World Journal of Medical Sciences 2 (2): 101-104, 2007
ISSN 1817-3055

Mechanical Lysis of *Candida* Cells for Crude Protein and Enzymatic Activity Estimation: Comparison of Three Methods

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Abstract: Cells of ten *Candida* strains were subjected to disruption using three methods-glass beads, sonicator and French pressure cell press separately and results compared for efficiency of each method. The French pressure cell press gave the highest protein yield and glass beads, the lowest (mean of means = 5.6, 40.0, 0.6 µg/ml, respectively, for the three methods). Enzymatic activity was also highest for the French pressure cell press and lowest for glass beads (mean of means = 0.94 mg/min/mg protein).

Key words: *Candida* · mechanical lysis · enzyme · activity · protein

INTRODUCTION

Yeast cell wall contains complex substances consisting of glucans [1].

Disruption of yeast cells can be achieved by several methods among which are use of Blender, liquid homogenization (for example, French cell press), sonication, freeze/thaw and manual grinding with mortar and pestle [2]. Each of these methods has at least a disadvantage as far as DNA typing and enzymatic activity is concerned [3]. Some of these disadvantages include denaturation of protein, reproducibility, destruction of important cell material, loss of enzymatic activity and cost [3, 4]. Although chemical lysis involving treatment of cells with enzymes is also sometimes employed, mechanical methods (grinding with glass beads, by sonication and liquid homogenizer such as the French press), are still commonly used [1, 5]. The purpose of this study was to compare the efficiency of the three methods for crude protein production and activity of an endoproteinase.

MATERIALS AND METHODS

Acid-washed glass beads are added to cells to facilitate crushing while spinning the cells. The sonicator uses pulsed, high frequency sound waves to agitate and lyse cells; this ultrasonic treatment is applied in multiple short bursts to sample immersed in an ice bath. A French press consists of a piston which is used to apply high pressure to a sample volume of 40-250ml, forcing it through a tiny hole in the press. Two passes are required for efficient lysis.

Molecular methods of detecting fungal pathogen in clinical samples requires a method that sufficiently lyses fungal cells [3] and maintains high enzyme activity. Protease is an arspartic (vacuolar) endoproteinase with the PEP4 structural gene [6]. Endoproteinases are present in most yeasts, including *Candida albicans* [7,8]. Arspartic proteases have been implicated in the pathogenesis of *Candida* [9,10] and have been used previously to characterize *Candida* strains [11,12].

Isolation and identification of *Candida* strains: Ten strains of *Candida* isolated from genital swabs collected from patients diagnosed to have urinary tract infection in the Microbiology Department of the University of Benin Teaching Hospital, UBTH, Benin City, Nigeria were used. The strains were purified by repeated subculturing on Saboraud Dextrose Agar, SDA (Siffin, Berlin) at 37°C and identified by conventional laboratory methods (colony and cell morphology, germ tube production in serum, chlamydospores production on Corn Meal Agar) and the CHROM-Agar method. The strains were kept at ambient temperature and were further identified with the HiCandida Kit (Himedia, India) at the Indian Institute of Chemical Biology, IICB, Kolkata, India and stored in McCartney bottles at 0-4°C. Subcultures were made from the stock when required.

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Growth of cells: Each of 5ml broth in a test tube was inoculated with a *Candida* strain and incubated over-night under aeration at 35°C. Then 50 ml of this over-night culture was transferred into 100 ml broth in a 250ml conical flask. The 250 ml culture was incubated for 48 hours, with shaking (producing stationary phase cells) since the levels of most endoproteinase increase as cells approach stationary phase [6,13].

Disruption of cells: Sonicator: *Candida* cultures grown as described above were centrifuged at 4°C for 15 minutes using Sorvall RC 5B Plus (Kendro, USA) at 2500g and maintained at 0-4°C on ice. Then 10ml buffer was added to 4g (wet weight of cells) to resuspend the cells. Cells were then subjected to ultrasonic disruption at 003 RMS (at 0-4°C ten times, each time for 30 seconds followed by 2 minutes of incubation in ice). Supernatant was collected after centrifugation for 15 min at 1650g and at 0-4°C, until use. Three replicates of each strain were used and experiment was done twice.

French cell press: Cells were harvested as described above and washed twice in ice-cole water. Then 4g (wet weight) cells were suspended in 10ml buffer and crushed at 18,000 Psi pressure, maintaining the temperature at 0-4°C. This was followed by centrifugation at 4°C, 1650g for 15 min. The residue was discarded while the supernatant was used for enzymatic activity. Three replicates of each strain were used and experiment was done twice.

Glass beads: Cells (4g wet weight harvested as described above) were subjected to disruption after suspending in 10 ml buffer and adding 10g glass beads using a Cyclo mixer (Remi, India). Cells were subjected to disruption ten times each time for 30 seconds with an interval of 2 min of incubation in ice. At the end of the exercise, centrifugation was done as before, while maintaining the mixture at 4°C. Estimation of protein in the supernatant and protease activity were done as before. Three replicates of each strain were used and experiment was done twice.

Determination of protein content: The method of Bradford was followed using Biorad protein assay reagent. (Biorad, USA). Bovine Serum Albumin, BSA (New England BioLabs) was used as standard. Optical Density (OD) was read at 595nm. Samples were in triplicates and mean OD values were calculated.

Protease a activity: The method described earlier [6] was used. Briefly, 100ml of extract collected by each of the three methods above and maintained at 0-4°C was used for the assay. A 2ml haemoglobin solution was added to 100ml extract in a tube and incubated at 37°C and at predetermined time-points of 0,15 and 30 min, 0.4ml of mixture was transferred into a fresh tube containing IN perchloric acid, shaken briefly at 1650g for 5 min at room temperature, in triplicates. A 0.1ml aliquot was added to 0.1ml sodium hydroxide. This solution was used to determine enzymatic activity according to earlier work [14].

Student *t*-test was used to analyse the statistical significance of the differences in protein concentrations and enzymatic activities.

RESULTS AND DISCUSSION

Protein concentrations and enzymatic activities of cell lysate varied significantly (Table 1). The amount of protein recovered for French cell press was the highest, followed by sonicator and glass beads (mean of means = 5.6, 4.0 and 0.6ug/ml respectively) as shown in Fig. 1. Also, activity of PrA was highest for French Cell press (mean = means = 3.0mg/min/mg protein) and least for Glass beads (mean of means = 0.94 mg/min/mg protein) shown in Fig. 2. Denaturation of protein is often a problem in cell lysis. Generally, in mechanical disruption, individual cells are disrupted at different times and so the composition of the released materials differs with time [2].

Table 1: Mean protein concentrations and enzymatic activities of cell lysate of ten *Candida* species disrupted by three methods. Values are means of two independent experiments, with each treatment in triplicates

	Mean protein conc (mg/min/mg protein)			Mean enzymatic activity (μg/ml)		
Candida strain	Sonicator	French press	Glass beads	Sonicator	French press	Glass beads
1	3.14	4.96	0.45	2.3	2.2	0.7
2	3.70	5.20	0.50	2.4	2.8	1.0
3	3.62	5.20	0.52	1.9	2.2	0.7
4	4.70	6.02	0.67	2.6	2.5	1.9
5	3.30	4.50	0.50	3.0	2.8	0.6
6	3.80	5.28	0.50	3.0	2.7	0.8
7	3.05	5.56	0.40	3.5	3.5	0.9
8	4.85	6.68	0.70	3.5	3.4	1.0
9	3.60	4.82	0.50	3.1	3.1	0.8
10	5.70	7.30	0.80	4.5	4.5	1.0

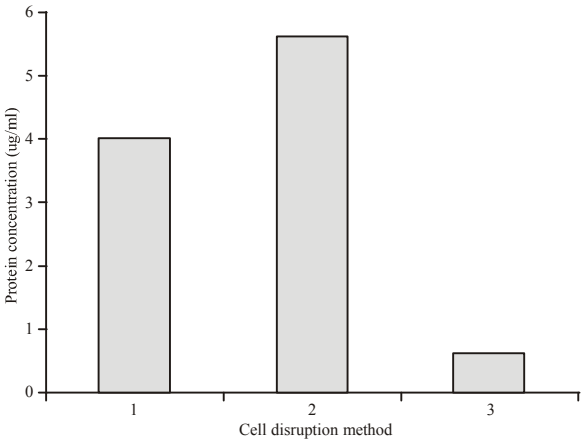

Fig. 1: Amount of protein recovered from lysate of *Candida* cells disrupted by different methods. 1 = Sonicator; 2 = French pressure cell press; 3 = Glass beads. Values are means of mean protein concentrations of ten *Candida* strains

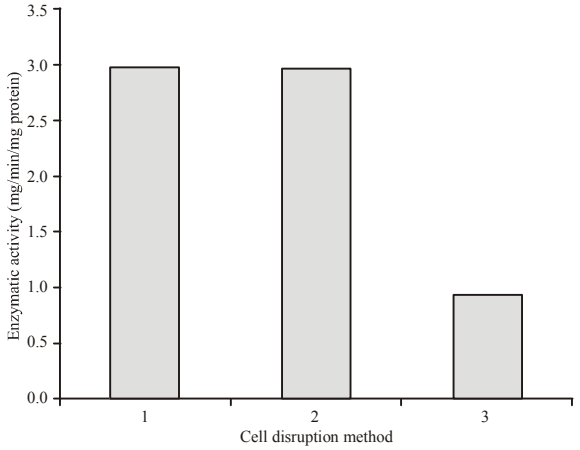

Fig. 2: Enzymatic activity of lysate of *Candida* cells disrupted by three different methods. 1 = Sonicator; 2 = French cell press; 3 = Glass beads. Values are means of mean enzymatic activities of ten *Candida* strains

Low yield of protein from yeast cells disrupted with glass beads have been reported previously [5, 15] and this has been attributed to the ability of the extracted proteins to non-specifically bind to the glass beads [5]. It was easier and more efficient to maintain the temperature at 0-4°C for sonication and French press than for the glass beads and as a result enzymatic activity was greatly lost in the glass beads method. It has been reported [4] that progressive loss in enzymatic activity (maltase, phosphatase) when yeast cells were crushed in a system containing glass beads. Although sonicator and French press gave the best results, both are expensive to procure, with the latter being more expensive. There was no significant difference in protein amounts with sonicator and French press though the values were constantly higher for French cell press.

Documented reports on protein recovery for *Candida* are scanty [3, 16]. Although glass beads method is still commonly used as it is relatively cheap, this study has shown that it is far less efficient than the other methods. The sonicator, if used with extra care to keep the system temperature low, would be as efficient as the French press. Bacerra *et al.* (2001) reported that Sonication was most efficient for the recovery of protein in *Kluyverromyces lactis*, a yeast [13]. In the report, sonicator also yielded over 10-fold more protein and activity than glass beads; stationary phase cells were used as in our study. This further supports the efficiency of sonicator we have reported here.

CONCLUSION

This study has shown that the sonicator is as efficient as the French pressure cell press in the mechanical lysis of *Candida* cells for crude protein extraction and enzymatic activity determination. The use of glass beads produces very little protein and leads to loss of protein activity.

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