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Isolation and Identification of Glucose Oxidase Hyper Producing Strain of *Aspergillus niger*

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AN and WA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors IUH and HM managed the analyses of the study, helped in paper write up and managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: Glucose oxidase is an enzyme with large scale applications in various industries. It is also used in several diagnostic kits which makes it medically important as well. Our aim was to isolate indigenous glucose oxidase hyper producing strain of *Aspergillus niger* from different soil samples of Punjab, Pakistan.
Study Design: An experimental study.
Place and Duration of Study: Institute of Industrial Biotechnology, GC University, Lahore from March 2011 to July 2012.
Methodology: Two hundred and seventy nine fungal strains were isolated from soil of different localities of Punjab. Isolates were screened for glucose oxidase production using submerged fermentation. Glucose oxidase hyper producer isolate was identified using morphological and molecular techniques i.e. 18S rDNA. DNA was isolated and amplified using PCR. Gene sequencing was done and homology analysis was studied. Rate of glucose oxidase production was also analysed.
Results: Glucose oxidase hyper producing isolate was identified as *A. niger* A247 strain. This strain gave best reproducible results (145.22 ±0.034 U/g of cell mass) after 72 hrs of fermentation at 30ºC and at a medium pH of 7.2.
Conclusion: Our results indicate the natural ability of *A. niger* to produce Glucose oxidase in large quantity instead of using genetic manipulation techniques.

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Keywords: Glucose oxidase; Aspergillus niger; Hyper producer; 18S r DNA; Enzyme.

1. INTRODUCTION

Aspergillus niger is a filamentous fungus belonging to phylum Ascomycota. It produces microscopic conidia on conidiophores that are produced asexually. Hyphae possess septa and are hyaline. They are supported at their base by foot cells from which conidiophores originate. It possesses long, double-walled, smooth and colorless to brown conidiophores [1]. The major morphological distinction of *A. niger* from other species of *Aspergillus* is the presence of carbon black or dark brown spores on biseriate phalides, which are arranged in a globose head radiating from a vesicle conidiophore. It is commonly found in mesophilic environments such as soil, plants and enclosed air environments. It is capable of surviving in various environments, it is not only a xerophilic fungus, but is also a thermo tolerant organism. It is because of this property that it exhibits a high tolerance to freezing temperatures [2].

Aspergillus niger fermentation is generally recognized as safe by the United States Food and Drug Administration under the Federal Food, Drug, and Cosmetic Act [2] so the metabolites produced by *A. niger* can be used in food and medical industries without any objection. β-D-glucose: oxygen oxidoreductase (EC 1.1.3.4) commonly known as glucose oxidase is the enzyme which has the capacity to oxidize glucose. It is an important enzyme due to a vast number of applications in various fields. Most important application of glucose oxidase is in diagnostics, as it is utilized on commercial scale in colorimetric diagnostic kits for the determination of glucose in blood, serum or plasma. It is used for the removal of glucose or oxygen to improve flavour, colour, texture and shelf life of various products in the food industry as well. Recently, glucose oxidase has also been used in biofuel cells and for the purpose of preservation of food [3]. It is also used in textile industry and environmental monitoring [4]. Glucose oxidase is also used for gluconic acid production and food preservation because of the production of hydrogen peroxide [5] and in the detection of glucose released from liposomes in liposomal immunoassays [6].

2. MATERIALS AND METHODS

2.1 Isolation of Fungal Strains

Fungal strains were isolated from soil samples collected from different localities of Punjab i.e. Lahore and three different sugar mills and their vicinities i.e. Patoki Sugar mill (80 km from Lahore), Haseeb Waqas Sugar mill (Nankana Sahab, 106 km from Lahore) and Ashraf Sugar mill, Bahawalpur (440 km from Lahore). Soil suspensions were prepared by adding 1 gram of soil sample into 9 ml of sterile saline solution in a test tube and vortexed for 15 mins. Serial dilutions were prepared up to 10^{-6} and spread over potato dextrose agar (PDA) plates which were incubated for 5 days at 30°C. The colonies showing resemblance with *A. niger* were picked up and sub-cultured on PDA slants and stored at 4°C. Preliminary identification of isolates was done on the basis of hypahal and spore morphology [7].

2.2 Screening of Fungal Isolates

2.2.1 Inoculum development

Ten ml of sterilized distilled water was transferred to each slant having profused conidial growth on its surface. Sterilized inoculating needle was used to break the clumps of conidia. The tube was vortexed to make homogenous mixture of conidial suspension. The number of conidia was counted with the help of Haemocytometer slide bridge (Neubauer improved, precicder HBG. Germany). The concentration of the final suspensions were adjusted to approximately 1.6x 10^7 spores per ml.

2.2.2 Submerged fermentation

Glucose oxidase fermentation was carried out using submerged fermentation technique having 25 ml of medium in 250 ml Erlenmeyer flasks. A modified fermentation medium (M-8) consisting of Glucose 6 %, peptone 0.3 %, $(NH_4)_2HPO_4$ 0.04 %, KH_2PO_4 0.0188 %, $MgSO_4$.7H2O 0.0156 %, $CaCO_3$ 3.5 % [8] was used. Medium was sterilized and after cooling the medium at room temperature, 1.0 ml of conidial suspension was aseptically transferred to each flask. The flasks were incubated in shaking incubator for 72 hrs at 30°C and 200 rpm. After incubation, fermented broth was acidified with HCl, prior to filtration, in order to convert insoluble $CaCO_3$ to soluble $CaCl_2$. The mycelia were filtered through cheese cloth and washed twice with deionized water and then freeze dried to constant weight. Known quantity of biomass was homogenized in known volume of citrate phosphate buffer (pH 7.0). The disrupted cell mass was centrifuged at 6000 rpm for 10 min. The extract obtained was used for the estimation of glucose oxidase [9] and total protein [10]. The fermentation broth was used to estimate residual sugars in the medium [11].

2.3 Glucose Oxidase Assay

2.5 ml O-dianisidine, 0.3 ml glucose and 0.1 ml peroxidase solution (1 mg/ml) were added to the cuvette with the help of a micropipette and allowed to equilibrate at 25ºC and was zeroed at 436 nm. Then 0.1 mL of sample extract was added to the cuvette and mixed the reaction mixture thoroughly. The absorbance per minute was determined using 7 cycles of 00.30 seconds each, using spectrophotometer (Model: 7200 Aquarius, Cecil CE, England). ΔA/min over liner portion of reaction was measured.

$$\text{Activity of glucose oxidase} = \frac{\Delta OD/min \times TV \times \text{dilution factor}}{8.3 \times SV \times \text{cell mass (g)}}$$

Where:

$$\Delta OD/min = OD2 - OD1 / T2 - T1$$

TV = Total Volume (3.0 ml)

8.3 = millimolar extinction coefficient for O-dianisidine (oxidised) at 436 nm.

SV = Sample Volume (0.1ml)

2.4 18S rDNA identification

2.4.1 Isolation of DNA

DNA of the fungi was isolated using the protocol previously reported by [12] with slight modifications. In particular, chopping of fungal mat (0.3-0.5 g on dry mass basis) was done using pestle and mortar with liquid nitrogen. Chopped material was transferred to 50 ml centrifuge tube containing 5 ml of extraction buffer (200mM tris-Cl of pH 8.5, 250 mM NaCl, 25mM EDTA and 0.5 % SDS) along with 2.5 ml of 3M sodium acetate of pH 5.2. Centrifuge tube was placed at -20°C for 10 min. After that centrifugation was done at 8000rpm for 20 min and supernatant was transferred to a new tube and equal volume of isopropanol was added to the tube. After 5 min again centrifugation was done to get pellet of DNA. Pellet of DNA was washed thrice using ethanol. After washing pellet was re dissolved in 200 µl of deionized water.

2.4.2 Polymerase chain reaction (PCR)

PCR conditions and primers for amplification of 18S rDNA were used as reported by Melchers et al. [13]. The primers were; Forward; 5'- CCTGGTTGATCCTGCCAGTA- 3' and Reverse; 5' – GCTTGATCCTTCTGCAGGTT- 3'. The PCR mixture used was containing 1.4mM $MgCl_2$, 0.2mM dNTPs, 50 pmol of each primer,0.5 µg of DNA and 3 units of Taq polymerase. The PCR system (Biometra®) was used for amplification. Initial denaturation, annealing and extension steps were performed at 95, 52 and 72°C, respectively. Analysis of the PCR products was performed by electrophoresis on 1% agarose gels using standard conditions according to Sambrook and Russel (2001).

2.4.3 Gene sequencing

The 18S rDNA product was extracted from agarose gel with the help of gel extraction kit (Fermentas). The PCR product was sent to Institute of Biochemistry and Biotechnology (IBBT), University of Veterinary and Animal Sciences (UVAS) Lahore for sequencing.

2.5 Homology Analysis

Multiple sequence alignment of obtained sequence was done using Clustal W2 at www.expasy.ch comparing with some sequences of *Aspergillus* species taken from NCBI data bank. Neighbour joining method was used to prepare dendogram in order to find homology as reported by Saitou and Nei and Jukes and cantor [14,15] using MEGA 5.0 computer software.

2.6 Statistical Analysis

Computer software Costat, cs6204W.exe was used for the statistical analysis [16]. Significance difference among replicates has been presented as Duncan's multiple range tests in the form of probability (p) values.

3. RESULTS AND DISCUSSION

3.1 Isolation and Screening

Two hundred and seventy nine fungal isolates were isolated from soil i.e. W1 to W150 isolated from Lahore and its localities and A 151 to A 279 isolated from three different sugar mills of Punjab. These isolates were morphologically identified as *Aspergillus niger* and were screened for their capability of producing glucose oxidase (Table 01) using submerged fermentation with modified M-8 medium. Three isolates i.e. A241, A222 and A247 showed significant biomass and maximum glucose oxidase production respectively as compared to their sister isolates (Table 01). These three isolates were selected and re cultured repeatedly under same conditions and it was found that A247 showed reproducible results with maximum production of GOD i.e. 145.22 ±0.034 U/g of cell mass. Hence A247 was selected for further studies.

Table 1. Screening of different strains of *A. niger*

Strain	Cell mass (g/25ml of medium)	Glucose oxidase activity (U/g of cell mass)	Strain	Cell mass (g/25ml of medium)	Glucose oxidase activity (U/g of cell mass)
W1	0.74±0.048	18.60±0.13	**W141**	1.32±0.019	43.29±0.14
W2	0.77±0.025	31.98±0.10	**W142**	2.10±0.097	77.32±0.30
W3	1.0±0.04	39.65±0.05	**W143**	NIL	NIL
W4	1.45±0.095	51.35±0.10	**W144**	1.85±0.135	62.75±0.28
W5	1.33±0.0436	43.89±0.07	**W145**	1.07±0.064	66.38±0.25
W6	1.09±0.062	67.09±0.15	**W146**	2.04±0.158	28.51±0.11
W7	0.89±0.026	79.56±0.07	**W147**	1.05±0.087	35.10±0.20
W8	1.07±0.080	73.46±0.15	**W148**	NIL	NIL
W9	0.92±0.064	67.13±0.31	**W149**	1.28±0.032	71.44±0.25
W10	1.54±0.087	59.87±0.23	**W150**	NIL	NIL
W11	1.87±0.038	51.23±0.07	**A151**	1.15±0.13	52.25±0.12
W12	1.13±0.146	36.78±0.10	**A152**	1.03±0.10	61.33±0.20
W13	0.94±0.115	57.78±0.22	**A153**	1.54±0.15	78.41±0.12
W14	1.91±0.185	31.90±0.17	**A154**	1.25±0.08	63.17±0.25
W15	0.86±0.058	111.56±1.47	**A155**	1.19±0.15	42.25±0.10
W16	1.51±0.112	28.34±0.31	**A156**	0.077±0.17	54.71±0.10
W17	2.08±0.173	11.09±0.10	**A157**	1.04±0.15	59.01±0.12
W18	1.05±0.045	82.46±0.25	**A158**	1.31±0.08	65.63±0.05
W19	0.96±0.020	75.90±0.30	**A159**	1.55±0.11	44.94±0.18
W20	1.14±0.061	55.78±0.22	**A160**	0.098±0.04	66.12±0.20
W21	1.29±0.035	71.34±0.07	**A161**	1.04±0.18	58.93±0.12
W22	0.85±0.016	47.89±0.10	**A162**	1.21±0.08	55.12±0.10
W23	1.43±0.10	78.43±0.09	**A163**	1.47±0.15	92.57±0.13
W24	0.91±0.06	67.89±0.16	**A164**	1.33±0.04	57.19±0.20
W25	1.09±0.038	41.98±0.11	**A165**	1.84±0.18	82.15±0.07
W26	1.34±0.031	23.87±0.21	**A166**	1.01±0.07	44.12±0.11
W27	1.06±0.098	46.23±0.13	**A167**	1.15±0.13	67.15±0.03
W28	1.36±0.058	67.12±0.05	**A168**	1.37±0.06	76.38±0.21
W29	0.96±0.028	35.30±0.10	**A169**	1.14±0.15	48.21±0.12

Table 1 continues……..

W30	1.08±0.038	44.21±0.25	**A170**	0.85±0.07	39.90±0.18
W31	1.23±0.065	21.67±0.03	**A171**	1.21±0.15	62.69±0.13
W32	1.61±0.028	67.90±0.12	**A172**	1.24±0.06	61.78±0.20
W33	0.94±0.072	43.98±0.07	**A173**	2.24±0.15	120.41±0.12
W34	1.47±0.062	73.90±0.20	**A174**	1.07±0.01	45.17±0.21
W35	1.58±0.065	47.32±0.06	**A175**	1.23±0.17	55.19±0.27
W36	0.95±0.019	81.49±0.10	**A176**	NIL	NIL
W37	0.06±0.007	9.43±0.081	**A177**	1.65±0.15	62.34±0.23
W38	1.84±0.104	19.63±0.30	**A178**	1.29±0.05	66.11±0.20
W39	1.24±0.123	61.59±0.26	**A179**	2.14±0.15	78.51±0.12
W40	0.90±0.026	32.83±0.12	**A180**	1.46±0.08	55.26±0.25
W41	1.23±0.102	NIL	**A181**	1.15±0.13	42.77±0.23
W42	1.30±0.01	54.19±0.21	**A182**	1.07±0.06	67.28±0.15
W43	1.26±0.105	61.03±0.15	**A183**	1.44±0.18	78.50±0.12
W44	0.85±0.022	78.49±0.12	**A184**	0.085±0.08	45.70±0.05
W45	1.37±0.050	49.41±0.11	**A185**	1.77±0.13	68.15±0.23
W46	1.57±0.020	59.21±0.15	**A186**	1.17±0.06	56.38±0.15
W47	1.08±0.074	112.92±0.11	**A187**	2.14±0.15	98.41±0.12
W48	1.35±0.101	78.82±0.17	**A188**	1.71±0.08	77.21±0.25
W49	1.20±0.087	66.39±0.15	**A189**	NIL	NIL
W50	1.03±0.112	53.21±0.13	**A190**	1.79±0.15	61.73±0.23
W51	0.089±0.004	NIL	**A191**	1.27±0.06	69.36±0.20
W52	0.88±0.029	75.83±0.11	**A192**	2.04±0.18	58.51±0.12
W53	1.063±0.083	71.39±0.09	**A193**	1.47±0.08	47.10±0.20
W54	NIL	NIL	**A194**	1.76±0.13	64.75±0.23
W55	1.907±0.042	66.39±0.13	**A195**	2.15±0.06	89.30±0.16
W56	1.267±0.061	61.02±0.12	**A196**	1.71±0.15	32.77±0.21
W57	0.98±0.083	91.77±0.18	**A197**	0.076±0.18	41.02±011
W58	1.18±0.058	63.88±0.20	**A198**	1.14±0.13	49.38±0.11
W59	1.05±0.090	44.12±0.11	**A199**	1.38±0.16	38.20±0.21
W60	1.86±0.089	29.98±0.21	**A200**	1.22±0.09	61.82±0.20
W61	NIL	NIL	**A201**	NIL	NIL
W62	NIL	NIL	**A202**	1.85±0.13	69.21±0.28
W63	NIL	NIL	**A203**	1.69±0.24	57.83±0.21
W64	1.19±0.004	57.62±0.20	**A204**	1.36±0.09	51.62±0.21
W65	1.60±0.053	39.53±0.26	**A205**	0.088±0.13	14.53±0.11
W66	0.61±0.017	NIL	**A206**	1.91±0.11	66.71±0.15
W67	1.09±0.076	51.05±0.12	**A207**	1.08±0.054	31.83±0.24
W68	NIL	NIL	**A208**	1.02±0.10	28.43±0.13
W69	1.29±0.091	64.29±0.27	**A209**	1.36±0.12	71.93±0.19
W70	1.02±0.094	60.48±0.13	**A210**	1.29±0.15	53.77±0.23
W71	1.86±0.102	82.50±0.18	**A211**	1.14±0.054	89.30±0.23
W72	1.24±0.026	59.63±0.312	**A212**	2.12±0.18	78.41±0.31
W73	1.38±0.082	17.98±0.16	**A213**	NIL	NIL
W74	1.30±0.084	33.87±0.26	**A214**	NIL	NIL
W75	1.48±0.066	16.83±0.11	**A215**	NIL	NIL
W76	0.99±0.080	61.04±0.13	**A216**	NIL	NIL
W77	1.89±0.010	73.65±0.12	**A217**	0.049±0.107	22.93±0.21
W78	0.67±0.031	25.37±0.12	**A218**	0.031±0.231	30.59±0.203
W79	NIL	NIL	**A219**	1.04±0.119	56.29±0.14

Table 1 continues……..

W80	NIL	NIL	**A220**	1.135±0.168	71.51±0.20
W81	0.58±0.031	47.27±0.21	**A221**	1.132±0.190	119.37±0.24
W82	1.51±0.009	7.78±0.16	**A222**	1.44±0.13	136.20±0.17
W83	1.29±0.042	5.09±0.06	**A223**	1.87±0.10	50.49±0.21
W84	1.29±0.063	37.49±0.15	**A224**	0.87±0.066	59.32±0.18
W85	NIL	NIL	**A225**	1.34±0.135	67.21±0.24
W86	NIL	NIL	**A226**	1.68±0.064	85.71±0.087
W87	0.87±0.012	82.33±0.15	**A227**	1.91±0.071	59.31±0.142
W88	1.12±0.061	73.41±0.21	**A228**	1.27±0.178	76.81±0.19
W89	0.96±0.109	105.18±0.08	**A229**	1.11±0.103	42.19±0.098
W90	1.27±0.027	79.32±0.11	**A230**	NIL	NIL
W91	0.99±0.093	55.20±0.19	**A231**	0.061±0.21	19.77±0.11
W92	1.23±0.050	74.77±0.23	**A232**	0.078±0.11	21.98±0.206
W93	1.09±0.027	86.16±0.26	**A233**	1.08±0.14	75.19±0.271
W94	1.23±0.071	63.90±0.22	**A234**	1.19±0.172	31.70±0.056
W95	NIL	NIL	**A235**	1.37±0.13	61.33±0.28
W96	NIL	NIL	**A236**	0.098±0.23	59.69±0.16
W97	NIL	NIL	**A237**	1.72±0.15	53.29±0.23
W98	0.11±0.008	3.99±0.02	**A238**	1.31±0.117	68.71±0.174
W99	1.54±0.110	27.41±0.25	**A239**	1.68±0.23	79.46±0.19
W100	1.80±0.059	Nil	**A240**	1.90±0.71	101.03±0.136
W101	1.12±0.056	63.31±0.14	**A241**	2.27±0.18	129.48±0.112
W102	0.75±0.030	81.67±0.09	**A242**	1.41±0.105	65.93±0.056
W103	1.35±0.057	44.06±0.08	**A243**	0.058±0.044	31.47±0.169
W104	NIL	Nil	**A244**	1.179±0.12	44.39±0.183
W105	1.74±0.130	51.42±0.23	**A245**	1.63±0.069	48.62±0.207
W106	1.12±0.060	33.97±0.11	**A246**	NIL	NIL
W107	0.36±0.035	12.79±0.20	**A247**	1.97±0.021	145.22±0.034
W108	2.09±0.025	58.43±0.09	**A248**	1.31±0.172	62.75±0.23
W109	1.91±0.042	66.14±0.10	**A249**	1.27±0.029	117.51±0.064
W110	0.75±0.02	45.09±0.30	**A250**	1.11±0.17	57.22±0.22
W111	1.69±0.021	59.53±0.72	**A251**	0.81±0.066	47.16±0.21
W112	NIL	NIL	**A252**	1.01±0.117	76.12±0.109
W113	NIL	NIL	**A253**	1.67±0.12	73.59±0.167
W114	0.32±0.021	41.73±0.04	**A254**	1.44±0.138	28.39±0.191
W115	0.27±0.007	66.32±0.13	**A255**	1.32±0.126	44.28±0.08
W116	0.65±0.025	61.93±0.15	**A256**	1.13±0.031	66.51±0.20
W117	1.40±0.002	56.27±0.08	**A257**	1.34±0.142	47.61±0.18
W118	1.17±0.134	71.01±0.01	**A258**	0.071±0.061	51.77±0.13
W119	1.45±0.060	47.29±0.28	**A259**	NIL	NIL
W120	0.71±0.019	16.30±0.20	**A260**	1.49±0.105	74.27±0.22
W121	1.677±0.070	53.69±0.17	**A261**	1.77±0.061	58.73±0.14
W122	0.90±0.013	51.48±0.22	**A262**	1.94±0.098	36.83±0.32
W123	1.38±0.046	43.22±0.16	**A263**	1.61±0.067	53.19±0.27
W124	1.55±0.046	61.36±0.05	**A264**	1.43±0.131	72.35±0.209
W125	0.21±0.006	30.91±0.15	**A265**	0.41±0.06	47.39±0.183
W126	0.46±0.025	42.57±0.04	**A266**	2.24±0.117	51.73±0.21
W127	NIL	NIL	**A267**	0.45±0.037	31.29±0.101
W128	0.68±0.041	19.42±0.20	**A268**	1.17±0.035	42.75±0.213
W129	1.38±0.167	34.17±0.14	**A269**	1.61±0.034	66.32±0.220

Table 1 continues……..

W130	1.34±0.035	57.25±0.05	**A270**	1.04±0.178	47.51±0.212
W131	1.49±0.065	41.61±0.09	**A271**	1.15±0.077	55.16±0.105
W132	0.18±0.003	5.07±0.030	**A272**	2.04±0.131	41.73±0.254
W133	NIL	NIL	**A273**	0.63±0.04	67.22±0.163
W134	2.05±0.130	17.40±0.14	**A274**	NIL	NIL
W135	0.12±0.006	37.52±0.25	**A275**	1.21±0.17	58.27±0.154
W136	NIL	NIL	**A276**	0.93±0.06	22.74±0.11
W137	NIL	NIL	**A277**	1.75±0.15	63.40±0.29
W138	0.02±0.002	NIL	**A278**	1.05±0.031	43.91±0.24
W139	0.33±0.031	21.73±0.11	**A279**	0.89±0.013	31.10±0.16
W140	0.67±0.400	27.00±0.05			

3.2 Identification of Isolate A247

3.2.1 Macro and microscopic properties

Colonies of isolate A247 grew rapidly on PDA medium at 30°C and reached 21-23 mm in diameter after 7 days. The colonies were initially white and quickly became black with the conidial production (Fig. 01). Hyphae found to be septate, double layered and hyaline. Conidiophores were 949-1060 µm in length, hyaline and finely roughened, smooth-walled and had conical terminal vesicles (42 µm in diameter), which supported a single row of phialides with 14-16 µm in length. Brown conidia (3.3-5.2 µm in diameter) in long chains were spherical to ovoid and smooth or rough. Similar type of finding was reported by Onion et al. [7] for the identification of *A. niger*.

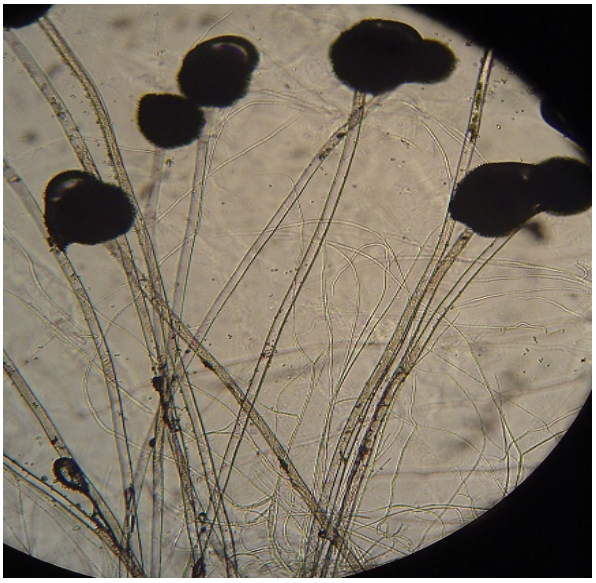

Fig. 1. Hyphae and conidiophores (40X)

3.2.2 18S rDNA identification

Chromosomal DNA of A247 isolate was isolated. The PCR was done to amplify the 18S rDNA which resulted in 1.8 kb band. This amplified fragment was then sequenced using

forward primer and a 648 base pair sequence was obtained. Multiple alignment of this sequence was carried out by comparing 19 other known sequences of *Aspergillus* species obtained from NCBI data bank. The results of multiple alignments were expressed as dendrogram (Fig 02). Results confirmed the isolate A247 as *A. niger* as it showed 98.6% similarility with *A. niger* strains TR-H and ETYB-13.

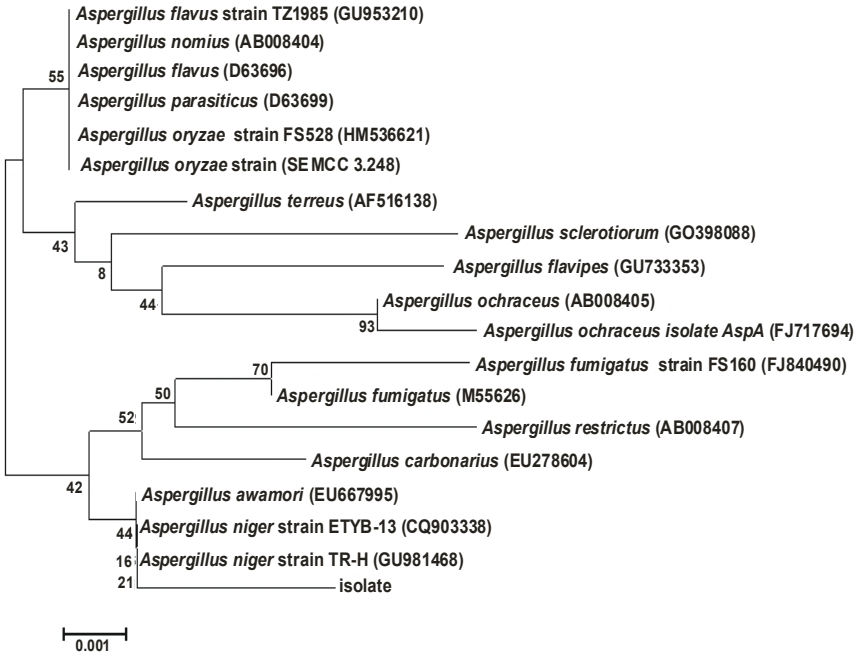

Fig. 2. Phylogenetic tree of *A. niger* A247 (isolate) with other species of Aspergillus genus.

3.3 Time Course of Glucose Oxidase Production

Time course of glucose oxidase production was studied up to 96 hrs using *A. niger* A247 strain and M-8 medium (Table 1). After 12 hrs of fermentation, considerable amount i.e. 0.36g of freeze dried cell mass was harvested and minute glucose oxidase activity was recorded (Fig 03). However, the cell mass and glucose oxidase activity was continued to increase with the passage of fermentation period and reached maximum at 72 hrs (1.97±0.01 g/25 ml) and (145.22 ± 0.03 U/g of cell mass), respectively. Total protein concentration was also increased on the same pattern and maximum protein production (3.98 mg/g of cell mass) was recorded after 72 hrs. Glucose oxidase production along with protein concentration remained stable with further increase in the time period and eventually started to decrease. This might be due to depletion of the nutrients from the medium or enzyme might start to denature. Similar types of results were reported with less activity (45.61 U/g of cell mass) of glucose oxidase as compared to our results by Hatzinikolaou and Macris, 1995. Activity of our enzyme is also greater by two folds than reported by Zubair et al. [17].

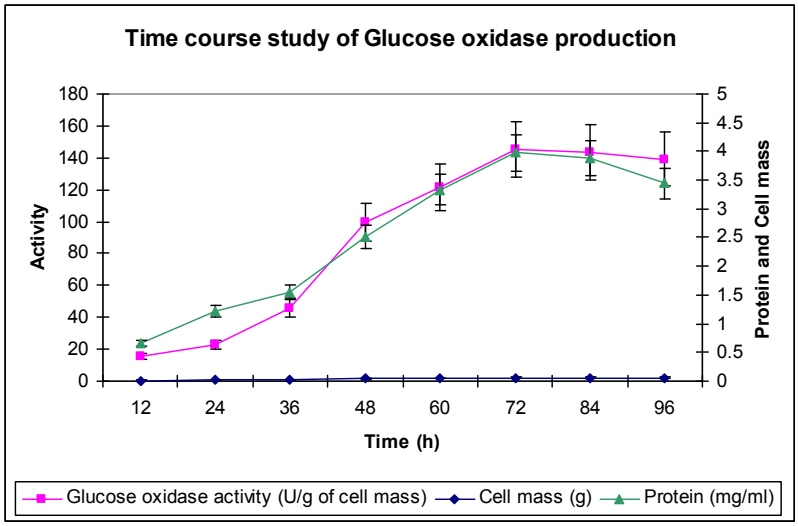

Fig. 3. Time course of Glucose oxidase, protein and cell mass production with *A. niger* A247.

Temperature 30°C; Agitation speed 200 rpm; pH 7.2. Y-error bars indicate the standard deviation (±SD) among the three parallel replicates, which differ significantly at p=0.05.

4. CONCLUSION

Our data suggests that locally isolated strain of *Aspergillus niger* is producing the enzyme glucose oxidase in large amount as compared to other isolated wild strains of *Aspergillus niger* reported in literature. This wild strain can be very beneficial in mass production of the particular enzyme at the industrial level.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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