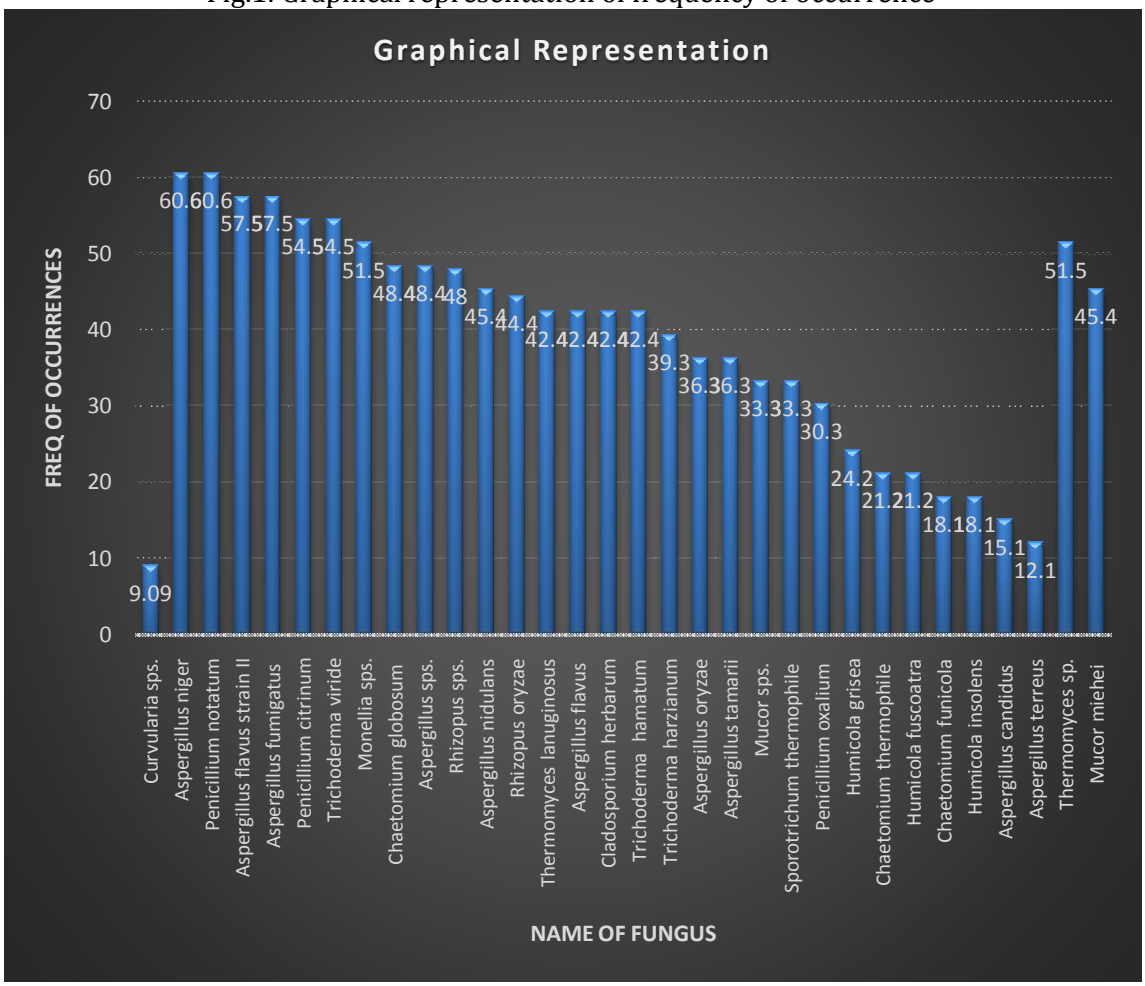
Table 2: Nature of fungi

S.no.	Name of Fungi	Mesophilic(M) or Thermophilic(T)
I.	**ZYGOMYCOTINA**	
1.	*Mucor miehei*	**M**
2.	*Mucor sps.*	**M**
3.	*Rhizopus oryzae*	**M**
4	*Rhizopus sps.*	**M**
5	*Rhizopus stolonifer*	**M**
II.	**ASCOMYCOTINA**	
6	*Chaetomium globosum*	**T**
7	*Chaetomium thermophile*	**T**
8	*Chaetomium funicola*	**T**
9	*Thermomyces sp.*	**T**
10	*Thermomyceslanuginosus*	**T**
III.	**DEUTROMYCOTINA**	
11	*Aspergillus candidus*	**M**
12	*Aspergillus flavus*	**M**
13	*Aspergillus sps.*	**M**
14	*Aspergillus flavus strain II*	**M**
15	*Aspergillus nidulans*	**M**
16	*Aspergillus oryzae*	**M**
17	*Aspergillus niger*	**M**
18	*Aspergillus tamarii*	**M**
19	*Aspergillus terreus*	**M**
20	*Aspergillus fumigatus*	**M**
21	*Cladosporium herbarum*	**M**
22	*Curvulariasps.*	**T**
23	*Humicola fuscoatra*	**T**
24	*Humicola grisea*	**T**
25	*Humicola insolens*	**T**
26	*Monellia sps.*	**M**
27	*Penicillium citrinum*	**M**
28	*Penicillium notatum*	**M**
29	*Penicillium oxalium*	**M**
30	*Sporotrichum thermophile*	**T**
31	*Trichoderma hamatum*	**M**
32	*Trichoderma harzianum*	**M**
33	*Trichoderma viride*	**M**

Table 3: Frequency of occurrence of different fungus

Sl.No.	Name of Fungus	Frequency of Occurrence (%)							Frequency %
I.	**ZYGOMYCOTINA**	Site-1	Site-2	Site-3	Site-4	Site-5	Site-6	Site-7	
1.	*Mucor miehei*	20	13	0	0	33	13	20	45.4
2.	*Mucor sps.*	18	27	9	0	36	9	0	33.3
3.	*Rhizopus oryzae*	21.4	10	14.2	21.4	7.14	0	28.5	44.4
4	*Rhizopus sps.*	31.2	6.25	12.5	18.7	0	12.5	12.5	48
5	*Rhizopus stolonifer*	0	37.5	0	0	0	37.5	25	24
II.	**ASCOMYCOTINA**								
6	*Chaetomium globosum*	25	0	25	18.7	0	31.2	0	48.4
7	*Chaetomium thermophile*	0	0	28.5	42.8	0	28.5	0	21.2
8	*Chaetomium funicola*	0	28.5	42.8	0	28.5	0	0	18.1
9	*Thermomyces sp.*	11.7	23.5	17.6	11.7	11.7	0	17.6	51.5
10	*Thermomyceslanuginosus*	21.4	14.2	0	21.4	0	28.5	14.25	42.4
III.	**DEUTROMYCOTINA**								
11	*Aspergillus candidus*	0	40	60	0	0	0	0	15.1
12	*Aspergillus flavus*	21.4	0	14.2	28.5	14.2	21.4	0	42.4
13	*Aspergillus sps.*	25	12.5	0	18.7	18.7	6.25	18.7	48.4
14	*Aspergillus flavus strain II*	21.05	21.05	0	15.7	24.3	15.7	0	55.5
15	*Aspergillus nidulans*	20	0	13.3	26.6	13.3	0	26.6	45.4
16	*Aspergillus oryzae*	33.3	0	16.6	25	0	25	0	36.3
17	*Aspergillus niger*	15	0	20	15	15	10	25	60.6
18	*Aspergillus tamarii*	0	25	0	33.3	16.6	25	0	36.3
19	*Aspergillus terreus*	40	20	0	0	0	0	40	12.1
20	*Aspergillus fumigatus*	26.3	15.7	0	21.05	15.7	21.05	0	57.5
21	*Cladosporium herbarum*	0	28.5	21.4	0	0	28.5	21.4	42.4
22	*Curvulariasps.*	0	33.3	0	66.6	0	0	0	9.09
23	*Humicola fuscoatra*	0	0	14.2	28.5	42.8	0	0	21.2
24	*Humicola grisea*	25	37.5	0	12.5	0	25	0	24.2
25	*Humicola insolens*	33.3	16.6	0	0	50	0	0	18.1
26	*Monellia sps.*	17.6	29.4	0	17.6	11.7	23.5	0	51.5
27	*Penicillium citrinum*	0	16.6	22.2	5.55	16.6	22.2	16.6	54.5
28	*Penicillium notatum*	20	15	25	10	5	20	0	60.6
29	*Penicillium oxalium*	20	30	0	20	20	0	10	30.3
30	*Sporotrichum thermophile*	0	27.2	18.1	0	18.1	27.2	9.09	33.3
31	*Trichoderma hamatum*	0	21.4	14.2	28.5	14.2	21.4	0	42.4
32	*Trichoderma harzianum*	23.07	0	30.7	15.3		30.75	5	39.3
33	*Trichoderma viride*	22.2	11.1	16.6	16.6	22.2	11.1	0	54.5

Fig.1: Graphical representation of frequency of occurrence



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