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Abstract

Pigments are compounds with highly diverse structures and wide uses, which production is increasing worldwide. An eco-friendly method of bioproduction is to use the ability of some microorganisms to ferment on renewable carbon sources. Wheat bran (WB) is a cheap and abundant lignocellulosic co-product of low recalcitrance to biological conversion. Microbial candidates with theoretical ability to degrade WB were first preselected using specific databases. The microorganisms were *Ashbya gossypii* (producing riboflavin), *Chitinophaga pinensis* (producing flexirubin), *Chromobacterium vaccinii* (violacein) and *Gordonia alkanivorans* (carotenoids). Growth was shown for each on minimal salt medium supplemented with WB at 5 $g.L^{-1}$. Activities of the main enzymes consuming WB were measured, showing leucine amino-peptidase (up to 8.45 $IU.mL^{-1}$) and β-glucosidase activities (none to 6.44 $IU.mL^{-1}$). This was coupled to a FTIR (Fourier Transform Infra-Red) study of the WB residues that showed main degradation of the WB protein fraction for *C. pinensis*, *C. vaccinii* and *G. alkanivorans*. Production of the pigments on WB was assessed for all the strains except *Ashbya*, with values of production reaching up to 1.47 $mg.L^{-1}$. The polyphasic approach used in this study led to a proof of concept of pigment production from WB as a cheap carbon source.

Keywords

Pigment production; Biorefinery; Wheat bran; Microorganisms; Lignocellulosic enzymatic activities; FTIR

Abbreviations

AA: Auxiliary Activity
A. goss.: *Ashbya gossypii*
CAZy: Carbohydrate Active Enzyme database
CAZyme: Carbohydrate Active Enzyme
CBM: Carbohydrate Binding Module
CE: Carbohydrate Esterase
C. pin.: *Chitinophaga pinensis*
C. vacc.: *Chromobacterium vaccinii*
FTIR: Fourier Transform InfraRed (spectroscopy)
G. alk..: *Gordonia alkanivorans*
GH: Glycoside Hydrolase
GT: Glycosyltransferase
LAP: L-Leucine-7-amido-4-methylcoumarin hydrochloride
OD: Optical density
ORFs: Open Reading Frames
PL: Polysaccharide Lyase

SD: Standard deviation
WB : Wheat bran

1. Introduction

Pigments are often described as compounds having the ability to absorb and reflect a specific part of the electromagnetic visible spectrum. With such a broad definition, pigments are ubiquitous in nature and have a huge variety of chemical structures, properties and uses (Ramesh et al. 2019). They are commonly used as food colorants, dyes in the textile or cosmetic industry, or for some specific biological activities such as antioxidant, antibacterial, or anticancer (Lagashetti et al. 2019).

Nowadays, natural organic pigments are seen as less harmful for the planet and for the health, and the global demand for these pigments is increasing. In 2018, the organic pigment market was estimated to be worth USD 3.5 billion, and it could reach around USD 4.8 billion by 2024 (MarketResearchFuture 2020). When regarding the market of specific pigment categories such as carotenoids, synthetic pigments are predominant (more than 80% of carotenoid syntheses is chemical) (Venil et al. 2020), because those are cheaper to produce.

Production of natural organic pigments from plant and animal extraction has some limitations. Those means of productions are dependent on seasonality (for plants), contents are low, and the extraction processes can be polluting at times (Capolupo and Faraco 2016). The use of microbial fermentation as a bioproduction process is increasing, as microbial growth is fast, independent on seasonality and requires overall less energy than plants and animals (Jorissen et al. 2020). Numerous organisms producing pigments have already been studied in yeasts (Johnson and Lewis 1979), fungi (Chiba et al. 2006), bacteria (Venil et al. 2013), and others (Sui et al. 2014).

A limiting factor for the fermentation approach is the important cost of growth media (Hong et al. 2011). Viable alternatives to lower this cost can be obtained by using the ability of some species to grow on agro-industrial co-products that are cheap and abundant (Sansinenea and Ortiz 2011). There is as a wide variety of pigments already being produced by microbial fermentation. In most of previous studies, agro-industrial co-products or wastes are mixed with rich growing medium to study pigment production (Silveira et al. 2008; Frengova and Beshkova 2009; Nguyen et al. 2020). Studies on the use of saccharides and phenols for pigment production by microorganisms are scarce. Lignocellulose,

which is cheap and abundant, is generally viewed as a promising candidate for bioproduction (Diaz et al. 2018; Sindhu et al. 2020).

The objective of this study was to evaluate the ability of various microorganisms from different species to produce pigments using a cheap lignocellulosic biomass, wheat bran (WB), as carbon source. Its production is estimated to be around 10^8 tons a year (Reisinger et al. 2013). It contains mainly starch (15-25%), cellulose (≈ 10%), arabinoxylans and glucans (10-25%) and proteins (≈ 15%) (Apprich et al. 2014). This co-product is of interest for bioconversion as its poor content in lignin makes it of low recalcitrance to biological conversion compared to other lignocellulosic compounds. WB was notably described as an interesting carbon source for the microbial production of hemicellulases with the bacterium *Thermobacillus xylanilyticus* (Rakotoarivonina et al. 2012; Rakotoarivonina et al. 2016).

For this study, the microorganisms were screened from databases and literature with two selecting criteria: (1) microorganisms already described as pigment producers, (2) microorganisms possessing genes coding for enzymes involved in WB components fractionation.

Four microorganisms producing each a different category of pigment were then selected: the bacterium *Gordonia alkanivorans* for carotenoids production (mostly canthaxanthin); the bacterium *Chitinophaga pinensis* for the production of flexirubin; the bacterium *Chromobacterium vaccinii* that produces violacein and deoxyviolacein (for readability, the production and quantification of both pigments will be referred to as "violacein" in the rest of the article); and the fungi *Ashbya* gossypii that produces riboflavin.

Historically, *A. gossypii* is the first organism used at the industrial scale for riboflavin production. It was isolated as a plant pathogen from cotton bolls (Aguiar et al. 2015). *C. pinensis* is a *Bacteroidetes* that was isolated from pine forest leaf litter (McKee and Brumer 2015), and *C. vaccinii* was isolated from cranberry roots and soil (*Vaccinium macrocarpon* Ait.) (Soby et al. 2013). Since those three species were isolated from plants, and regarding their Carbohydrate Active enzyme (CAZyme) profile, it was expected that they could be good candidates to valorise lignocellulose. The remaining strain of

100 interest, *G. alkanivorans*, was isolated from soils contaminated by tar and phenols and is mostly
101 known to degrade hydrocarbons (Kummer et al. 1999).

102 In this study, strains having the ability to degrade lignocellulose and produce pigments were chosen by
103 analysis of the CAZyme and MEROPS databases (dedicated to carbohydrate active enzymes and
104 proteases). The use of WB as carbon source was then studied by measuring specific enzyme activities
105 and analysing the WB composition with Fourier Transform InfraRed (FTIR) spectroscopy. Finally,
106 pigments were extracted according to already described protocols and their productions were
107 measured.

2. Materials and methods

2.1. Materials

Wheat bran (WB) in ground form (particle sizes: 0.8-2 mm) was kindly provided by BioWanze (Belgium). The strains were obtained from DSMZ (Leibniz Institute - German collection of microorganisms and cell cultures GmbH).

2.2. WB analysis

The sugar composition of WB was evaluated by HPAEC-PAD with a Dionex CarboPac PA-1 column (4 x 250 mm, Thermo Fisher Scientific, Waltham, USA), after acid hydrolysis of WB as previously described (Rémond et al. 2010). The quantification of starch was performed with the Total Starch HK Assay kit (K-TSHK, Megazymes, Bray, Ireland) WB protein content was evaluated with the Dumas method using a CHNS-O Elemental Analyser 'EA3000, Eurovector, Milan, Italia) of total nitrogen measurement after catalytic combustion according to the corresponding ISO norm (ISO 2016).

2.3. Bioinformatic analyses

Full genomes were available at GenBank (www.ncbi.nlm.nih.gov/genbank/) under the accession numbers **NC_005782.2** (*Ashbya gossypii*), **CP001699.1** (*Chitinophaga pinensis*), **CP017707.1** (*Chromobacterium vaccinii*) and **NZ_BACI00000000.1** (*Gordonia alkanivorans*). Full theoretical proteomes from the genomes were obtained using Augustus software (default parameters) installed on usegalaxy.org (Stanke et al. 2006). For total CAZymes predictions, theoretical proteomes were annotated using the dbCAN2 meta server (http://bcb.unl.edu/dbCAN2/, option HMMER) (Zhang et al. 2018). For the full proteasome, the BLAST MEROPS tool (Rawlings et al. 2009) for comparison to the MEROPS peptidase database was used (www.ebi.ac.uk/merops/). For the secreted proteins, the SignalP 5.0 server was used (http://www.cbs.dtu.dk/services/SignalP (Almagro Armenteros et al. 2019). Transmembrane regions were determined with the TMHMM Server v. 2.0 (http://www.cbs.dtu.dk/services/TMHMM/) (Krogh et al. 2001). The predicted secretome were then annotated using dbCAN2 (see above) and the MEROPS database in order to obtain respectively the secreted CAZYmes and proteasome.

2.4. Strains and culture media

Four strains were used in this study: *Ashbya gossypii* DSM 3485, *Chitinophaga pinensis* DSM 2588, *Chromobacterium vaccinii* DSM 25150 and *Gordonia alkanivorans* DSM 44369. Optimal growth media were used for growth on agar-plate and optimal liquid cultures. Optimal growth media compositions are available at the www.dsmz.de/ website. Optimal media were: Yeast medium (medium 186, *A. gossypii*); Cy-broth (medium 67, *C. pinensis*); Luria-Bertani (medium 381, *C. vaccinii*) and Trypticase Soy Broth (medium 535, *G. alkanivorans*). Minimal salt medium M3 (KH_2PO_4, 1.9 $g.L^{-1}$; Na_2HPO_4, 5.1 $g.L^{-1}$; $MgSO_4.7H_2O$, 0.1 $g.L^{-1}$; $(NH_4)_2SO_4$, 0.2 $g.L^{-1;}$ pH 7.2) was used for *A. gossypii* and *G. alkanivorans*. Minimal salt medium M9 (NH_4Cl, 1 $g.L^{-1}$; $Na_2HPO_4.2H_2O$, 6 $g.L^{-1}$; KH_2PO_4, 3 $g.L^{-1}$; NaCl, 0.5 $g.L^{-1;}$ pH 7.2) was used for *C. pinensis* and *C. vaccinii*.

2.5. Growth conditions

Inocula were prepared from differentiated colonies grown on agar plate. One colony was inoculated per erlenmeyer containing 50 mL of culture medium. Minimal media consisted of minimal salt medium supplemented with WB at 5 $g.L^{-1}$. Wet autoclave protocol: the WB and the minimal salt medium are mixed prior the autoclave program (20', 121°C, slow decrease in temperature and pressure). Dry autoclave protocol: the WB is sterilized within an erlenmeyer (autoclave program: 20', 121°C, immediate decrease in temperature and pressure afterwards) and sterilized minimal salt medium was added after. Agitation was of 100-150 rpm. Temperatures of growth were 30°C for *A. gossypii* and *C. pinensis* and 22°C for *C. vaccinii* and *G. alkanivorans*. Growths were followed by sampling 1 mL of culture medium and measuring the OD at 600 nm, except for *A. gossypii*. All data were collected in biological triplicates, where three separate cultures were inoculated then treated of the same manner.

2.6. Enzymes activities

2.6.1. Enzymes extraction

Enzymes extraction was achieved from the cultures in minimal medium. For α-amylase, xylanase and β-glucosidase activities, 1 mL of culture was lysed in microtubes containing ≈ 5 mm of zirconia-

160 coated silica-microbeads (11079101z, Biospec Products, Bartlesville, USA) with a vortex mixer for
161 10-20' at room temperature. Microtubes were then centrifuged and supernatants were collected. For
162 LAP activities, 1 mL of culture was centrifuged, and the supernatant was collected. Controls were
163 non-inoculated media incubated with the same conditions. All data for enzyme extraction were
164 collected in biological triplicates, with samples obtained from three separate cultures. Enzymatic
165 activity was expressed in international units (IU), where 1 IU is the quantity of enzyme required to
166 release 1 μmole of reducing sugar per min. Temperature for the reactions were the same as the growth
167 temperatures of the corresponding microorganisms.

168 *2.6.2. α-amylase and xylanase activities*

169 Those activities were assessed by measuring the reducing sugars liberated from xylans or starch by the
170 xylanase or amylase activities, respectively. Done according to the Kidby-Davidson methodology as
171 previously described (Rémond et al. 2010). 100 μL of enzyme extracts were incubated with 900 μL of
172 either native potato starch (1259, Merck, Darmstadt, Germany) or xylan from beechwood (4414.4,
173 Roth, Karlsruhe, Germany) at 0.5 % (w/v) in Tris-HCl, 10 mM, pH 7.5. 100 μL of the mix was added
174 to 1.5 mL of alkaline ferricyanide reactant after 0', 5' and 10' of reaction. Reactions were boiled 5
175 minutes, cooled down, and absorbances measured at 420 nm, 25°C.

176 *2.6.3. β-glucosidase activity*

177 This activity was assessed using *p*-nitrophenyl-β-D-glucopyranoside (*p*NP-Glc) (N7006, Sigma-
178 Aldrich, Darmstadt, Germany). 100 μL of enzyme extracts were incubated with 900 μL of *p*NP-Glc 10
179 mM in Tris-HCl, 10 mM, pH 7.5. The absorbance was measured at 401 nm for 15'.

180 *2.6.4. LAP activity*

181 This activity was assessed using L-Leucine-7-amido-4-methylcoumarin hydrochloride (LAP) (L2145,
182 Sigma-Aldrich, Darmstadt, Germany). Reaction were made with 1050 μL of Tris-HCl 10 mM, pH 7.0;
183 300 μL of LAP 200 μM in ultrapure water; 150 μL of enzyme extract. Reactions were incubated
184 between 10' and 3h in order to obtain significant activities without using all the LAP substrate.
185 Reactions were stopped with 10 μL of NaOH 1M. 250 μL of each reaction mix were dispatched 5

timed in a 96-well black microplate for fluorescence (MaxiSorp Nunc, Thermo Fisher Scientific, Waltham, USA). Fluorescence was measured; (excitation: 365 nm, emission: 450 nm, room temperature) (SpectraMax Gemini, Molecular Devices, San Jose, USA). Standard fluorescence curve were obtained with 7-Amino-4-methylcoumarin (7-AMC) (A9891, Sigma-Aldrich, Darmstadt, Germany).

2.7. FTIR analyses

FTIR analyses were performed on WB without fermentation (culture media were treated the same way as a fermentation) and on WB residues after the stationary growth phase was reached. After cultivation, supernatant was discarded, and the remaining WB was rinsed several times with water. WB was then dried at 50°C for at least 2 days. Attenuated Total Reflectance - Fourier Transform Infra-Red (ATR-FTIR) analyses were performed using a Nicolet 6700 FT-IR (Thermo Fisher Scientific, Waltham, USA) with ATR-diamond crystal (Smart iTR, Thermo Fisher Scientific, Waltham, USA). Each sample was measured at least 3 times (technical replicates). Spectra were treated with the OMNIC 8 software. A mean spectrum of the three readings was calculated; the baseline was corrected and the area of interest (≈ 2990-800 cm^{-1}) was normalized. The TQ analyst Z software was used to retrieve the absorbances associated to each peak of interest (cf. Table 1). Ratios of absorbances of fermented over non-fermented WB were calculated. All data were collected in biological triplicates, with samples obtained from three separate cultures.

2.8. Pigment extraction and quantification

All data for enzyme extraction were collected in biological triplicates, with samples obtained from three separate cultures grown in the same manner. Minimal media conditions were done with wet-autoclaved media.

2.8.1 Riboflavin (Adapted from (Ledesma-Amaro et al. 2015))

Samples of cultures at 210h (optimal medium) and 70h (minimal medium) of growth were collected. 1:1 culture:HCl 0.1 M were added and boiled at 100°C for 30'. Each sample was sonicated (Sonicator Vibra-Cell, 72410, Sonics, Newtown, USA) for 40', 20-40 Hz, with pulses of 4 seconds on/off, at

30°C. Lysates were centrifuged (room temperature) and supernatants collected. The lysates were placed in a lyophiliser (Cosmos 20k, Cryotec, S aint-Gély-du-Fesc, France) for a 1-2 days at -20°C. The solids were then resuspended in 2 mL of ultrapure water. Those 2 mL were centrifuged (room temperature) and the supernatant was collected. Riboflavin fluorescence was measured in 96-well black microplates (MaxiSorp Nunc, ThermoScientific, Waltham, USA) with a SpectraMax Gemini spectrophotometer (Molecular Devices, San Jose, USA) (excitation: 450 nm; emission: 350-650 nm).

2.8.2 Flexirubin (Adapted from (Venil et al. 2015))

20 mL of cultures at 96h of growth were collected then centrifuged (10', 8.10^3 rpm, 4°C). The supernatant was discarded, and the pellet rinsed with deionised water, then discarded after another centrifugation. 20 mL acetone 5% was added to the pellet and cells were sonicated (Sonicator Vibra Cell, 72410, Sonics, Newtown, USA) for 40', 20-40 Hz, with pulses of 4 seconds on/off, at 30°C. Lysates were centrifuged (room temperature) and supernatants were collected. Another sonication in 10 mL acetone 5% was performed and the supernatant collected. Butan-1-ol was added (ratio lysate:butan-1-ol 11:2) and vortexed. After a centrifugation the organic phase was collected. The butan-1-ol was then evaporated using a rotating evaporator (R-215, Büchi, Villebon sur Yvette, France) at 50°C, 25 mbar, rotation at 85 rpm. The pigments were resuspended in pure acetone, the optical density read at 450 nm and the concentration calculated with an extinction coefficient of 99.400 $mL.\mu mol^{-1}.cm^{-1}$ (Reichenbach et al. 1974).

2.8.3 Violacein (Adapted from (Kanelli et al. 2018))

Samples of cultures at 73h (optimal medium) and 73h (minimal medium) of growth were collected, centrifuged (room temperature), rinsed with Tris-HCl, pH 7.0, 10 mM followed by another centrifugation (room temperature) and the supernatant was discarded. 1 mL of DMSO (ACS reagent grade) was added to each pellet and the mix was vortexed (2'). Supernatant was collected after centrifugation (room temperature) then evaporated using a rotating evaporator (R-215, Büchi, Villebon sur Yvette, France) at 50°C, 4 mbar, and rotation at 85 rpm. Content was resuspended in absolute

ethanol. Absorbance was measured at 575 nm and concentration calculated with an ε of 0.05601 $mL.\mu g^{-1}.cm^{-1}$ (Mendes et al. 2001).

2.8.4 Carotenoids (Adapted from (Fernandes et al. 2018))

Samples of cultures at 168h of growth were collected. Cells were centrifuged (room temperature), supernatant was discarded and 1.5 mL DMSO (ACS reagent grade) were added. The mix was transferred into a microtube containing ≈ 5 mm of zirconia-coated silica-microbeads (11079101z, Biospec Products, Bartlesville, USA). Lysis was done by vortex mixer for more than 4h at room temperature. The microtubes were then centrifuged (4°C) and supernatants were collected. Another 0.5 mL DMSO was added for the optimal medium conditions, treated as before and collected. A mix of DMSO / Acetone / NaCl 20% / ethyl acetate (3/1/1/6) was done, mixed gently, the upper part collected after 1h and absorption measured at 477 nm. Concentrations were calculated with an ε of 0.20676 $mL.\mu g^{-1}.cm^{-1}$ (477 nm, canthaxanthin in ethyl acetate) (made from canthaxanthine, DRE-CA10947000, Dr Ehrenstorfer, Augsburg, Germany).

3. Results

3.1. CAZymes prediction

Strains for this study were selected in part on the theoretical enzymes arsenal required to degrade the polysaccharides present in WB: notably starch, cellulose, glucans and xylans. For this, the search for genes coding for CAZymes was performed. The CAZy database (Lombard et al. 2014) is often used to find potential enzymes with an activity of interest in a genome (Aguiar et al. 2013), or to perform transcriptome (Ben Ali et al. 2020) or metasecretome analyses (Jimenez et al. 2015).

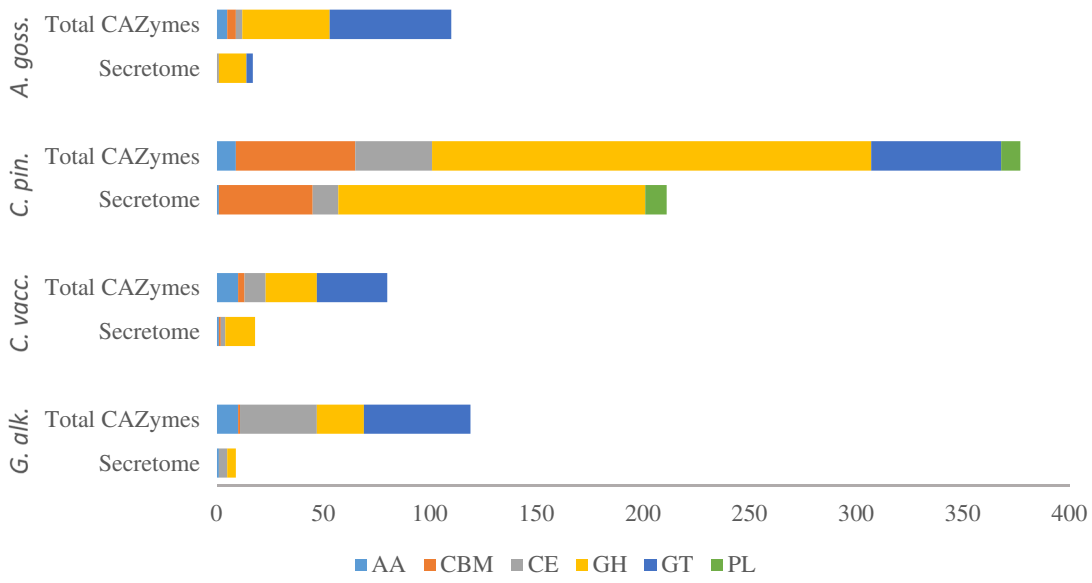


Figure 1 - Distribution of the CAZy classes predicted from the genomes of each selected pigment producer. Total CAZymes represent the entirety of the CAZy classes predicted from a genome, while the secretome corresponds to the predicted modules with signal peptides and no transmembrane region. AA: Auxiliary Activity; CBM: Carbohydrate Binding Module; CE: Carbohydrate Esterase; GH: Glycoside Hydrolase; GT: Glycosyl Transferase; PL: Polysaccharide Lyase.

Total amount of theoretical CAZymes in the genome represents 1.73% of the proteome for *C. vaccinii*, 2.24% for *A. gossypii*¸ 2.77% for *G. alkanivorans* and 4.64% for *C. pinensis*. CAZymes are classified in different classes. The repartition of the *in silico* predictions between these classes is shown in Figure 1. Glycosyl Transferases (GT) are highly predicted since they are generally involved in the

biosynthesis of the oligo- and polysaccharides (Breton et al. 2001). Glycoside Hydrolases (GH) represent 18.5-54.6% of the total predicted CAZy modules and are the most predicted ones in *C. vaccinii*. GHs mainly act on cell wall polysaccharides, and can have a role either in their biosynthesis or degradation (Minic 2008). Carbohydrate Esterases (CE) and Polysacharide Lyase (PL) classes are mainly directed towards degradation of polysaccharides (Lombard et al. 2010; Biely 2012) and are predominant in *G. alkanivorans* and *C. pinensis*, respectively. Carbohydrate binding modules (CBM) are more present in *C. pinensis* (14.9% of the total modules) than in the other strains. CBMs are responsible for the binding of a CAZyme to a carbohydrate, supposedly to its substrate (Bornscheuer et al. 2014). Auxiliary Activity (AA) modules, usually active on non-carbohydrate substrates such as lignins (Bornscheuer et al. 2014), are more present in *C. vaccinii* and *G. alkanivorans* (12.5 and 8.4% of the total modules, respectively).

Since only secreted enzymes can act on exogenous lignocellulosic compounds, secreted CAZymes were then studied (Table S1). Secreted enzymes were selected on the presence of a signal peptide sequence and the absence of transmembrane regions.

In *A. gossypii*, families GH5, GH17, GH31 and GH32 are probably involved in hydrolysis of carbohydrates (Chen et al. 2018). The others display wide specificities for polysaccharides. For *C. pinensis*, the most present families have the ability to hydrolyse hemicelluloses (GH43, GH29, GH78). This correlates with the high number of carbohydrate-binding modules (CBM) specific to hemicelluloses. CAZymes AA10, GH8 and CH15-CBM20 from *C. vaccinii* act on cellulose, xylans and starch, respectively. For *G. alkanivorans*, only the GH23 family has no probable lignocellulolytic activity.

3.2. Secreted peptidases analysis.

In parallel to the carbohydrate degrading enzymes, the ability to use another major constituent of the WB was screened. Since the protein fraction makes 15.5% of the dry mass of the WB (Apprich et al. 2014), strains were also selected onto their genetic potential of secreted peptidases that could be involved in the hydrolysis of WB proteins during growth. Only the secreted peptidases were screened,

since peptidase activities are a main metabolic activity in any given cell. This study was done by interrogating the MEROPS database (Rawlings et al. 2009), specialized in peptidases.

Table 1. Predicted secreted peptidases families. The number of predicted proteins belonging to a family is shown in the second column, and the main function associated with the members of this family according to the MEROPS website data is given. Results are ordered according to the families having the highest number of predicted proteins. For *C. pinensis*, only results with occurrences (Occ.) >1 are shown.

A. gossypii	Occ.	Fonction
A01A	4	Pepsin
S08A	2	Nutrition / Pathogenesis
S10	2	Structural role
C13	1	Legumain
C44	1	Autolytic - Transferase
M14A	1	Various
M20F	1	Degradation of proteins into amino acids
M28A	1	Various
M28E	1	Various

C. pinensis	Occ.	Fonction
S33	9	Use proline-rich substrates
S12	8	Bacterial cell wall
M01	6	Aminopeptidases
M28F	5	Various
M97	4	Unknown
S09B	4	Degradation of biological active peptides
S09X	4	Degradation of biological active peptides
S41A	4	Degradation of incorrectly synthesized prots
M14X	3	Various
M93	3	Unknown
S08A	3	Nutrition / Pathogenesis
S09D	3	Degradation of biological active peptides
C01A	2	Lysosome
C40	2	Bacterial cell wall
C56	2	Various
M13	2	External polypeptides
M20D	2	Degradation of proteins into amino acids
M23B	2	Bacterial cell wall
M38	2	Detoxification
M57	2	Unknown
S09A	2	Degradation of biological active peptides
S15	2	Casein degradation
S41B	2	Cytoplasmic proteolytic pathway
T03	2	Glutathione degradation
…	<1	Not shown

C. vaccinii	Occ.	Action
S53	3	Probable digestion of food proteins
C40	2	Bacterial cell wall
C82A	2	Bacterial cell wall
M23B	2	Bacterial cell wall
S09A	2	Degradation of biological active peptides
S09D	2	Degradation of biological active peptides
S12	2	Bacterial cell wall
C93	1	Unknown
M09A	1	Precursors
M09B	1	Precursors
M13	1	External polypeptides
M14A	1	Various
M16B	1	Mitochondrium and other
M23A	1	Bacterial cell wall (lysis of peptidoglycans)
M24B	1	Various
M35	1	Various
M48C	1	Various
S01E	1	Various
S09B	1	Degradation of biological active peptides
S13	1	Bacterial cell wall
S66	1	Bacterial cell wall
S82	1	Unknown
T03	1	Glutathione degradation

G. alkanivorans	Occ.	Action
S12	4	Bacterial cell wall
M23B	3	Bacterial cell wall
C82A	2	Bacterial cell wall
C40	1	Bacterial cell wall
C44	1	Autolytic - Transferase
M15D	1	Bacterial cell wall
M24A	1	Remove initiating methionine
M28A	1	Various
T03	1	Glutathione degradation

300 The most predicted family for *A. gossypii*, A1A, encodes for pepsin-like peptidases and has already
301 been found to be highly present in a lignocellulolytic organism (Aylward et al. 2013). Interestingly,
302 members of the family S8A (subtilisin family) are known for some of their pathogenicity in animals
303 and plants depending on the species (Segers et al. 1999).

304 Overall, there are more peptidases predicted in *C. pinensis* than in the three other strains of this study.
305 (104 peptidases for 48 families) (Table 1). The most predicted family, S33, is known to be active on
306 proline rich substrates, and therefore can have a nutritional role that gives selective advantages for the
307 strain. The S33 family has already been shown to be expressed differently on various lignocellulosic
308 biomasses in *Hypocrea jecorina* (Bengtsson et al. 2016). In the families predicted more than once,
309 there are no clear (M1, M28F, M14X) unknown (M97), or very specific biological roles (the other
310 classes). Only the S8A family has a known role in nutrition/pathogenesis. Regarding these results, a
311 moderate to high peptidase activity can be predicted for this strain.

312 The most predicted family (S53) in *C. vaccinii* has uncertain functions, but is believed to be part of the
313 "extracellular digestion of food proteins" (Table 1). This family was abundant in a lignocellulosic
314 organism and thought to potentially use plant proteins (Aylward et al. 2013). For the families only
315 predicted once, multiple candidates could be involved in the fractionation of proteins of the WB.
316 Those families have either unknown functions (C93 and S82), degrades small polypeptides (S9B), or
317 very diverse activities (M14A, M24B, M35, M48C, S1E). Therefore, no precise use of the protein
318 fraction of WB can be certainly predicted for *C. vaccinii*, but it still has interesting enzymes for such
319 activity.

320 Only 15 proteins were predicted representing 9 families for *G. alkanivorans* (Table 1). All of them
321 have defined major functions in the cell that could theoretically not be used for the degradation of WB
322 proteins. Only one family has a diverse range of activity in its members (M28A), so its function in
323 *Gordonia* cannot be predicted.

324 According to these results, the valorisation of the protein fraction of WB is theoretically possible for
325 *A. gossypii, C. pinensis* and *C. vaccinii* and less feasible for *G. alkanivorans*.

326

3.3. Growth kinetics of the different strains

A. gossypii forms pellets during growth, showed to reach 1-2 mm diameter on WB (Figures S1). Therefore it was not possible to follow OD at 600 nm, as it was the case for the other organisms, and growth was visually assessed. Both wet autoclave and dry autoclave protocols gave similar results. In its optimal medium *A. gossypii* grows beyond the formation of pellets and fills the entirety of the flask. Stationary phases were reached during the second week of culture.

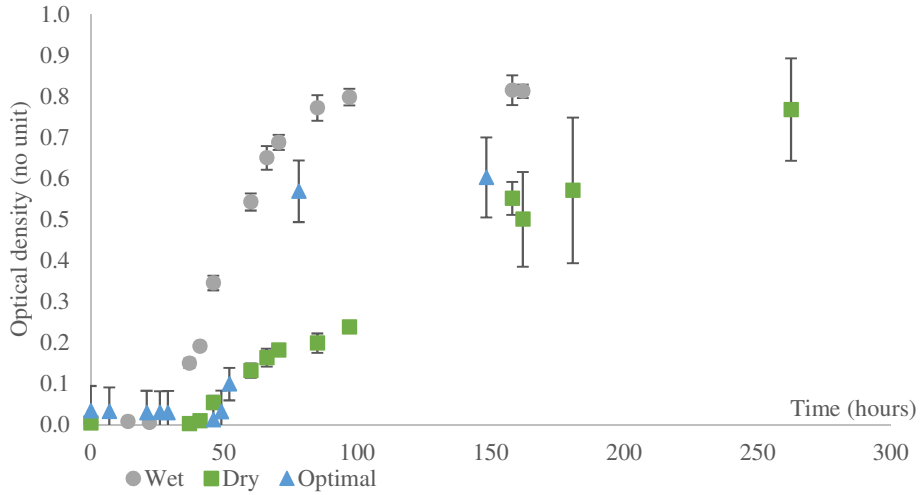
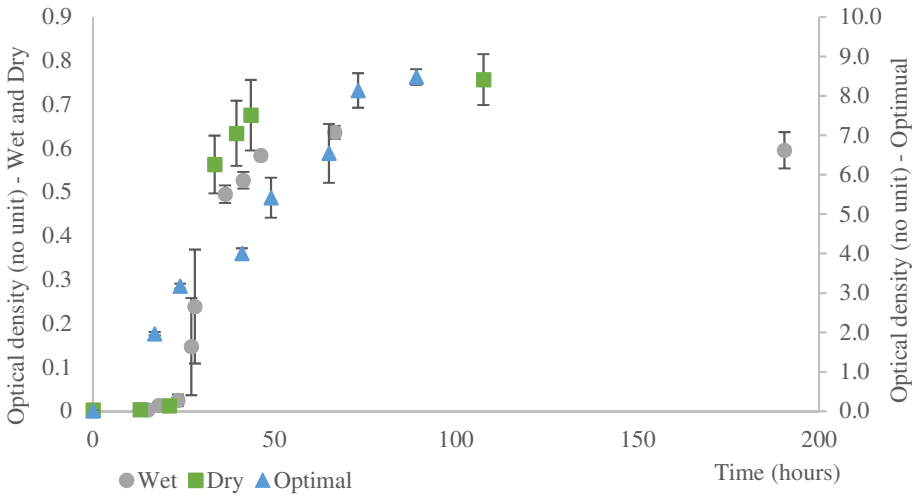


Figure 2.1 - Growths of *C. pinensis* were done at 22°C, 100 rotations per minute (rpm). Optical density was followed at 600 nm. Optimal medium was Cy-broth. n=3, brackets are SD (standard deviation).

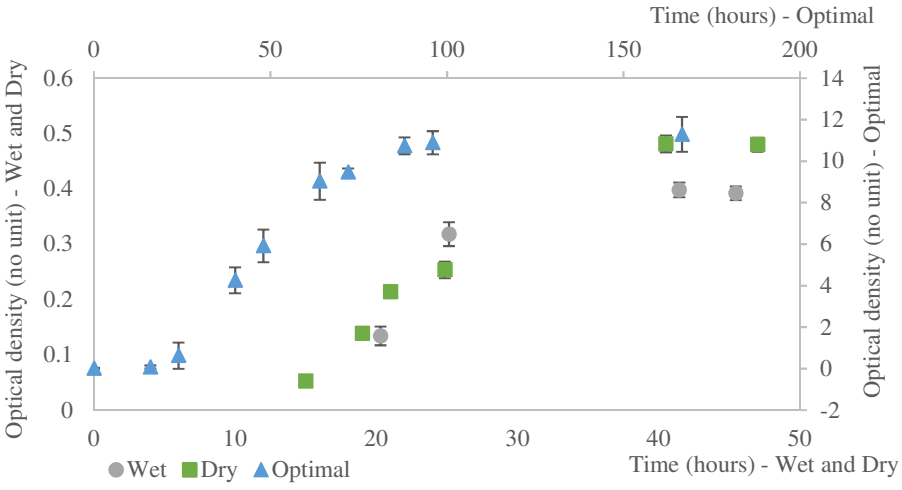
C. pinensis reached similar stationary phases for the three types of culture medium, but interestingly OD was slightly more important for WB than for the optimal medium (max. absorptions : optimal medium - 0.60 ± 0.03; dry autoclave - 0.77 ± 0.13; wet autoclave - 0.81 ± 0.02) (Figure 2.1). The growth on dry-autoclaved WB was significantly slower than the one on wet-autoclaved WB (stationary phases were reached at almost 150h and 100h, respectively), while the exponential phases of wet autoclave and optimal medium are very similar. These findings show that the wet autoclaving of WB might be necessary to improve *C. pinensis*' growth on this substrate.



344

345 **Figure 2.2 -** Growths of *C. vaccinii* were done at 30°C, 100 rpm. Optical density was followed at 600
346 nm. Optimal medium was LB. Ordinates were adapted depending on the growth medium for
347 readability. n=3, brackets are SD.

348 Growth kinetics of *C. vaccinii* on WB were not impacted by the autoclave protocol (Figure 2.2) and is
349 lower than optimal medium (max. OD : 8.48 ± 0.20; stat. phase reached at ≈ 70h). During the kinetics,
350 the WB was coloured in purple, showing that some violacein is secreted and binds to the substrate.



351

352 **Figure 2.3 -** Growths of *G. alkanivorans* were done at 30°C, 100 rpm. Optical density was followed at
353 600 nm. Optimal medium was Trypticase Soy Broth. Ordinates and abscissas were adapted depending
354 on the growth medium for readability. n=3, brackets are SD.

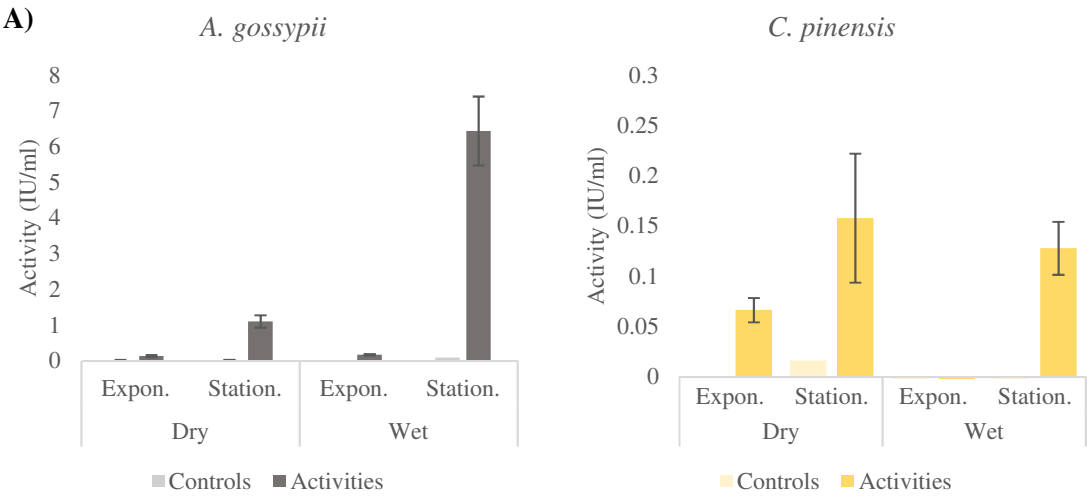
355 *G. alkanivorans* growth was also possible on WB, but was much more lower than its optimal medium
356 (wet : 0.31 ± 0.03; dry : 0,48 ± 0.01; optimum : 0.48 ± 0.01)

357 Overall, the type of autoclave has little impact on growth (except for *Chitinophaga*), and the
358 microorganisms reached early their stationary phase, which is quite low (ODs are always under 1). In
359 order to highlight which components from WB were used as carbon source by the strains, some
360 enzymatic activities produced by the strains during their growth were quantified, and the evolution in
361 composition of the WB was assessed by FTIR analysis.

362

363 **3.4. Enzymatic activities**

A)



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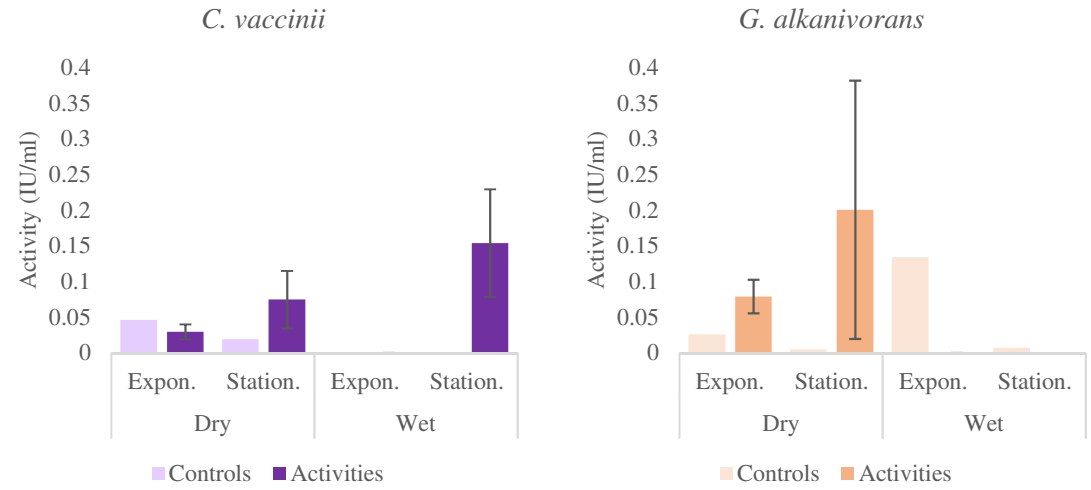

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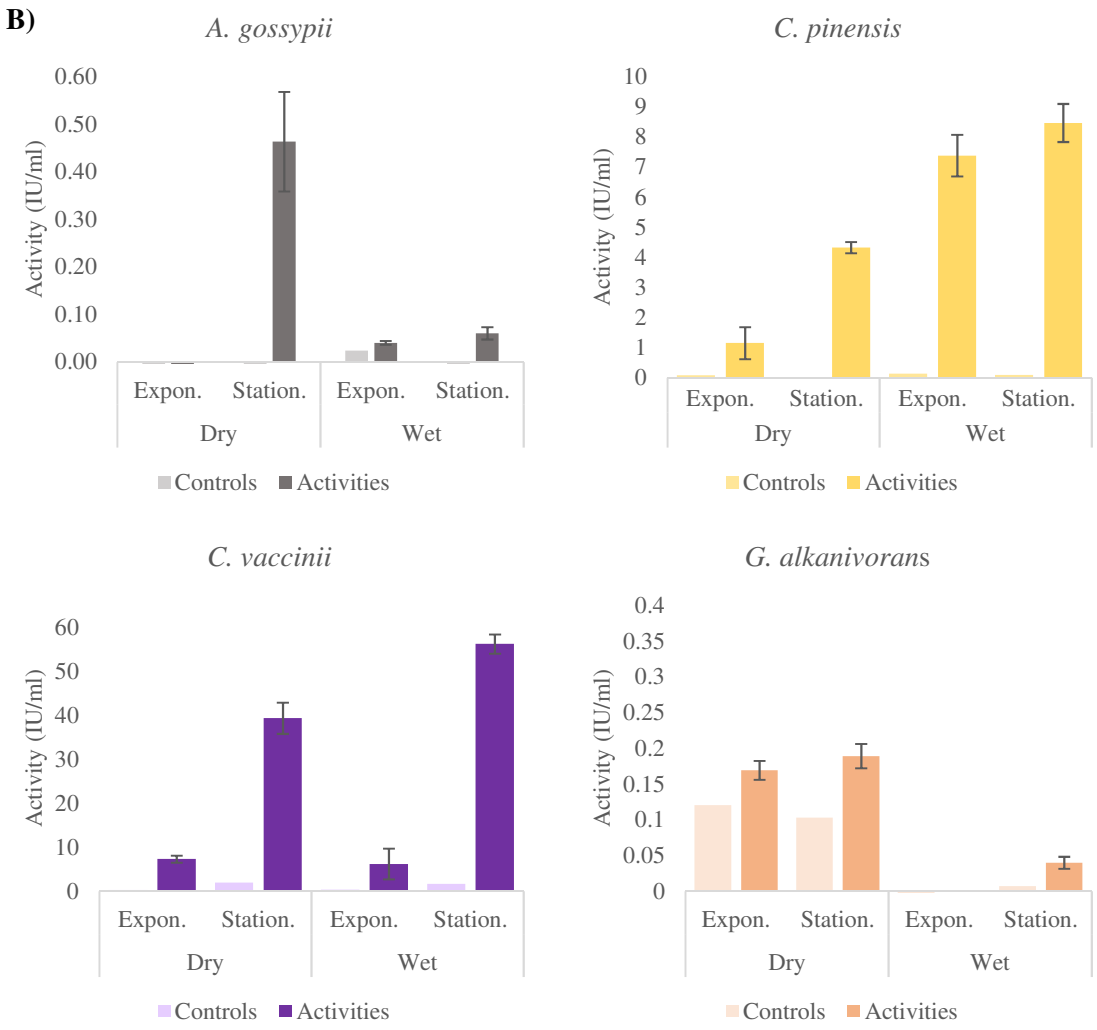


Figure 3 - Measured β-glucosidase and LAP activities for the four microorganisms. β-glucosidase was assessed for extra- and intra-cellular lysates combined. LAP was assessed for extracellular extracts only. Results are displayed according to the type of autoclave (dry or wet) and the growth phase (exponential or stationary). For a same condition, results obtained for controls (no microbial inoculation) are shown next to the results of inoculated cultures. n=3, brackets are SD. (A) - β-glucosidase activities. (B) - Extracellular LAP activities.

The saccharidic and protein content of the WB was precisely analysed according to the methods previously described. Its major components (in percent of dry weight) are glucose (33.11% ± 6.7), xylose (21.63% ± 2.13), protein (around 15.5%), arabinose (12.12% ± 1.61) and starch (11.67% ± 0.38). Measured enzyme activities were chosen regarding this composition. Xylanase, α-amylase and

β-glucosidase activities were measured for the mixed intra- and extra-cellular enzymatic activities. This allows an overview of the degradation mechanisms of WB displayed by the strains. Ligninolytic activities were not measured here, as WB has poor contents in lignin (less than 5% DM).

3.4.1. α-amylase

Starch is a polymer of glucose units linked by α-1,4 and/or α -1,6 linkages that can be enzymatically hydrolysed by the action of exo- and endo-α-amylases. No α-amylase activity was detected for the four strains.

3.4.2. β-glucosidase

Almost a third of the dry weight mass composition is made of glucosidic units. In WB, glucose is a constituent of cellulose, β-glucan and some hemicellulose. β-glucosidase enzymes are the final step for a strain to produce D-glucose from such polymers. Results for the glucosidase activity assay (Figure 3A) showed no activity for *G. alkanivorans*, and results were low for *C. vaccinii* (75 $mIU.mL^{-1}$ ± 40 for the dry-autoclave, stationary phase, with some controls having values of 46 $mIU.mL^{-1}$). Weak glucosidase activity was visible for *C. pinensis* (up to 158 $mIU.mL^{-1}$ ± 64). and important activities were measured for the stationary phases of *A. gossypii* (6.44 $IU.mL^{-1}$ ± 0.97, wet autoclave).

3.4.3. Xylanase

The WB used in this study has important amounts of xylose and arabinose (21.63% and 12.12% of dry weight, respectively), which are components of arabinoxylan, a highly present polymer in WB (Ebringerová and Heinze 2000). No xylanase activity was detected for any of the four strains.

3.4.4. Leucine aminopeptidase

As mentioned earlier, WB contains a non-negligible amount of proteins (15.5%). Fermentation on proteins is possible by some microorganisms (Lai et al. 2016), and proteolytic enzymes can be produced on agricultural wastes (Oliveira et al. 2006; Mať ať a et al. 2016). Leucine amino-peptidase (LAP) is an ubiquitous enzyme that release N-terminal amino-acids of proteins, with a preference for leucine, that is produced by numerous microbial strains (Gonzales and Robert-Baudouy 1996;

403 Wharam et al. 2008; Rahulan et al. 2012). LAP is secreted by each of the microorganisms in almost
404 every condition, except for *G. alkanivorans*, wet autoclave, exponential growth phase and *A. gossypii*,
405 dry autoclave, exponential growth phase (Figure 3B). *C. pinensis* and *C. vaccinii* showed high LAP
406 activities (maximums : 8.45 UI.mL^{-1} ± 0.63 and 56.29 IU.mL^{-1} ± 2.18, respectively). Their activities
407 were a bit lower when the microorganisms were grown on dry autoclaved WB. It was the opposite for
408 *A. gossypii* and *G. alkanivorans*, when an activity was measured.

409

410 **3.5. FTIR**

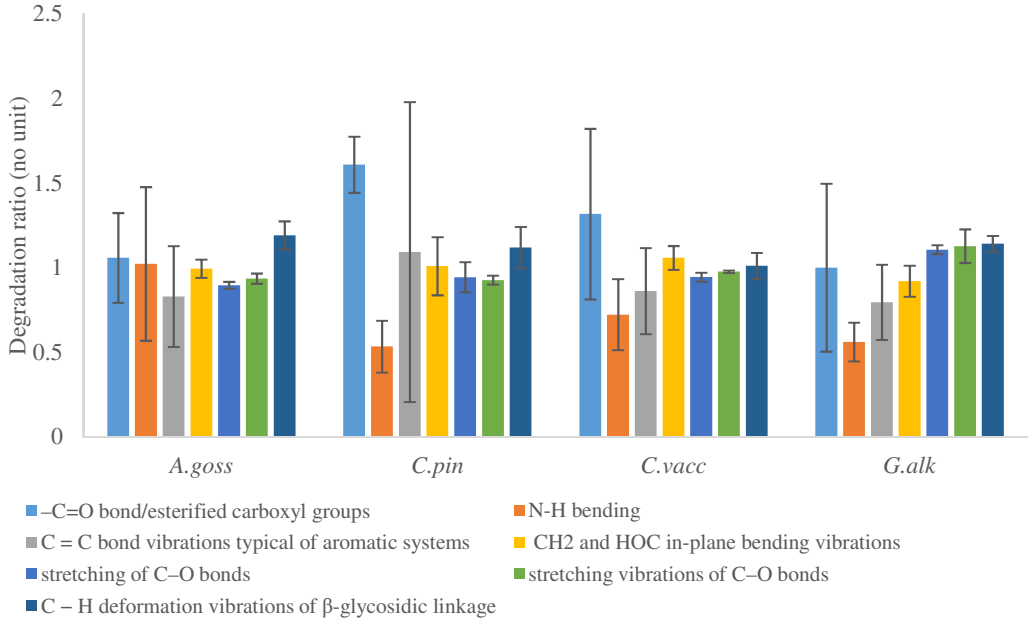
411 The use of FTIR is a relevant approach to highlight which components from lignocellulosic biomasses
412 are modified after an enzymatic or microbial conversion (Sills and Gossett 2012; Waghmare et al.
413 2014; Heidary Vinche et al. 2020). FTIR allows the association between some absorbances values
414 with specific chemical liaisons. By extension, this technique can correlate absorbances with specific
415 types of molecules constituted with these chemical liaisons. Absorbances used in this study and
416 associated molecules are presented in Table 2.

417 **Table 2** - Associations of absorbances measured at specific wavelengths using FTIR with specific
418 functional groups, and related wheat bran components (Fackler et al. 2011; Wang et al. 2013; Xu et al.
419 2013; Lun et al. 2017; Heidary Vinche et al. 2020).

Wavelength (cm^{-1})	1732	1540	1510	1370	1100	1030	896
Functional groups	–C=O bond/esterified carboxyl groups	N-H bending	C = C bond vibrations typical of aromatic systems	CH_2 and HOC in-plane bending vibrations	stretching of C–O bonds	stretching vibrations of C–O bonds	C – H deformation vibrations of β-glycosidic linkage
Associated molecules	Hemicellulose	Proteins (Amide II)	Lignin	Cellulose (mostly)	Phytate content	Polysaccharide	Cellulose (mostly)

420

421 Ratios of the absorbance values at a given wavelength of fermented WB over unfermented one were
422 measured for each organism. Ratio values inferior to 1 usually indicate a consumption of the element
423 during fermentation. Ratio values over 1 can either indicate a specific enrichment, more accessibility
424 due to a degradation of surrounding components (Dong et al. 2019; Zhang et al. 2020), or (for proteins
425 mostly) newly synthesised molecules



426

427 **Figure 4 -** Degradation ratios determined by FTIR analyses and associated to specific type of
428 molecular linkages. Each ratio is made with the Total Attenuated Reflectance value measured at a
429 specific wavelength of dried WB and normalised. Ratios correspond to the value obtained with
430 microorganisms growth over the value of WB treated with the same conditions but not inoculated with
431 microorganisms. WB were wet- autoclaved. n=3, brackets are SD.

432 Overall, FTIR results indicate few changes in the composition of the WB after microbial growth
433 (Figure 4). For *A. gossypii*, degradation ratios lower than 1 for stretching of C-O bonds (phytate
434 content, 0.90 ± 0.05) and stretching vibrations of C-O bonds (polysaccharides, 0.93 ± 0.03) were
435 observed. The C-H deformation ratio attributed to cellulose is increasing (1.23 ± 0.11) but the other
436 ratios attributed to cellulose and hemicellulose do not variate. This means that no lignocellulose
437 variation occurred from the microbial fermentation of WB. For *C. pinensis*, there is a moderate
438 decrease in the stretching vibrations of C-O bonds (polysaccharides, 0.93 ± 0.03). Parallel to this
439 decrease, the -C=O bound/esterified carboxyl group ratio is clearly increasing (hemicellulose, 1.61 ±
440 0.17), indicating a change in the lignocellulose content. There is no evolution in the cellulose,
441 hemicellulose, lignin and overall polysaccharide contents in *C. vaccinii*. An enrichment was measured
442 for *G. alkanivorans* corresponding to the stretching of C-O bonds (phytate content, 1.11 ± 0.03).
443 Another weak enrichment can be observed for one peak of cellulose (C-H deformation vibrations of

444 beta-glucosidic linkages, 1.14 ± 0.05) but not for the other (CH_2 and HOC in-plane bending
445 vibrations).

446 The 1540 cm^{-1} absorption associated to proteins is the most changing of FTIR values observed for all
447 strains in this study, except for *A. gossypii* for which there is no apparent variation (*C. pinensis:* 0.53 ±
448 0.15; *C. vaccinii*: 0.72 ± 0.21; *G. alkanivorans*: 0.56 ± 0.11). This clearly indicate a variation in the
449 protein content of the WB.

450

3.6. Pigment production

After reaching their stationary growth phases, pigments were extracted and concentration measured using specific protocols. Production of pigments was achieved for *C. pinensis, C. vaccinii* and *G. alkanivorans* on a lignocellulosic substrate, but not for *A. gossypii* (Table 3). Growth on their respective optimal medium led to pigment production, as it has already been demonstrated in the literature. As expected, pigment production was more important on optimal medium. Still, *C. pinensis* is not a strong flexirubin producer, and can produce almost half of its pigment on WB compared to the optimal medium (0.15 ± 0.09 compared to 0.27 ± 0.02). To assess the quality of the purification, an absorption spectrum was made on each extract. The observed molecular absorption coefficient (ε_{max}) were those expected, and the pics were isolated and characteristic of the pigments.

Table 3 - Pigment concentration produced by the microorganisms and extracted with specific extractions and determined using absorbances at specific wavelengths. Concentrations are given in $mg.L^{-1}$. Results are compared between growth on optimal medium and specific minimal medium supplemented with WB at 5 $g.L^{-1}$. Mean values are given here. n=3.

	Riboflavin (*A. gossypii*)		Flexirubin (*C. pinensis*)		Violacein (*C. vaccinii*)		Carotenoids (*G. alkanivorans*)	
	Extracted ($mg.L^{-1}$)	SD	Extracted ($mg.L^{-1}$)	SD	Extracted ($mg.L^{-1}$)	SD	Extracted ($mg.L^{-1}$)	SD
Optimal medium	59.04	15.2	0.27	0.02	20.82	4,68	1.45	0.5
Min. medium with WB	0	-	0.15	0.09	1.47	0,29	0.07	0.02

4. Discussion

4.1. CAZymes prediction and secreted peptidases analysis.

All four genomes had CAZy families predicted to degrade starch, cellulose or hemicellulose (Table S1). The number of total and secreted CAZymes predictions (Figure 1) could be compared to other CAZy predictions. *Clostridia phytofermentas* is known for its good lignocellulolytic activities. It has 161 CAZymes predicted in total from its genome, which is in the same range as the organisms in this study, and has a smaller proteome compared to them (3926 proteins, compared to 4259-6997 predicted genes) (Tolonen et al. 2011). *C. pinensis* gives better predictions, with 325 theoretical total CAZymes and 211 secreted. Another example can be found in the filamentous fungi *Pycnoporus cinnabarinus*, which is known to have a great lignocellulosic activity and 315 predicted CAZymes (Levasseur et al. 2014). Even though predictions for *A.* gossypii, *C. pinensis*, *C. vaccinii* and *G. alkanivorans* are lower, the analysis of their secreted CAZymes families showed potentially interesting lignocellulosic activities.

Few studies on the fractionation of agro-industrial co-products focus on the fermentation on the protein content. With this *in silico* study of secreted peptidases, this article propose an interesting new approach for the selection of strains recycling plant biomasses. This, added to the types of pigments that they were supposed to produce, encouraged the selection of these organisms for the rest of the study.

4.2 Growth kinetics of the different strains

In the literature, when growth of *A. gossypii* is studied on a sole carbon source, it is usually on monosaccharides such as glucose, fructose or mannose (Demain 1972). The use of wastes as carbon sources is rare and is almost exclusively studied for lipids-rich wastes (corn oil, rapeseed oil, soybean oil) (Demain 1972; Park et al. 2007) As expected, and regarding the size of the pellets that were formed, growth on WB is not optimal and lower than those on pure monosaccharides. Thermal action at high pressure under water vapour might induce a destructuration of the plant cell walls that could improve their use as carbon source by this strain as it was described previously for other strains

493 (Biswas et al. 2015). This can explain the growth of *C. pinensis* depending on the type of autoclave.
494 However, this was not observed for the three other strains. Previous studies of the use of
495 polysaccharides as sole carbon source showed that *C. pinensis* was unable to grow on starch and
496 cellulose (McKee and Brumer 2015). This strain was also reported to use polysaccharides and
497 lignocellulosic biomasses such as glucomannans (maximum OD = 1.45), curdlan (maximum OD =
498 0.71), wheat arabinoxylan (maximum OD = 0.26) or spruce wood (maximum OD = 0.1) when added
499 to M9 medium (McKee et al. 2019).

500 As a relatively recent discovered species (Soby et al. 2013), no data are available for growth of *C.*
501 *vaccinii* on monosaccharides and polysaccharides. This study is the first to show its ability to grow on
502 a lignocellulosic biomass.

503 Growth of *G. alkanivorans* on alternative carbon source has been reported for sugar beet molasses
504 (Alves and Paixao 2014), artichoke juice (Pacheco et al. 2019) and recycled paper sludge (Alves et al.
505 2008). This study is the first demonstrating the use of lignocellulosic biomass for carotenoid
506 production by *G. alkanivorans*.

507 **4.3. Enzymatic activities**

508 As an element of the upcoming discussion, it has to be noted that there is no data available in the
509 literature for *C. vaccinii* regarding its consumption of starch, xylan, cellulose, as for any of the related
510 enzyme activities.

511 *4.3.1. α-amylase*

512 A previous study in *C. pinensis* showed its inability to hydrolyse starch (Sangkhobol and Skerman
513 1981). For *A. gossypii*¸ α-amylase has already been measured with an activity of ≈ 0.02 IU (dry weight
514 of cell)$^{-1}$ on a solidified agar-starch substrate (Ribeiro et al. 2013). In the article introducing *G.*
515 *alkanivorans* (Kummer et al. 1999), potato starch was hydrolysed by the strain, but no enzymatic
516 activity value was given. Interestingly, *C. vaccinii* has the potential to secrete a CAZyme combining
517 both a starch hydrolysing activity (GH15) and a module binding this substrate (CBM20). Therefore, it

518 is possible that WB is not sufficient to trigger some α-amylase activity in *A. gossypii*, *C. pinensis* and
519 *C. vaccinii*.

520 *4.3.2. β-glucosidase*

521 A semi-quantitative analysis for β-glucosidases (Kummer et al. 1999) showed a weak but positive
522 activity for *G. alkanivorans*, which contrasts with the results of this article. *C. pinensis* was previously
523 demonstrated to be unable to use cellulose (McKee et al. 2019). β-D-glucosidase activities in *C.*
524 *pinensis* could then act on oligosaccharides that might probably be derived from another polymer than
525 cellulose. *A. gossypii* was found early to be unable to degrade cellulose (Ribeiro et al. 2012), but β-
526 glucosidase activity was measured after growth on carboxymethylcellulose-agar (Ribeiro et al. 2013).
527 Since one of its predicted CAZymes (GH5) might specifically target cellulose in the crystalline state,
528 the state of cellulose could have a significant impact on the production for some of its enzymes.

529 *4.3.3. Xylanase*

530 For *G. alkanivorans*, it was predicted that xylose consumption does not occur regarding previous
531 articles (Kummer et al. 1999; Alves and Paixao 2014). *C. pinensis* is the only strain which was shown
532 to have the ability to grow on wheat arabinoxylan and wood glucoronoxylans (McKee and Brumer
533 2015). Growth on xylose led to a stationary phase at ≈ 0.4 (OD, no unit) [ibid.], which is a bit lower
534 than the mean 0.83 observed for the wet and dry autoclaved WB in this study.

535 *4.3.4. Leucine Amino-Peptidase*

536 *In silico* secreted proteasome analysis showed that all these strains had proteolytic potential, therefore
537 LAP activity, being ubiquitous, had definite chances to be observed. In the literature, no LAP
538 activities were previously measured for those four strains.

539 **4.4. FTIR**

540 For the four strains, no clear cellulolytic, hemicellulolytic or ligninolytic effect was visible
541 individually. Overall, the evolution of a single ratio does not have a clear effect on the others.
542 Decreases and increases in the FTIR absorbance values with fermentation seem more to indicate the

543 complex interaction between all the components of the WB. That is also why no clear correlation
544 between the *in silico* predictions and the FTIR results can be made.

545 Proteins are heterogeneously displayed in the different WB layers, with varying structures and
546 activities (Balandrán-Quintana et al. 2015). Thus, even if the interactions between the proteins of the
547 WB and its other components has not received major attention yet, multiple types of linkages can be
548 expected. This could explain the fact that the decreases in the 1540 cm^{-1} ratios does not have the same
549 effect on the other FTIR ratios depending on the strain. Ratios were unchanged for *C. vaccinii*, there
550 were slight specific enrichment for the 1100 cm^{-1} and 896 cm^{-1} bands in *G. alkanivorans*, and specific
551 enrichment only for 1732 cm^{-1} with *C. pinensis*. FTIR measurements seem to confirm that LAP is not
552 the main enzyme responsible for protein degradation of the WB, as the highest LAP activities for wet-
553 autoclaved WB (*C. vaccinii*) did not lead to the lowest 1540 cm^{-1} ratio. Also, LAP activity was
554 detected with a low level for *G. alkanivorans* and can probably not be responsible for the important
555 decrease in proteins contents within WB. Since LAP is not the only proteolytic activity in a given
556 organism, this activity in itself cannot be used to predict accurately the degradation of the protein
557 fraction of the WB. Though, it is still an interesting indicator of a general secreted proteolytic activity.

558 FTIR analysis confirms some changes in the state of the WB, with a clear indication of the use of its
559 protein content.

560 **4.5. Pigments production**

561 Concentrations in organic matter are quite important within the four optimal media (more than 15 $g.L^{-1}$).
562 In comparison, concentration of WB in minimal salt medium was set to 5 $g.L^{-1}$, mostly for
563 homogenisation reasons during cultures. The structure and the nature of the components are quite
564 different from those in an optimal medium. WB components are also less readily available for
565 consumption than those of an optimal medium. Therefore, yields of pigments were logically expected
566 to be lower on WB than optimal media. Nevertheless, it was demonstrated the possible use of a cheap
567 and abundant lignocellulosic co-product as carbon source for pigments production by some
568 microorganisms already known to produce them.

569 *4.5.1. Riboflavin production*

570 *A gossypii* is known to be a strong producer of riboflavin (Kato and Park 2012). On a rich medium,
571 wild type strains can produce this pigment up to 5.2 $g.L^{-1}$ (Sugimoto et al. 2009). Riboflavin
572 overproduction is expected to appear when the culture reaches its stationary phase and the growth rate
573 declines (Kato and Park 2012). Since overproduction is known to be triggered by a variety of stresses
574 (nutritional, oxidative, …) (Averianova et al. 2020), it can be hypothesized that the low amount of
575 microorganisms (Figure 3) at the end of the cultures was not enough to trigger these stresses.

576 *4.5.2. Flexirubin production*

577 Potent applications of flexirubin are its anti-cancer properties and treatment of tuberculosis (Venil et
578 al. 2016). This study is the first to our knowledge to quantify a flexirubin production by *C. pinensis*. In
579 comparison, the highest flexirubin production recorded in the literature was 521.64 $mg.L^{-1}$, obtained
580 by *Chryseobacterium artocarpi* CECT 8497 (Venil et al. 2015). Liquid pineapple waste has also been
581 used as a mean to reduce the overall cost of production for the same species, and yielded 200 $mg.L^{-1}$ of
582 flexirubin (Aruldass et al. 2016). Still, flexirubin quantification in the literature is often given in dry
583 weight of pigment after solvent evaporation so comparisons have to be done cautiously.

584 *4.5.3. Violacein production*

585 *C. vaccinii* is known to produce both violacein and deoxyviolacein by a process of quorum sensing
586 (Soby et al. 2013). Violacein has many interesting properties for bioproduction, as anticancer,
587 antibacterial, antifungal and anti-leishmanial agent (Kothari et al. 2017). A previous attempt to
588 quantify violacein production in *C. vaccinii* in Nutrient Broth medium gave 975 $\mu g.L^{-1}$ (Palaniswamy
589 and Vishnu 2016). *Chromobacterium violaceum* was used on immobilized sugar-cane bagasse in a
590 column system alone to produce 150 $mg.L^{-1}$ of violacein (Ahmad et al. 2012). The use of liquid
591 pineapple waste 10% (v/v) in rich Nutrient Broth medium for this strain led to an increase of 46 $mg.L^{-1}$
592 compared to NB alone (Aruldass et al. 2015).

593 *4.5.4. Carotenoids production*

Carotenoids are widely used as food colorants, and their consumption is linked to multiple health benefits (Chandi and Gill 2011). Overall, *G. alkanivorans* gave poor results for carotenoid production (Table 3). Carotenoid production by *G. alkanivorans* has not been studied on sole lignocellulosic components. However, the *Gordonia* genera is well known for its carotenoid production (Arenskotter et al. 2004). *G. alkanivorans* is one of the best producers of carotenoids, with one of its highest production being of 2.5–3.1 mg.(g of dry cell weight)$^{-1}$ in a minimum salt medium containing glucose and sulphate (Fernandes et al. 2018). Therefore, an efficient release of glucose from cellulose could greatly impact carotenoid production. Also, is has to be noticed that since the use of light is important for carotenoid production in *Gordonia* species (Fernandes et al. 2018), adjusting this factor can be essential for improving productivity.

6. Conclusion

In the end, this study shows that known pigment producers from the literature can be rationally chosen on their ability to use renewable carbon sources as a substrate. A precise knowledge of the components in a substrate allows to interrogate specific enzyme databases to select potent candidates able to degrade and feed on it. Structural studies of the substrate with FTIR combined to enzymatic assays are interesting tools to analyse complex changes in the composition of the fermented substrate. More importantly, this study contributes to the discovery of new methods for the renewable production of molecules of interest. Best candidates from this study will be chosen for a deeper analysis of their consumption of WB, in order to improve biomass recycling and pigment production.

Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Acknowledgments

The authors thank la "Fondation de France" and la "Fondation du site Paris-Reims" for its financial support to this project.

The authors are grateful to the French Region Grand Est, Grand Reims and the European Regional Development Fund (ERDF) for the financial support of the chaire AFERE.

Funding sources had no involvement in the collection, analysis or interpretation of data, in the writing of the report or in the decision to submit the article for publication.

Declaration of competing interest

The authors declare no conflict of interest.

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