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Bioprospecting of a phyllospheric microorganism-*Aspergillus niger* for crude xylanase enzyme production

Suganya W
Assistant Professor in Botany, Government Arts College Coimbatore, Tamil Nadu, India

Abstract
Filamentous fungi are predominantly present in the phyllospheric region of the plant. The present study aims in the Biosprospection of phyllospheric microorganisms *Aspergillus niger* for crude Xylanase Enzyme production. The fungus isolated were then subjected to grow on different cultural conditions like Culture medium, pH, Temperature and incubation periods in order to optimize the conditions that is well suited for the maximum enzyme production. Czapek's Dox medium with pH 6.5, temperature 35° C and Incubation period 144 hrs showed the maximum crude xylanase production.

Keywords: bioprospection, phyllosphere, xylanase

Introduction
Phyllosphere is one of the major microbial habitat on the earth, that provides shelter to diverse and complex microbial communities like bacteria, yeast, fungi, actinomycetes, algae, protozoa etc. The leaf surface contains different types of stimulatory and inhibitory substances that regulate the microbial colonization on Phyllosphere. Leaves constitute a very large microbial habitat. The microbial communities of leaves are diverse and include many different genera of bacteria, filamentous fungi, yeasts, algae, and, less frequently, protozoa and nematodes. Filamentous fungi are considered transient inhabitants of leaf surfaces, being present predominantly as spores, whereas rapidly sporulating species and yeasts colonize this habitat more actively (Andrews *et al.*, 2000) [1].

Most of the fungal isolated were belongs to the class Ascomycetes having septate hyphae and asexual spores like conidiospore (micro and macro). Determination of colonization frequency (CF %) indicated that the dominant fungal genera on the phyllosphere were the members of *Penicillium, Aspergillus and Fusarium*. Although bacterial community numerically dominated the leaf surface, different types of filamentous fungi, molds, yeast and other sporulating fungal species belongs to the class Ascomycetes and Deuteromycetes also actively colonize on *P. bombycina* phyllosphere (Bhuyan *et al.*, 2013) [4]. Microbial biodiversity become an integral part of the human welfare because of their significant role in agriculture, industry, medicine, food industry, textiles, biotransformation and bioremediation. In the present study, also an attempt has been made to study the varied uses of phyllospheric microorganisms in industries.

Materials and Methods
Flora of varied regions are collected through field visits namely Top slip, Maruthamalai hills, Siruvani, Vellingiri hills and Tamil nadu Agricultural University Coimbatore. Small portion of the plant (namely leaf, Stem, Flowers) according to the need were aseptically placed in the polythene bags, levelled carefully and brought to the laboratory. A modified leaf washing technique was adopted to isolate the phyllospheric as well as the phylloplane mycoflora. Disc of 3 mm diameter were cut randomly from leaves of each variety with sterile cork borer. The discs were placed in 250 ml conical flask containing 100 ml sterile distilled water and shake for 20 minutes to get a homogenous suspension. One ml suspension was pipette out in to sterilized petriplates. The plates were poured with Potato dextrose media, Peptone dextrose agar medium containing rose bengal and streptomycin (Martin, 1952) [8], Czapeks dox agar medium, Malt Agar Medium and Nutrient broth medium and mixed thoroughly. It was kept undisturbed in dust free chamber at room temperature for 7 days. On incubation, the fungal colonies and the bacterial colonies were observed and identified on the basis of morphological and reproductive characters (Gilmam, 1957, Subramanian CV 1971, Barnett 1972) [5, 13, 3].

Bioprospection
The isolates that are isolated has been bioprospected for their applications in the industry. As xylanase enzyme have plenty of applications in industries, an attempt has been made to test the organisms for xylanase production. From the literature surveyed *Aspergillus niger* has been extensively studied for the xylanase enzyme production. Hence in the present study *Aspergillus niger* was chosen as the fungal source for crude enzyme production.

Crude Xylanase production
The method of Bailey *et al.* (1992) [2] has been used for estimating the amount of xylanase. Oat spelt xylan was used as the substrate for xylanase assay and the amount of xylose released was measured by DNS method of Miller (1959) [10].

Culture Medium

The following media's namely Czapeks dox medium, Potato Dextrose Agar medium, Potato Dextrose broth medium, Mandel and Reese medium, Yeast extract medium and Carter and Bull medium were used for standardisation of the medium for crude xylanase enzyme production.

Effect of pH, Temperature and Incubation Period

The culture medium (Czapeks dox) were subjected to different pH conditions using buffers with a pH range of 3.5-9 and it was inoculated with the mycelial spores of *Aspergillus niger* and incubated for 6 days to find out the suitable pH for maximum enzyme production. The medium was incubated at different temperatures of 25ºC, 30 ºC, 35 ºC, 40 ºC, 45 ºC. and the organisms were then subjected to different incubation periods namely 96hrs, 120hrs, 144hrs, 168hrs, 192 hrs. in order to standardize the optimum cultural conditions for the maximum enzyme production.

Results and Discussion

Microbes dominate world not only as plant and animal pathogens, but also as a source of food and other useful products and more importantly as the critical components of natural and agricultural ecosystems. Presently, microbes have contributed much to the development of various industrial chemicals, materials and processes, agri-food commodities and human health products.

In the present study, the organism was subjected to different cultural conditions like medium, pH, temperature, incubation period – in order to standardise the best cultural conditions for the maximum crude enzyme production. The fungus was inoculated in five different media's namely Czapeks dox medium, Potato Dextrose broth medium, Mandel and Reese medium, Yeast extract medium and Carter and Bull medium. It was inferred that Czapek's dox medium showed the maximum crude enzyme production. (Table -1).

In general, acidic pH of cultural medium was found to be favourable for the xylanase production. The optimum pH for the xylanase production was pH 4-4.9 in *Arachinotus* sps pH 5-5.5 in *Penicillium janthinellum* (Palma *et al.*, 1996) [11] *Aspergillus japonicas* (Maria and Samia 2005) [9] pH 5.0 in *Fusarium* (Gupta *et al.* 2009) *Aspergillus brasilensis* and *Aspergillus niger* (Pederson *et al.* 2007) [12] and *Aspergillus ficuum* (Lu *et al.* 2008) [7]. In the present study also the pH of Czapeks dox medium was maintained using buffers with a pH range of 3.5-9 and it was inoculated with the mycelial spores of *Aspergillus niger* and incubated for 6 days to find out the suitable pH for maximum enzyme production. The results are presented in the Table-2. It was inferred that pH 6.5 was found to be the best pH for maximum Crude xylanase enzyme production.

Each organism prefers to have temperature optima for enzyme production. In the present study also an attempt was made to find out the effect of temperature of the culture medium on xylanase production by *Aspergillus niger*. The medium was incubated at different temperatures of 25ºC, 30 ºC, 35 ºC, 40 ºC, 45 ºC. It was inferred that at 35 ºC maximum enzyme production was recorded. (Table-3) The organisms were then subjected to different incubation periods namely 96hrs, 120hrs, 144hrs, 168 hrs, 192 hrs. The incubation period of 144 hrs showed the maximum crude enzyme production. (Table -4).

Table 1: Crude Xylanase production by *Aspergillus niger* using Different medium

S. No	Name of the Medium	Xylanase Production U/ml
1	Czapeks dox medium	0.22 ± 0.01^{a}
2	Potato Dextrose broth medium,	0.16 ± 0.01^{b}
3	Mandel and Reese medium	0.04 ± 0.00^{d}
4	Yeast extract medium	0.06 ± 0.01^{b}
5	Carter and Bull medium	0.08 ± 0.01^{d}

Values given in each cell is the mean ± SD of three replicates [a-g] Mean values within a column with no common superscript differ significantly ($p<0.05$).

Table 2: Crude Xylanase production by *Aspergillus niger* using Different pH Conditions

S.No	pH	Xylanase Production U/ml
1	3.5	0.01 ± 0.00^{f}
2	4.0	0.04 ± 0.02^{f}
3	4.5	0.08 ± 0.03^{e}
4	5.0	0.03 ± 0.01^{f}
5	5.5	1.5 ± 0.46^{c}
6	6.0	4.1 ± 0.36^{b}
7	6.5	5.96 ± 0.31^{a}
8	7.0	2.27 ± 0.05^{d}
9	7.5	0.07 ± 0.01^{f}
10	8.0	0.05 ± 0.01^{f}
11	8.5	0.02 ± 0.01^{f}
12	9.0	0.02 ± 0.01^{f}

Values given in each cell is the mean ± SD of three replicates [a-g] Mean values within a column with no common superscript differ significantly ($p<0.05$)

Table 3: Crude Xylanase production by *Aspergillus niger* using different temperature

S. No	Temperature (Degree celsius)	Xylanase Production U/ml
1	25	0.23 ± 0.01^{d}
2	30	1.82 ± 0.41^{b}
3	35	4.85 ± 0.42^{a}
4	40	0.87 ± 0.48^{c}
5	45	0.13 ± 0.02^{e}

Values given in each cell is the mean ± SD of three replicates[a-g] Mean values within a column with no common superscript differ significantly ($p<0.05$)

Table 4: Crude Xylanase production by Aspergillus niger using Different Incubation Period

S. No	Incubation period (hrs)	Xylanase Production U/ml
1	96	0.36 ±0.05d
2	120	1.79 ±0.33c
3	144	3.86 ±0.42a
4	168	0.78 ±0.46b
5	192	0.13 ±0.02e

Values given in each cell is the mean ± SD of three replicates[a-g] Mean values within a column with no common superscript differ significantly ($p<0.05$)

Conclusion

The phyllospheric isolate –*Aspergillus niger* proved to be the best isolate for crude xylanase enzyme production. Since the

enzyme is of crude form, it is cost effective and can be bio prospected for varied other applications in food, fodder, textile paper industries etc.

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