The effect of phytohormones on the growth, cellulose production and pellicle properties of *Gluconacetobacter xylinus* ATCC 53582

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Abstract

Gluconacetobacter xylinus is a plant-associated bacterium best studied for its cellulose production. Bacterial cellulose is important in facilitating plant-microbe interactions but little is known about the effect that exogenous phytohormones have on bacterial cellulose synthesis or the growth of *G. xylinus.* We characterized the growth, development and effect on pellicle characteristics caused by exogenous indole-3-acetic acid (IAA), gibberellic acid (GA), abscisic acid (ABA) and zeatin (Z) over a range of concentrations (1 nM to 100 μM). These phytohormones are plant growth regulators known to be involved plant development including fruit ripening and stress tolerance. Each of these hormones stimulated *G. xylinus* growth and influenced its pellicle characteristics. Exogenous IAA had the greatest effect on *G. xylinus* pellicles. Growth in IAA produced thin pellicles with very little cellulose. In general, pellicle wet weight was inversely proportional to the bacterial cellulose yield when cultures were grown in the presence of ABA, suggesting ABA influenced pellicle density and hydration. The crystallinity index, CI (IR) of cellulose produced in the presence of each phytohormone over a variety of concentrations was determined by Fourier transform infrared spectroscopy. The observed effect on cellulose crystallinity was concentration and hormone dependent. GA caused the greatest alterations in crystallinity with the highest CI (IR)=0.94 at 1 μM and the lowest CI (IR)=0.47 at 500 nM. Endogenous production of hormones by *G. xylinus* was investigated by high performance liquid chromatography of extracts prepared from both cell pellets and culture supernatants. We found *G. xylinus* synthesized GA, ABA and Z but did not produce IAA.

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Key words: cellulose, phytohormones, abscisic acid, gibberellic acid, indole-3-acetic acid, zeatin, crystallinity, *Gluconacetobacter xylinus*.

Acknowledgements: the authors wish to thank F. Gaspari for assistance with FTIR.

Conference presentation: part of this paper was presented at the *3rd International Conference on Acetic Acid Bacteria. Vinegar and Other products*, 2012 Apr 17-20, Cordoba, Spain (http://www.uco.es/aab2012/).

Funding: UOIT-Start-up funds and Natural Sciences and Engineering Research Tools and Instruments (NSERC-RTI) grant to J.L. Strap.

Contributions: JLS conceived and designed the experimental work, contributed to the experimental work and co-wrote the manuscript; OQ, HS, AL carried out experimental work and contributed to writing the manuscript.

Conflict of interests: the authors declare no potential conflict of interests.

Received for publication: 1 August 2012.
Revision received: 14 October 2012.
Accepted for publication: 18 October 2012.

Acetic Acid Bacteria 2013; 2(s1):e7
doi:10.4081/aab.2013.s1.e7

Introduction

Gluconacetobacter xylinus is a gram-negative bacterium that can be isolated from rotting fruits and vegetables[1,2] however the mechanisms involved in this plant-microbe interaction are not known in detail. *In vitro, G. xylinus* produces a cellulose pellicle at the air/liquid interface of broth-grown cultures. The cellulose biofilm produced by this organism has been the subject of intense investigation due to its purity, mechanical strength, and high crystallinity.[3-11] These properties make bacterial cellulose useful for a variety of industrial applications.[3,12-15] Despite decades of study, the complete cellulose biosynthetic pathway has not yet been elucidated; there is still much to learn about its regulation, export mechanisms and assembly into the crystallized form.

We recently identified a small molecule, pellicin, capable of disrupting crystalline cellulose formation by *G. xylinus*.[16] Given the importance of bacterial cellulose for plant-microbe interactions[17-20] it was of interest to investigate whether phytohormones are also capable of influencing bacterial cellulose crystallinity as this will have implications for attachment, growth and survival of plant-associated microbes.

In this study, representative phytohormones known to play a key role in ripening and senescence of fruits and vegetables were chosen: gibberellic acid (GA), abscisic acid (ABA), the auxin indole-3-acetic acid (IAA), and the cytokinin zeatin. In plants, auxins stimulate cell wall synthesis, cell enlargement, cell division and importantly delay leaf senescence and fruit ripening.[21] At high concentrations, IAA has been shown to reduce root growth.[22] Gibberellins, a large class of phytohormone comprised of more than one hundred compounds,[23] have been implicated in the stimulation of both cell division and cell elongation in plant stems[22,24] and serves an important role in primary root growth by regulating cellulose deposition.[25] In grapes, exogenous application of gibberellins induce fruit setting and fruit growth.[24] Cytokinins are

adenine derivatives that function in many aspects of plant growth and development including the induction of cell division in fruits and shoots as well as the delay of senescence.[26,27] ABA opposes the activity of the other classes of phytohormone exerting an inhibitory effect on plant growth. ABA is also involved in fruit ripening.[28] Moreover, exogenous ABA treatment of grapevines has been shown to enhance the activity of cellulase promoting the decomposition of cellulose[29] leading to fruit softening.

While there have been many reports on the effect of bacterially produced phytohormone-like compounds on plants,[30-35] very few studies have focussed on the effect that plant hormones have on the growth and biofilm production of bacteria. Therefore, the purpose of the present study was to examine the effect that phytohormones have on the growth and development of *Gluconacetobacter xylinus* ATCC 53582 and in particular, to determine the effect that these hormones have on pellicle characteristics.

Materials and Methods

Chemicals

Zeatin (Z) and ABA were purchased from BioBasic (Markham, ON, Canada). GA and IAA were purchased from BioShop (Burlington, ON, Canada).

Bacteria, growth medium and culture conditions

Gluconacetobacter xylinus ATCC 53582 was grown in Schramm-Hestrin (SH) medium[8] either on a rotary shaker at 30°C or under static conditions. To obtain a uniform suspension of *G. xylinus* cells lacking cellulose, 0.1% (v/v) filter-sterilized cellulose (Sigma, St. Louis, MO, USA) was added to cultures. Growth curve data was obtained in 96-well microtitre plates inoculated with a total volume of 150 μL SH medium containing 0.1% cellulose and either dimethylsulfoxide (DMSO) or ABA, GA, IAA or Z dissolved in DMSO and appropriately diluted in SH broth to give concentrations of 0-100 μM. The addition of DMSO does not alter the growth rate of *G. xylinus* under the growth conditions used in this study.[16] The inoculum was prepared by harvesting 3-day old, cellulase-digested *G. xylinus* cultures by centrifugation at 3000 x *g* for 10 min at 4°C, washing once with 0.1% saline and twice in room temperature SH broth. The starting inoculum was adjusted to an optical density at 600 nm of 0.05 in SH broth. Culture plates were incubated at 30°C with shaking at 150 rpm. Optical density was recorded at 600 nm using a Bio-Rad xMark™ Microplate Absorbance Spectrophotometer (Bio-Rad Laboratories Ltd., Mississauga, ON, USA). Growth was followed for 204 h. Data from three biological replicates each with six technical replicates were averaged. Growth rates were calculated for each treatment and normalized against *G. xylinus* grown in the absence of phytohormone to facilitate comparison.

Pellicle characteristics

To investigate the effect of phytohormones on the *G. xylinus* pellicle, the bacterium was cultivated under static conditions at 30°C in 24-well plates containing 2 mL SH medium supplemented with either ABA, GA, IAA or Z in concentrations ranging from 1 nM to 100 μM. The inoculum was prepared and standardized as described above. All media had an initial pH of 6.3. Three biological replicates, each with six technical replicates were used for data collection. The pH of culture supernatants was recorded after pellicle removal at the end of seven days of static growth.

The thickness of *G. xylinus* pellicle was measured without the removal of water at the time of harvest for each treatment. Pellicles from each replicate were photographed from the side and the thickness measured using Macnification software (Orbicule, Inc, Belgium) at three different positions on each pellicle; the values were averaged.

Bacterial cellulose yield was determined by first recording the pellicle wet weights and then treating with 0.1 N NaOH at 80°C for 20 min.[36] Treated pellicles were washed in water until neutral, then dried in an oven at 55°C to constant weight.

Crystallinity of pellicles formed by *G. xylinus* under each of the phytohormone treatments was assessed using Fourier transform infrared (FTIR) spectroscopy. Replicate pellicles from each treatment that had been NaOH-treated, washed and dried were used for FTIR analysis on a Perkin Elmer Precisely Spectrum 100 FTIR spectrometer with an horizontal attenuated total reflectance sampling accessory in triplicate using 10 scans with a resolution of 4 cm^{-1} for each measurement in the range of 4000 to 650 cm^{-1}. Background correction was performed prior to collecting sample data. The crystallinity index, CI (IR) was calculated as $A_{1437}/_{895}$ as previously described.[37]

Detection of endogenous *G. xylinus* hormones

Gluconacetobacter xylinus was grown in SH medium with and without 0.05% (w/v) L-tryptophan under agitated conditions. The cellulose pellicle was digested overnight by addition of 0.1% (v/v) filter-sterilized cellulase. The cultures were then centrifuged at 3000 x *g* for 15 min at room temperature. Extraction and detection of the free and the bound forms of abscisic acid, GA, IAA and Z from *G. xylinus* cell pellets and culture supernatants were performed as previously described[31,38] with minor modifications. Briefly, the culture supernatant was transferred to a separate glass vial whereas the cell pellets were washed in 0.85% sterile saline solution and resuspended in 10 mL sterile water in a glass vial. The cell fraction was extracted with 20 mL of methanol:chloroform:ammonium hydroxide (12:5:3 v/v/v). The aqueous phase was centrifuged at 3000 x g for 15 min to remove cell debris. The clarified aqueous phase was transferred to a clean glass vial. The cell-derived aqueous phase and the culture supernatants were adjusted to pH 2.5 and extracted three times with ethyl acetate to obtain a final volume of 25 mL; the organic fraction contains the free form of IAA, GA and abscisic acid. The aqueous phase was then adjusted to pH 7 and the samples were extracted three times with ethyl acetate. The organic fraction was expected to contain free Z. The resultant aqueous phase was adjusted to pH 11, hydrolyzed for 1 h at 70°C, cooled to room temperature, then the pH was reduced to 7 and extracted three times with ethyl acetate. The organic fraction was expected to contain the bound form of Z, if present. Next, the pH of the aqueous layer was reduced to pH 2.5 to extract the bound forms of IAA, GA and ABA in the ethyl acetate fraction. Once extractions were complete, the samples were evaporated to dryness, solubilized in 2 mL of 30% acetonitrile, filtered through 0.2 mm filters and analyzed using a Shimadzu Prominence high-performance liquid chromatography (HPLC) system equipped with a photodiode array detector using a 5 mm apHera™ 150×4.6 mm C18 column (Sepulco) at a flow rate of 0.8 mL/min with isocratic elution in acetonitrile-water (26:74). Standard solutions of (+/-) ABA, GA, IAA and Z were used as reference. Absorbance was monitored at 265 nm for ABA and Z, 280 nm for IAA and 208 nm for GA as described previously.[31,39]

Culture supernatants of *G. xylinus* grown in either SH or SH amended with 0.05% (w/v) L-tryptophan were assayed for the production of IAA using the method of Gordon and Weber[40] as modified by Sarwar and Kremer[41] in which culture filtrate was reacted with Salkowski reagent in a microplate. A pink-colored product after 30 min was taken to be a positive result. Quantitation of IAA equivalents was determined by reading the absorbance at 530 nm.

Microscopy

Colonial morphology changes due to varying concentrations of phytohormones were assessed using an Amscope dissecting microscope equipped with a digital camera.

Statistical analysis

All experiments were performed in triplicate. Each biological replicate was comprised of six technical replicates. The statistical analysis was performed using regular two-way ANOVA with Bonferroni post-tests to compare the means. The analysis was performed using GraphPad Prism software, version 5.0 (GraphPad Software, La Jolla, CA, USA). The significance level reported is $P<0.05$.

Results

Growth of *G. xylinus* in broth cultures is affected by plant hormones

Gluconacetobacter xylinus was grown in liquid culture in increasing concentrations of ABA, GA, IAA, and Z (Figure 1). *G. xylinus* grew to higher optical densities when grown in media containing phytohormone for all concentrations tested compared to untreated controls. The growth rates of *G. xylinus* grown in the presence of 100 nM, 500 nM, 5 µM, and 50 µM were significantly higher ($P<0.05$) than for cultures grown in the absence of ABA (Figure 1A and B). Cultures grown under these concentrations of ABA grew 1.45 times faster than untreated cultures. Very low concentrations (1 nM–50 nM) of ABA had no significant effect on growth rate.

GA-grown cultures reached stationary phase more quickly than cultures grown in any of the other plant hormones tested, reaching the end of logarithmic phase at 110 h (Figure 1C). GA increased the growth rates of *G. xylinus* at all concentrations, but a significant increase was observed for 5 nM to 50 nM, 500 nM, 5 µM, 50 µM and 100 µM. The greatest stimulation of growth rate was observed for 5 nM, which increased the growth rate 1.6 times that of control cultures, while for 50 nM stimulated cultures to grow 1.5 times faster than control cultures (Figure 1D).

Notably, cultures grown in 100 µM IAA started to lyse by 150 h (Figure 1E); such a precipitous decline in culture health was not observed for any other phytohormone treatment. The growth rates of cultures supplemented with IAA were significantly stimulated at concentrations of 5 nM, 100 nM, 5 µM, 10 µM and 100 µM with the latter having the greatest effect by causing an increase in the growth rate to 1.5 times that of control cultures (Figure 1F).

Z significantly increased the growth of *G. xylinus* (Figure 1G). Z stimulated the growth rate of cultures at concentrations of 1 nM to 50 nM, 5 µM and 50 µM by 1.6, 1.4, 1.5 and 1.4 times that of untreated control cultures, respectively (Figure 1H).

Effect of phytohormones on *G. xylinus* pellicle formation

The effect that select phytohormones had on the bacterial cellulose production of *G. xylinus* was determined by examining pellicle thickness, wet weight and cellulose yield. To facilitate comparisons between treatments, measurements were normalized against pellicle properties derived from untreated cultures. The only concentration of ABA to significantly affect pellicle thickness was 50 nM (Figure 2A). At this concentration, pellicles were 0.76 times thinner than pellicles produced by control cultures. The thinnest pellicles were observed at a GA concentration of 100 µM while 5 nM GA caused *G. xylinus* to produce pellicles 1.2 times thicker than pellicles produced by untreated cultures (Figure 2A). IAA had the most pronounced effect on pellicle thickness with most concentrations resulting in thinner pellicles compared to controls. IAA treatment resulted in thinner pellicles (Figure 2A), that were less hydrated (Figure 2B) and that contained the least cellulose (Figure 2C) of all the phytohormone treatments. In contrast, Z had no significant effect on pellicle thickness (Figure 2A) but did significantly affect the wet pellicle weight at low concentrations ranging from 10 nM to 10 µM (Figure 2B). ABA caused *G. xylinus* to produce pellicles of greater wet weight compared with the other three hormones (Figure 2B). Bacterial cellulose yield was only increased in ABA-grown cultures at concentrations of 1 nM, 5 nM and 500 nM with the maximal yield being 1.12 times that of the cellulose yield obtained for control cultures. The cellulose yield for cultures grown in the presence of GA were only significantly affected for three concentrations. At 5 µM, the cellulose yield was 1.2 times that of controls, at 1 µM the yield was 1.1 times that of control and at 100 µM, the yield dropped to 0.9 times that of the control cellulose yield (Figure 2C). Only a single concentration of Z, 10 nM, had a significant effect on bacterial cellulose yield (Figure 2C).

To examine whether extracellular pH changed in response to plant hormone treatment, the final pH of *G. xylinus* culture supernatants of cultures grown under static conditions in the presence and absence of plant hormones was measured. In general, the pH of culture supernatants after seven days of static growth was lower for higher concentrations of hormone, from 10 µM to 100 µM compared to untreated cultures. The converse was observed for low concentrations, between 1 nM and 50 nM, which were observed to have higher pH compared to cultures grown in the absence of phytohormone (Figure 3).

Structural characterization of pellicles produced in the presence of phytohormones

To characterize the influence phytohormone treatment had on the structure of cellulose produced by *G. xylinus,* we calculated the crystallinity index, CI (IR) based on FTIR spectra.[37] The CI (IR) of pellicles produced in the absence of phytohormone was 0.8. ABA, GA, IAA and Z affected the crystallinity of pellicles formed in their presence in a concentration-dependent manner. There was an inverse relationship observed for the crystallinity of pellicles produced in the presence of Z and the crystallinity of pellicles produced in the presence of GA. At concentrations of Z that decrease the pellicle crystallinity, the crystallinity of GA-produced pellicles was greater. For example, at 500 nM, the CI (IR) of Z was 0.87 while for GA the CI (IR) was 0.47, the lowest crystallinity observed for hormone-treated pellicles (Figure 4). ABA had the least effect on pellicle crystallinity except for intermediate concentrations (50 nM to 100 nM) which exhibited a decreased CI (IR) of 0.55 (Figure 4).

Morphological changes caused by growth of *G. xylinus* on phytohormones

The colonial morphology of *G. xylinus* was profoundly affected by growth on solid medium supplemented with phytohormones (Figure 5). Colonies of *G. xylinus* formed in the absence of exogenous phytohormones were ~ 0.5 cm in diameter, irregular in form, off-white in color, convex in elevation and were filiform in margin. Overall, colonies were larger (≥1 cm) when grown in the presence of >500 nM phytohormone. Cellulose formation on solid medium was affected by the presence of phytohormone as well. For example, at 500 nM, ABA and IAA grown *G. xylinus* did not produce cellulose but rather formed smooth colonies. Cultures grown on GA barely produced cellulose at 500 nM while Z-grown colonies exhibited more pronounced cellulose production (Figure 5). Colonies formed in the presence of 500 nM ABA were pearlescent compared to colonies formed in the presence of the other phytohormones. The form of *G. xylinus* colonies at 500 nM GA and IAA were round compared to the irregular form observed in the presence of the other phytohormones. The elevation of the colonies varied depending on exposure to phytohormone. For example, unexposed colonies were convex as were colonies formed in the presence of Z, but were raised in the presence of 500 nM GA, crateriform on 500 nM IAA and flat on 500 nM ABA. The margin of colonies in 500 nM IAA were entire but were undulate for ABA and Z at the same concentration; this differed

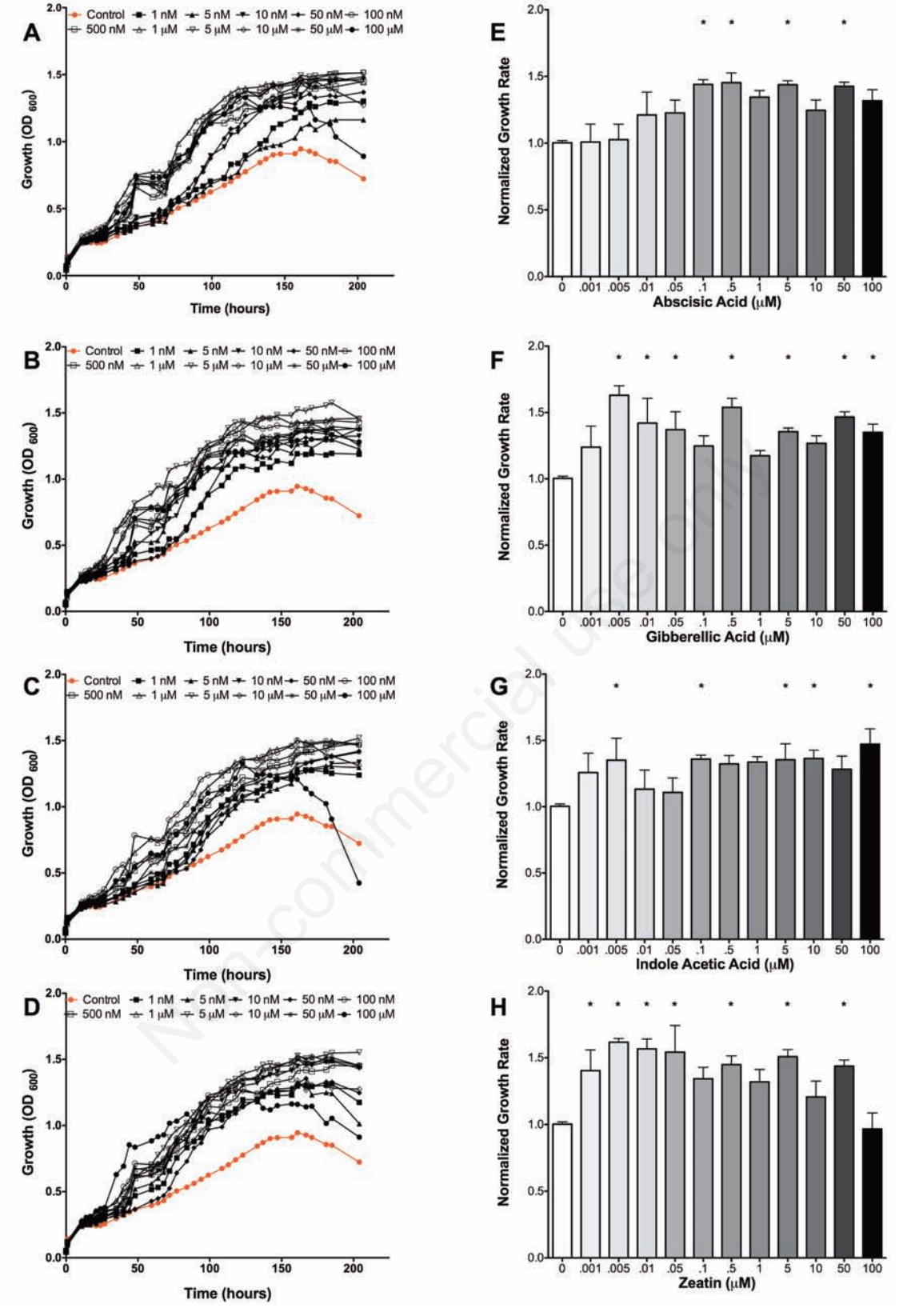

Figure 1. Phytohormones stimulate the growth of *G. xylinus*. Phytohormones did not affect the viability of *G. xylinus* when grown at 30°C in Schramm-Hestrin broth containing 0.1% cellulase in the presence and absence of phytohormones. Growth curves of *G. xylinus* containing varying concentrations of (A) abscisic acid, (B) gibberellic acid, (C) indole-3-acetic acid, and (D) zeatin. Growth rate of *G. xylinus* in the presence of varying concentrations of (E) abscisic acid, (F) gibberellic acid, (G) indole-3-acetic acid, and (H) zeatin normalized to the growth rate calculated for untreated cultures. Data show the mean for three biological replicates each with six technical replicates. Error bars shown are the standard error of the mean. Statistical analysis was performed using ANOVA with Bonferroni post-tests to compare the means. The significance level reported is P<0.05 (*).

from the filiform margins observed for the untreated *G. xylinus* and for colonies formed in the presence of GA.

Endogenous hormone production by *G. xylinus*

In this study, exogenous phytohormones were found to affect the growth, colonial morphology, and pellicle characteristics of *G. xylinus*. Since this is a plant-associated bacterium, we sought to determine whether this bacterium endogenously produces any phytohormone-like compounds. Under the culture conditions used in this study, no IAA was detected in either cell pellets or culture supernatants of *G. xylinus* by colorimetric assays or after extraction and HPLC analysis. Free GA (Figure 6A) free ABA and free Z (Figure 6B) were detected. To our knowledge, this is the first report of endogenous GA, ABA and Z production by *G. xylinus.*

Discussion

Bacteria involved in plant-microbe interactions have been reported to synthesize hormones such as ABA, GA, IAA and Z.[27,33,42,43] The functional role of hormone synthesis by microorganisms is not fully understood. Even less is known about the influence exogenous phytohormones exert on bacterial growth and development or their impact on plant-microbe interactions. We report here that exogenous phytohormones influence the growth, development and pellicle characteristics of *G. xylinus*, including cellulose crystallinity. Furthermore, we found that under the culture conditions used in this study, *G. xylinus* ATCC 53582 produced endogenous GA, ABA and Z. Notably, IAA was not detected in either cells or culture supernatants.

Gluconacetobacter xylinus is known to be associated with rotting fruits.[1,2] The phytohormones chosen in this study play a role in the ripening and senescence of fruits and vegetables. In plants, ABA serves to induce senescence while auxins, giberellins and cytokinins can delay senescence in a concentration-dependent manner.[28,44-46] IAA is a plant growth promoting hormone synthesized by many plant growth promoting bacteria including *Gluconacetobacter diazotrophicus.*[35,47] In contrast to *G. diazotrophicus, G. xylinus* does not synthesize IAA; however, exogenously supplied, this hormone greatly influenced growth and development. We observed that when *G. xylinus* was grown in the presence of IAA, pellicles were thin (Figure 2A) and of low cellulose content (Figure 2C). This result can be explained in context of a plant-microbe interaction: elevated auxin levels in the plant delay ripening and senescence,[28,48-50] as a result, the sugars available for bacterial cellulose synthesis will be lower compared to ripened fruit. Exogenous IAA impedes *G. xylinus* cellulose synthesis yet stimulates growth rate so that cellular density is at a peak when the fruit ripens and glucose becomes more readily available. During ripening, IAA production ceases,[51] alleviating the inhibition of bacterial cellulose synthesis. For example, immature strawberry seeds secrete auxins but when ripening starts IAA production stops.[51] We further speculate that *G. xylinus* may metabolize exogenous IAA reducing its effective concentration so that ripening can commence. There are several examples of plant-associated bacteria capable of degrading auxins.[33,52,53] The capacity of *G. xylinus* to utilize auxins as carbon source will be explored in future investigations.

GA stimulated the growth of *G. xylinus* (Figure 1). Growth stimulation by this hormone has been observed for other bacteria including *Azotobacter, Pseudomonas* and *Lactobacillus.*[23] At higher concentrations, exogenous GA increased the bacterial cellulose wet weight (Figure 2B) and yield (Figure 2C). Furthermore, GA had profound effect on the crystallinity of *G. xylinus* pellicles, depending on concentration. GA has been shown to stimulate endo-β-1,4-xylanase activity

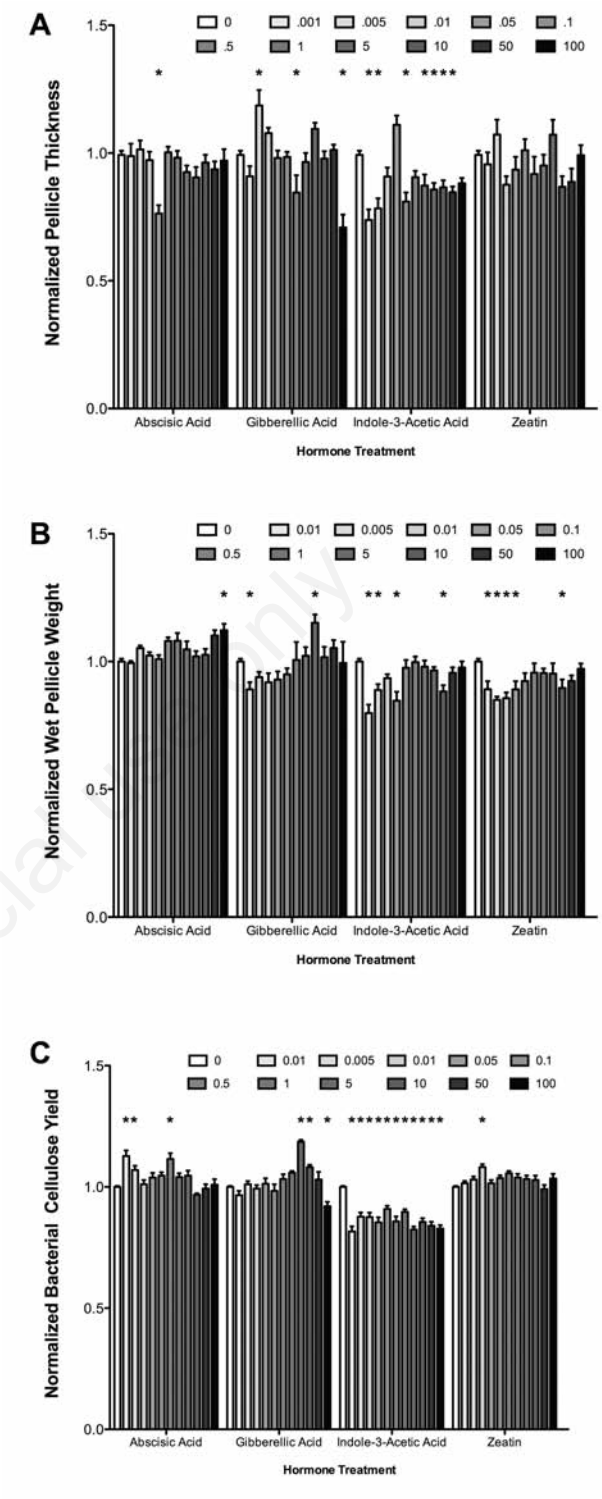

Figure 2. Phytohormones influence the physical pellicle characteristics and bacterial cellulose yield of *G. xylinus*. *G. xylinus* was grown under static conditions at 30°C in Schramm-Hestrin broth for 7 days in the presence and absence of the phytohoromones abscisic acid, gibberellic acid, indole-3-acetic acid, and zeatin. (A) Normalized pellicle thickness, (B) normalized pellicle wet weight, and (C) normalized bacterial cellulose yield. The data is normalized to the data obtained for untreated cultures to facilitate comparisons. Data show the mean for three biological replicates each with six technical replicates. Error bars shown are the standard error of the mean. Statistical analysis was performed using ANOVA with Tukey post-tests to compare the means. The significance level reported is P<0.05 (*).

in barley[54] leading to the solubilisation of stored carbohydrate. We found that *G. xylinus* is capable of endogenous synthesis of GA (Figure 6). Notably, *G. diazotrophicus,* a nitrogen-fixing acetic acid bacterium synthesizes this hormone as well but synthesis is carbon source-dependent.[34]

Z stimulated the growth (Figure 1), pellicle thickness (Figure 2A), bacterial cellulose yield (Figure 2C) and crystallinity (Figure 4). Interestingly, we observed an inverse relationship between the crystallinity of pellicles produced in the presence of Z and in the presence of GA. The physiological implications of this relationship for cellulose producing plant-associated bacteria require further investigation.

While there is a paucity of information in the literature on the effect that phytohormones have on bacterial growth, IAA and GA were both found to stimulate the growth of *Azospirillum* sp.; the extent of growth

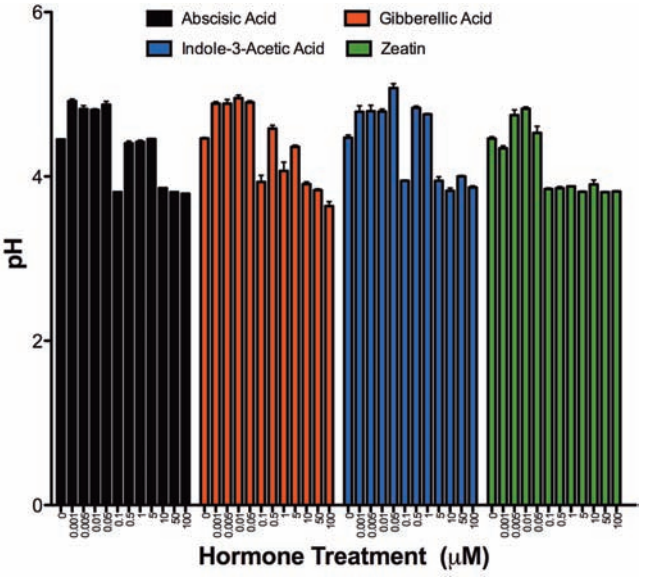

Figure 3. Phytohormones influence the final culture pH of *G. xylinus*. The supernatant pH of *G. xylinus* cultures grown statically in the presence and absence of phytohormones for seven days at 30°C in Schramm-Hestrin broth were measured after pellicle removal. Data show the mean for three biological replicates each with six technical replicates. Error bars shown are the standard error of the mean.

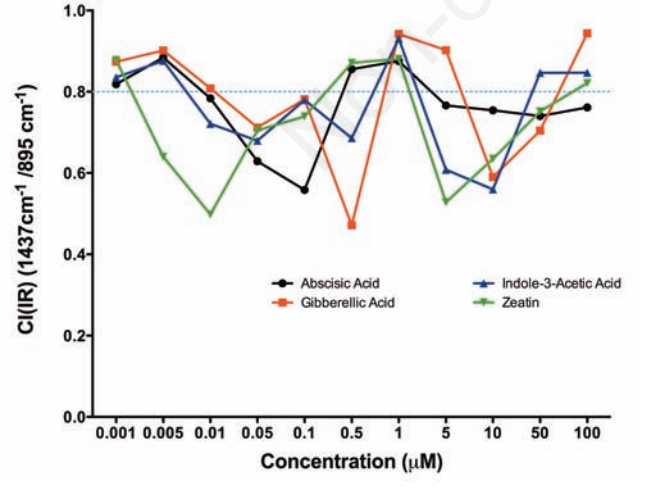

Figure 4. Growth in the presence of phytohormones influences *G. xylinus* pellicle crystallinity. The crystallinity index, CI (IR) for *G. xylinus* pellicles produced when grown for seven days under static conditions at 30°C in Schramm-Hestrin broth in the presence of the phytohoromones abscisic acid, gibberellic acid, indole-3-acetic acid, and zeatin. The blue dashed line shows the CI (IR) of untreated pellicles (0.80) for comparison.

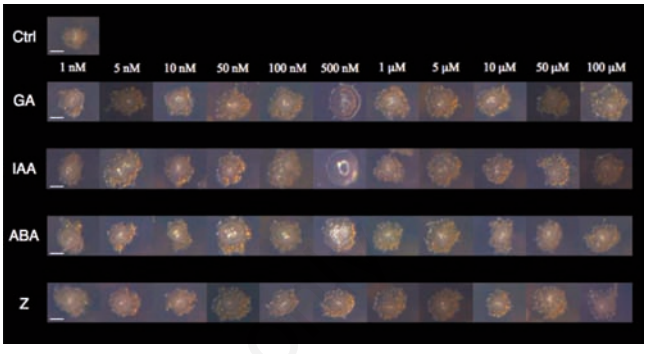

Figure 5. Phytohormones alter the colonial morphology of *G. xylinus*. Representative colonial morphologies exhibited by *G. xylinus* grown on Schramm-Hestrin agar at 30°C in the presence and absence of increasing concentrations of gibberellic acid (GA), idole-3-acetic acid (IAA), abscisic acid (ABA) and zeatin (Z). Photographs were taken after five days of growth. Scale bar represents 0.5 cm.

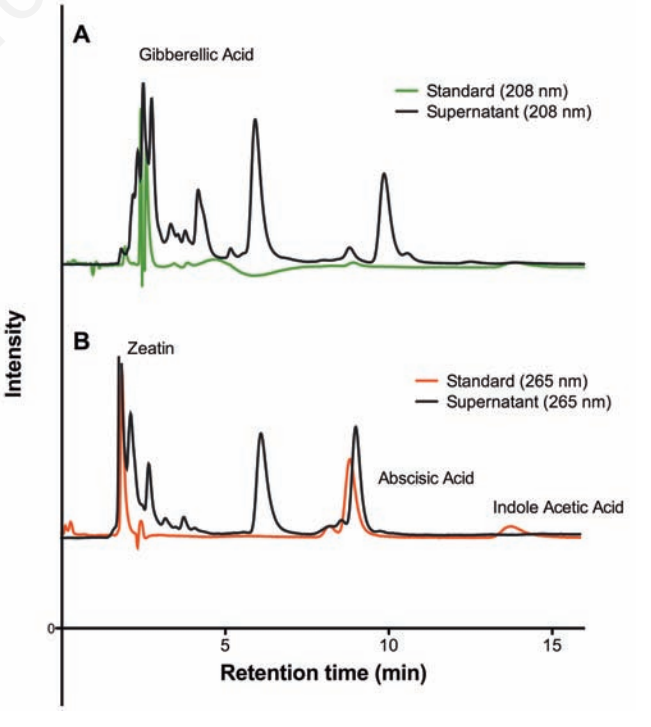

Figure 6. *Gluconacetobacter xylinus* produces endogenous phytohormones. High performance liquid chromatography of culture supernatant extracts showing endogenous (A) gibberellic acid and endogenous (B) abscisic acid and zeatin production analyzed at the optimum wavelength for each hormone as described in the Materials and Methods section. No indole-3-acetic acid was detected in *G. xylinus* cultures.

stimulation was hormone, bacterial strain and growth medium dependent.[55] Interestingly, in the same study, the cytokinin, kinetin was found to inhibit *Azospirillum* growth at high concentrations. We did not observe any growth inhibition of *G. xylinus* by the cytokinin, Z used in this study.

The pH of culture supernatants was affected by phytohormone concentration. Lower pHs were observed for higher hormone concentrations (10 μM to 100 μM) compared to untreated cultures (Figure 3). Whether the pH differences observed were due to acetic acid production or due to excretion of other organic acids was not determined in this study.

Exogenous ABA increased the growth rate of *G. xylinus* (Figure 1). In general, pellicle wet weight (Figure 2B) was inversely proportional to the bacterial cellulose yield (Figure 2C) suggesting ABA influenced pellicle density and hydration. Pellicle crystallinity was moderately influenced by exogenous ABA with the lowest crystallinity index observed at a concentration of 100 nM compared to untreated pellicles which exhibited a crystallinity index of 0.80. The endogenous synthesis of ABA by *G. xylinus* (Figure 6) may serve to encourage fruit ripening. In grapes, the most important hormone involved in ripening is ABA.[45] ABA has been demonstrated to suppress resistance to necrotrophic pathogens.[56] We speculate that the increase in cellulose yield observed for *G. xylinus* in response to this hormone shields its food source against other competitive microbes as has been demonstrated in apple-slice colonization studies.[1]

Conclusions

In conclusion, this research provides insight into the effects of phytohormones on the growth, cellulose production and cellulose structure in the model cellulose-producing bacterium *G. xylinus*. Furthermore, we report for the first time, endogenous production of GA, ABA and Z by *G. xylinus.* The results presented here have tremendous implications for understanding the cellulose synthesis of *G. xylinus* and identifying key factors that affect the interaction of this organism with plants.

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