

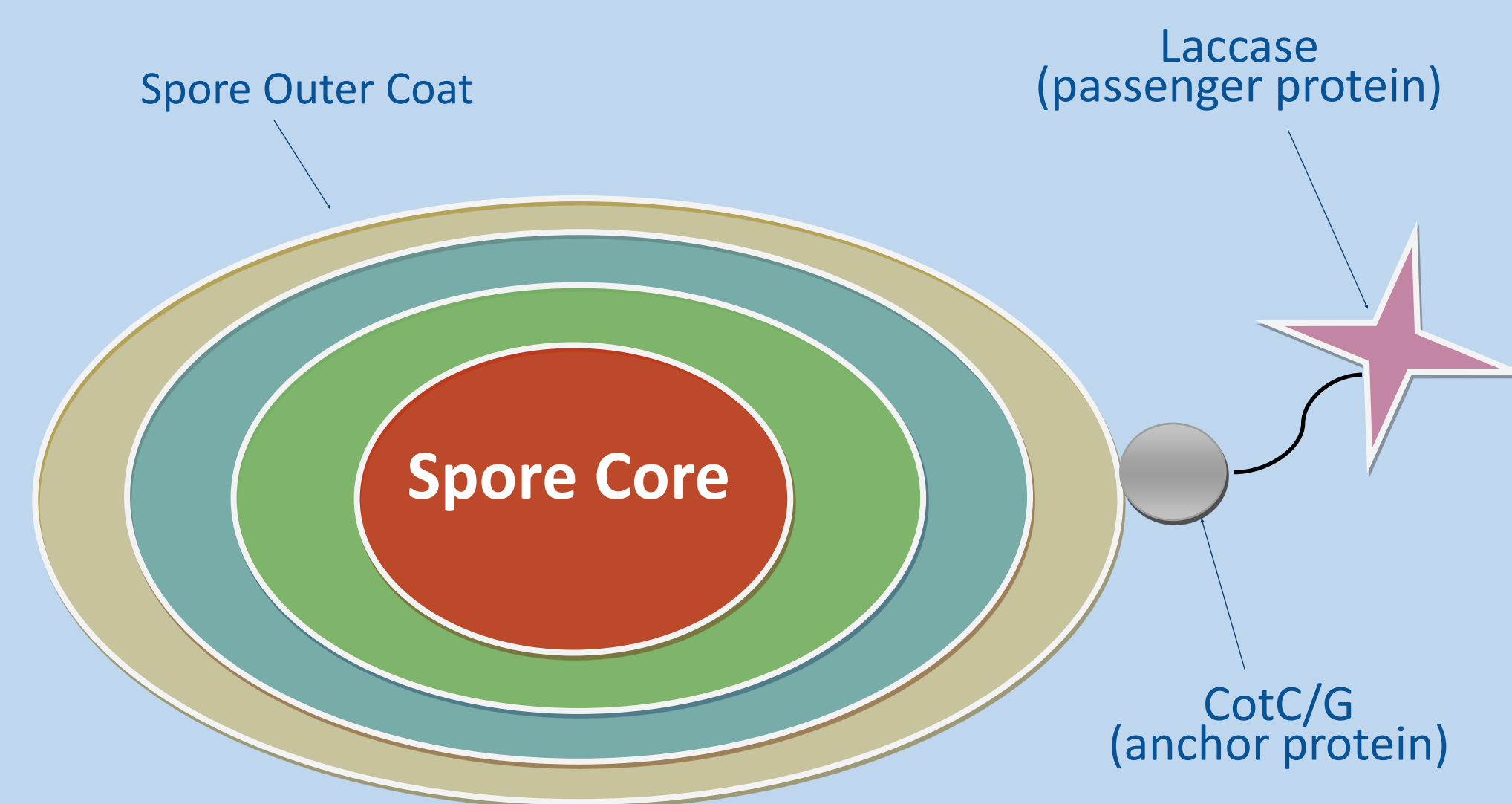


Display of Laccase on Bacterial Spore Surface for Improved Stability

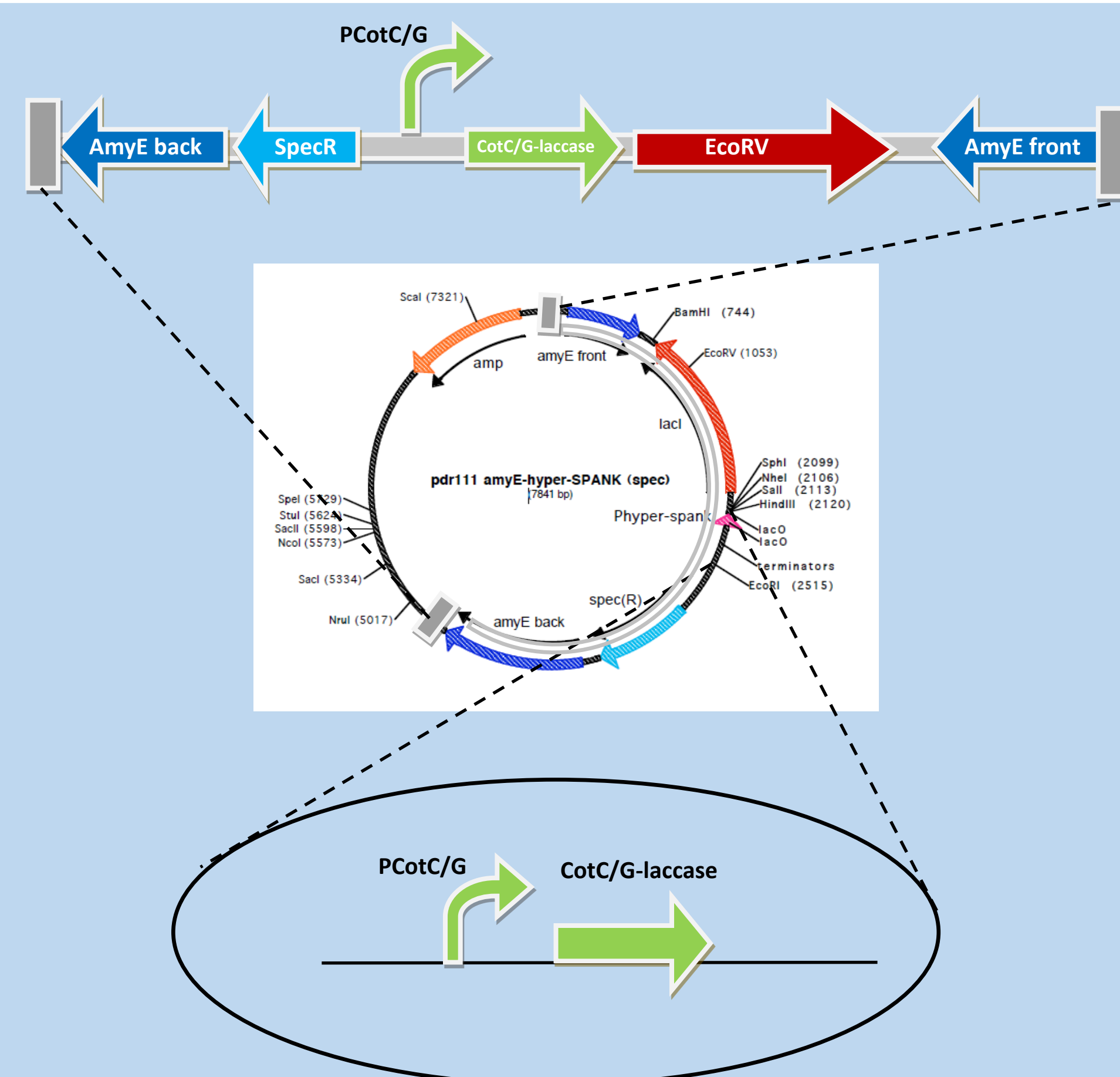
Halie White, Tony Castagnaro and Kang Wu
Department of Chemical Engineering

Background

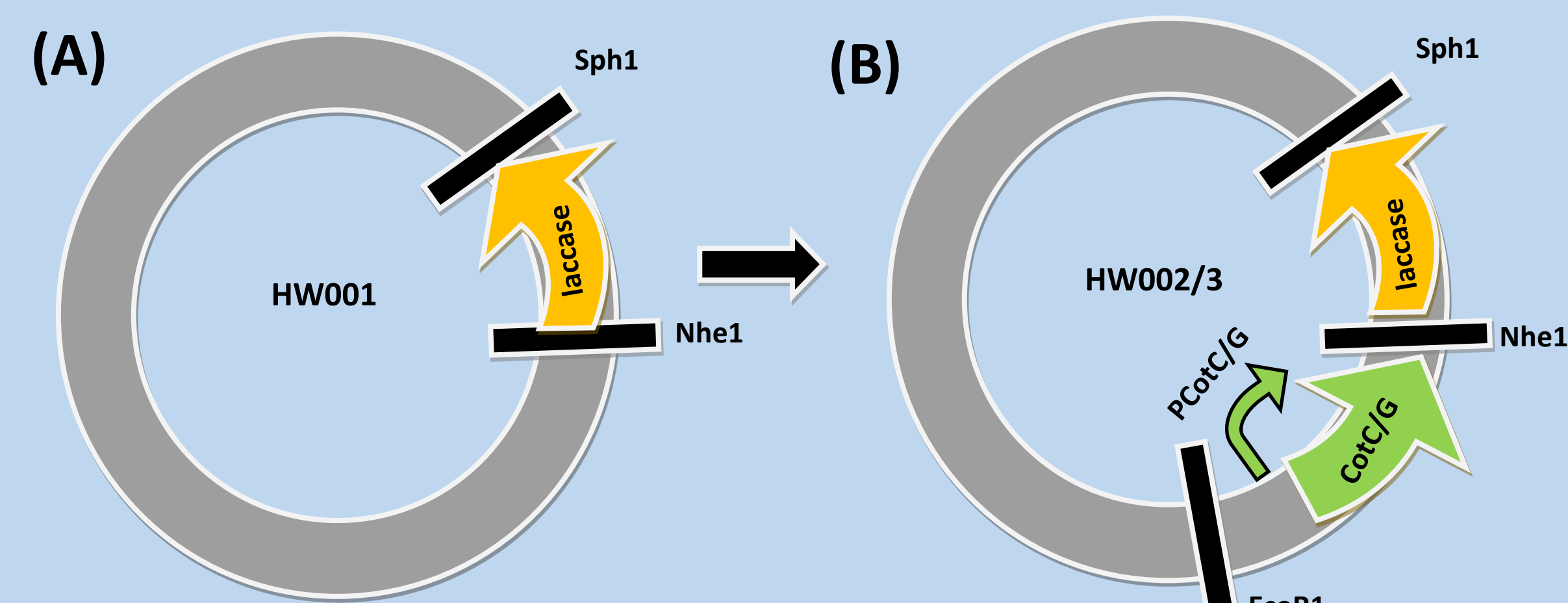
Laccases are copper-containing enzymes that oxidize a wide range of substrates and can be potentially used for biomass deconstruction or bioremediation. Currently commercial laccases are produced from fungi and enzymes from fungi are generally not as stable as those from bacteria. The goal of this project is to develop an easy-to-produce, recoverable, and stable laccase displayed on spore surface. The spore is chosen due to its ability to withstand extreme temperatures, radiation and chemicals, and can be stored at room temperature for years.



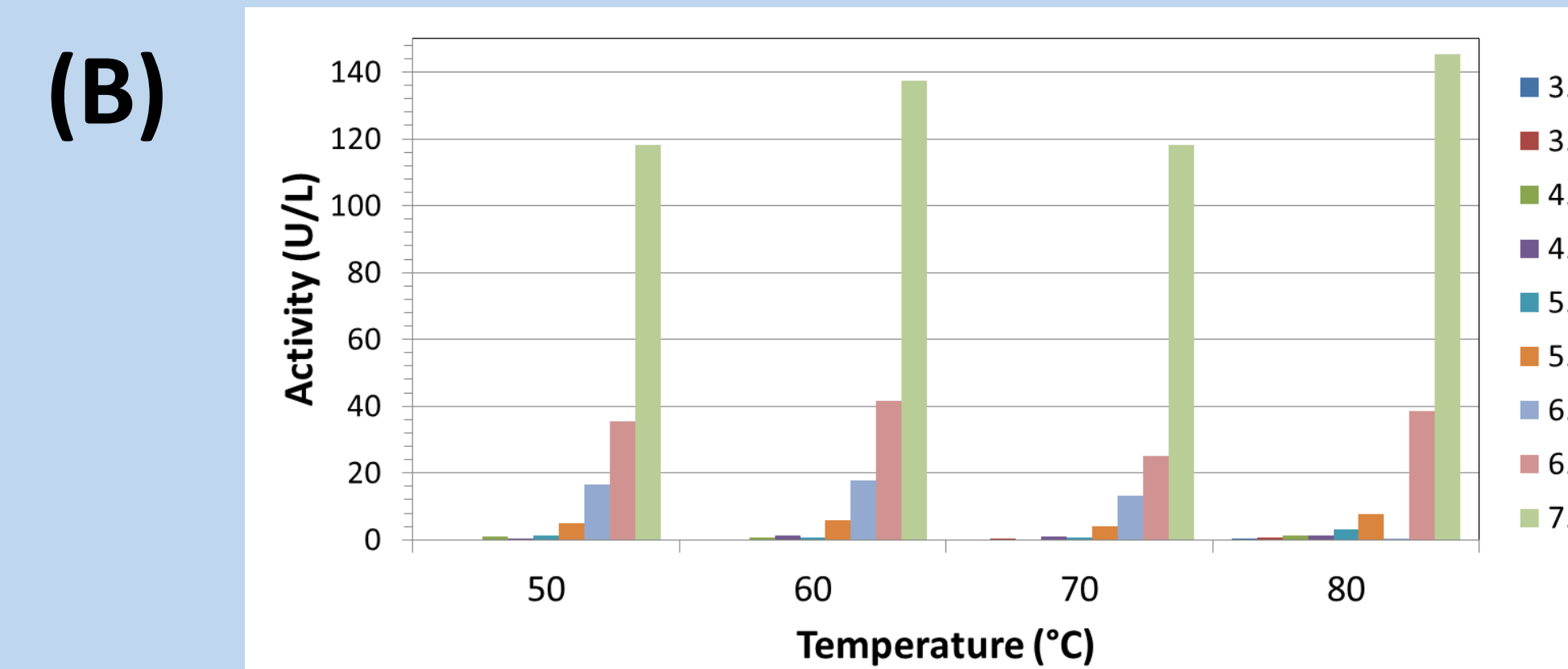
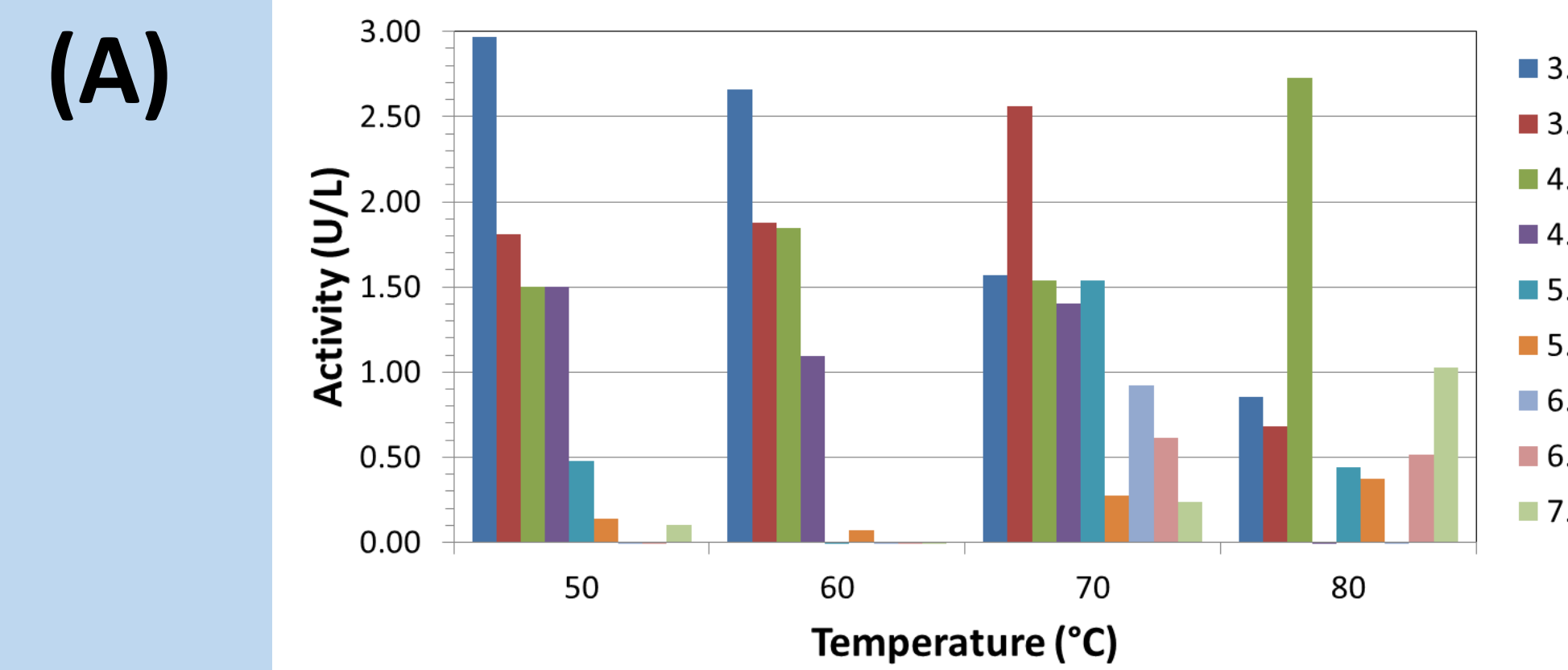
Experimental Design



Promotor and anchor/passenger protein are inserted into PDR111 and then integrated into the chromosome of *Bacillus subtilis*

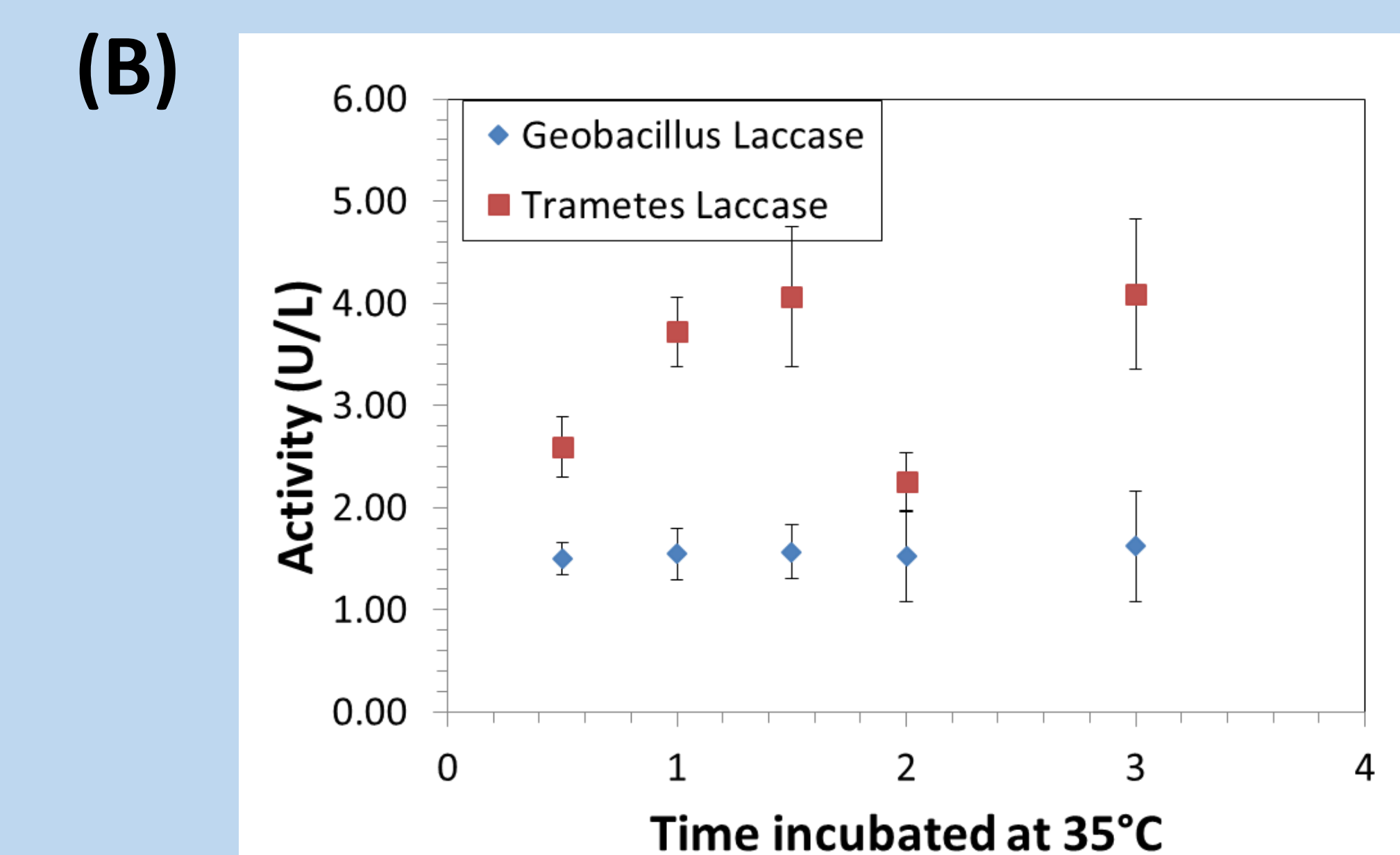
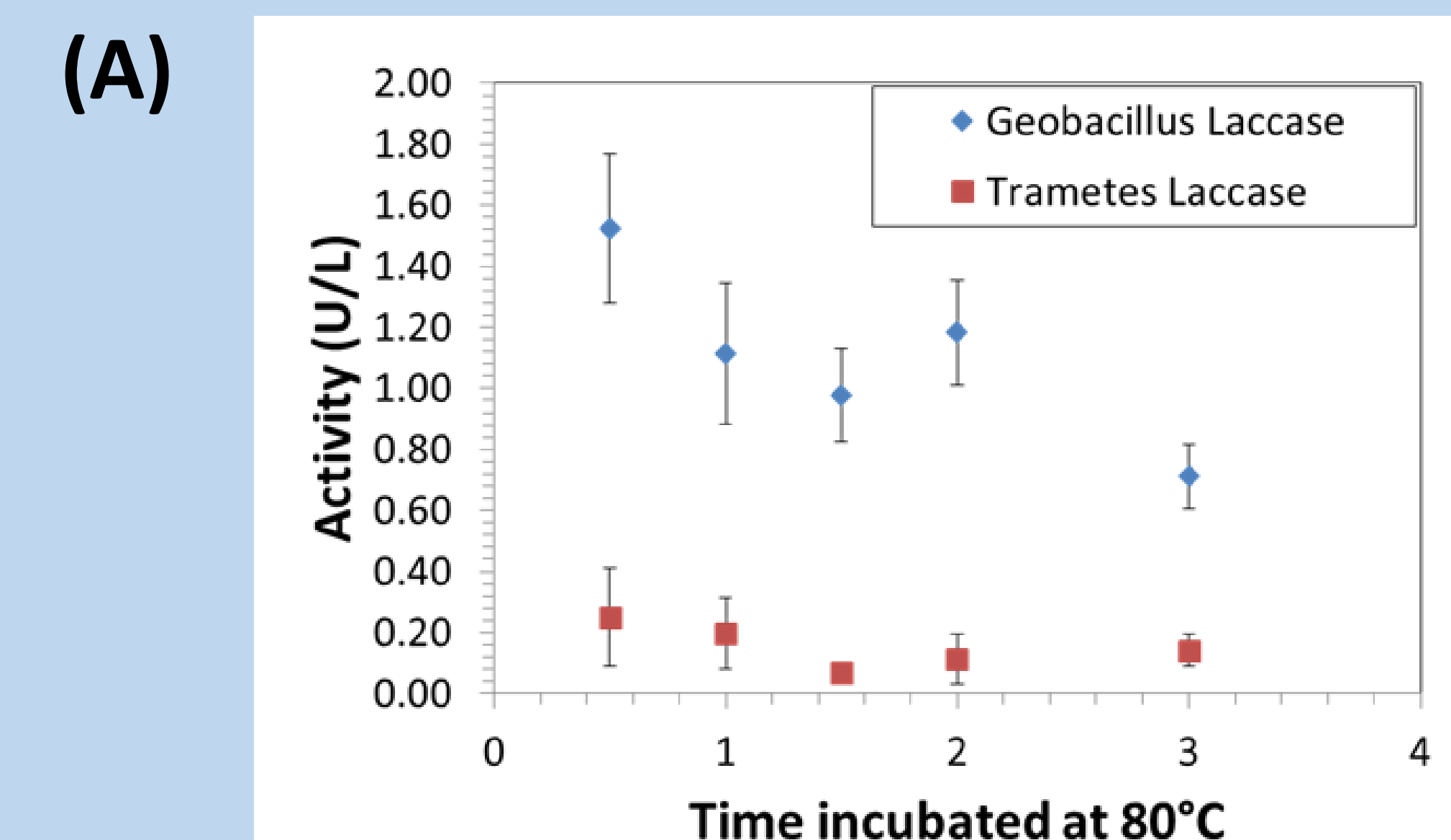


Laccase inserted into PDR111 at cutting sites Nhe1 and Sph1 (A); Gene CotC or CotG and their corresponding promoter is inserted into plasmid HW001 at cutting site EcoR1 and Nhe1 (B), resulting in pHW002 and pHW003



The activity at various temperatures of laccase from thermophiles *Geobacilli* in ABTS at pH values ranging from 3 to 7 (A); The activity at various temperatures of laccase from thermophiles *Geobacilli* in 2,6-dimethoxyphenol at pH values ranging from 3.0 to 7.0 (B)

Results



Activity of commercial fungal laccase and *Geobacillus Thermoglucosidasius* at 80°Celsius (A); Activity of commercial fungal laccase and *Geobacillus Thermoglucosidasius* at 35°Celsius (B)

Fungal laccase is unstable at 80°C whereas the laccase characterized from gram-positive and thermophilic *Geobacillus Thermoglucosidasius* is stable.

Future Work

- * Complete strain construction
- * Compare laccase on spore surface with fungal laccase and *Geobacillus thermoglucosidasius* laccase
- * Examine spore integrity
 - Viability
 - Resistance to solvents, temperature, and pH

References

1. Kim, Junehyung, and Wolfgang Schumann. "Display of Proteins on *Bacillus Subtilis* Endospores." *Cellular and Molecular Life Sciences* 66.19 (2009): 3127-136. Web.
2. Madhavi, Vernekar, and S. S. Lele. "Laccase: Properties and Applications." (n.d.): n. pag. Web.