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# Study of Effect of Various Carbon Sources on Biomass and Extracellular Protein of *Aspergillus Fumigatus Strain* (Mtcc 1811)

KEYWORDS: Aspergillus fumigatus Biomass Extracellular protein

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**ABSTRACT** *The filamentous fungus Aspergillus fumigatus is well-known as a producer of primary metabolites and extracellular proteins. Extracellular proteins of filamentous fungi are important for biotechnological and biomedical applications. Aspergillus fumigatus not only comprises an important class of organisms that have significant commercial relevance to the biotechnology industry, but also is a fungal pathogen in immunocompromised patients. Thus, in this study, we analyzed the biomass and extracellular proteins of Aspergillus fumigatus under different culture conditions using sucrose, glucose, lactose or xylose as a main carbon source. The range of variation of biomass was from 14.24 gm/lit to 9.34 gm/lit when grown on 3.0% of glucose as sole carbon source at pH 4 and on 4.0% sucrose as carbon source at pH 7 respectively. Upon variation of carbon source concentration, the highest amount, 155.88µg/40ml of extracellular protein was produced when 3.5% of lactose was used as sole carbon source at pH 5.*

## INTRODUCTION

The widespread use of fungi in different biotechnological processes can be attributed to their intrinsic characteristics as they are relatively easygoing organisms and most of them can be grown in fomenters in a quite cheap and easy way. Fungi are eukaryotes, and thus valuable expression hosts for proteins requiring elaborate posttranslational modification. Moreover, they produce a very large array of secondary metabolites, some of which have important activities. They can secrete an impressive amount of extracellular enzymes and proteins, generally referred to as secretome, which represents a powerful biochemical toolkit for the catalysis of a great number of valuable reactions. Aspergillus species such as A. niger and A. oryzae are known for their exceptional ability to secrete large amounts of homologous enzymes. For decades they have been commonly exploited as commercial production organisms for a variety of enzymes [1, 2].

Fungi secrete extracellular proteins or enzymes to enable them to harvest nutrients from the environment. Extracellular proteins of *Aspergillus terreus* grown on different carbon sources [3] as well as the ability of xylanolytic enzymes produced by *Aspergillus fumigatus* and *Aspergillus niveus* on cellulose pulp have been investigated [4]. In the case of pathogenic fungi the extracellular proteins can also be pathogenesis factors. Therefore the knowledge of secretome not only opens path for study of pathogenesis but also for diagnosis [5, 6, 7]. The secretion pattern in different conditions will give an insight for efficient production of specific secreted proteins [8] and lighten the different prospects in industrial production of such proteins. In this study, we investigated the effect of different carbon sources on production of biomass and secretory proteins under variation of pH.

## MATERIALS & METHODS

### Fungal culture

The fungal strain *Aspergillus fumigatus* strain (MTCC 1811) was obtained from Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh. The culture was grown in Czapek Dox (CD) medium (0.3% $NaNO_3$, 0.05% KCl , 0.05% $MgSO_4 \cdot 7H_2O$, 0.001% $FeSO_4 \cdot 7H_2O$ ,0.1% $K_2HPO_4$ and 3% Sucrose). The amount of sucrose was replaced by three different carbon sources namely glucose, lactose and xylose. Each experiment was performed in triplicate. 100 ml Flasks containing 50 ml Czapek dox medium for each sugar were inoculated with $10^6$ spores $ml^{-1}$ suspension. The flasks were incubated at 30 °C in a rotary shaker at 125 rpm for five days. The effect of pH was also studied for various carbon sources. The concentration variation of 3%, 3.5% and 4% of different carbon sources was also studied.

### Cell dry weight determination

Culture was centrifuged at 4,000 × *g* for 15 minutes. The supernatant was filtered though a dried, pre-weighed filter paper (Whatman No.1). The mycelia pellet was also transferred onto the filter paper. This was followed by washing with distilled water twice and then drying at 110 °C till the constant weight was achieved [9]. The weight was measured and the mycelia mass dry weight was calculated as a difference of two weights. Cell culture filtrate was used for protein analysis.

### Determination of protein concentration

Protein content of the culture filtrate was estimated by Lowry method [10] using Bovine serum albumin as standard. 1 mg /ml stock solution is prepared and from that stock solution various dilutions ranging from 0.1 mg/ml -1.0 mg/ml were prepared and standard plot was performed.

## RESULTS

In this study, we compared the effect of different carbon sources on protein secretion. This is useful for identifying changes in extracellular protein expression under different experimental conditions.

**Table 1** Effect of different carbon sources on biomass and extracellular protein concentration at various pH.

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| Experiment Name | pH | Biomass dry weight (gm/lit) Mean±SD | Total Amount of Protein in culture filtrate (µg/ 40 ml) Mean±SD |
|---|---|---|---|
| CD Sucrose | 4 | 12.86±0.40 | 144.78±2.77 |
| CD Sucrose | 5 | 12.34±0.43 | 147.49±3.86 |
| CD Sucrose | 6 | 9.65±0.32 | 139.82±6.23 |
| CD Sucrose | 7 | 8.67±0.21 | 129.01±5.76 |
| CD Lactose | 4 | 12.05±0.26 | 154.94±2.55 |
| CD Lactose | 5 | 11.75±0.18 | 153.67±4.86 |
| CD Lactose | 6 | 9.91±0.10 | 132.89±7.47 |
| CD Lactose | 7 | 9.94±0.06 | 131.16±3.62 |
| CD Xylose | 4 | 12.33±0.14 | 139.24±5.34 |
| CD Xylose | 5 | 11.99±0.11 | 137.14±2.34 |
| CD Xylose | 6 | 11.87±0.14 | 126.18±5.41 |
| CD Xylose | 7 | 10.63±0.12 | 116.39±6.73 |
| CD Glucose | 4 | 14.24±0.12 | 144.54±1.69 |
| CD Glucose | 5 | 13.40±0.22 | 147.92±6.37 |
| CD Glucose | 6 | 11.53±0.30 | 137.02±5.73 |
| CD Glucose | 7 | 9.94±0.08 | 127.36±2.85 |

Values are the mean±standard deviation of three independent experiments.

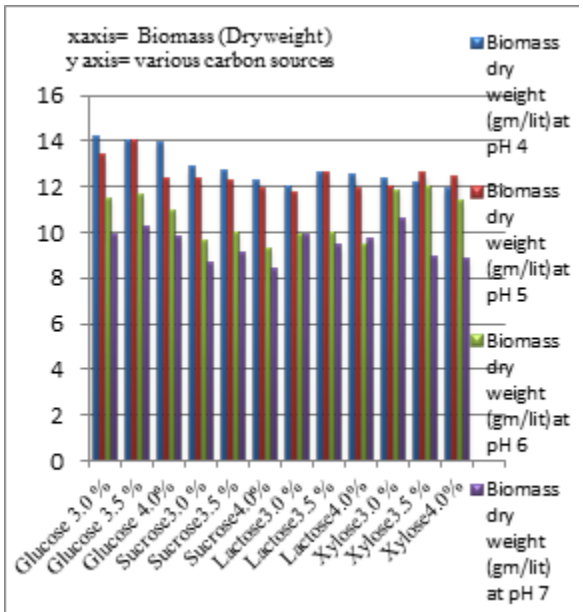


**Figure-1** Comparisons of effect of different concentration of various carbon sources at pH 4, 5, 6 and 7 on biomass produced.

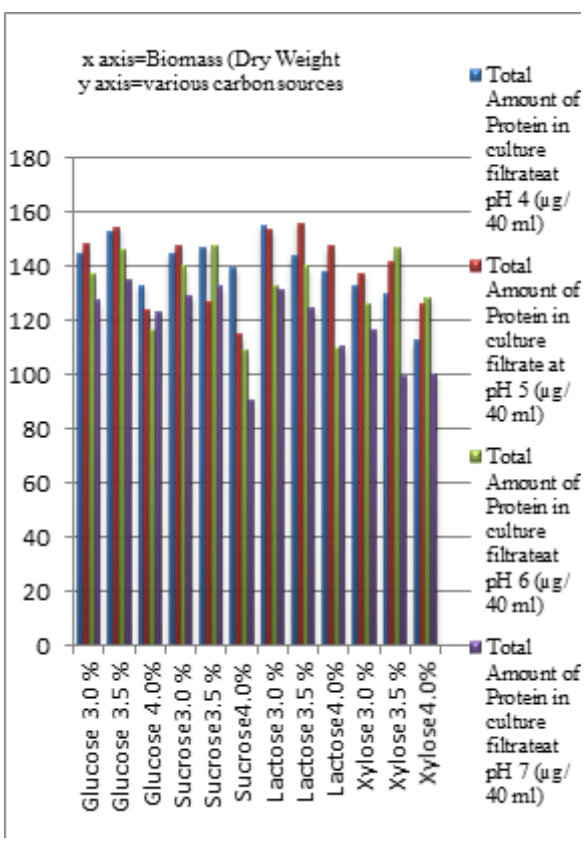


**Figure-2** Comparisons of effect of different concentration of various carbon sources at pH 4, 5, 6 and 7 on extracellular proteins produced.

The highest production of biomass, 14.24±0.12gm/lit, was observed when glucose was used as sole carbon source at pH 4. However, with relatively lower biomass of 12.05±0.26 gm/lit, the amount of secreted protein was highest, 154.94±2.55µg/40 ml, in presence of lactose at

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pH 4 (Table 1). The lowest amount of biomass of 9.34 gm/lit was produced with 4.0% sucrose as carbon source at pH 7 (Figure-1). Further, upon variation of carbon source concentration, the highest amount, 155.88µg/40 ml of extracellular protein was produced when 3.5% of lactose was used as sole carbon source at pH 5 (Figure-2).

## DISCUSSION

The comparison of the biomass produced and protein content in various culture conditions revealed that the fungal growth and extracellular protein production is influenced by factors like the carbon source [3], its concentration and the pH of the medium. The range of variation of biomass was from 14.24±0.12gm/lit to 9.34 gm/lit when grown on 3.0% of glucose as sole carbon source at pH 4 and on 4.0% sucrose as carbon source at pH 7 respectively. The largest biomass is not an indication of highest extracellular protein production as happened at pH 4 when grown in the CD glucose.

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