Product Information

MycoPolyzyme

For use with yeast and fungal lysis, free of DNA contaminants, suitable for Microbiome research

SAE0200

Product Description

Metagenomics is a field of basic and applied research which looks at all DNA that has been isolated directly from given single samples (like environmental samples or biological organisms).[1,2] Metagenomics enables studies of microbes from extreme environments, which have been historically difficult to isolate, culture, and study.[3] Metagenomics has revealed the existence of novel microbial species.[4] Applications of metagenomic studies include public health data analysis,[5,6] discovery of novel proteins, enzymes and natural products,[7,8] environmental studies,[9,10] and agricultural investigations.[11,12]

Studies of microbial communities have been revolutionized by the widespread adoption of culture-independent analytical techniques such as 16S rRNA gene sequencing and metagenomics. Since DNA contamination during sample preparation is a major problem of these sequence-based approaches,[13] DNA extraction reagents free of microbial DNA contaminants are essential.

MycoPolyzyme for yeast and fungi is a mix of two enzymes, lyticase and chitinase, which may be used to lyse samples[14-18] for applications like microbiome studies. Since fungi have more rigid cell walls than bacteria, a specific enzyme mixture is needed for fungi lysis to achieve sufficient DNA extraction.

This purified MycoPolyzyme product undergoes strict quality control testing to ensure the absence of detectable levels of contaminating microbial DNA, using 35 cycles of PCR amplification of 16S and 18S rDNA with universal primer sets.

Precautions and Disclaimer

This product is for R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store the product at –20 °C.

Preparation Instructions

Solutions of MycoPolyzyme can be prepared in DNA-free water (Cat. No. MBD0025) or PBS (pH 7.5) without EDTA, calcium, or magnesium. Prepare the solution at 5 mg/mL. Store in aliquots at –20 °C.

Procedure

Given the diversity of samples for microbiome studies that involve yeasts and fungi, optimal sample preparation and treatment must be experimentally determined. The susceptibility of fungal cells to lytic enzymes will depend on the species, environmental conditions, as well as growth stage.[19] While effective methods have been reported to enhance lytic enzyme activity, such as adding 2-mercaptoethanol, DTT, and surfactants (SDS),[20] there are instances when harsh conditions are not desired, like spheroplast/protoplast preparation. For these instances, enzymatic lysis is a common sample preparation method that can be combined with DNA extraction protocols, including bead beating or SDS treatment, if a more intense method of lysis is required. In general, spheroplast/protoplast preparation will require lower lytic enzyme concentration than complete lysis.

For optimal results, proteinases (such as Proteinase K) and chelators (such as EDTA) should not be used with MycoPolyzyme. It is recommended to treat samples with MycoPolyzyme for at least 40 minutes.

The following is a sample protocol for treatment of a frozen, pure culture of *Candida albicans* grown into the stationary phase, to represent a microbiome sample that is difficult to lyse. This protocol can be used as a guide for steps ahead of additional lysis methods, such as bead beating or SDS treatment.

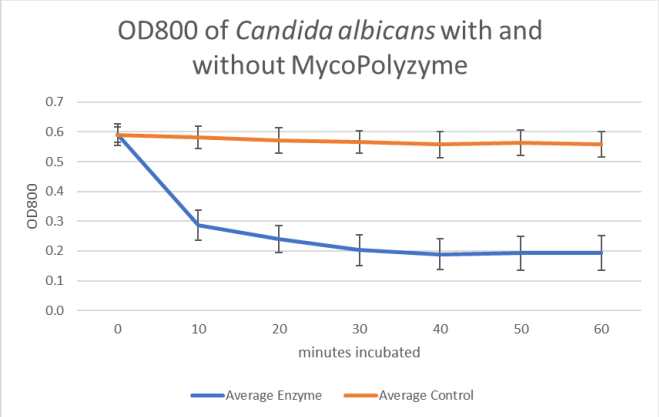
1. Grow *Candida albicans* for 18 hours submerged in YM media supplemented with dextrose (Cat. No. G8270 or equivalent).
2. Pellet culture by centrifugation. Wash pellet 2× with PBS. If necessary, the pellet can be frozen after this step for later use.

3. Add 1 mL of PBS (pH 7.5) without EDTA, calcium, or magnesium (such as Cat. No. D8537 or equivalent) to 5 mg MycoPolyzyme to create a 5 mg/mL stock solution of MycoPolyzyme.
4. Resuspend the pellet in sterile water, to achieve a hypotonic solution which will induce the lysis of spheroplasts. If protoplast formation is desired, PBS or sorbitol solution is recommended instead of water. Dilute the pellet until the OD_{800} is < 0.8.
5. Add 600 µL of 5 mg/mL MycoPolyzyme to 13 mL of diluted culture.*
6. Incubate the sample at 35 °C, 250 rpm, along with a negative control with no enzyme.
7. Measure the OD_{800} with a spectrophotometer every 10 minutes for at least 40 minutes, up to 1 hour.
8. Calculate the Lysis Percentage according to the following formula:

   Lysis Percentage = [(OD_{800} of Reference – OD_{800} of Reaction Mixture) × 100] / [Initial OD_{800} of Reference]
9. Once maximal sample lysis is reached, continue with the next step of the extraction protocol.

* The MycoPolyzyme needed for maximal lysis will vary depending on the sample type. These recommendations are based on experiments performed by our experts. It is recommended to adjust the amount and concentration for different sample types and experimental protocols.

A



B

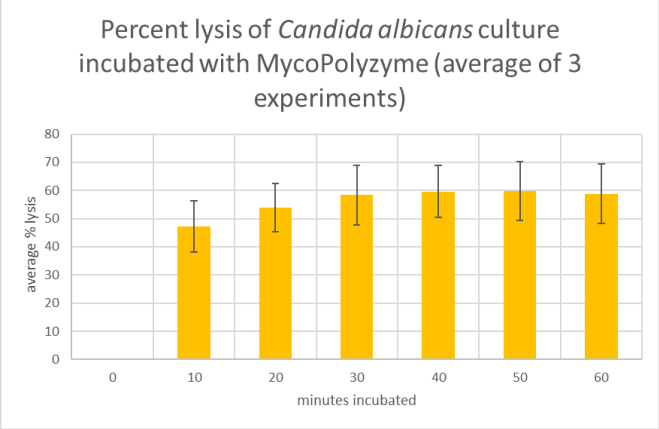


Figure 1. Frozen *Candida albicans* culture was treated with MycoPolyzyme. Data include 3 different experiments (n = 7). **A**. A decrease in OD indicates an increase in cell lysis. **B**. Data are expressed as % cell lysis compared to the control with no MycoPolyzyme. Both the samples treated with MycoPolyzyme and the negative controls were incubated at 35 °C.

The treatment data show that incubation time affects lysis percentage. Thus, it is recommended to determine empirically specific conditions before a larger experiment. In this case, lysis is shown as early as 10 minutes, with maximum lysis reached by 40 minutes.

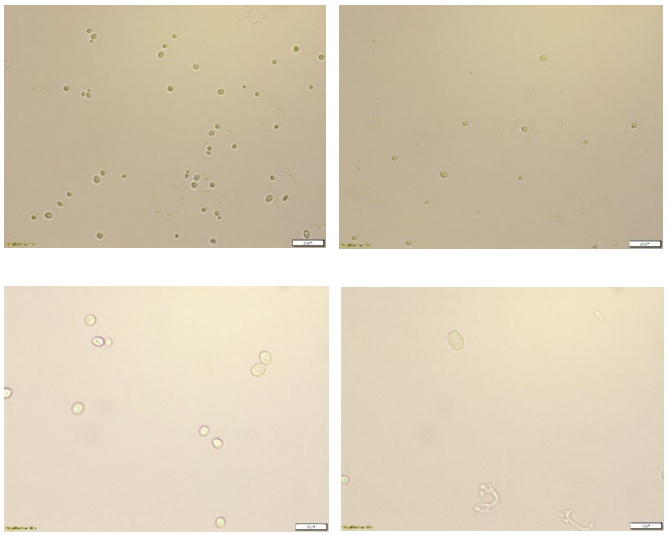


Figure 2. Microscopic image of *Candida albicans* showing cell lysis after 1 hour incubation at 35 °C. Left side: without MycoPolyzyme (negative control). Right side: treated with MycoPolyzyme. Upper row: 40×. Bottom row: 80×.

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