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β-Galactosidase release kinetics during ultrasonic disruption of *Lactobacillus acidophilus* isolated from fermented *Eleusine coracana*

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ABSTRACT

The indigenous isolate of *Lactobacillus acidophilus* obtained from fermented *Eleusine coracana* was used as the source of intracellular β-galactosidase. Release of this enzyme from the bacterial cells is a challenging step for its recovery and further downstream processing. This paper reports the release kinetics of β-galactosidase by ultrasonication and survival rate of *L. acidophilus* as a function of acoustic power. A maximum of 360.3 U/g DCW of lactase was recovered with a release rate constant, K_a, of 0.454/min at an acoustic power of 50 W. The experimental data was fitted to Doulah's equation to determine the kinetic constant β and decipher the pattern of lysis during the ultrasonication process. Here we also hypothesize that due to local heat generation during the ultrasonication process, the enzyme was translocated from the cytoplasmic space in to the periplasm.

Key words: β-Galactosidase; Ultrasonication; Cell disruption; Acoustic power; Release kinetics; Location factor

1. Introduction

Lactobacilli are known to show diverse behaviour and have gained attention for production of a variety of enzymes. An indigenous strain of *Lactobacillus acidophilus* isolated by us from fermented *Eleusine coracana* was found to be a potential source of intracellular β-galactosidase (Akolkar et al., 2005). Since such intracellular enzymes are of delicate nature, the disruption protocol must be carefully standardized to attain efficient disruption without denaturation and loss of the released intracellular products (Farkade et al., 2005). Several mechanical methods, such as ultrasonication, high pressure homogenization and bead mill, have been used for cell disruption to release intracellular protein products (Farkade et al., 2005).

Ultrasonication is one of the most commonly employed cell disruption methods on a laboratory scale particularly due to ease in its operation, simple equipment requirement and efficient disruption results (Ho et al., 2006). The mechanism of cell disruption is associated with the phenomenon of cavitation (Harisson, 1991; Middelberg, 1995), which is a combination of formation, growth and collapse of the vapour-filled bubbles created by high-intensity (ultrasound above 20 kHz) sound waves. According to Doulah (1977), the collapse of the sound waves causes release of large amount of energy in the form of elastic waves, which in turn disintegrate in to eddies. Depending on the size of eddies, the energy is either dissipated to the cells in the surrounding medium or it merely results in motion of the cells within the medium thereby determining the overall pattern of cell disruption. The efficacy of the cell disruption process is not only dependent on the acoustic power of the sound waves, but is also dependant on the physical strength of the cell wall of the microorganisms (Sauer et al., 1989) and intracellular location of the enzyme (Hettwer and Wang, 1989).

However, cell disruption by ultrasonication poses some serious problems, like, non-specific cell wall disruption, high heat generation and prolonged operation time leading to generation of harmful free radicals. These effects are largely dependant on the acoustic power at which the process is carried out and the time for which the cell suspension is subjected to the ultrasound waves. Due to such drawbacks, it is necessary to study the kinetic release of the enzyme and total protein at various acoustic power and develop a standard protocol ensuring minimal loss during recovery of the enzyme.

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Nomenclature

F_n	fraction of the number of cells disrupted
t	sonication time (min)
α	kinetic constant
β	kinetic constant
R_a	lactase released at time t (U/g DCW)
R_{am}	maximum amount of lactase released (U/g DCW)
K_a	lactase release rate constant (/min)
R_p	total protein released at time t (mg/g DCW)
R_{pm}	maximum amount of total protein released (mg/g DCW)
K_p	total protein release rate constant (/min)

In this paper, we decipher the pattern of ultrasonic cell disruption of indigenous *L. acidophilus* cells, as per the theory proposed by Doulah (1978). The paper also reports the release kinetics of β-galactosidase and total protein during the ultrasonication process as a function of acoustic power in order to standardize a cell disruption protocol ensuring maximum release and minimum enzyme loss. The rate constants for the release of the enzyme and protein were further used for the calculation of the location factor of β-galactosidase (Umakoshi et al., 1998).

2. Materials and methods

2.1. Maintenance of the culture and preparation of seed culture

Indigenously isolated *L. acidophilus* (Akolkar et al., 2005) used in this study was maintained on MRS slants and was sub-cultured every month. A seed culture was prepared by inoculating 100 mL MRS broth with a fresh slant (24 h old). The broth was incubated for 18 h at 25 °C under stationery conditions. The cells were harvested in sterile centrifuge tubes by centrifugation at 5000 rpm (2850 × *g*) for 15 min at 15 °C using Remi Centrifuge. The cell pellet was suspended in sterile saline such that the resulting optical density was 1.0, when measured at 660 nm wavelength on a spectrophotometer (Spectronic Genesys 5 UV–vis spectrophotometer, Thermo Electron Corp., USA). This cell suspension was used as the seed culture.

2.2. Production of β-galactosidase

Production of β-galactosidase by *L. acidophilus* was carried out by growing them in a medium containing 10 g/L lactose, 20 g/L yeast extract, 10 g/L mycological peptone, 50 mg/L magnesium sulphate and 25 mg/L manganese sulphate from Himedia Labs, Mumbai, India, and 4 g/L tri-ammonium citrate, 2.5 g/L potassium acetate and 4 g/L dipotassium hydrogen phosphate from S.D. Fine Chemicals, Mumbai, India, at 25 °C for 24 h under stationary conditions. The pH of the medium was adjusted to pH 6.5 before sterilization. The cells were then harvested by centrifugation at 5000 rpm (4420 × *g*) at 15 °C for 15 min using Beckman J2-MC Centrifuge, USA (Rotor JA 10). The harvested cells were washed twice by resuspending the cells in distilled water followed by centrifugation at 10,000 rpm (12,100 × *g*) at 15 °C for 10 min using Beckman J2-MC Centrifuge, USA (Rotor JA 20).

2.3. Ultrasonication process

The cell pellet obtained after washing was suspended in 100 mM phosphate buffer (pH 7.0) so as to prepare a 5% (wet weight) cell suspension before subjecting them to the ultrasonic lysis process. 15 mL of this cell suspension was subjected to ultrasonication using the Branson Sonifier S 450A, USA, with a 1/2″ diameter tapped biohorn that delivers ultrasonic sound at a constant frequency of 20 kHz. The ulrasonication process was carried out at varying acoustic power of 10, 30 and 50 W at a constant duty cycle of 70%. The samples were kept in an ice bath to prevent over heating of the cell suspension during the lysis process. Aliquots were removed at intervals of 3, 6, 9, 12 and 15 min. A part of the aliquot was diluted appropriately and was spread on MRS agar plates to obtain the survival rate during lysis process. The cell density was measured at 660 nm. The remaining part of the aliquot was centrifuged for 20 min at 12,000 rpm (16,500 × *g*) using Plastocraft Superspin R-V/FA, India at 5 °C and the resulting cell-free supernatant was analyzed for the release of the enzyme and total protein.

2.4. Analytical methods

2.4.1. Assay of β-galactosidase

β-Galactosidase activity was measured by a modified method of Dickson (Dickson and Markin, 1980) involving the hydrolysis of substrate o-nitrophenyl-β-galactopyranoside (ONPG) to o-nitrophenol (ONP). The cell-free supernatant was appropriately diluted to 1 mL using 100 mM phosphate buffer (pH 7.0) for the assay. 0.2 mL ONPG solution (2 mg/mL), prepared in the same buffer was added to the enzyme solution. The mixture was incubated in a water bath at 50 °C for 10 min and the reaction was then stopped by adding 1 mL (100 g/L) Na_2CO_3 solution. The yellow colour of ONP obtained as a result of ONPG hydrolysis was measured at 420 nm (Spectronic Genesys 5 UV–vis spectrophotometer, Thermo Electron Corp., USA) using ONP as the standard.

The amount of ONP released per min by the cell-free supernatant was directly proportional to the quantity of enzyme released. One unit of enzyme was defined as μmoles of ONP released per min, per g DCW under the specified conditions using ONPG as the substrate.

2.4.2. Protein estimation

The total soluble protein (mg/g DCW) estimation was carried out by the modified Folin–Lowry method (Lowry et al., 1951) with bovine serum albumin (BSA) as a standard.

2.4.3. Measurement of cell density and dry cell weight

The cell density of aliquots removed after ultrasonication for different time periods was measured spectrophotometrically (Genesys UV–vis spectrophotometer) at 660 nm.

The total survival percentage was measured by spread plate method. The ultrasonicated aliquots were appropriately diluted and spread on plates containing MRS agar. The number of colonies was counted after incubating the plates for 24 h at 37 °C. Only plates containing colony counts between 30 and 300 were considered.

1 mL of the cell suspension was centrifuged in pre-weighed ependorffs and the pellet obtained was dried at 70 °C for 24 h to determine the dry cell weight.

All the experiments were carried out in triplicates and the results shown are an average of the same.