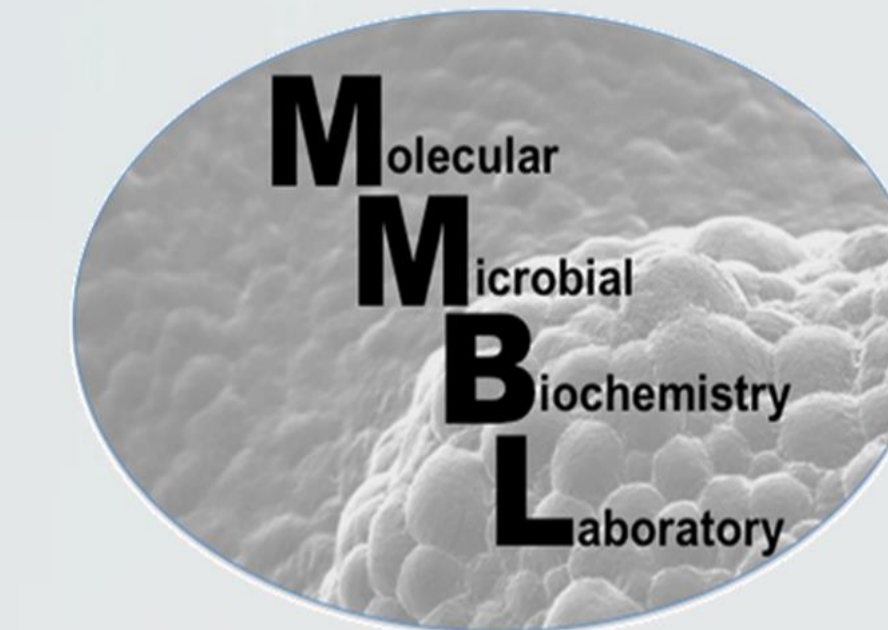


Influence of Plant Associated Compounds on Bacterial Cellulose Production

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Abstract

Gluconacetobacter species grow in close association with plants and are of interest for their ability to synthesize cellulose pellicles. The aim of this work was to identify possible regulatory influences of plant associated compounds (PACs) as well as their potential to serve as alternative carbon sources on cellulose production by *Gluconacetobacter xylinus* ATCC 53582 and *Gluconacetobacter hansenii* ATCC 23769. Cultures were inoculated in Schramm-Hestrin (SH) medium with and without glucose supplemented with 0.5%, 0.05%, or 0.005% (w/v) xylan, lignin, cellobiose, colloidal chitin, chitosan, and polyethylene glycol (PEG) of molecular weight 1000, 4000 and 8000. Pellicle size, wet weight, and dry weight as well as pH of culture broths were measured after 7 days of static growth at 30°C. *G. xylinus* cultures consistently produced larger pellicles than *G. hansenii*. Interestingly, *G. xylinus* also reduced the pH of the medium to a greater extent than *G. hansenii*, which are known to reproduce faster, providing some insight into the energy requirements of these bacteria with respect to the synthesis of the biopolymer cellulose. This greatly impacts our ability to manipulate cellulose production and composition for industrial applications.

Introduction

Cellulose is a naturally synthesized, linear polysaccharide of β-D-glucopyranose units with unique physical and chemical properties. In papermaking, it is used for ultra-strength paper and provides a strong enough matrix for electronic paper. Cellulose can be used as a food additive for dietary fibre and has many uses in the medical field including wound dressings, bone grafting, and blood dialysis (Hoenich 2007). As a global incentive, it can be used as a feedstock for biofuel production.

It is found in nearly every known plant and is the most abundant organic polymer on Earth, however effective extraction of plant cellulose is difficult due to the presence of the plant cell wall compounds hemicellulose and lignin. Bacterial cellulose (BC) is far superior to plant cellulose as it is pure, and therefore free from these compounds. It also displays higher mechanical strength and crystallinity, in the form of a micro fibril network (George et al. 2005). Expensive, inefficient production of BC prevents wide scale industrial approval; improving methods of synthesis through the study of biochemical pathways is therefore a necessity.

Gluconacetobacter sp. has long been used as a model organism for studies on bacterial cellulose synthesis. This study concentrates on *Gluconacetobacter xylinus* ATCC 53582 and *Gluconacetobacter hansenii* ATCC 23769 for their high rate of cellulose production and rapid proliferation, respectively. These gram negative bacteria use cellulose as a biofilm for colonization while growing on rotting fruits (Williams and Cannon, 1989) and are commonly responsible for spoilage of wine. When grown in liquid culture they form cellulosic pellicles at the liquid-air interface, entrapping cells where oxygen is abundant.

In this study, pellicles formed by *Gluconacetobacter sp.* grown in the presence of compounds commonly associated with plants and fruits were characterized in order to elucidate biochemical mechanisms that regulate cellulose synthesis in these bacteria. Furthermore, this knowledge will improve our ability to manipulate cellulose production and composition for industrial applications.

Methodology

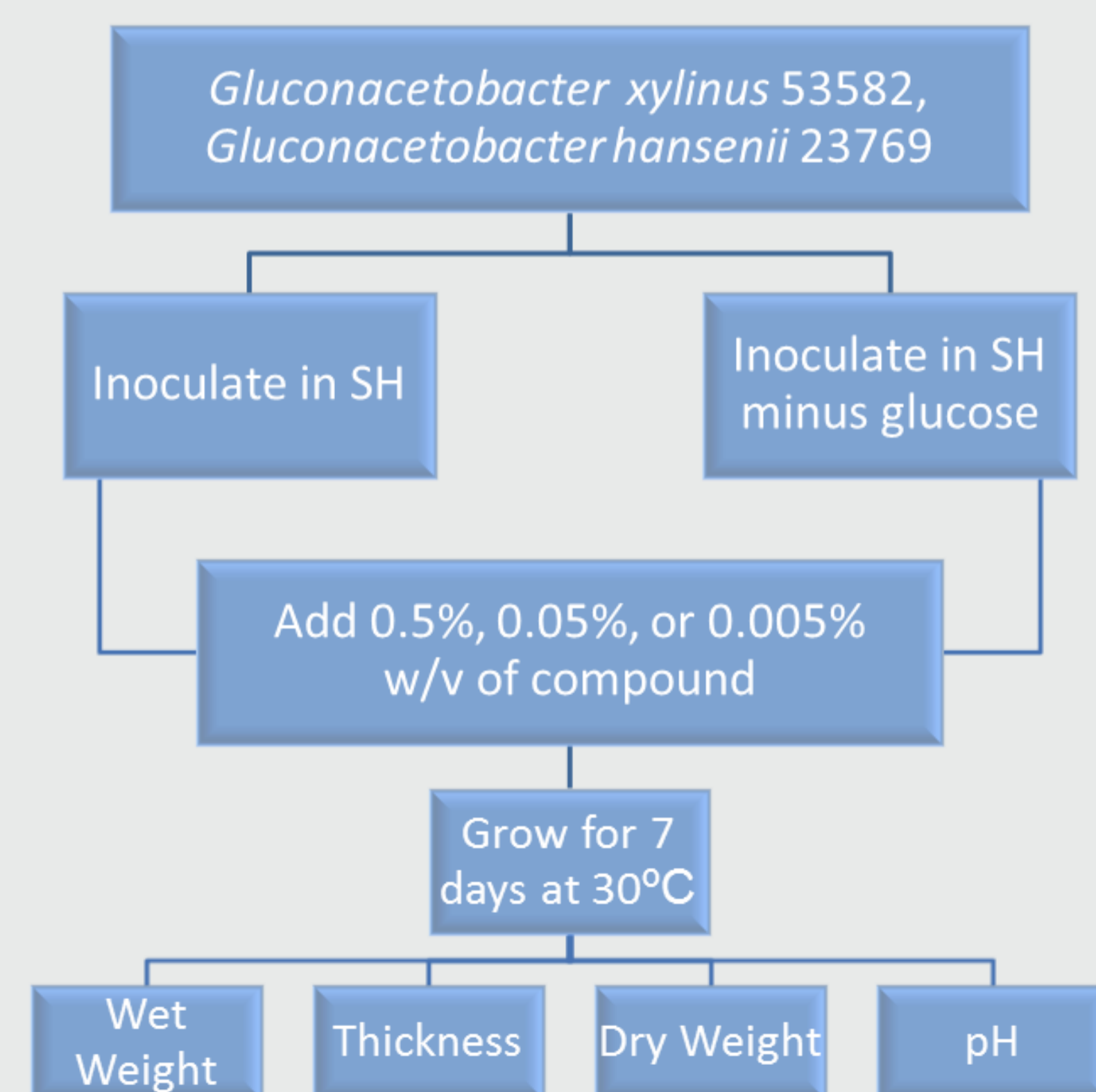


Figure 1. Schematic for characterization of pellicles grown in various PACs.

Results

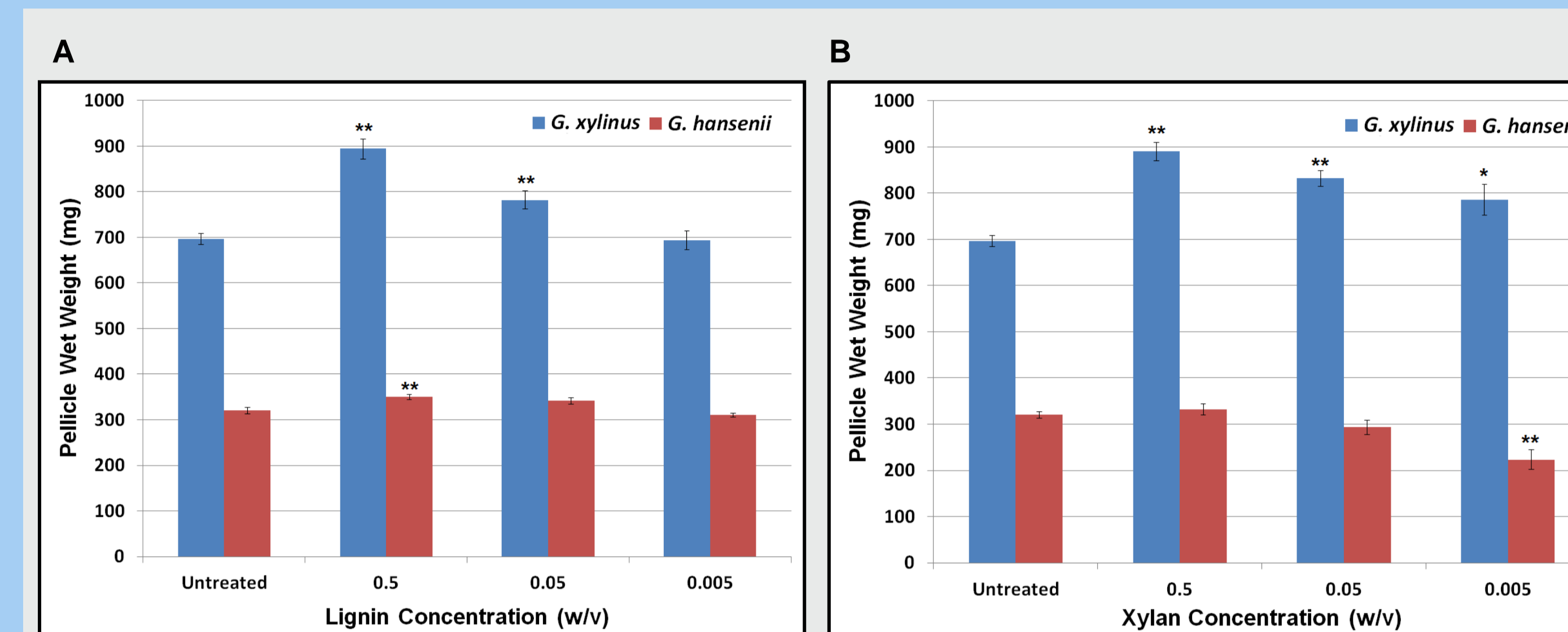


Figure 2. Concentration dependent influence on wet weight of pellicles produced by *G. xylinus* and *G. hansenii* in the presence of **A) lignin (0.005% - 0.5% w/v)** and **B) xylan (0.005% - 0.5% w/v)** at 30°C in Schramm-Hestrin broth. Data show the mean for six technical replicates. Error bars shown are standard error of the mean. The significance levels reported are $p < 0.01$ (**) and $p < 0.05$ (*).

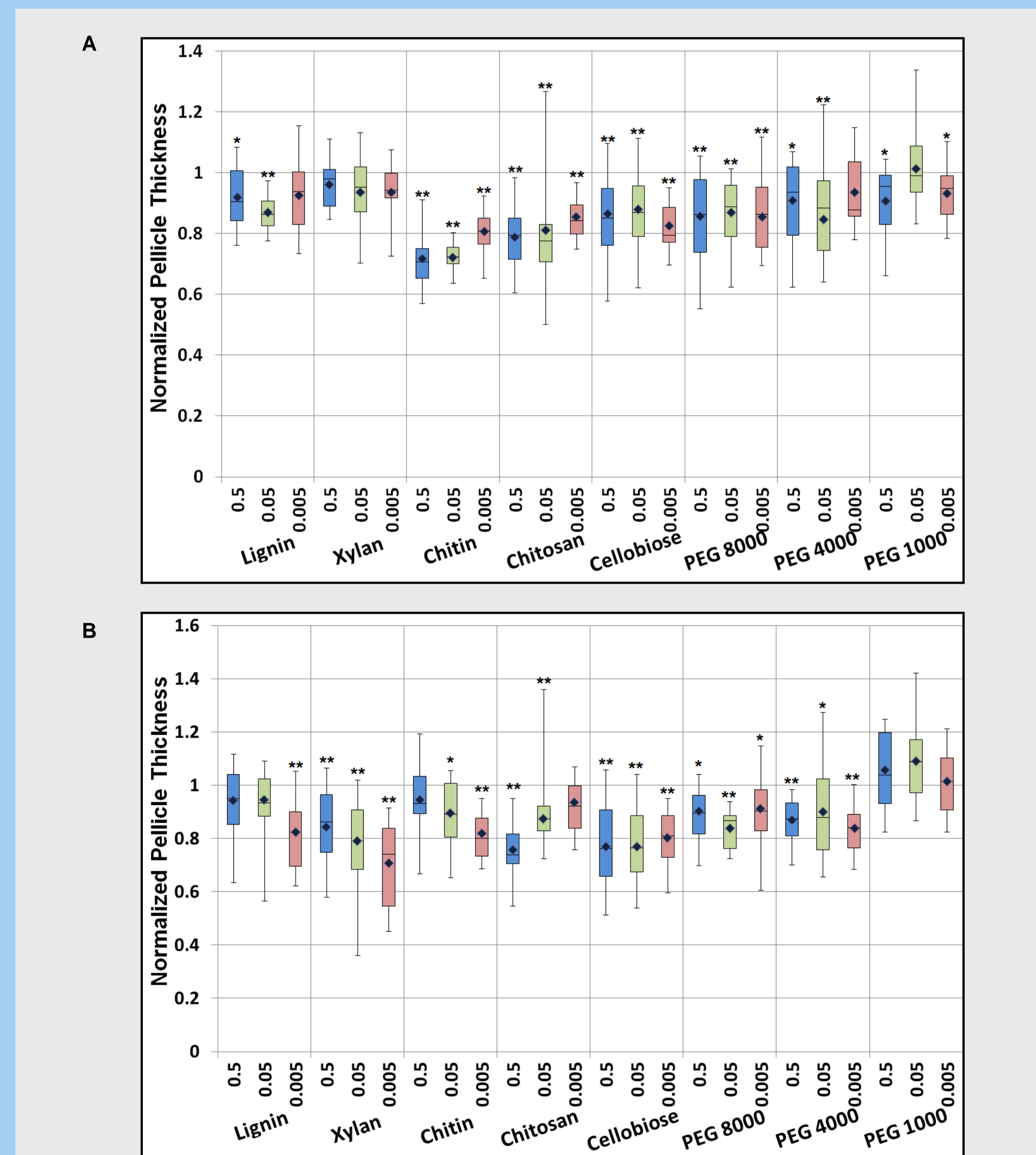


Figure 3. PACs influence amplitude and regularity of pellicle thickness. Box and whisker plots of pellicle thickness of **A) *G. xylinus*** and **B) *G. hansenii*** pellicles grown in the presence of various PACs at concentrations of 0.5% - 0.005% (w/v). Each box represents 6 technical replicates, each measured at three loci. Pellicle thickness was measured using ImageJ software. Data was normalized to untreated controls. The significance levels reported are $p < 0.01$ (**) and $p < 0.05$ (*).

Results

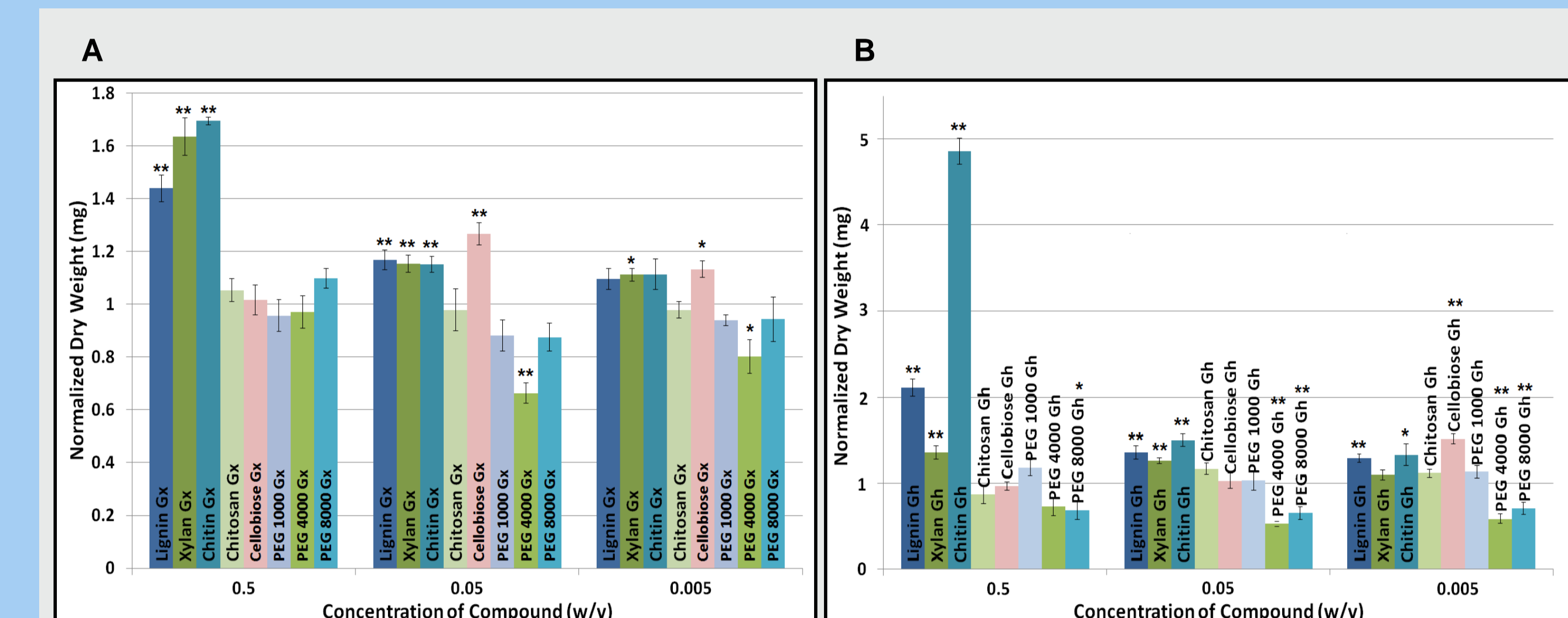


Figure 4. Pellicle weights are influenced by PACs. Dry weight of **A) *G. xylinus*** and **B) *G. hansenii*** pellicles grown in the presence of various PACs at concentrations of 0.5% - 0.005% (w/v). Data show the mean for six technical replicates normalized to untreated controls. Error bars shown are standard error of the mean. The significance levels reported are $p < 0.01$ (**) and $p < 0.05$ (*).

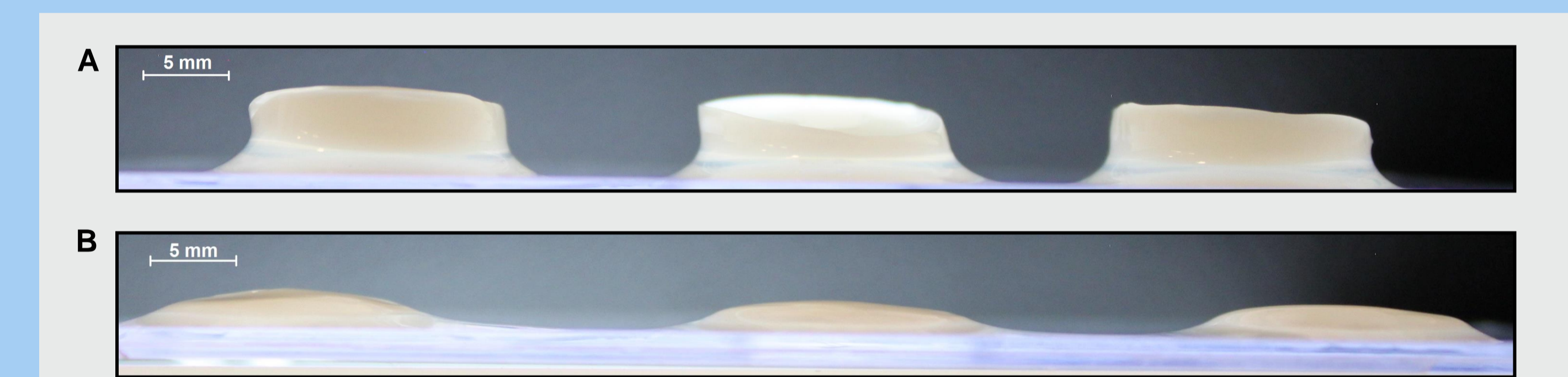


Figure 5. Pictures of untreated **A) *G. xylinus*** and **B) *G. hansenii*** hydrated pellicles depicting contrast in pellicle size.

Discussion/Conclusion

1. Lignin and xylan, both components of plant cell walls, statistically increased pellicle wet weight for *G. xylinus* in a dose-dependent manner (Figure 2). These may act to trigger pellicle formation as they would indicate the presence of plant material.
2. Polyethylene glycol of molecular weight 8000 statistically increased pellicle wet weights for *G. hansenii*.
3. Due to a water retaining amorphous cellulose present beneath the crystalline pellicle, the wet weight of pellicles was nearly 50% volume of the 2 mL of medium present in culture wells.
4. Pellicles retained insoluble materials such as chitin and chitosan. These PACs reduced pellicle thickness in *G. hansenii* (Figure 3A) and increased dry weight of pellicles (Figure 4). This carryover dramatically increased pellicle dry weight when *G. hansenii* was grown in 0.5% chitin.
5. *G. xylinus* pellicles were significantly larger than *G. hansenii* pellicles (Figure 5).

References

George J, Ramana KV, Sabapathy SN, Jagannath JH, Bawa AS (2005) Characterization of chemically treated bacterial (*Acetobacter xylinum*) biopolymer: some thermo-mechanical properties. *Int J Biol Macromol* 37:189-194

Hoenich NA (2007). Cellulose for medical applications: past, present, and future. *BioRes* 1: 270-280.

Williams WS & Cannon RE (1989). Alternative environmental roles for cellulose produced by *Acetobacter xylinum*. *Appl Environ Microbiol* 55: 2448-2452.

Acknowledgements

This work was supported by a Natural Sciences and Engineering Research Council of Canada Discovery Grant to J.L. Strap and the UOIT University Works program.