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Research Paper

A Comparison of Protein Extraction Methods Using Organic Solvents for Secretome of *Aspergillus Fumigatus* Strain (MTCC 1811)

Biology

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ABSTRACT

Sample preparation is a fundamental step in proteomic methodologies. Protein precipitation is frequently used to concentrate proteins and to remove interfering compounds. Various methods for protein precipitation are applied, which rely on different principles, have been compared for precipitation of extracellular proteins of Aspergillus fumigatus strain (MTCC 1811) as Aspergillus species comprise strains of medical and industrial importance. Secretory proteins were extracted using acetone, 2-propanol and chloroform/methanol when grown under different carbon sources. It was found that chloroform/methanol delivered the highest protein recovery of 86.78±0.57%, 85.55±0.59%, and 85.27±1.07% when lactose, glucose and sucrose respectively were used as sole carbon source. The lowest percentage recovery of extracellular proteins was 67.68±1.44%, when proteins were extracted using acetone from the filtrate of culture grown under sucrose as sole carbon source.

Introduction:

Filamentous fungi have received attention for protein production because of their high secretion capability and eukaryotic posttranslational modifications. *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]. Sample preparation is crucial for conducting reliable proteomic analysis [3, 4]. Samples should have a high-protein concentration and be free of salt and other interfering components, such as detergents, nucleic acids, lipids, *etc* [5, 6]. Precipitation is widely used for processing of biological molecules such as proteins to concentrate and fractionate the target molecule from various contaminants [7]. For example, in the biotechnology industry, protein precipitation is used to eliminate contaminants commonly contained in blood [8]. The first step is usually homogenisation or sonication followed by protein precipitation and solubilisation in a suitable buffer. Chloroform, methanol, acetone and isopropanol are common organic solvents used as protein precipitating reagents [9].

Fungi secrete extracellular proteins or enzymes to enable them to harvest nutrients from the environment. In the case of pathogenic fungi these enzymes can also be pathogenesis factors. The knowledge of secretome not only opens path for study of pathogenesis but also for diagnosis [10, 11, 12]. The secretion pattern in different conditions will give an insight for efficient production of specific secreted proteins [13]. It will lighten the different prospects in industrial production of such proteins. In this study, we investigated the efficiency of various methods for extracellular protein precipitation of *Aspergillus fumigatus* strain (MTCC 1811). Proteins were extracted using acetone, 2-propanol and chloroform/methanol when grown under different carbon sources. Some proteins were lost during sample preparation; precipitation followed by re-solubilisation in sample solution rarely gives a 100% yield.

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 medium under three different carbon sources, glucose, lactose and sucrose. 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. Culture was then centrifuged at 5,000 × g for 15 min, and the supernatant was filtered through a dried Whatman (No.1) filter paper and filtrate was used for protein analysis.

Protein precipitation

Three different precipitation procedures were used: acetone, 2-propanol and a mixture of chloroform and methanol.

Chloroform/methanol precipitation

Experiments were performed at room temperature. Four volumes of methanol were added to one volume of the protein sample, and the mixture was vortexed. One volume of chloroform was then added, and the mixture was vortexed. The sample was centrifuged at 10000×*g* for 5 min and the aqueous methanol layer was removed from the top of the sample. The proteins remained at the phase boundary between the aqueous methanol layer and the chloroform layer. Four volumes of methanol were added, and the mixture was vortexed. The sample was spun at 10000×*g* for 15 min. The supernatant was removed without disturbing the pellet, and the pellet was air dried.

Acetone precipitation

Experiments were performed at 4°C. Four volumes of ice-cold acetone containing 20 mM DTT were added to one volume of protein sample. The mixture was vortexed and incubated at –20°C for 1 h. This was followed by centrifugation at 10000×*g* for 15 min at 4°C. The supernatant was discarded and the pellet was air dried.

2- propanol precipitation

Equal volume of filtrate was combined with 100% isopropanol. After vigorous mixing (1min) the samples were incubated overnight at 4oC. Then it was centrifuged at 10000g for 15 min. The supernatant was removed carefully without disturbing the pellet, and the pellet was air dried.

Determination of protein concentration

The protein concentration of the re-solubilised samples was determined in triplicate using the Lowry assay [14]. The efficiency of precipitation was determined as a ratio of the protein concentration before and after precipitation. The results presented are an average of three experiments.

Results:

In order to obtain reliable, reproducible and significant data proper sample preparation is crucial, particularly in comparative proteomic studies where minor differences between experimental and control groups are often meaningful [15]. In this study, we compared different carbon sources on protein secretion. These methodologies are useful for identifying changes in extracellular protein expression under different experimental condi-

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tions. It was found that chloroform/methanol delivered the highest protein recovery of 85.55±0.59%, 2-propanol 79.50±0.35% and acetone 67.84±0.49% when glucose was used as carbon source [**Table 1**].

Table 1 Percentage of secretory protein recovery after various precipitation procedures when glucose was used as sole carbon source.

Precipitation method	Protein amount before precipitation(µg)	Protein amount after precipitation(µg)	Percentage of recovery (%)
Acetone	144.54±1.69	98.06±1.20	67.84±0.49
2- propanol	144.54±1.69	114.9±1.71	79.5±0.35
Chloroform/ methanol	144.54±1.69	123.66±1.49	85.55±0.59

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

Table 2 Percentage of secretory protein recovery after various precipitation procedures when lactose was used as sole carbon source.

Precipitation method	Protein amount before precipitation(µg)	Protein amount after precipitation(µg)	Percentage of recovery (%)
Acetone	154.94±2.55	107.16±2.25	69.15±0.51
2- propanol	154.94±2.55	125.1±2.12	80.73±0.135
Chloroform/ methanol	154.94±2.55	134.6±1.82	86.78±0.57

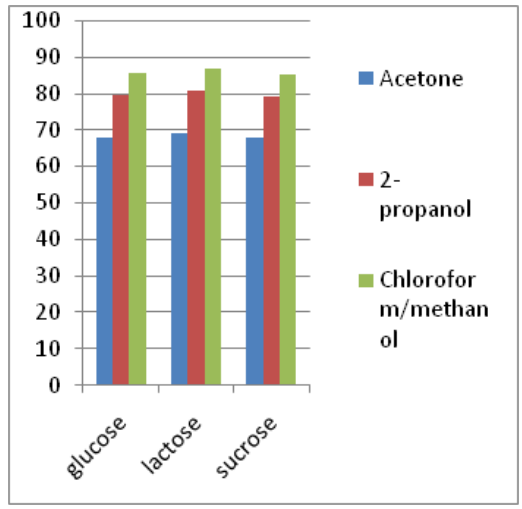
Values are the mean±standard deviation of three independent experiments.

Table 3 Percentage of secretory protein recovery after various precipitation procedures when sucrose was used as sole carbon source.

Precipitation method	Protein amount before precipitation(µg)	Protein amount after precipitation(µg)	Percentage of recovery (%)
Acetone	144.78±2.77	98.06±1.20	67.68±1.44
2- propanol	144.78±2.77	114.26±1.59	78.92±0.60
Chloroform/ methanol	144.78±2.77	123.43±1.27	85.27±1.07

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

Figure 1 Comparison of the precipitation efficiency of different methods for various carbon sources.



When lactose was used as sole carbon source percentage recovery was of 86.78±0.57% for chloroform/methanol, for 2-propanol it was found to be 80.73±0.13% and acetone showed 69.15±0.51% recovery [**Table 2**]. Percentage recovery of extracellular proteins was 85.27±1.07% for chloroform/methanol, for 2-propanol it was found to be 78.92±0.60% while acetone showed 67.68±1.44% [**Table 3**]. The trend of precipitation efficiency was similar in the three different culture conditions [**Figure 1**].

Discussion

The comparison of protein content in three filtrate revealed that the acetone method produced the lowest efficiency for protein precipitation. Reduced protein loss (fewer proteins in supernatants) occurred with the 2-propanol and methanol/chloroform methods. The methanol/chloroform method yielded the lowest loss of proteins, as determined by analysis of the medium filtrate. The acetone method yielded low precipitation efficiency in comparison to precipitation with methanol/chloroform. The study by Ewelina Fic *et al* also showed that the precipitation with chloroform/methanol delivered the highest protein recovery for proteomic analysis [16]. However, as has been shown by Garcia-Rodriguez S *et al* [6] acetone precipitation following incubation with DTT gave the highest protein recovery. In summary, it is important that the chosen protein precipitation method is able to effectively concentrate samples and eliminate contaminants; however, precipitation procedures rarely yield complete recovery.

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