Comparison

Zymolyase™ vs Lyticase & Glusulase

Work Performed by:

Dr. David Burden
Biotechnology Training & Consulting, Inc.
P.O. Box 348, Lebanon, NJ 08833, USA

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For orders or tech service contact amsbio

ams biotechnology (europe) ltd – info@amsbio.com www.amsbio.com
☎ (UK) +44(0)1235 828200 ☎ (CH) +41(0)91 604 55 22 ☎ (DE) +49(0)69 779099

PART ONE

A Comparison of Zymolyase, Lyticase, and Glusulase

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 minutes, when used at 300 U/ml. Lyticase required a 70 minute 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 minutes, while for *Saccharomyces* a longer incubation period of 60 minutes 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.

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 five times to 60 U/ml. Conclusively, Zymolyase can be used at a more dilute concentration for practical applications that competitive enzymes. This lower "user level" makes Zymolyase more cost effective that Lyticase, while Glusulase fails to reach comparable activity.

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 hydrolyses glucans to glucose, thus further degrading the cell wall. Present in lesser amounts are protease and mannose which act on glycoproteins and mannans, respectively. The combined action of these four enzymes allows for efficient protoplast formation by the Zymolyase mixture.

Protocol for Protoplast Formation

The following protocol is a modified version of the Invitrogen protocol and 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 µl 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 µl is required for this protocol.
- Water – Sterile, molecular biology grade.
- 5% SDS in water.

Protocol

1. Prepare actively growing yeast be 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 necessary to dilute the yeast in the 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, the 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 10 ml of sterile water. The cells can be vortexed to assure they are suspended homogenously. Transfer the cells to a sterile 15 ml Falcon screw capped tube.

5. Centrifuge 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 in 10 ml of 1 M Sorbitol and again pellet the yeast by centrifuge at 1500 x g for 5 minutes.

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 µl of Zymolyase and incubate at 30°C. Protoplasts are formed within 60 minutes, depending on the concentration and batch of Zymolyase.

9. Measuring protoplast efficiency is accomoplished 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 protoplasts 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 µl of yeast cells from the 15 ml tube. Add 800 µl 5% SDS and mix. Measure and record the OD of this mixture. This represents 0% protoplasts or 100% intact cells.

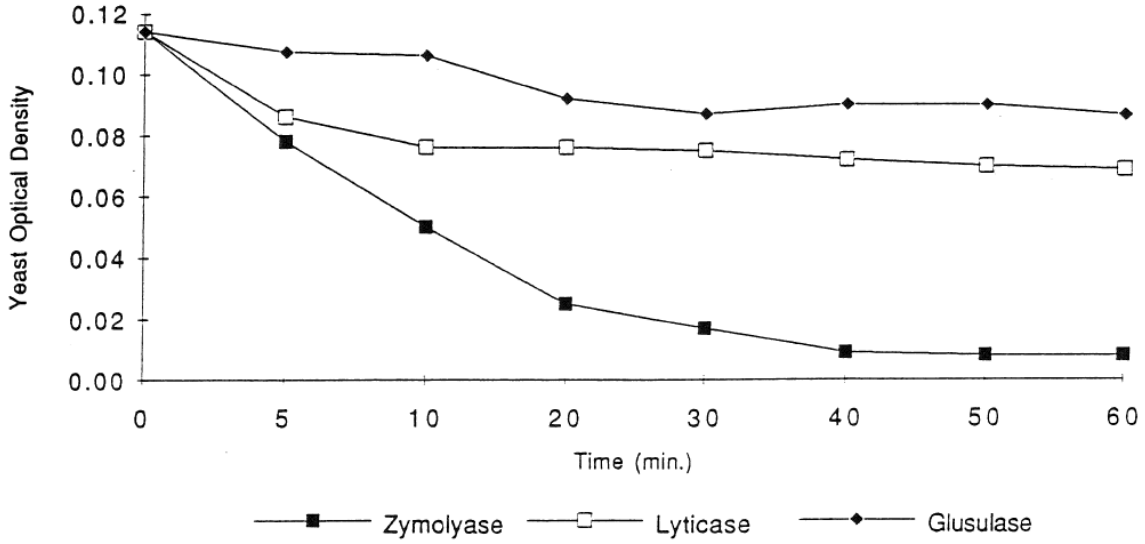
10. Add 2 ml of 1 M sorbitol to seven tubes. Yeast and SDS will be added to these tubes during a time course assay to measure protoplast formation. To the 1.6 ml of yeast, add 2.5 µl of Zymolyase and incubate at 30°C. A water bath or heat block arranged near the spectrophotometer is a useful arrangement.

11. At 5, 10, 20, 30, 40, 50, and 60 minutes, remove 200 µl of yeast from the reaction, add it to a tube of sorbitol and mix. Add 800 µl 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.

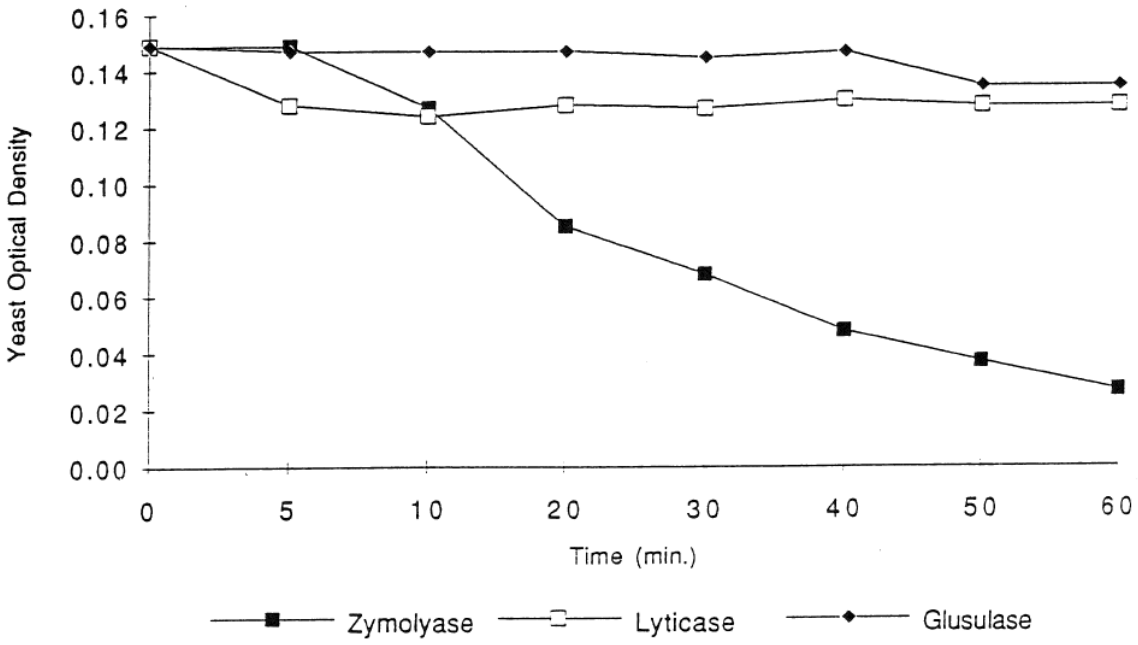
12. The total efficiency and time course for protoplast efficiency can be determined by plotting OD vs. time. To calculate efficiency, divide the OD at each time point by the starting OD, the multiply by 100. For transformation experiments, normally the yeast culture must be treated so that 70% protoplast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

13. To protoplast the remaining yeast, add 13 µl 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.

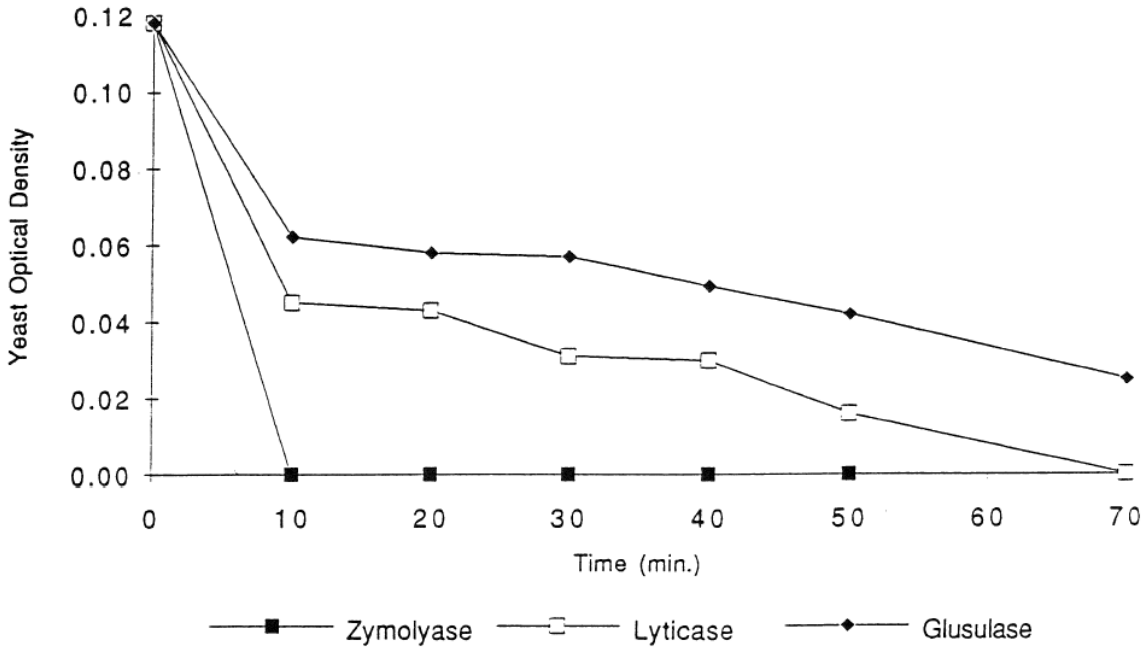
Protoplast Formation of Pichia using Zymolyase, Lyticase, and Glusulase (enzymes used at 60 U/ml)



Protoplast Formation of Saccharomyces using Zymolyase, Lyticase, and Glusulase (enzymes used at 60 U/ml)



Protoplast Formation of Saccharomyces using Zymolyase, Lyticase, and Glusulase (enzymes used at 300 U/ml)



PART TWO

A Comparison of Methods and Reagents for the Transformation of *Saccharomyces cerevisiae*

I. Summary

The transformation of yeast can be accomplished by several methods, which include treatment of the yeast with lithium cations and by protoplast fusion. The efficiency of these methods vary greatly as do the effect of individual reagents on these methods. For instance, the quality of polyethylene glycol (PEG) on the efficiency of DNA uptake in the protoplast transformation method is known to be critical, with researchers testing the PEG lots much the same way cell culturists test serum. This study compares lithium cation and protoplast fusion transformations of yeast, in addition to a comparison of Zymolyase and Lyticase as the cell wall degrading enzyme for the protoplast transformation of yeast.

II. Materials and Methods

Yeast Strain

Saccharomyces cerevisiae INVSc1, from Invitrogen, was used for all transformation. The yeast phenotype is *MATα his3Δ1 leu2 trp1-289 ura3-52*. Transformation experiments will make use of the leu^- phenotype and selection of transformants using *LEU2* complementation.

Plasmid DNA

The plasmid Yep351/A/M was used as the control for DNA for the transformation experiments. This plasmid contains the *LEU2* and thus allows for leucine complementation for the selection of transformants. This plasmid also encodes an easily detectable marker for verification. The plasmid contains the *ADC1* promoter fused to the *MEL1* coding region. This results in the expression of *MEL1* and synthesis of α-galactosidase on glucose. α-Galactosidase is easily measured using a variety of colorimetric substrates, as with the enzymatic cleavage of X-α-Gal.

Transformation of *Saccharomyces cerevisiae* by the Lithium Cation Method

Materials

YPD Broth
TE Buffer
100 mM Lithium Acetate in TE Buffer, sterile
Plasmid DNA
PEG 4000, 50% in water, sterile
1 M Sorbitol in TE buffer
Leu^- selective agar plates: 1% glucose, 0.67% yeast nitrogen base, 1 M sorbitol

Protocol

1. Culture yeast in YPD broth (2% glucose, 2% peptone, 1% yeast extract) with aeration overnight at 30°C. The culture should have approximately 1 x 10^7 cells/ml.
2. For each transformation, pellet yeast from 4.5 ml of broth by centrifuging for 5 minutes at 3000 rpm.
3. Resuspend the yeast in 4 ml TE buffer and centrifuge at 3000 rpm for 5 minutes.
4. Carefully resuspend the yeast in 3 ml of 100 mM lithium acetate, TE and shake gently at room temperature for 30 minutes.
5. Centrifuge the yeast for 5 minutes at 2000 rpm. Carefully remove the supernatant and resuspend the yeast in 100 µl of lithium acetate/TE and transfer to a 1.5 ml microfuge tube. The final density of the yeast is approximately 5 x 10^8 cells/ml.
6. Add up to 10 µg of plasmid to the yeast in a volume not greater than 10 µl. Incubate at 30°C without shaking for 30 minutes.
7. Add 300 µl of 50% PEG, mix and incubate at 30°C for 1 hour without shaking.
8. Heat shock the cells for 5 minutes at 42°C.
9. Immediately centrifuge the cells for 20 seconds. Remove the PEG solution and resuspend the cells in 1 ml of sorbitol/TE solution.
10. Plate the cells on selective media. Allow the plates to dry before inverting to incubate.

Transformation of *Saccharomyces cerevisiae* by the Protoplast Method

Materials

1 M Sorbitol, 25 nM EDTA, ph 8, 50 mM DTT – The sorbitol/EDTA is prepared separately and sterilized. Filtered sterilized 1 M DTT stock solution (molecular biology grade) is prepared and frozen in 500 µl aliquots. The DTT is added to 9.5 ml of sorbitol/EDTA just prior to use.
1 M Sorbitol – sterilize by autoclaving.
1 M sorbitol, 1 mM EDTA, 10 mM sodium citrate buffer, pH 5.8 (for Zymolyase)
1 M sorbitol, 1 mM EDTA, 10 mM Tris buffer, pH 7.5 (for Lyticase)
Zymolyase in water – 60 U/ml
Lyticase in water – 1200 U/ml
1 M Sorbitol, 10 mM tris, pH 7.5 10 mM $CaCl_2$
20% PEG/10 mM tris, pH 7.5, 10 mM $Cacl_2$ (freshly prepared)
Leu^- selective agar plates: 1% glucose, 0.67% yeast nitrogen base, 1 M sorbitol.
Leu^- selective top agar tubes: 10 ml of 1% glucose, 0.67% yeast nitrogen base, 1 M sorbitol in a large foam stoppered test tube. Melt and store at 47°C when using.
5% SDS in water

Protocol

1. Streak cryopreserved yeast on a YPD agar plate (1% yeast extract, 2% peptone, 2% dextrose, 2% agar) and culture 24-48 hours at 28-30°C.

2. Inoculate 100 ml of YPD (1% yeast extract, 2% peptone, 2% dextrose) from an isolated colony into a 500 ml flask with yeast from the YPD plate. Culture the yeast overnight at 28°C with shaking (250-300 rpm).

3. Measure the OD_{520} of the yeast culture the following morning using sterile YPD to zero the spectrophotometer. If necessary, 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. Alternatively, dilute the yeast to an OD of 0.2 and continue culturing. When the culture 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. Thoroughly resuspend the yeast pellet in 10 ml of sterile water. Transfer the cells to a sterile 15 ml screw capped tube.

5. Centrifuge the yeast at 1500 x g for 5 minutes at room temperature. Decant and save the yeast pellet.

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

7. Resuspend the yeast cells in 10 ml of 1 M sorbitol. Pellet the yeast by centrifuging at 1500 x g for 5 minutes.

8a. 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 add 2.5 µl of Zymolyase. Proceed to step 9. Save the remaining cells.

8b. Resuspend the cells in 10 ml of sorbitol/citrate buffer (1 M sorbitol, 1 mM EDTA, 10 mM Tris buffer, pH 7.5). To assess the time required and efficiency of protoplast formation, transfer 1.6 ml of cells to a small tube and add 2.5 µl of Lyticase. Proceed to step 9. Save the remaining cells

9. Following the addition of cell wall lytic enzyme (i.e., either Zymolyase or Lyticase) incubate the cells at 30°C. remove 200 µl aliquots of treated cells at 0, 5, 10, 20, 30, 40, 50, and 60 minutes and add the aliquot to 2 ml of 1 M Sorbitol. Add 800 µl 5% SDS, mix, and measure cell lysis by culturing clearing at OD_{800}. The spectrophotometer should be blanked against 1 M sorbitol.

10. The efficiency and time required for protoplast formation can be determined by plotting OD_{800} of the treated yeast cells vs. time. To calculate the efficiency, divide the OD_{800} at each time point by the starting OD_{800}, then multiply by 100. A protoplast efficiency of 70% is needed for transformation.

11. To protoplast the remaining yeast, at 13 µl either Lyticase or Zymolyase to their respective tubes and incubate for the determined time to reach 70% protoplast efficiency. Handle the protoplasts carefully since they are fragile and will easily rupture.

12. Centrifuge the protoplasts at 750 x g for 10 minutes at room temperature. Decant and save the pellet.

13. Gently resuspend the protoplasts in 1 ml of 1 M sorbitol. Centrifuge at 750 x g at room temperature.

14. Resuspend the protoplasts in 5 ml of 1 M Sorbitol, 10 mM Tris, pH 7.5, 10 mM $CaCl_2$.

15. For each transformation, aliquot 100 µl of the protoplasts into sterile 15 ml tubes. Add 10 µg of YEp351/A/M control plasmid and incubate at room temperature for 10 minutes.
16. Add 1.0 ml of fresh 20% PEG/10 mM Tris, pH 7.5, 10 mM $CaCl_2$ to the protoplasts, mix gently, and incubate at room temperature for 10 minutes.
17. Centrifuge the protoplasts at 750 x g for 10 minutes at room temperature and carefully decant the 20% PEG/10 mM Tris, pH 7.5, 10 mM $CaCl_2$. Invert the tube and drain excess solution.
18. Gently resuspend the protoplasts in 1 m of 1 M sorbitol.
19. Add 100 µl of protoplasts to 10 ml of molten top agar and pour onto the selective agar plates. Allow the top agar to solidfy.
20. Invert plates and incubate at 28-30°C. Transformed cells should appear in 4-6 days.

III. Results and Discussion

The transformation of *Saccharomyces cerevisiae* with YEp351/A/M was successful. The efficiency of the transformation was affected by both the method employed, and in the case of protoplast formation, the lytic enzyme used (i.e., Zymolyase and Lyticase). The protoplast transformation method was significantly more efficient than lithium cation transformation, with the enzyme employed for protoplast formation also having a significant effect. When Zymolyase was used for protoplast formation, efficiency was 652 transformants/10 µg plasmid DNA. This efficiency dropped considerably when Lyticase was used, which yields 53 transformants/10 µg. The transformation of yeast with lithium cations was relatively inefficient with 10 µg of plasmid yielding 6 transformants.

The efficiencies of each of the transformation methods employed can be correlated to the difficulty and cost of the technique. Protoplast transformation though relatively efficient, does require removal and regeneration of the yeast cell wall which can often be difficult. This is especially true when a variety of yeast strains are being transformed. Lithium acetate transformation is relatively easy to perform, however ease in the procedure results in loss in the relative efficiency of the transformation.

The effect of lytic enzyme source on protoplast efficiency is easily demonstrated with the differences observed between transformation using Lyticase and Zymolyase. Previous experiments comparing Lyticase and Zymolyase show that the specificity of these enzymes differs significantly. Lyticase is often ineffective in completely lysing cells, thus demonstrating limited lytic specificity. Zymolyase is a mixture of several carbohydrases (i.e., ß-1,3 glucan laminaripentaohydrolase, ß-1,3 glucanase, protease, and mannase) all which work in conjunction for efficient cell wall degradation.

The success of protoplast transformation is two fold: 1) sufficient cell wall must be removed so to allow fusion between protoplasts, and 2) adequate cell wall must remain in order to successfully regenerate the complete cell wall. It is feasible that Zymolyase contains the correct enzymatic activities to allow for both of these conditions when yeast are partially protoplasted (i.e., to 70%). Lyticase could conceivably be under or over degrading a particular cell wall component which either prevents protoplast fusion or subsequent cell wall regeneration.

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PRODUCT SPECIFICATIONS

Zymolyase®, purified from culture fluid of *Arthrobacter luteus*, has strong lytic activity against living yeast cell walls to produce protoplast or spheroplast of various strains of yeast cells. Essential enzyme lytic activity of Zymolyase® is β-1, 3-glucan laminaripentaohydrolase, which hydolyzes glucose polymers linked by β-1, 3-bonds and produces laminaripentaose. Zymolyase® is reported to be a complex enzyme of Zymolyase A, β-1, 3-glucan laminaripentaohydrolase and Zymolyase B, alkaline protease, which may change the structure of the yeast cell wall to facilitate penetration of Zymolyase A. Zymolyase A alone was unable to lyse yeast cell walls. There are two preparations of Zymolyase®, Zymolyase®-20T and 100T, having lytic activity of 20,000 units/gram and 100,000 units/gram respectively. Zymolyase®-20T is ammonium sulphate precipitate while Zymolyase® 100T is a further purified preparation by affinity chromatography. Lytic activity varies depending on strains, fermentation conditions and growth phases of yeast substrate.

Form: Lyophilized powder

Purification:
Zymolyase®-20T: $(NH_4)_2SO_4$ precipitation
Zymolyase®-100T: Affinity Chromatography

Activity:
Zymolyase®-20T: 20,000 units/gram
Zymolyase®-100T: 100,000 units/gram

Essential enzyme: ß-1,3-glucan laminaripentaohydrolase

Other activities contained:

	Zymolyase®-20T	Zymolyase®-100T
ß-1,3-glucanase	ca. 1.5 x 10^6 units/g	ca. 1.0 x 10^7 units/g
protease	ca. 1.0 x 10^4 units/g	ca. 1.7 x 10^4 units/g
mannanase	ca. 1.0 x 10^6 units/g	ca. 6.0 x 10^4 units/g

Contaminants: Trace amounts of amylase, xylanase, phosphatase. No DNase, RNase detected

Optimum pH & temperature: pH 7.5, 35°C (for lysis of viable yeast cells)
pH 6.5, 45°C (for hydrolysis of yeast glucan)

Stable pH: 5~10

Heat stability: The lytic activity is lost on incubation at 60°C for 5 minutes.

Specificity (lytic spectrum)[5]: Ashbya, Candida, Debaryomyces, Eremothecium, Endomyces, Hansenula, Hanseniaspora, Kloeckera, Kluyveromyces, Lipomyces, Metschikowia, Pichia, Pullularia, Torulopsis, Saccharomyces, Saccharomycopsis, Saccharomycodes, Schwanniomyces, etc.

Activity: SH compound such as cystein, 2-mercaptoethanol of dithiothreitol

Stability: No loss of activity was found after storage for 1 year at 4°C

PROPERTIES OF ZYMOLYASE

Lytic Spectrum by Zymolyase®

1) **Susceptible strains in low concentration (0.2 units/ml)**
Ashbya, Endomyces, Kloeckera, Kluyveromyces, Pullularia, Saccharomyces

2) **Susceptible strains in high concentration (2.0 units/ml)**
Candida, Debaryomyces, Eremothecium, Hansenula, Hanseniaspora, Lipomyces, Metschikowia, Saccharomycopsis, Saccharomycodes, Schizosaccahromyces, Selenozyma, Trigonopsis,Wickerhamia

3) **Susceptibility depending on strains**
Bretanomyces, Cryptococcus, Nadsonia, Pichia, Rodosporidium, Schwanniomyces, Stephnoascus, Torulopsis

4) **No susceptible strains**
Bullera, Pityrosporum, Rhosotorula, Sporidiobolus, Sporobolomyces, Stetigmatomyces, Trichosporon

ASSAY FOR ENZYME ACTIVITY

Unit Definition
One unit of lytic activity is defined as that amount which indicates 30% of decrease in absorbance at 800 nm (A_{800}) of the reaction mixture under the following condition.

Reaction mixture

Enzyme Solution:	0.05-0.1 mg/ml for Zymolyase®-20T 0.012-0.024 mg/ml for Zymolyase®-100T	1 ml
Substrate:	Brewer's yeast cell suspension (2 mg dry weight/ml)	3 ml
Buffer:	M/15 Phosphate buffer, pH 7.5	1 ml
Distilled water:		1 ml

Procedure
After incubation for 2 hours at 25°C with gentle shaking, A800 of the mixture is determined. As a reference, 1 ml of distilled water is used instead of enzyme solution.

Calculation
Percentage decrease in A_{800} = (A_{800} of reference - A_{800} of reaction mixture) x 100/initial A_{800} of reference when 60% of A_{800} decrease, equivalent to 2 units, is observed in the reaction system, the brewer's yeast cells are completely lysed, namely 1 unit of Zymolyase®-20T or Zymolyase®-100T lyses 3 mg dry weight of brewer's yeast.

PRECAUTIONS ON USE:
1) Avoid using nitrocellulose filters and use of material other than nitrocellulose, when sterilizing. Zymolyase may be adsorbed on nitrocellulose membranes.
2) Zymolyase, especially Zymolyase® -100T, may not be completely dissolved in buffers. Use Zymolyase as suspension.
3) When sterilized, Zymolyase is used in a concentration higher than 0.05%, prepare 2% Zymolyase solution in buffers containing 5% glucose, filter the suspension and dilute the solution with the appropriate buffer.

APPLICATIONS:
- Protoplast/spheroplast preparation
- Yeast cell fusion
- Transformation of yeast cells
- Yeast genetics

STORAGE:
Stable for least 1 year at 2°C. When stored at 30°C for 3 months, about 70% of the lytic activity is lost in Zymolyase®-20T and 90% in Zymolyase®-100T.

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NOTE:
This product is for laboratory use only - not for drug, household or other uses.

Cat. Number	Description	Package Size	Price
120491-1	Zymolyase®-20T	1 g (20KU/g)	£ 145 / 180 € / CHF 260
120493-1	Zymolyase®-100T	500 mg (100KU/g)	£ 540 / 750 € / CHF 1080