CASE STUDY BACTERIA

*Extraction of labile enzymes from gram-positive bacteria with the **FastPrep**™ **System***

Overview

To demonstrate the capacity of the **FastPrep System** to deal with otherwise difficult to lyse bacteria, we chose to study two gram-positive bacteria. These two species, Bacillus amyloliquefaciens and Staphylococcus aureus 3A, are extremely resistant to classical cell rupture by methods such as sonication. These bacteria are used as a source for the production of two restriction enzymes, Bam HI and Sau 3AI respectively. The instability of these enzymes is well-known, and special conditions during purification and storage are necessary to maintain their biological activity. It is clear that these enzymes have to be purified under the most stringent conditions in order to prevent denaturation by factors such as proteases, heat, chemical agents, and others.

Materials and Methods

Lysis of Bacillus amyloliquefaciens with a standard lysing procedure involving sonication was compared with a modified **FastProtein Blue Kit** protocol (Cat N°: 6550-400).

Staphylococcus aureus 3A with its extremely thick cell wall is resistant to sonication. Normally, for successful large-scale extraction of production size volumes of Sau 3AI, use of the French Press is necessary; alternatively, protoplasts can be prepared. Here we utilize a small-scale sonication protocol for comparison with a modified **FastProtein Blue Kit** protocol.

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.

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 s bursts for Bacillus amyloliquefaciens and 60 s in 5 s bursts for Staphylococcus aureus 3A.
- **FastPrep:** The **FastProtein Blue matrix** was used. Tubes containing the lysing matrix and sample were prechilled at 4° C then mixed. Samples are homogenized with the **FastPrep instrument** at speed 6.0 for 40 s for Bacillus amyloliquefaciens and at speeds 4.0 and 6.0 for 20 s and 40 s 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 OD_{260} and activity.

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Results

1. Lysis

Bacillus amyloliquefaciens. Figure 1 shows that the optimal lysis of cells is achieved after 3 x 40 s treatments in the **FastPrep instrument** at speed 6.0 (red line), while equivalent lysis by sonication required 9 x 40 s bursts for equivalent quantities of cells (OD_{260} of sample diluted 100 times) (blue line). This means that for Bacillus amyloliquefaciens, the **FastPrep** produces a more complete lysate in approximatively one-third of the time. This time-saving feature also reduces the amount of time the extracted material is exposed to the denaturating conditions required to lyse the bacteria.

Staphylococcus aureus 3A. Figure 2 indicates that optimal lysis of cells is achieved after 3 x 40 s treatments in the **FastPrep instrument** compared with 10 x 5 s bursts (every other time point shown) of sonication for the equivalent quantity of cells (OD_{260} of sample diluted 200 times). This shows that for Staphylococcus aureus 3A, 3 x 40 s treatments was optimal for the release of extracted material (green line), while even ten 5 s sonication bursts released almost no material (blue line). The relative ease of lysis for the **FastPrep** method is clearly more effective than sonication, and is much faster and easier to perform than the classical French Press technique (data not shown).

2. Activity testing

Bacillus amyloliquefaciens. The activity of lysed samples from Bacillus amyloliquefaciens was tested on λ DNA. 10 µl of each supernatant was diluted five times in Bam HI storage buffer and then 2 µl was mixed with 1 µg of DNA in a 50 µl reaction volume and incubated at 37° C for 30 min. The reaction was then observed by agarose gel electrophoresis followed by ethidium bromide staining (see Figure 3). Results show that all **FastPrep** samples retained Bam HI activity, even at the shortest processing time of 40 s.

Staphylococcus aureus 3A. The activity of lysed samples from Staphylococcus aureus 3A was tested on λ DNA. 2 µl of each supernatant was mixed with 0.6 µg of DNA in a 25 µl reaction volume and incubated at 37° C for 30 min. The reaction was then observed by agarose gel electrophoresis followed by ethidium bromide staining. No restriction endonuclease activity was observed from any of the sonicated samples, and limited activity was observed from samples processed at speed 4 in the **FastPrep instrument**. However, samples that were processed at speed 6 showed increased activity, with the highest level of activity at 4 x 40 s (data not shown).

Figure 1: *Bacillus amyloliquefaciens*

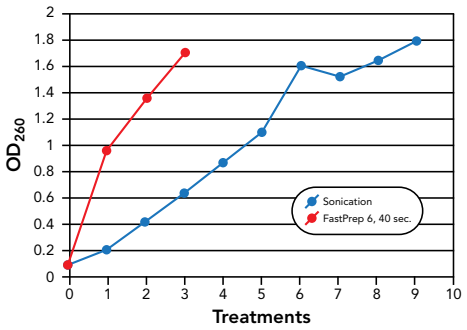


Figure 2: *Staphylococcus aureus 3A*

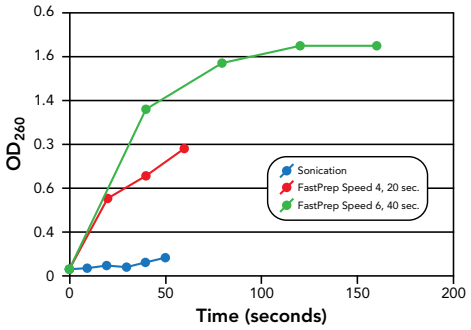
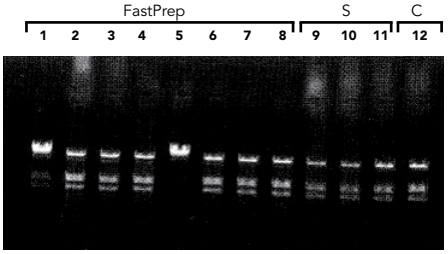


Figure 3: *Aqarose gel electrophoresis with ethidium bromide staining*



Lanes 1 to 8 correspond to the samples processed in the FastPrep: 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 s, 3 and 7 at 2 x 40s, 4 and 8 are 3 x 40 s. Lanes 9, 10 and 11 correspond to sonication samples (S) taken at 4 x 40 s, 7 x 40 s and 9 x 40 s, respectively. Lane 12: λ DNA cut by purified Bam HI (C).

Conclusion

These experiments clearly show that the **FastPrep 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.