USBiological
Life Sciences

A Comparison of Zymolyase, Lyticase, and Glusulase

Discussion

Zymolyase is very efficient at forming yeast protoplasts while not hindering the regeneration of those protoplasts into viable cells. Both Lyticase and Glusulase at 300 U/ml can only approach the activity of Zymolyase diluted fives times to 60 U/ml. Conclusively, Zymolyase can be used at a more dilute concentration for practical applications than competitive enzymes. This lower "user level" makes Zymolyase more cost effective than Lyticase, while Glusulase fails to reach comparable activity. A cost/benefit analysis of Zymolyase and Lyticase shows that 1000 U of Zymolyase costs $8.00 or $6.00, for 100T and 20T preparations, respectively, while an equivalent amount of Lyticase, as measured by activity, would cost over $13.00. The high activity of Zymolyase is attributed to its composition, as it is a mixture of lytic enzymes as opposed to a purified enzyme, as is Lyticase. Zymolyase contains four enzymes each of which attack a different cell wall polymer. Generally, the yeast cell wall consists of four major components, namely 1) branched beta 1- 3 glucans (33%), substituted beta 1- 3 glucans (21%), glycoproteins (30%), and mannans (16%). Zymolyase includes enzymes which act on each of these polymers. The principle enzyme is beta-1, 3 glucan laminaripentaohydrolase which fragments cell wall glucans into pentamers. The second key enzyme is beta-1, 3 glucanase which hydrolyzes glucans to glucose, thus further degrading the cell wall. Present in lesser amounts are a protease and mannase which act on glycoproteins and mannans, respectively. The combined action of these four enzymes allows for efficient protoplast formation by the Zymolyase mixture.

Summary

Zymolyase, Lyticase and Glusulase were compared as reagents for yeast cell wall degradation for protoplast formation. These enzymes were tested on Saccharomyces cerevisiae and Pichia pastoris. Of these three enzymes, Zymolyase had the greatest activity by forming 100% protoplasts within 10 min. when used at 300 U/ml. Lyticase required a 70 min. incubation period to form 100% protoplasts while Glusulase failed to be completely effective, reaching only 80% protoplast formation. At lower concentrations of the enzyme, i.e, 60 U/ml, protoplast formation for Pichia was nearly complete at 40 min., while for Saccharomyces a longer incubation period of 60 min. was required. At lower enzyme concentrations, both Lyticase and Glusulase failed to yield significant quantities of protoplasts. Both yeasts treated with Zymolyase were tested for their ability to regenerate cell walls and form viable yeast colonies. When 94% of Pichia cells were converted to protoplasts, 38% of the protoplasts regenerated when plated in a 1M sorbitol/YPD agar medium. For a culture of Saccharomyces in which 89% of the cells were protoplasted, 62% were capable of regenerating cell walls. These percentages are high and useful to researchers who transform yeast and perform protoplast fusions.

Protocol for Protoplast Formation

The following protocol can be used for either Saccharomyces or Pichia. It is suggested that 300 U/ml Zymolyase be used if yeast cells are to be lysed for nucleic acid purification and 60 U/ml for yeast transformation and protoplast fusion protocols.

Materials

1 M Sorbitol, 25 mM EDTA, pH 8, 50 mM DTT -
The sorbitol/EDTA can be prepared separately and sterilized. A 1 M DTT stock solution (molecular biology grade) should be filter sterilized and frozen in 500 ul aliquots. Add the DTT aliquot to 9.5 ml of sorbitol/EDTA just prior to use.
1 M Sorbitol - Sterilize by autoclaving.
1 M Sorbitol, 25 mM EDTA, pH 8.0
1 M sorbitol, 1 mM EDTA, 10 mM sodium citrate buffer, pH 5.8
Zymolyase in water- Either 60 U/ml (for transformation) or 300 U/ml (for cell lysis). No more than 20 ul is required for this protocol.
Water - Sterile, molecular biology grade.
5% SDS in water

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Protocol

1. Prepare actively growing yeast by streaking on a YPD agar plate (1% yeast extract, 2% peptone, 2% dextrose, 2% agar). For best results, use freshly cultured yeast.

2. Using aseptic technique, inoculate 100 ml of YPD (1% yeast extract, 2% peptone, 2% dextrose) in a 500 ml flask with yeast from the YPD plate. Culture the yeast overnight at 28°C with shaking (250-300 rpm). Yeast may also be cultured at room temperature, but generation times are reduced. Yeast will not grow well above 32°C.

3. Measure the OD (@600 nm) of the yeast culture the following morning. Use YPD to zero the spectrophotometer. It may be necessary to dilute the yeast in YPD in order to attain a readable OD (i.e., between 0.1 and 1.0). If the yeast OD is between 0.2 and 0.3, then harvest the cells as described below. Otherwise, dilute the yeast to an OD of 0.2 and return the culture to the shaker. Monitor the culture OD over the next couple of hours. When the OD reaches 0.3, harvest the cells by centrifuging at room temperature for 10 minutes at 1500 x g. Pour off the supernatant and save the yeast pellet.

4. Resuspend the yeast pellet in 10ml of sterile water. The cells can be vortexed to assure they are suspended homogeneously. Transfer the cells to a sterile 15 ml Falcon screw capped tube.

5. Centrifugation the yeast at 1500 x g for 5 minutes at room temperature. Pour off the supernatant and again save the pellet.

6. Resuspend the cells in 10 ml of fresh 1 M Sorbitol, 25 mM EDTA, pH 8, 50 mM DTT, and immediately pellet the yeast by centrifuging at 1500 x g for 5 minutes at room temperature.

7. Resuspend the yeast cells 10ml of 1 M sorbitol and again pellet the yeast by centrifuging at 1500 x g for 5 min.

8. Resuspend the cells in 10 ml of sorbitol/citrate buffer (1 M sorbitol, 1 mM EDTA, 10 mM sodium citrate buffer, pH 5.8). To assess the time required and efficiency of protoplast formation, transfer 1.6 ml of cells to a small tube and proceed below. Otherwise, if the entire tube is to be treated, add 15 ul of Zymolyase and incubate at 30°C. Protoplasts are formed within 60 min., depending on the concentration and batch of Zymolyase.

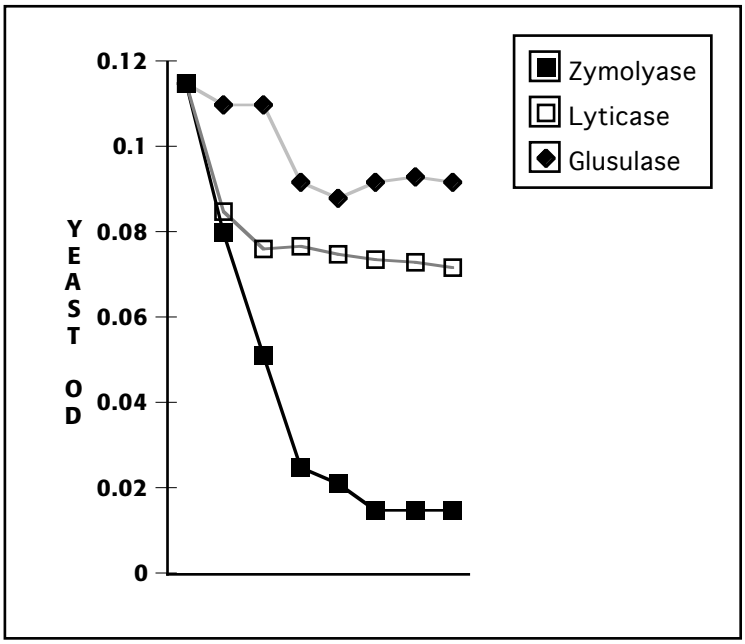
9. Measuring protoplast efficiency is accomplished by monitoring cell lysis spectrophotometrically. Zymolyase is added to the yeast suspension, followed by removal of aliquots which are mixed with 1 M sorbitol and 5% SDS. Intact yeast will survive the SDS while protoplasts will lyse. Measuring the OD of the intact yeast is a measurement of protoplast formation (i.e., by examining what cells remain). Set the spectrophotometer to 800 nm and zero with sorbitol/citrate buffer. In order, mix 2 ml of 1 M sorbitol and 200 ul of yeast cells from the 15 ml tube. Add 800 ul 5% SDS and mix. Measure and record the OD of this mixture. This represents 0% protoplasts or 100% intact cells.

10. Add 2 ml of 1 M sorbitol to seven tubes. Yeast and SDS will be added to these tubes during a time course assay to measure protoplast formation. To the 1.6 ml of yeast, add 2.5 ul of Zymolyase and incubate at 30°C. A water bath or heat block arranged near the spectrophotometer is a useful arrangement.

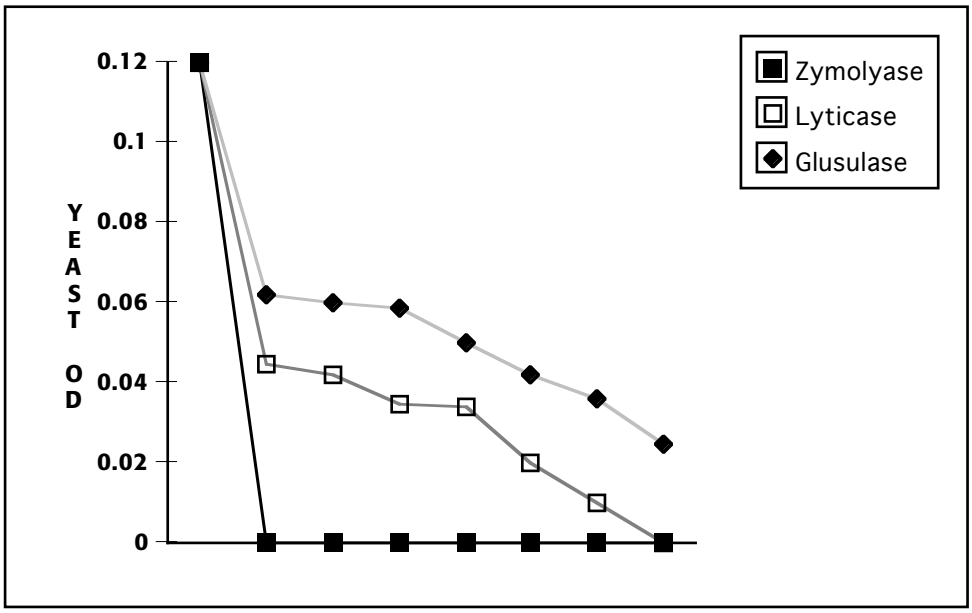
11. At 5, 10, 20, 30, 40, 50, and 60 min., remove 200 ul of yeast from the reaction, add it to a tube of sorbitol and mix. Add 800 ul of 5% SDS, mix, and measure the OD. For accurate lysis, measure the OD immediately. Adequate time is available between time points to make these measurements.

12. The total efficiency and time course for protoplast efficiency can be determined by plotting OD vs. time. To calculate efficiency, divide the OD at each time point by the starting OD, then multiply by 100. For transformation experiments, normally the yeast culture must be treated so that 70% protoplast efficiency is attained.

13. To protoplast the remaining yeast, at 13 ul Zymolyase to the 15 ml tube and incubate for the determined time. Once protoplasts are formed, handle the cells carefully since they are fragile and will rupture easily. Do not vortex or dilute with hypertonic solutions (e.g., water) if intact protoplasts are needed. Alternatively, protoplasts can easily be lysed with detergents or by osmotic shock.



Protoplast Formation of Pichia
Zymolyase, Lyticase and Glusulase
[60units/ml]



Protoplast Formation of Saccharomyces
Zymolyase, Lyticase and Glusulase
[300units/ml]

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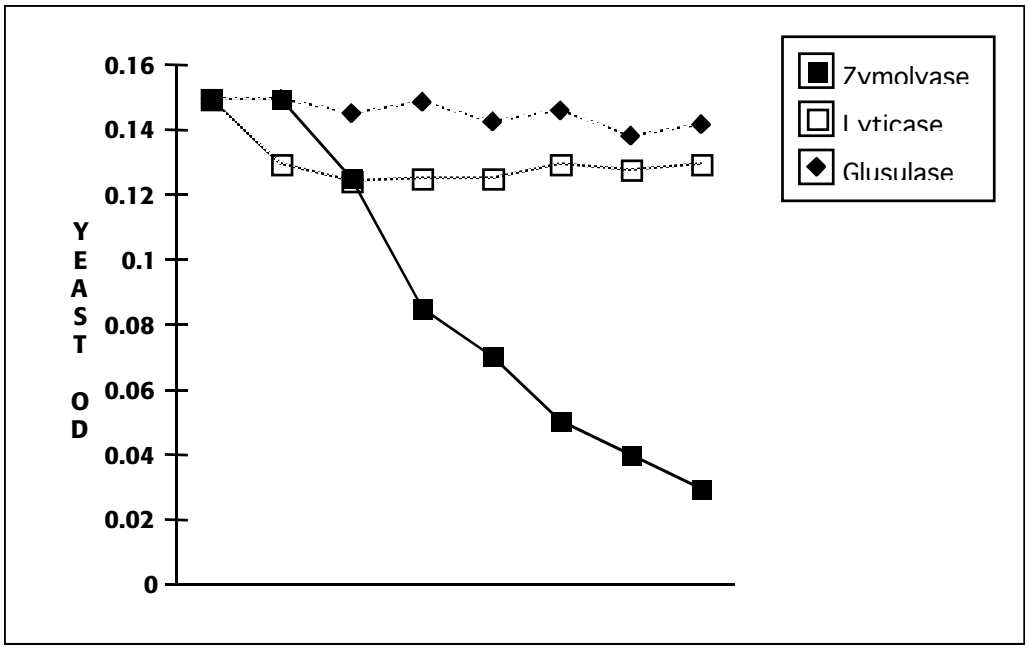

Protoplast Formation of Saccharomyces
Zymolyase, Lyticase and Glusulase
[60units/ml]

For further information and a sample of Zymolyase, please contact:

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