Response to Comment on "Revealing Nature's Cellulase Diversity: The Digestion Mechanism of *Caldicellulosiruptor bescii* CelA"

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Gusakov critiques our methodology for comparing the cellulolytic activity of the bacterial cellulase CelA with the fungal cellulase Cel7A. We address his concerns by clarifying some misconceptions, carefully referencing the literature, and justifying our approach to point out that the results from our study still stand.

Gusakov (*1*) argues that the pH used in our study (*2*) for the enzymatic assays of Cel7A was not within the range of optimal pH values for this enzyme and that the substrate loading data were missing. He provides various references that we believe are hard to interpret, because they do not show curves representing activity versus pH. However, we have found such data in a paper by Boer *et al.* (*3*), according to which *Trichoderma reesei* Cel7A at pH 5.5 retains 95 to 96% of the activity it had at pH 5.0 [see figure 5 in (*3*)]. Nevertheless, we realized that there was a typographical error in the supplementary material (SM) of our paper, stating that our assays were performed at pH 5.5; the Cel7A assays were in fact run at pH 5.0, and the SM has been corrected. Regarding the substrate loading, the data in figure 1 in our paper were measured using 10 mg glucan/ml [1% (w/v)] of buffer for the Avicel experiments and comparable loadings for the other substrates. The original SM specified that the mass loading of the substrate was 1% for carboxymethyl cellulose (CMC), Avicel, and oat spelt xylan but did not explicitly clarify that it was similar for all the assays.

Gusakov also argues that the experiments to compare the activity of CelA/β-glucosidase with Cel7A/β-glucosidase should have been conducted instead of referencing published work. We feel that the articles we referenced for this discussion were sufficient in this regard because they evaluated the effects of β-glucosidases on the performance of Cel7A using a wide range of β-glucosidase loadings (*4*–*6*). Additionally, our manuscript focused on the characterization of CelA, and the comparison to Cel7A was only conducted without the presence of β-glucosidases. Given that our Cel7A assays were actually conducted at pH 5.0, we feel that the addition of β-glucosidase could not provide more than a 30% improvement.

Gusakov also argues that our results would be less dramatic if other mixtures or individual cellobiohydrolases, thought to be more efficient than those produced by *T. reesei,* were to have been used instead of Cel7A. However, the point of our paper was to compare single enzymes, not mixtures of enzymes, and in doing so, we selected the most commonly used single enzyme in commercial preparations, *T. reesei* Cel7A. We chose to compare purified enzymes because the variability in microbial broths can be considerable.

Additionally, Gusakov mentions a patent showing the existence of a cellobiohydrolase I (CBH I) more active than the enzyme from *T. reesei*. This patent compares *T. reesei* CBH I and *Penicillium funiculosum* CBH I on a time-to-target basis—in other words, time to achieve a certain level of conversion (*7*). If we compare enzyme activities in terms of endpoint conversion only, as done in our study, we can use figure 5 from the patent to determine that the total endpoint conversion is ~78% for the *P. funiculosum* native CBH I and 63% for the *T. reesei*–expressed CBH I. This increased performance represents a 25% improvement in activity over *T. reesei* CBH I and is small in comparison with the two-fold increase in conversion claimed in the patent using this metric. We can therefore assume that if *P. funiculosum* CBH I (with E1) had been used instead of *T. reesei* CBH I, the extent of conversion would be about 25% higher—i.e., from 30% to 38%. This level of activity increase does not change the conclusion of the paper. Also, if we were to apply the metric used in the patent—i.e., time-to-target performance—to CelA, we would find that CelA is much more than twice as active as *T. reesei* Cel7A/E1 (33% conversion achieved in 38 hours instead of 168 hours).

Gusakov proposes that when we comment on the molar efficiency of CelA, we should account for the fact that CelA has two catalytic domains. Although this could be another valid performance comparison for CelA and Cel7A, it is not a valid molar comparison. On a molar basis—i.e., one mole of Cel7A enzyme compared with one mole of CelA enzyme—our calculations are correct. In the context of this work, two metrics are important: enzyme loading based on mass (for industrial use) and on moles of protein. Regarding Gusakov's suggestion that the enzyme concentration could have been determined spectroscopically instead of with the bicinchoninic acid (BCA) assay, we chose to use the Pierce BCA assay, a well-known commercial product, for the sake of consistency both internally and externally. Any over- or underestimation of protein concentration was applied to all the proteins tested. The Bradford method was used initially only to quantify the raw broth. Gusakov's citation to the McMillan paper

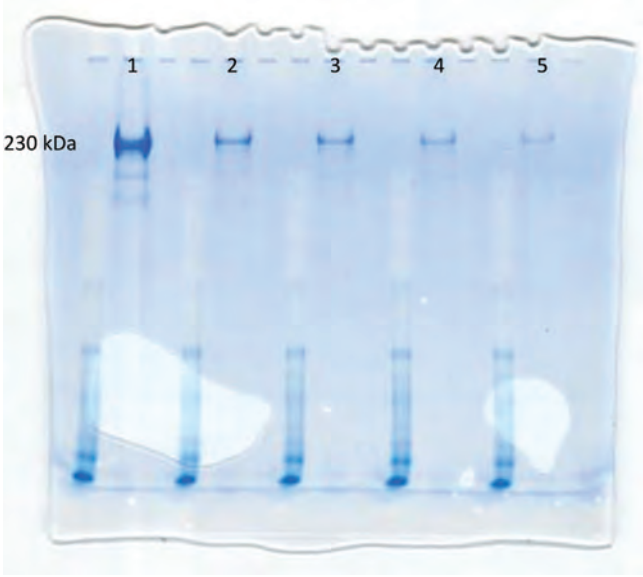

Fig. 1. Sodium dodecyl sulfate (SDS) gel showing the protein CelA before (lane 1) and after (lanes 2 to 5) size-exclusion chromatography.

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(*8*) is not relevant in this case, as those authors noticed a big difference between Bradford and BCA assays of test broths, not single enzymes. We believe that as long as the concentrations are measured using a consistent method, the relative difference from one enzyme to the next is a valid result. It is also well known that the use of 280-nm absorption measurements to quantify concentration is subject to large errors if the proteins being assayed lack tryptophan residues.

Finally, Gusakov wonders why the electrophoretic data for the purified CelA were not included in the manuscript. This is a valid concern, and we have included the electrophoretic data here, showing a clean single band for CelA (Figure 1). The purification scheme—including the final size exclusion chromatography (SEC) step—is used to remove the truncated or fragmented CelA forms that are about half the size of CelA.

References and Notes

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