Gaining insight into the biomass degradation of *Populus* caused by *C. bescii* growth with surface characterization

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Abstract

Calicibacteriaceae *C. bescii* is a cellulolytic/chemicellulolytic anaerobic bacterium that is considered for biomass conversion because it is a heat-tolerant bacterium which efficiently degrades non-prehydrolyzed biomass. It is reported that *C. bescii* is able to grow up to 90°C on untreated plant biomass and to degrade crystalline cellulose/xylan on untreated plant biomass such as switchgrass (SG) and poplar. Surface characterization of biomass during *C. bescii* growth provides insights into the biomass recalcitrance. To understand the chemical and physical changes on the surface of biomass with *C. bescii* growth, we employed ToF-SIMS. As a model substrate, cross-sections of a juvenile *Populus* stem (ca. 80 μm thick) were used. The sectioned poplar stem was incubated with/without *C. bescii*. Fresh juvenile poplar stems (ca. 1.3 cm length) were debarked and incubated with *C. bescii* for 5 different times in order to understand the *C. bescii* penetration mechanism. The poplar stems after *C. bescii* growth (i.e., positive control) were cross-sectioned to 50 μm thick slices and collected from top to center (i.e., 120 sections/sample). These sectioned samples can be used to observe the level of cell wall degradation depending on the vertical depth by SEM and ToF-SIMS. Here, we found that *C. bescii* growth conditions partially affect cell wall damage, but the damage should not change chemical composition on the surface of cell wall. We also found that *C. bescii* can penetrate into the short wood stick through the lumen area and result in severe cell wall damage at vertical center area.

1. Experimental conditions to measure cell wall degradation during *C. bescii* growth

- **Negative Control**
  - *C. bescii* inoculum was incubated with 2% inoculum obtained from overnight growth culture.

- **Positive Control**
  - *C. bescii* inoculum was incubated with 2% inoculum obtained from overnight growing culture.

2. Topographical & chemical changes on the surface during *C. bescii* growth

- **Topographical changes by SEM**
  - **Negative Control**
    - Visual: Top and vertical centers show severe cell wall damages compared to vertical middle area.
  - **Positive Control**
    - Visual: Top and vertical centers show severe cell wall damages compared to vertical middle area (green dots)

- **Chemical changes by ToF-SIMS**
  - **Negative Control**
    - Cellulose intensity on the surface of negative control samples is low.
  - **Positive Control**
    - Cellulose intensity on the surface of positive control samples is high.

Conclusions

- **Conditions for *C. bescii* growth cause some cell wall damage.**
  - Negative sample images by SEM show partial cell wall damage.
  - However, these damages may not affect chemical composition change on the surface.
- **C. bescii** penetrates the *Populus* wood stick.
  - *C. bescii* causes severe cell wall damage.
  - *C. bescii* should penetrate the poplar wood stick through lumen area. Consequently, there are severe cell wall damages at 5mm below surface.
  - Semi-quantitative ToF-SIMS results also show lower cellulose intensity at vertical center.