

Case study - Bacteria

Extraction of labile enzymes from gram-positive bacteria with the FastPrep® System.

Montserrat Zieger, Q Biogene.

Overview

- **Keywords:** Protein extraction, gram-positive bacteria, restriction enzyme
- **Aim of the study:** Demonstrate the capacity of the FastPrep® System to deal with otherwise difficult to lyse bacteria.
- **Application:** Restriction enzymes extraction
- **Sample name:** *Bacillus amyloliquefaciens* and *Staphylococcus aureus* 3A
- **Material:** FastPrep-24™, FastProtein™ Blue Kit containing Lysing Matrix B tubes

Protocol and Parameters

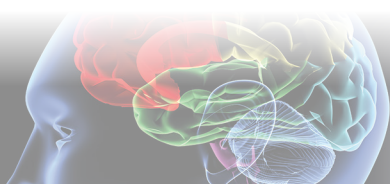
1. Cell density.

- **Sonication:** Bacterial suspensions of 0.2 g wet weight (w/w) and 0.15 g (w/w) per ml of buffer for *Bacillus amyloliquefaciens* and *Staphylococcus aureus* 3A, respectively.
- **FastPrep®:** Bacterial suspensions of 0.1 g (w/w) and 0.4 g (w/w) per ml and 0.15 g (w/w) per ml of buffer for *Bacillus amyloliquefaciens* and *Staphylococcus aureus* 3A, respectively.

2. Disruption.

- **Sonication:** Bacteria are disrupted at 50% maximum intensity (large tip) for *Bacillus amyloliquefaciens* and 20% maximum intensity (small tip) for *Staphylococcus aureus* 3A with a Branson Sonicator B30. Temperature is maintained at 4 ° - 5 °C by cooling in an ice salt water bath. Sonication was continued for 10 min in 40 sec. bursts for *Bacillus amyloliquefaciens* and 60 sec. in 5 sec. bursts for *Staphylococcus aureus* 3A.
- **FastPrep®:** The FastProtein™ Blue matrix was used. Tubes containing the lysing matrix and samples were prechilled at 4 °C then mixed. Samples are homogenized with the FastPrep-24™ instrument at speed 6.0 for 40 sec. for *Bacillus amyloliquefaciens* and at speeds 4.0 and 6.0 for 20 sec. and 40 sec. respectively for *Staphylococcus aureus* 3A. The tubes were returned to the ice bath. Homogenization and chilling was repeated for all time points.

At each time point a 50 µl sample was taken, centrifuged for 5 min at 4 °C in a benchtop centrifuge and tested for OD₂₆₀ and activity.

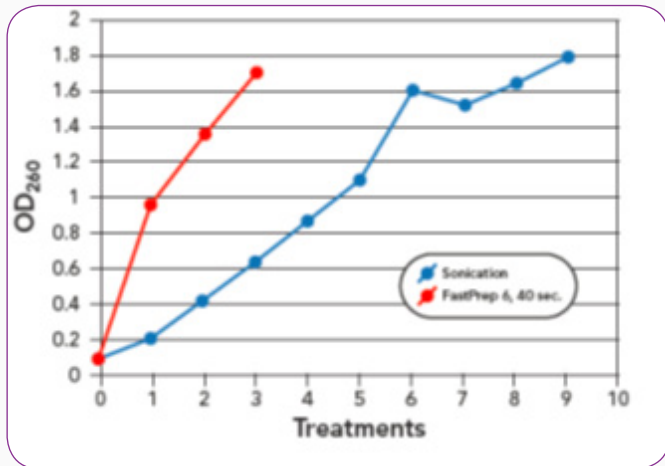


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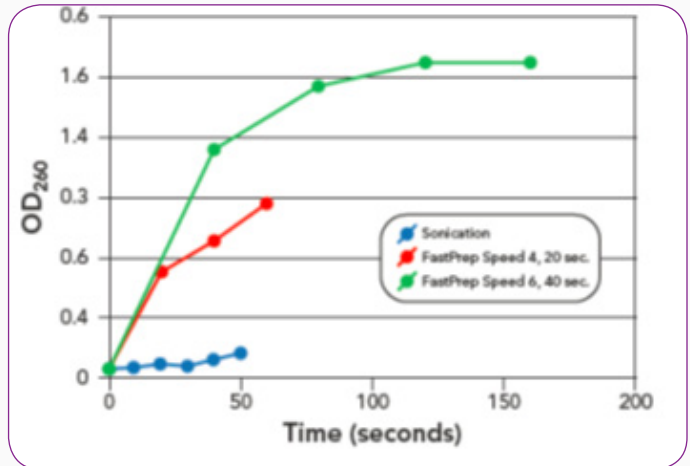
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Results

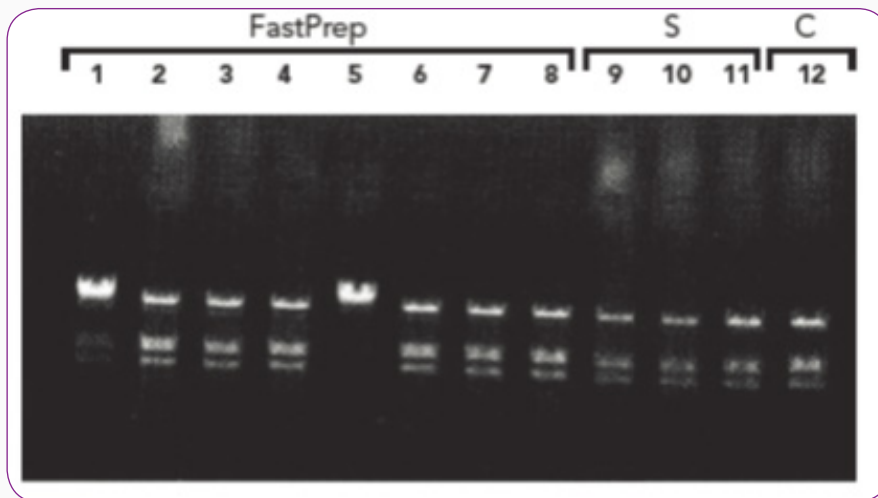
Bacillus amyloliquefaciens



Staphylococcus aureus 3A



Agarose gel electrophoresis with ethidium bromide staining



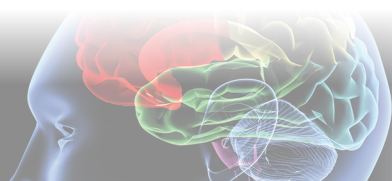
Lanes 1 to 8 correspond to the samples processed in the FastPrep-24™: 1 to 4 are at 0.4 mg/ml and 5 to 8 at 0.1 mg/ml. 1 and 5 at time 0, 2 and 6 at 40 sec., 3 and 7 at 2 x 40 sec., 4 and 8 are 3 x 40 sec. Lanes 9, 10 and 11 correspond to sonication samples (S) taken at 4 x 40 sec., 7 x 40 sec. and 9 x 40 sec., respectively. Lane 12: λ DNA cut by purified Bam HI (C).



Conclusion

These experiments clearly show that the FastPrep-24™ instrument using FastProtein™ Blue matrix can be used to successfully extract unstable enzymes from gram positive bacteria. Even in cases where sonication can release active materials (such as the *Bacillus amyloliquefaciens* experiments here), the lysing time can be reduced by approximately 60%. For samples like *Staphylococcus aureus* 3A that require longer and less efficient methods of lysis (such as French Press), the FastPrep® method offers clear advantages for extraction of active proteins.

Successful sample preparation using the MP Biomedicals FastPrep® product line has been highlighted in thousands of scientific articles. To access articles and other materials, visit www.mpbio.com/FastPrepLibrary.



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