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Degradation of lignocellulose by different bacterial and fungal co-cultures

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ABSTRACT

Long seen as non-valorisable waste, agricultural co-products are increasingly used in biorefinery processes. Co-culture appears as new trend for to improve the degradation of lignocellulose and improve the production of bioproducts. The goal of the study was to setup inter-domain co-cultures with high capabilities of lignocellulose degradation using a pluridisciplinary approach combining bioinformatics, enzymology, transcriptomics. Different individual lignocellulolytic strains: *Trichoderma reesei* QM6a and three bacteria (*Streptomyces coelicolor* A3(2), *Rhizobium* sp.XylPr11 and *Sphingobacterium prati* AraPr2 affiliated from different phyla) were used in that study . Synergic activities have been observed and quantified in co-culture conditions, particularly for xylanases and peroxidases activities. The enzymatic activities for the co-cultures in the most interesting co-culture (*T. reesei QM6a/S. coelicolor A3(2)*) reached more up to 2 IU/mL and 430 IU/mL respectively for the xylanase and peroxidase. Furthermore, ATR-FTIR analysis showed a real impact of co-culture condition on the substrate compared to the monoculture specially for hemicellulose degradation. Transcriptomics of *S. coelicolor* A3(2) either in mono or co-culture showed a relative similar pattern profile whatever the condition analysed with a specific overexpression of certain CAZyme genes involved in glycolysis due to the hydrolytic role played by the fungal partner. This work provided the proof of concept for technological feasibility, pertinence and usefulness of interdomain co-culture.

Introduction

The advantage of lignocellulose compared to other renewable energies (solar, hydroelectric, wind) is that in addition to its energy capacities, it has a strong chemical potential. The lignocellulose constitutes an excellent source for high potential production biofuel, biomolecules, or bioenergy (Chandel et al., 2018; de Lima Brossi et al., 2016). Cellulose is the most used plant polymer in various fields: paper industry, materials (cellophane), are with the products of its fermentation (bioethanol) the main examples (Sundarraj and Ranganathan, 2018). Hemicelluloses can also be used to produce bioethanol, but also high value-added sugars such as xylitol (pharmaceutical and agro-industrial applications), or organic acids (lactic acid, fumaric lactic acid, fumaric acid, succinic acid) (Ji et al., 2012). In terms of chemical potential, lignin seems to be the most promising in terms of future applications due to its chemical diversity of its composition, but it is also the least used component of biomass.

Lignocellulose has indeed high molecular potential (Rana et al., 2018; Torres et al., 2020), but it is complicated to fully utilize and valorize. Plant cell walls have evolved over time to withstand biotic and abiotic stresses, i.e., environmental conditions and attacks by pathogens. This phenomenon is called recalcitrance and is due to many parameters of lignocellulose. There is a very high diversity in lignocellulose composition (hemicellulose, lignin and cellulose) between species in the plant kingdom (Escarnot et al., 2010; Sundarraj and Ranganathan, 2018), but also between different tissues composing the same organism (lignin content is higher in straw than in bran in wheat, for example) (Zoghlami and Paës, 2019). Considered separately, each polymer brings recalcitrance: the degree of polymerization and crystallinity of cellulose, the branching and diversity of hemicelluloses, the protective layer formed by pectin, and the chemical and structural complexity of complexity of lignin. When they interact together to form plant cell walls, the recalcitrance is strongly increased since their interactions will form very solid and refractory structures because of the large variety of bonds between the components.

In order to make lignocellulose usable, the pre-treatment step of the biomass is essential. Drastic pre-treatments (physical and chemical) are traditionally used in industries but can be polluting. Since the development and improvement of enzymatic techniques, more and more industries are using this approach. In the same way, hybrid pre-

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treatments, mixing enzymatic with physical-chemical techniques are increasingly attractive, since they limit the disadvantages of each technique and increase the yields (Zhao et al., 2012; Zoghlami and Paës, 2019). The efficiency of the enzyme system remains limited and the quantity of enzymes required is large, which has a negative impact on economic and environmental sustainability (Jørgensen and Pinelo, 2017). The global enzyme market is estimated to be worth several tens of billions of euros, and the cost of enzymes in biomass fractionation for bioethanol production in 2012 represents >15 % of the selling price of the latter (Humbird et al., 2011). New alternatives therefore need to be found to obtain cocktails inspired by efficient, low-cost lignocellulolytic ecosystems.

In soils and in nature in general, the lignocellulose degradation is dependent on a multitude of factors such as the physico-chemical conditions (humidity, temperature, nature of the soil), the type lignocellulose (botanical origin, plant composition, density) as well as the richness and diversity of the microorganisms present in the ecosystems (Condron et al., 2010). The degradation of lignocellulose is therefore a time-, substrate-, and actor-dependent dynamic (Cortes-Tolalpa et al., 2017). Abiotic mechanisms (rainfall, alternating seasonal conditions, etc.) will lead to physico–chemical constraints and leach soluble elements. The biotic actors of the different levels catalyze the degradation thanks to the biological mechanisms that which allow them to use the organic matter as a substrate. It is important to specify that the degradation of organic matter does not stop at a "breaking of complex polymers into compounds of low molecular weight easily metabolized" by an individual, it is rather a question of a close collaboration between different participants (Feldman et al., 2017). The cooperation will be improved by a complementarity of the metabolic pathways of the actors and the exchange of metabolites (Cortes-Tolalpa et al., 2017). This cooperation can make intervene microorganisms form different kingdoms such as brown rot fungi (use cellulose and leave lignin which they cannot degrade), and white rot fungi (consume all plant polymers, the substrate is bleached), white rot fungi (consumes all plant polymers, substrate is bleached (Hatakka, 1994) and bacteria that will have a role in mineralization of nutrients through more specific intracellular systems (Condron et al., 2010).

Since no single microorganism can perform it alone, co-cultures are relevant biotechnological approaches (Jones and Wang, 2018). Co-culture is a process inspired by natural microbial communities under controlled and simplified conditions. It has at least two participants and uses biological phenomena such as competition, synergy and elicitation between members. Co-cultures can also be described as consortia or synthetic communities. Interactions between microorganisms can be negative, neutral or positive. A positive, or even neutral, interaction is preferable for the establishment of co-cultures. The use of co-cultures allows to increase the enzymatic productions. Several explanations exist: (A) a greater diversity of enzymes is produced, allowing a more efficient and complete degradation, this is the enzymatic synergy (Taha et al., 2015), (B) chemical interaction (interacting molecules, elicitors, secondary metabolites), as well as the sharing of metabolic pathways allow for emulation of microbial development, this is growth synergy (Ren et al., 2015). In addition, co-cultures provide the opportunity to activate certain genes to produce molecules of interest (Ueda and Beppu, 2017; UEDA et al., 2000). Co-cultivation is a method that is fully in line with the bioeconomy because of its ability to lignocellulosic biomass and by the possibility of developing "one-pot" processes. Indeed, the pooling of several metabolic pathways from different microorganisms allows to reduce the number of fermenters to be used in industry (Boruta et al., 2023; Chandel et al., 2018).

Although advantageous, the "competition" between the partners also has disadvantages. Indeed, a co-culture requires a certain balance, one of the strains must not dominate the co-culture. Maintaining the stability and robustness of the synthetic community as well as controlling the competition as well as controlling competition are the main hurdles to overcome in this process (Jones and Wang, 2018). Setting up co-cultures is relatively feasible at the laboratory scale, but is much more complicated at an industrial scale (Goers et al., 2014). The establishment of microbial lignocellulolytic co-cultures is currently in full swing, whether between intrakingdom (between 2 *Clostridium* strains (Zhang et al., 2018) or interkingdom microorganisms either: 1) between a lignocellulolytic bacteria *Clostridium phytofermentas* and a yeast *Saccharomyces cerevisiae* (Zuroff et al., 2013), 2) or a lignocellulolytic bacterial species (*Streptomyces* sp.) and a lignocellulolytic fungi (*Aspergillus niger*) (Detain et al., 2022).

Fermentative bioconversion of lignocellulosic biomass by μorganisms can be achieved using several strategies: separate enzymatic hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), and consolidated bioprocessing (CBP) (Verardi et al., 2020). In SHF, enzymatic hydrolysis and fermentation reactions are carried out in separate bioreactors. SSF and CBP technologies combine enzymatic hydrolysis and fermentation in a single reactor, reducing overall production time and operating costs.

The objective of the manuscript will be to develop co-culture protocols by setting up new interdomain co-cultures with different fungal (*T. reesei* QM6a (Novy et al., 2019)) and bacterial strains able to fractionate the biomass (*S.* A3(2) (Besaury et al., 2022)) or isolated from lignocellulolytic ecosystems in our laboratory (*S. prati AraPr2* (Besaury et al., 2021)) and *Rhizobium* sp.XylPr11 (unpublished) in order to develop microbial cocultures with particularly high lignocellulolytic performance. Those microbial partners have been identified previously as main lignocellulose degraders and harbour a wide variety of lignocellulose degrading enzymes and are members of interest for the bio-industry (Cortes-Tolalpa et al., 2020; Detain et al., 2022; Fonseca et al., 2020; Jackson et al., 2017). This manuscript is the first milestone towards the deciphering the interactions between microbial partners and determining the parameters for a robust, resilient and performant co-culture for lignocellulose degradation. This manuscript in clearly involved in the field of bioeconomy which consists in the valorization of the biomasses to produce molecules of high industrial interest and use biological processes as an alternative to the chemical, physical and enzymatical which are not green and can be costly. This manuscript will enable the bioconversion of lignocellulosic biomass directly as a carbon source using the co-culture of micro-organisms (as CBP then), without the need for biomass pre-treatments and, in the very long term, will reduce the cost of producing molecules. Moreover, this study represents the opportunity to produce robust, efficient lignocellulolytic enzymatic cocktails obtained from those synthetic microbial communities that will be less expensive compared to commercial ones mainly produced using expensive glucose as a carbon source (Fonseca et al., 2020).

Material and methods

Substrate preparation

Wheat bran (WB) (0.5–2 mm) was provided by the ARD society (https://www.a-r-d.fr/) and the compoisition was obstained from a previous study (Rémond et al., 2010). The WB contained 16 % of dry matter (DM) of arabinose, 19 % DM of glucose, 26 % DM of xylose, 1.1 % DM of galactose, 11.6 % DM of starch, 15 % DM of protein and presents a lignin content of 5 % DM. The% of glucose can be attributed to cellulose and mixed β-glucans present in WB.

Growth media and inoculation of the microbial partners

In order to characterize the ability of the strains to grow on wheat bran, 250 mL flasks containing 50 mL of M3 media 1X (KH_2PO_4 1.9 g/L, Na_2HPO_4 5.1 g/L, $MgSO_4.7H_2O$ 0.1 g/L, $(NH_4)_2SO_4$ 0.2 g/L) [24] supplemented with dry wheat bran up to 5 g/L were used. After sterilization, 1 mL of a $Ca(NO_3)_2 \cdot 4H_2O$ solution at 25 g/L and 1 mL of a trace element solution were added sterically . Those last 2 solutions were autoclaved and filtered at 0.2 μm respectively and added sterically in the

flasks. The trace element was purchased from Sigma Aldrich with subsequent composition (EDTA: 570 mg/L, $CuSO_4$. $5H_2O$:10 mg/L, AlK $(SO_4)_2 \cdot 12H_2O$: 20 mg/L, H_3BO_3:10 mg/L, Na_2MoO4. $2H_2O$: 10 mg/L, Na_2SeO_3: 1 mg/L, Na_2WO_4 . $2H_2O$: 10 mg/L, $NiCl_2$. $6H_2O$: 20 mg/L, $MgSO_4$. $7H_2O$: 3000 mg/L, $MnSO_4$. H_2O: 500 mg/L, NaCl: 1000 mg/L, $NH_4Fe(SO_4)_2 \cdot 12H_2O$:170 mg/L, $Co(NO_3)_2$. $6H_2O$: 100 mg/L,$CaCl_2 \cdot 2H_2O$:100 mg/L, $ZnSO_4$. $7H_2O$:100 mg/L, https://www.sigmaaldrich.com/FR/fr/product/sigma/mbd0056). 1 ml of bacterial liquid culture and $10^{\hat{}6}$ spores of *T. reesei QM6a* were inoculated into the flasks. To determine this value of $10^{\hat{}6}$ spores, the fungal strain was grown on PDA solid medium for one week at 30 °C until germination. The spores were then washed with Tween80, concentrated and counted on a Thoma cell and diluted if necessary. In order to compare the physiology of the strains, cultures were grown using 40 mM glucose as the carbon source.. All flasks were shaken for 240 h at 100 rpm at 30 °C. The experiments were performed as 3 biological triplicates.

Degradation of the substrate ATR-FTIR (Attenuated total reflectance Fourier transform infrared) spectroscopy analyses

ATR-FTIR analyses were conducted on WB without fermentation (with culture media treated the same way as during fermentation) and on WB residues after the stationary growth phase was reached. Following cultivation, the supernatant was discarded, and the remaining WB was rinsed several times with sterile ultrapure water. The WB was then dried at 50 °C for at least 2 days. ATR-FTIR analyses were carried out using a Nicolet 6700 FTIR spectrometer (Thermo Fisher Scientific, Waltham, USA) with an ATR-diamond crystal (Smart iTR, Thermo Fisher Scientific, Waltham, USA). Each biological replicate was measured at least three times (technical replicates). Spectra were processed using the OMNIC 8 software. A mean spectrum of the three readings was calculated, the baseline was corrected, and the area of interest (approximately 2990−800 cm^{-1}) was normalized. Ratios of absorbances of fermented to non-fermented WB were calculated. All data were collected for 3 biological triplicates, with samples obtained from three separate cultures. This protocol is often used in the laboratory (Besaury and Rémond, 2022; Cassarini et al., 2021).

Enzymatic activities of the mono and co-cultures

In order to extract the enzymes from the microbial cultures, 1 mL of culture was collected and the cells were lysed using FastPrep and lysing matrix B at 6.5 G during 4 times * 40 s (MpBiomedicals, France). All the lignocellulolytic activities were measured for each biological triplicate measured all along the growth curve. The activities were expressed as mIU (or IU) of enzymatic activity /mL.

Xylanolytic activity was evaluated as previously described [26] with 0.1 mL of extracted proteins mixed with birchwood xylan (Sigma Aldrich France) at 0.5% w/v in 50 mM phosphate buffer, pH 7.5 at 30 °C for 10 min. Enzymatic activity was expressed in international milliunits (mIU), where 1 IU is defined as the quantity of enzyme required to release 1 μmole of reducing sugar per min.

Total phenol-oxidase/peroxidase activities were evaluated as in [27]; for this, 0.1 mL of extracted proteins was mixed in a total volume of 2 mL with 8 mM of pyrogallol, 1 mM of EDTA and 0.5 mM of H_2O_2 at 30 °C during 10 min and measured at 420 nm.

Arabinofuranosidase activity by determining the hydrolysis rate of *p*-nitrophenyl α-l-arabinofuranoside (0.5 mM) and xylosidase activity was measured by quantifying the rate of hydrolysis of *p*-nitrophenyl-β-xylopyranoside (0.5 mM) in the same buffer in 1 mL reaction containing 900 μL of buffer (50 mM sodium phosphate buffer, pH 7.5) and 0.1 mL of culture supernatant. β-d-glucosidase activity were measured in the same conditions using as substrate *p*-nitrophenyl -β-d-glucopyranoside. The extinction coefficient of *p*NP in the measurement conditions was 15,850 M^{-1} cm^{-1}. Experiments were measured using the absorbance at 401 nm for 5 min at 30 °C and using recording spectrophotometer (Uvikon 933).

Genomic sequencing, assembly and annotation of total and secreted CAZymes

The strain *Rhizobium* sp.XylPr11 (isolated previously in the laboratory from an agricultural soil supplemented with wheat bran) was grown in 25 mL Luria Bertani media. After microbial growth, genomic DNA was extracted using PureLink Genomic DNA Mini Kit (Invitrogen, France) according to manufacturer's instructions. DNA was quantified using the ND-1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, USA). DNA quality was controlled after electrophoresis on a 0.8 % (w/v) agarose gel made in Tris–acetate-EDTA buffer (TAE buffer). Genomic DNA was sequenced using a NovaSeq system (IlluminaPE150, 100 × coverage) and was performed by Novogen (UK). The sequence data files were filtered for quality using FastQC (Andrews, 2010) and then de novo assembled by IDBA-UD (Peng et al., 2012); default parameters were used for all software. The quality and completeness of those newly obtained genomes was studied by using checkM (Parks et al., 2015) using the default parameters. The reference genome of *Rhizobium* sp.XylPr11 was deposited at GenBank under the following accession number (PRJNA1014381). The genome sequences from *T. reesei* QM6a and *S. coelicolor* A3(2) downloaded from GenBank (respective accession numbers: GCF_000167675.1 and GCA_000203835.1). The genome sequences of *S. prati AraPr2* was already available in our laboratory (GCA_013167215.1). The presence and diversity of the Carbohydrate Active enZyme (Cantarel et al., 2008) was demonstrated by using the online resource dbCAN (http://csbl.bmb.uga.edu/dbCAN/annotate.php). The CAZyme secretome of the fungus and bacteria was analysed by using the SignalP (Almagro Armenteros et al., 2019) software in order to identify the proteins with a secretion signal to the extracellular medium. The TMHMM server (Krogh et al., 2001) was used to identify the presence or absence of transmembrane domains in the sequence of these proteins. Only the sequences with no TMHMM topology were obtained and finally submitted to the dbCAN meta server in order to determine the CAZy secretome (Huang et al., 2018).

Transcriptomic analysis of S. coelicolor A3(2) in mono and co-culture

Regarding the transcriptomic analysis, bacterial pellets were sentto Novogene (United Kingdom) and RNA was extracted according to their laboratory procedures. Briefly, the same mass of mycelium was collected, mixed with Trizol at room temperature for 5 min, and then stored in liquid nitrogen for subsequent RNA extraction. The concentration of RNA was measured by UV detection at 260 nm, with ddH2O as the blank control. Sequencing was performed using the Nugen Universal Prokaryotic RNA-Seq Library Preparation Kit on an Illumina Novaseq6000. Reads were then quality checked using .FastQC (Andrews 2010). The high-quality reads remaining were mapped against the genome using Bowtie 2 (Langmead and Salzberg, 2012) and "very-sensitive local" parameter. Mapping of the reads to the genome were summarized at the CDS level by using the software Htseqcount (Anders et al., 2015) and the annotation file in GFF3 format from *S. coelicolor* A3(2). Differential gene expression was analysed using the Sartools package (Varet et al., 2016) and more specifically Deseq2. CDS with *p*-values $< 0{,}05$ and $\log_2$Foldchange > 2 were considered as differentially expressed.

Statistical analysis

The values given in this report are the means of the biological triplicates, with error bars corresponding to the standard deviation of this mean. Significance was tested with ANOVA model to determine variation in the lignocellulose degradation whereas mixed model ANOVA was performed to determine modifications in term of lignocellulolytic activities.

Results and discussion

Setting up co-cultures appears to be a solution to enhance biomass degradation and reduce financial and environmental costs of other processes (physico–chemical, mechanical…). There are several explanations for this: (A) a greater diversity of enzymes is produced, enabling more efficient and complete degradation, known as enzymatic synergy (Taha et al., 2015), (B) chemical interaction (interacting molecules, elicitors, secondary metabolites), as well as the sharing of metabolic pathways, enables emulation of microbial development, known as growth synergy (Ren et al., 2015). The number of publications dealing with co-cultures to produce biomolecules (Llamas et al., 2023) has increased recently but not manty targeted the elicitation of lignocellulolytic pathways specially with members from different domains.

The fungal strain used in this manuscript was *T. reesei* QM6a. The *Trichoderma* genus, which belongs to the Ascomycetes, is found in the soil and more specifically in the rhizosphere. in the rhizosphere. In the literature, members of this genus are frequently referred to as a promising solution for biocontrol as part of a more reasoned and sustainable approach to agriculture (Harman et al., 2004). It can be used against phytopathogenic fungi as an antifungal agent (Guzmán-Guzmán et al., 2019). However, this characteristic, which could be a hindrance to the establishment of co-culture, is less present for the strain used during the course: *T. reesei* QM6a. Indeed, clusters involved in the synthesis of mycoparasitic metabolites are absent from the genome of this species. However, it is interesting to note that contact with the hyphae of another filamentous fungus results in an increase in the capacity to degrade cellulose for *T. reesei* QM6a (Guzmán-Guzmán et al., 2019). Industrially, *T. reesei* QM6a is one of the largest producers of lignocellulolytic enzymes (Bischof et al., 2016). Like any microorganism, this strain will produce different types of enzyme cocktails depending on its substrate (Novy et al., 2019). *Trichoderma* has been used in co-cultures for the production of lignocellulolytic enzymes but mainly with other fungi (*Aspergillus, Monascus* and *Coprinus* among others (Sperandio and Filho, 2021)) and not with μorganismes from other domains of the tree of life

S. coelicolor A3(2) was used as a bacteria partner and belongs to Actinobacteria which ubiquitous Gram-positive bacteria, many of which form mycelium. This phylum is of particular importance in biotechnology, since two-thirds of antibiotics (as well as anticancer, anti-helminthic and antifungal drugs) are produced by these bacteria (Barka et al., 2016). Because they live alongside many other (micro)organisms, *Streptomyces* (which belongs to the Actinobacteria) have a high capacity to produce secondary metabolites, particularly antimicrobials and antifungals (Barka et al., 2016). *S. coelicolor* A3(2), model strain among the *Streptomyces*, harbours numerous carbohydrate degradation pathways and several CAZymes, which allow an efficient lignocellulose degradation which requires the involvement of several unexpected CAZymes which have been characterized previously in our laboratory (Besaury et al., 2022). *Streptomyces* members have been co-cultured with bacterial and fungal partners in order to either elicit the production of new secondary metabolites but also to establish consolidated bioprocesses for the production of molecules of interest such as bioplastics (Boruta et al., 2023; Kumar et al., 2023).

The last strains used in this study were *S. prati AraPr2* (Besaury et al., 2021) and *Rhizobium* sp.XylPr11 and were isolated in our laboratory from agricultural soils incubated with wheat straw (Besaury et al., unpublished). The strain *S. prati AraPr2* has been characterized a new bacterial species involved in the lignocellulose fractionation which contained an high number of CAZymes (Besaury et al., 2021). Strains affiliated to the *Sphingobacterium* species were found and showed ability to degrade different lignocellulosic biomass (Cortes-Tolalpa et al., 2020; Jiménez et al., 2016; Wang et al., 2023) and that they can interact with fungal partners. *Sphingobacterium* appears as a good candidate in the literature for the setup of co-cultures regarding the lignocellulose degradation due to its ability to degrade hemicellulose but also exchange possible vitamin and metabolites with other microbial partners (Cortes-Tolalpa et al., 2020; Wang et al., 2023). Members of the *Rhizobium* genus and more generally of the Alphaproteobacteria have been recognized isolated recently as able to metabolize lignin and lignin-like compounds and harbor an important collection of both lignin-oxidizing and carbohydrate-hydrolyzing enzymes which show their ability to be involved in degradation of lignocellulosic biomasses (Jackson et al., 2017). To our knowledge, no co-culture have been established including a *Rhizobium* sp. member regarding the lignocellulose degradation.

Table 1 gives the basic information on the genomes of our microbial strains of interest. The genome of the *T. reesei* QM6a was 33.4 Mbp while the bacteria have smaller genomes (between 5.6 and 9.1 Mpb). However, although the genome of the bacteria used was 3.6 to 6 times smaller than the fungi, the number of gene coding proteins was <2 times smaller between the fungi and the bacteria. Indeed, the number of gene coding proteins between *S.coelicolor* A3(2) and *T. reesei* QM6a was in the same order of magnitude as that of the fungi (8128 to 9111 respectively) whereas the number was lower for the 2 remaining bacteria (respectively 5174 and 5112 for *Rhizobium* sp.XylPr11 and *S. prati AraPr2*).

The CAZyme number was higher in the fungi (386 CAZymes) compared to the 3 other bacteria; indeed a gradient of CAZymes s is present in our bacterial dataset with respectively 336, 297 and 123 CAZymes for *S.coelicolor* A3(2), *S. prati AraPr2* and *Rhizobium* sp. XylPr11. For comparison, the reference genome of *E.coli* contains 4242 coding sequences and 92 CAZymes, i.e. a bacterium with no lignocellulolytic potential has 2.17 % of its proteins focused on carbohydrate metabolism. Indeed, metabolic pathways of simple sugars (lactose, glucose) require enzymes that can be classified as CAZymes. The obtained total number of CAZyme were higher compared to well-known bacteria lignocellulose degraders such as *Catenulispora acidiphila* DSM 44928 (253 CAZymes) but less than in certain *Acidobacteria* (Coluccia and Besaury, 2023). In terms of percentage, the CAZyme percentage compared to gene coding-proteins was higher in *S. prati AraPr2* (5.81 %) compared to the other microorganisms. The second microbial strain was *T. reesei* QM6a with 4.24 % followed by *S.coelicolor* A3(2) with 4.13 %. The obtained values in terms of relative abundance of CAZyme for some strains are superior compared to well-known degraders such as members of *Bacteroides* (3.41 %), or *Cellvibrio* (3.38 %) (Grondin et al., 2022).

By looking at the amount of secreted CAZymes, 2 bacteria showed higher numbers compared (respectively 229 and 192 secreted CAZymes for *S. prati AraPr2* and *S.coelicolor* A3(2)) compared to *T. reesei* QM6a (191 secreted CAZymes). This led to an higher percentage of secreted CAZymes for those 2 bacteria (4.48 % and 2.3 % respectively for *S. prati AraPr2* and *S.coelicolor* A3(2)) compared to the fungal strain (2.1 %). The remaining μorganism *Rhizobium* sp.XylPr11 presented for each CAZyme category (Total CAZymes number, Total secreted CAZymes,% CAZymes Total and% secreted CAZymes) the lowest value among the 4 micoorganisms in our dataset.

CAZyme content

The diversity of structures, compositions and bonds of components forming lignocellulose has led microorganisms, during evolution, to produce large panels of enzymes capable of degrading it (Manavalan et al., 2015). To characterize the specific lignocellulolytic genomic potential of the microbial strains used, each genome was annotated according to the CAZyme database. CAZymes are classified according to the CAZy database into five classes (Fujimoto, 2013): glycoside hydrolases (GH), glycosyltransferases (GT), polysaccharide lyases (PL), carbohydrate esterases (CE) and auxiliary activities (AA). Carbohydrate-active enzymes often display a modular structure with non-catalytic modules appended to the enzymes above which are the Carbohydrate-Binding Modules (CBMs) and reinforce the adhesion to carbohydrates.

The total CAZyme distribution (comprising the intra- and extracellular CAZymes) was studied for the 4 microbial strains of our dataset (Fig. 1). The distribution of CAZyme classes within was relatively

Table 1
Genomic characteristics (%GC, genome size) and distribution and CAZymes among the strains.

Strains	Genome size (Mbp)	% GC	Gene coding proteins	Total CAZymes number	Total secreted CAZymes	% CAZymes Total	% secreted CAZymes	% CAZymes secreted among the total CAZymes
T. reesei QM6a	33.4	52.82	9111	386	191	4.24	2.10	49.48
S. coelicolor A3 (2)	9.1	72	8128	336	192	4.13	2.36	57.14
Rhizobium sp. XylPr11	5.6	58.53	5174	123	33	2.38	0.64	26.83
S. prati AraPr2	6	40.38	5112	297	229	5.81	4.48	77.10

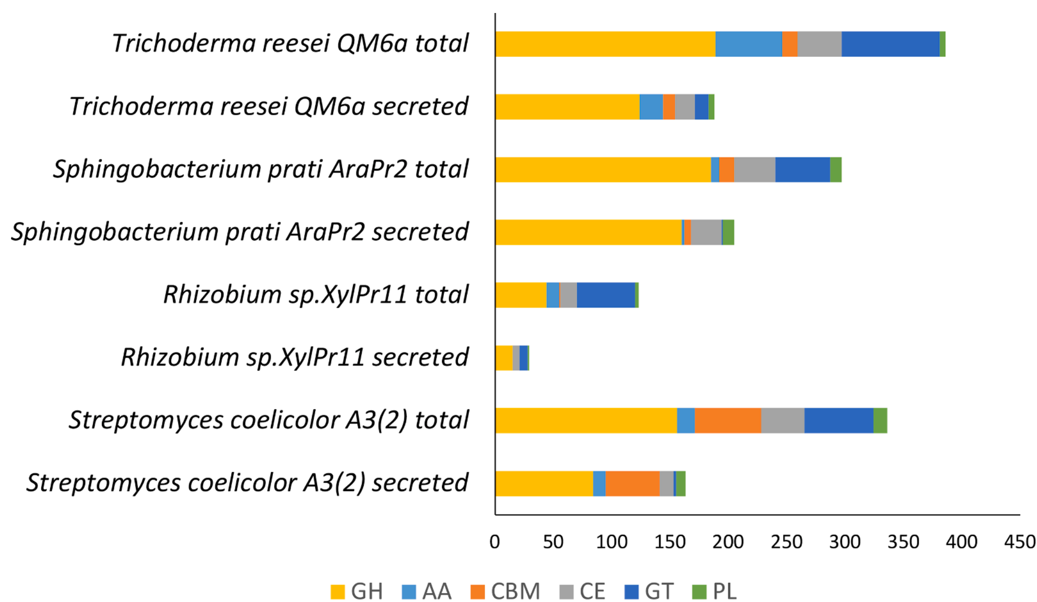

Fig. 1. Bar graph comparing putative total CAZymes and hypothetically secreted CAZymes for each strain used.

homogeneous. GHs was the most abundant CAZyme and represented between 35.8 % of the whole CAZyme content for *Rhizobium* sp.XylPr11 and 62.3 % for *S. prati AraPr2*. GHs hydrolyze the glycosidic bonds between two carbohydrates, or between a carbohydrate and a non-carbohydrate residue. The total GH number was relatively between the strain *S. prati AraPr2* (185 GHs) compared to the well described cellulose and lignocellulose fungal degrader strain *T. reesei* QM6a (189 GHs) which is one of the largest producers of lignocellulolytic enzymes (Bischof et al., 2016). Interestingly, the proportion of GH in the total CAZyme content was higher for *S. prati AraPr2* (62.3 %) compared to *T. reesei* QM6a (49 %). The GHs was the first CAZyme class among the most secreted with% of secreted GHs among the total CAZymes higher than 50 % implying than those strains have the ability and capacity to secrete lignocellulolytic enzymes and that they are involved in the degradation of the lignocellulosic biomass.

The second most abundant CAZyme class was the glycosyl transferase (GTs) among our microbial dataset. GTs are involved in the biosynthesis of saccharide chains and have debranching activities. The% content of GTs varied between 40.7 % and 15.8 % respectively for *Rhizobium* sp.XylPr11 and *S. prati AraPr2*. Regarding the secretion, GT proteins are the least secreted class for all the strains (with the exception of *Rhizobium* sp.XylPr11) which are most often involved in a disconnection role and are therefore mainly involved in and associated with intracellular mechanisms (Breton et al., 2001).

A main difference among the microbial strains consisted in the distribution of Auxiliary Activity enzymes (AAs) which group together enzymes that act on lignins and polysaccharides (LPMO or Lytic Polysaccharides MonoOxygenases) through redox mechanisms (Cantarel et al., 2008). 57AAs were present in the strain *T. reesei* QM6a whereas only 15, 11, and 7 AAs were present for *S.coelicolor* A3(2), *Rhizobium* sp. XylPr11 and *S. prati AraPr2*. The same trend was observed for the secretion of this CAZyme class with 20AAs secreted for *T. reesei* QM6a and <11 copies for the 3 remaining bacterial strains.

Another difference in our dataset consisted in the presence of CBM which was overrepresented in the bacterial strain *S.coelicolor* A3 (2with 57 CBMs in total and 46 secreted among them. The second strain which harbored the most CBM was *T. reesei* QM6a with 13 CBMs and 10 secreted. Carbohydrate Binding Modules (CBMs), which are not enzymes, increase the efficiency of the other CAZymes (Shi et al., 2023). The 2 remaining bacterial strains had much lower total and secreted CBMs.

For the Carbohydrate Esterase (CEs), the total number of CEs was relatively similar among 3 microbial strains (*S. prati AraPr2, S.coelicolor* A3(2) and *T. reesei* QM6a with respectively 35, 37 and 38 CEs). The abundance of CEs was lower for *Rhizobium* sp.XylPr11 with only 14 CEs present. The CEs catalyze the hydrolysis of the carbohydrate esters. For the last CAZyme class, the distribution of Polysaccharide Lyases (PLs) mainly cleave bonds between acids and polysaccharides was relatively similar among the microbial strains of our dataset.

Overall, the results regarding the CAZyme content showed that the strains harbored a high amount of total and secreted CAZyme which is a first milestone towards the setup of a performant lignocellulolytic cocultuure (Detain et al., 2022)

CAZyme diversity

The heat map presented here associates CAZymes with their substrates, which provides a better view of the lignocellulolytic potential of our strains (Cortes-Tolalpa et al., 2020) and does not only refer to the CAZyme classification (**Supplementary Figure 1**). *S. prati AraPr2* has

the widest range of CAZymes according to this ranking with 62 different CAZyme families, followed by *S. coelicolor* A3(2) with 53 different families. The fungal strain *T. reesei* QM6a is in the same order of magnitude whereas with 42 different CAZyme classes whereas *Rhizobium* sp.XylPr11 is well below with only 32 different CAZyme classes. Lignocellulose and its components being complex from a structural and chemical point of view, the different microorganisms need numerous CAZymes to be able to degrade them. Overall, the results showed that 3 microbial strains (*S. prati AraPr2, S. coelicolor* A3(2) and *T. reesei* QM6a) harbored a complete repertoire of CAZyme for the breakdown of lignocellulose whereas *Rhizobium* sp.XylPr11 does not. Those 3 microbial strains have indeed a complete repertoire which will allow potentially the degradation of cellulose, starch, hemicellulose. No enzyme were affilated to the degradation of lignin for the bacterial strain *S. prati AraPr2*.

Some GHs are common to the 4 strains of our microbial dataset, such as GH13 which are involved in the degradation of starch or GH16 involved in the degradation of hemicelluloses. On the other hand, some CAZymes will be found only in one strain like GH127 associated with hemicelluloses specific to *S. prati AraPr2*. Some families of CAZymes are specific to our 3 bacteria (CBM48 which have glycogen-binding function and are appended to GH13 modules), or to our fungi (AA2(encoding for peroxidase) or GH37 (encoding for trehalase)).

The main difference between the 3 prokaryotic and the eukaryotic strain was related to the absolute abundance of CAZymes for certain ones. Indeed, for most of the CAZymes common to the 4 strains, *T. reesei* QM6a will have one to several dozen while the bacteria will have less than ten. The example can be given for GH3 (13 copies for *T. reesei* QM6a and respectively 9, 3, 1 copies for *S. coelicolor* A3(2), *S. prati AraPr2* and *Rhizobium* sp.XylPr11) The same observation was done for other CAZymes having a role in degradation such as GH16 (involved in the cellulose degradation), AA3 (which belong to the glucose-methanol-choline (GMC) oxidoreductases family) or CE10 (encoding for arylesterase or carboxyl esterase).

A significant difference was observed in the abundance of CBMs between *S. coelicolor* A3(2) and *T. reesei* QM6a as mentioned before. The diversity of CBMs in *S. coelicolor* A3(2) (which was represented by 14 different CBM families) is dominated by 12 copies of CBM2 exhibit binding capabilities to crystalline cellulose or xylan. 12 copies of CBM13 were also resent in the genome of *S. coelicolor* A3(2) which is related to the high abundance of CBM13 in the bacterial domain which account for 4.9 % among the CBMs. CBM 13 can interact with xylanases, endoglucanases, chitinases, galactosidases and arabinosidases (Fujimoto, 2013). 6 copies of CBM32 were detected which have an interaction with the saturated non-reducing end of oligosaccharides such as pectin or mannans (Mizutani et al., 2012). CBM48 represent up to 20.1 % of the bacterial domain and 5 copies in *S. coelicolor* A3(2) which will attach to various linear and cyclic glucans.

On the other hand, the diversity of CBMs was only represented by 6 families for *T. reesei* QM6a with CBM1 as the most abundant with 3 copies which are involved in the binding of cellulose. Other CBM1 from *T. reesei* QM6a have been reported as cellulolytic enzymes that strongly bind to lignin (Tokunaga et al., 2019). 4 different CBM families (CBM24, CBM42, CBM43 and CBM66) were present as 2 genes copies on *T. reesei* QM6a genome which have interactions with glucan, arabinofuranose, β-1,3-glucan and fructans.

CAZyme common core

A Venn diagram describes the richness and diversity of the CAZymes brought by each organism during the mono and co-culture (**Supplementary Figure 2**). To do so, CAZyme families with several iterations (such as CE10 which was present 21, 9, 4 and 6 times which were present respectively for *T. reesei* QM6a, *S. coelicolor* A3(2), *S. prati AraPr2* and *Rhizobium* sp.XylPr11) were only considered once (reducing then the number of CAZymes in the genomes).

For the co-culture *T. reesei* QM6a and *S. coelicolor* A3(2), 168 CAZymes families were present in total spread out as follow: 52 CAZymes families were shared between whereas each microbial partner harbored respectively 56 and 60 CAZymes family the *S.coelicolor* A3(2) and *T. reesei* QM6a. Due to the higher diversity of CAZyme encountered in the *S.coelicolor* A3(2) genome, a less common core was found for the other co-cultures with only 40 and 26 CAZymes families (respectively *Sphingobacterium prati AraPr2* and *Rhizobium* sp.XylPr11); consequently, the proportion of unique CAZyme families brought by the fungi was higher in the remaining co-cultures. Overall, the abundance of CAZymes families was the same in the co-cultures associating the fungus and *S. coelicolor* A3(2) and *S. prati AraPr2* (169 and 168 families) but was lower with the strain *Rhizobium* sp.XylPr11 (142 CAZymes families). The Venn Diagram clearly shows that the metabolic diversity is improved in the co-culture due to the capacity of each individual partner to bring its respective unique CAZyme families which is a key objective for the set-up of a microbial co-culture. However, this represents the genomic potential of the co-culture but does not imply that all the metabolic activities associated will be expressed in the co-culture.

Lignocellulolytic enzymatic activity assays

Several activities (amylase, xylanase peroxidase, and debranching activities such as β-xylosidase, acetyl-esterase, α-arabinofurosidase) were measured through the growth of the different monoculturemonocultures and co-cultures in order to assess their lignocellulolytic potential and a possible elication of enzymatic activities. Overall, only xylanase (Fig. 2) and peroxidase (Fig. 3) activities were detected whatever the sampling time and the number of microbial partners.

Regarding the xylanase activity, *T. reesei* QM6a exhibited the highest one with approximately 1.75 IU/mL from 72 h to 240 h. The activity of the bacterial strains (as monoculture) was always confused with the background noise and was considered then none. For the co-culture, *Rhizobium* sp.XylPr11 and *Sphingobacterium prati AraPr2* presented an inhibitory effect on the xylanase activities towards *T. reesei* QM6a. In fact, the co-cultures have respectively 9 and 200 times less xylanase activity than the fungal strain with values lower than 200 mIU/mL whatever the sampling time of the co-culture. Regarding the co-culture between *S.coelicolor* A3(2) and *T. reesei* QM6a, *S. coelicolor* A3(2) did not behave in the same way than the 2 other bacterial strains. Indeed, the xylanolytic activities were the same for between *T. reesei* QM6a and the *S.coelicolor* A3(2)/*T. reesei* QM6a co-cultures for 72H and 96H but tend to decrease by 50 % at 240 h. This loss of activity may be linked to the disappearance of the substrate linked to enzymatic activities or the release of compounds that are more easily assimilated by the two microorganisms.

For the peroxidase activities of the co-cultures, the results showed than the peroxidase activities were mainly carried in the co-culture by the bacterial strains. Indeed, the results for the fungal strain *T. reesei* QM6a revealed that no peroxidase activity was detected higher than 3 IU/mL at 240H whereas higher values were detected for *S. coelicolor* A3 (2) (135 IU/mL at 240H), *Rhizobium* sp.XylPr11 (13.9 IU/mL at 240H) and *S. prati AraPr2* (39 IU/mL at 240H). Overall, the results show that the co-cultures have a higher peroxidase activity compared to the monoculture. This is particularly true for the co-culture *T. reesei* QM6a/ *S. coelicolor* A3(2) which consisted in the highest activity obtained at 96 h at around 425± 130 IU/mL (p-value < 0.05). This value was significantly higher compared to the respective monocultures *T. reesei* QM6a and *S. coelicolor* A3(2)). The enzymatic activity of this co-culture then fell back to values close to those of the *S. coelicolor* A3(2) monoculture at the end of the co-culture (135 IU/mL).

For the monocultures of *Rhizobium* sp.XylPr11 and *S. prati AraPr2*, the activities detected were around 20 IU/mL and for their co-culture, around 40 IU/mL at each time point; however no significant results were detected between the mono and co-culture which do not allow to affirm a synergistic phenomenon.

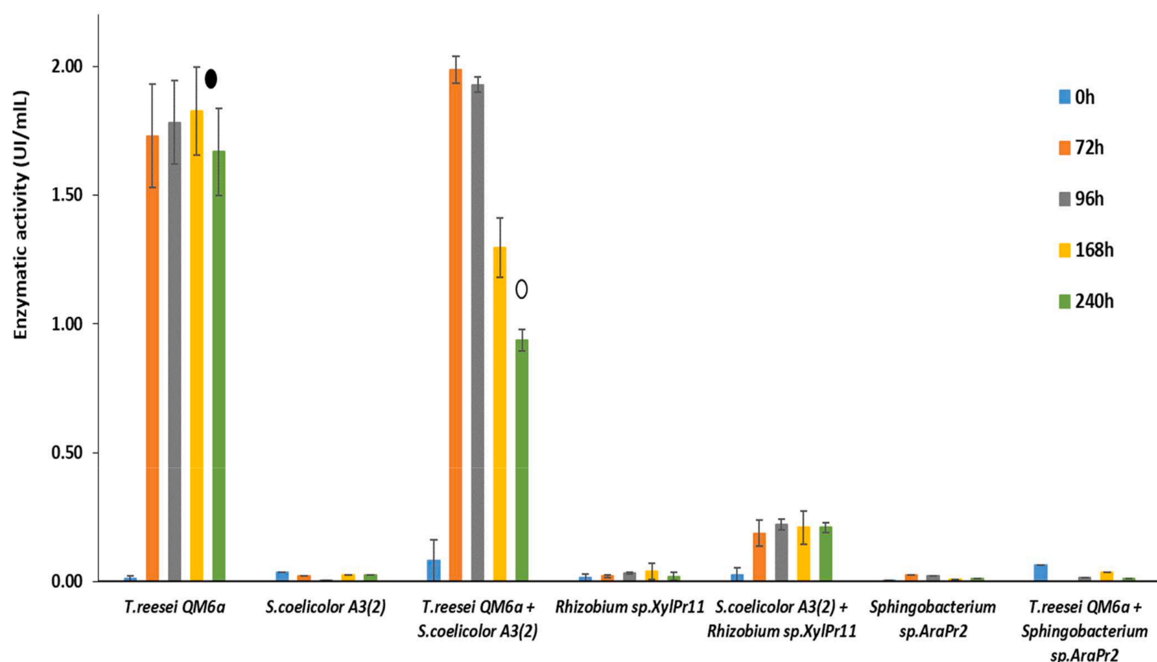

Fig. 2. Xylanase activities measured for co-cultures of *T. reesei* QM6a and the three bacterial strains, as well as monoculture controls at times 0, 72, 96, 168 and 240 h (● and ○ significantly different at $p \leq 0.05$, Student's t.test).

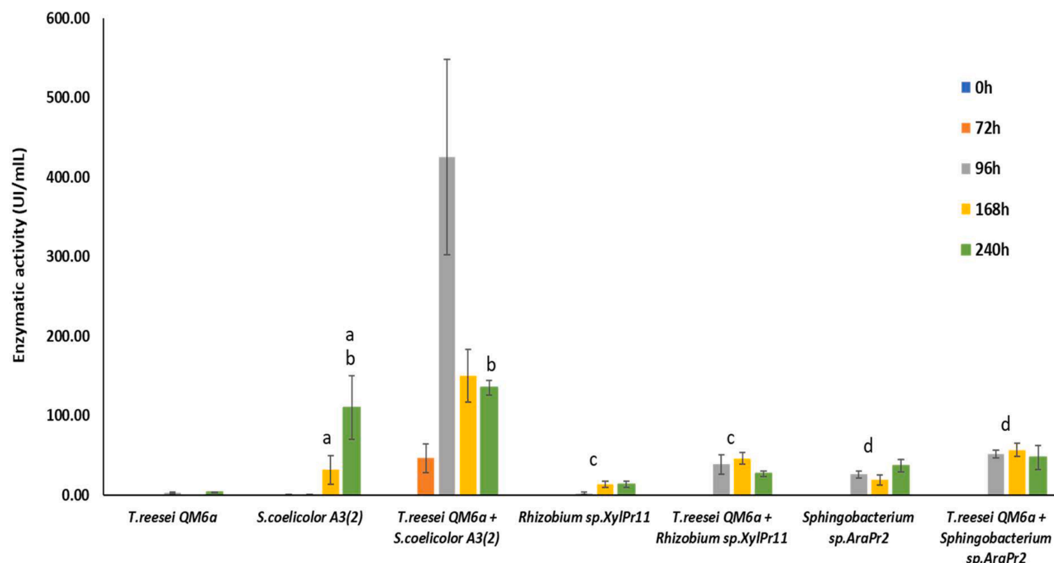

Fig. 3. Peroxidase activities measured for co-cultures of *T. reesei* QM6a and the three bacterial strains, as well as monoculture controls at times 0, 72, 96, 168 and 240 h (means with the same letters are not significantly different at $p \leq 0.05$, ANOVA mixed models, $n = 3$).

Overall, co-cultures seem to have different effects depending on the enzymatic activities observed: co-cultures with *Rhizobium* sp.XylPr11 and *S. prati AraPr2* tend to inhibit xylanase activities but increase peroxidase activities. A difference in term of enzymatic activities is present between the co-cultivation of *T. reesei* QM6a and *S. coelicolor* A3 (2) and the single cultivation of *T. reesei* QM6a. This difference can be due maybe: (1) to a slower increase of the fungi which could result in a lower production of enzyme, (2) the fact that the bacteria will attach to the substrate resulting in less availability of attachment for the fungi leading to a lower activation of transcription and production of enzyme. This might be correlated to the high presence of CBMs in the bacterial strain compared to the fungal strain. Indeed, The CBM families typically exhibit elevated substrate specificities, ensuring precise anchoring of CBMs to their corresponding substrates. The interactions among essential amino acids located within the binding sites of CBMs and their respective substrates lead to the proximity of substrates to the catalytic domains of CAZymes. Consequently, this proximity enhances enzymatic activities (Armenta et al., 2017; Shi et al., 2023).

Peroxidases act in the first stages of lignin oxidation (extracellular fractionation), which is why their ligninolytic activities were tested here (Gold and Alic, 1993). They represent a small group of enzymes among many others enabling the extracellular fractionation of lignin. Because lignin is so complex, the enzymes potentially responsible for its fractionation are tested on simpler molecules. Here, culture supernatants were tested using a phenolic molecule based on the literature: 1,2,3-trihydroxybenzene (also named Pyrogallol (PYGL)) (Bach et al., 2013).

Activities measured with this test do not necessarily represent activity on native lignin. The test performed only shows the presence of peroxidases, however it should be noted that the results obtained for these activities agree with the literature as Actinobacteria (*S. coelicolor* A3(2)) are known to produce extracellular peroxidase activities (Brown and Chang, 2014). Nevertheless, supplementation of lignolytic tests may prove interesting results, whether with other enzymatic activities (laccases, oxidases or reductases), or on other substrates more closely resembling native lignin subunits (syringaldehyde, biphenolic molecules).

Other enzymatic activities linked to other biomass components have been tested (amylase for starch, FPU test for cellulose), but the techniques used were not sensitive enough for culture supernatants. In fact, the enzymatic activities obtained may be lower than the activities found in the literature, partly because the strains used are natural and unmodified and partly because the conditions are not optimal for enzyme production (mineral medium with a complex carbon source, with no substrate supplementation to increase the various enzymatic activities). In the future, the use of genetically modified strains belonging to these different microbial genera with superior lignocellulolytic activities will be considered to obtain better degradation of biomass. Genetically modified *T. reesei* QM6a has been obtained recently which harbors an higher cellulolytic activity compared to the wild one (reaching up to 22.33 IU/mL, FPase) (Li et al., 2023). *Streptomyces* members represent also hosts for the production of industrially relevant enzymes such as lignocellulolytic ones (Vojnovic et al., 2024) and new enzymes have been identified such as laccase, produced and characterized recently (Kumar et al., 2020; Sidar et al., 2024)

Effects of co-cultures on the substrate

The overall modification of the substrate was observed after autoclaving it for the blank and compared to the different mono and co-cultures by Fourier transform infrared spectroscopy. Each peak indicates a specific functional group (linkages) present in the chemical composition of wheat bran. Peaks displaying major differences were particularly investigated which were at: (1) 1730 cm^{-1} (–C═O bond/esterified carboxyl groups) and can be attributed to cellulose and hemicellulose groups, (2) 1510 cm^{-1} attributed to C═C bond vibrations typical of aromatic systems and (3) 896 cm^{-1} related δ C—H rocking (related to the cellulose-hemicellulose interaction (Alemdar and Sain, 2008; Besaury and Rémond, 2022; Fackler et al., 2011; Sisti et al., 2021; Xu et al., 2013). The degradation ratios for each of the carbon functions listed above are presented in the Fig. 4. The ratios of <1 show degradation of the bond associated with the peak, due to consumption of the of the polymer. Conversely, ratios greater than 1 correspond to greater exposure of the associated with the peak and not an enrichment.

Fig. 4 shows the modification of the lignocellulosic substrate after the co-cultures co-cultures with *T. reesei* QM6a and the three bacteria, as well as their monoculture controls. Different patterns were detected depending on the culture done. Indeed, *T. reesei* QM6a tend to degrade more the cellulose/hemicellulose compared to the other compounds (with a ratio of 0.87 ± 0.27 for the –C═O bond/esterifed carboxyl group) which is in agreement with the results of the enzymatic activities for the xylanase with values reaching up almost 2 IU/mL throughout the culture. The *T. reesei* QM6a/*S. coelicolor* A3(2) co-culture improved the –C═O bond/esterified carboxyl groups which is attributed to cellulose and hemicellulose groups (the ratio of the associated peak was 0.50 ± 0.04 (p-value < 0.05 compared to the *T. reesei* QM6a monoculture); this result is in line with the xylanase activities measured for both conditions. On the opposite, the cellulose (corresponding to the peak at 896 cm^{-1}) was more exposed (peak ratio 1.28 ± 0,13) which is also in accordance with absence of cellulase activity detected. Overall, the results for the co-cultures showed that the presence of *Rhizobium* sp. XylPr11 and *S. coelicolor* A3(2) tend to increase the hemicellulose degradation whereas the presence of *S. prati AraPr2* inhibited it compared to the monoculture.

Rhizobium sp.XylPr11 and *Sphingobacterium prati AraPr2* have two different behaviours with respect to wheat bran: *Rhizobium* sp.XylPr11 degrades and consumes hemicelluloses (corresponding to a ratio of 0.75 ± 0.09 for the wavelength at 1730 cm^{-1} (–C═O bond/esterified carboxyl groups)), leaves lignin untouched (with a ratio for the associated peak at 1510 cm^{-1} of 1.09 ± 0.25) and exposes cellulose (with a ratio of 1.31 ± 0.13 for the wavelength at 896 cm^{-1}). *S. prati AraPr2*, on the other hand, exposes hemicelluloses and cellulose, with associated peak ratios of 1.63 ± 0.31 and 1.34 ± 0,21 (respectively at 1730 cm^{-1} and 896 cm^{-1}) . Surprisingly, with only 30 mIU/mL of peroxidase activity, this bacterium seems to be able to degrade lignin, the degradation ratio being 0.78 ± 0.24 (wavelength at 1510 cm^{-1}). Bioinformatics indicate that *S. prati AraPr2* secretes only two CAZymes of the AA class: an AA3 (family including cellobiose dehydrogenases and aryl alcohol/

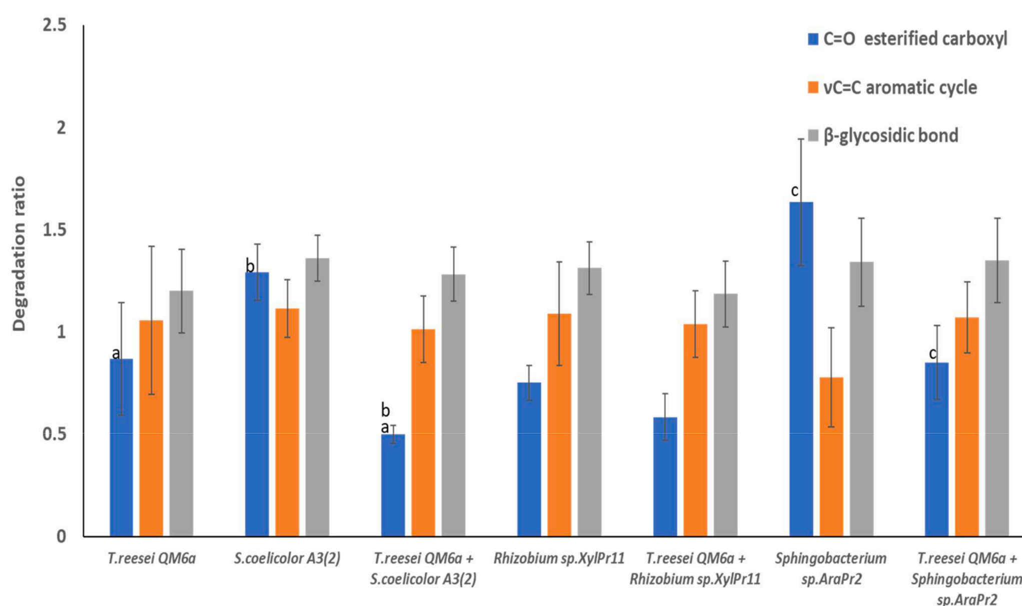

Fig. 4. Degradation ratios of each of the carbon functions present in wheat bran after 240H growth for the mono and co-cultures (a,b,c show the statistical differences one microbial partner and the same microbial partner in the co-culture ($p < 0.05$; ANOVA test, $n = 3$).

glucose/pyranose/oxidases) and an AA6, which is more likely to be involved in lignin degradation. This last family includes 1,4-benzoquinone reductase, which has a known role in the biodegradation of aromatic compounds (Akileswaran et al., 1999). AAs are mainly responsible for degrading of lignin, but other enzymes may of course be involved in lignolytic mechanisms.

Several bonds and therefore several visible FTIR peaks can be associated with a polymer, but not all these bonds will necessarily be impacted by microorganism cultures. Moreover, a polymer can be both exposed (through the degradation of other polymers) and consumed by the microorganisms, resulting in a ratio close to 1 when the two effects are cancelled out. Hence the importance of combining bioinformatics, enzymatic tests and looking at the state of the substrate post-culture. It would be preferable to carry out more enzymatic tests and combine several analytical techniques to have a more precise and global view of the effects of co-cultures on lignocellulosic biomass.

Transcriptomics of S. coelicolor A3(2) in mono and co-culture with T. reesei QM6a

The data quality of the RNA-seq data showed that most of the reads presented a high quality with low error rates and high percentages of Q_{30} confirming that the data are valid and usable. Many reads were detected with an average mapping rate of 4.81 % for the bacterial strain and 2.12 % for the co-culture (**Supplementary Table 1**).Based on the different results obtained previously, a transcriptomic approach was conducted to identify the transcripts involved in the metabolism of *S. coelicolor* A3(2) either in monoculture or co-culture with the fungal strain *T. reesei* QM6a. The selection for this bacterial strain was due to the fact that: (1) more CAZymes were present in the *S. coelicolor* A3(2) genome compared to the other strains; (2) *S. coelicolor* A3(2) enhanced the degradation ratios associated to hemicellulose with the fungal strain based on ATR-FTIR experiment; (3) the *T. reesei* QM6a/*S. coelicolor* A3 (2) co-culture exhibited the highest enzymatic values among all the co-cultures with a dramatically increase of the peroxidase activity (mainly brought by *S. coelicolor* A3(2)) and a conservation of the xylanase activity (mainly brought by *T. reesei* QM6a). The study of both transcriptomes (fungal and bacterial) of this co-culture would have given more data about the interaction, communication and lignocellulolytic mechanisms involved and decipher the degradation of the biomass. However, we decided to focus only on the transcriptome of the bacterial strain *S. coelicolor* A3(2) due to the budget dedicated to this study but moreover due to the exceptional increase of peroxidase activity in the co-culture for which the strain *S. coelicolor* A3(2) was responsible This would allow to identify possible CAZymes transcripts involved in the lignocellulose degradation that were only expressed in co-culture compared to the monoculture due to the interaction with the fungi.

Correlation between the 6 samples was performed to analyze the similarity profile of the gene expression level (Fig. 5). Correlation of the gene expression levels between samples plays an important role to verify reliability and sample selection, which can not only demonstrate the repeatability of the experiment but estimate the differential gene expression analysis as well. The higher the correlation coefficient of the sample (up to 1), the closer the expression pattern is. The results showed a high similarity between the biological replicates of the same condition with at least correlation coefficient matrix of 0.819. Moreover, comparison between the samples from different conditions showed also high similarities with at the minimum correlation coefficient matrix valueof 0.763. Those data would thus suggest that the strain *S. coelicolor* A3(2) tend to have a similar pattern of activity whatever the strain is in mono or co-culture.

The annotation of the transcripts in both conditions (based on the GO (Gene onthology database)) was performed to compare the metabolic functions overexpressed in *T. reesei* QM6a/*S. coelicolor* A3(2) co-culture compared to *S. coelicolor* A3(2) monoculture (**Supplementary Figure 3**). GO is a major bioinformatics classification system to unify the presentation of gene properties across all species. It includes three main branches: cellular component, molecular function and biological process. The results showed that no significant enrichment is observed at a high level of ontology (metabolism pathway). This is confirmed by the Pearson correlation analysis described previously with high similarities. However, several processes were more present in the co-culture

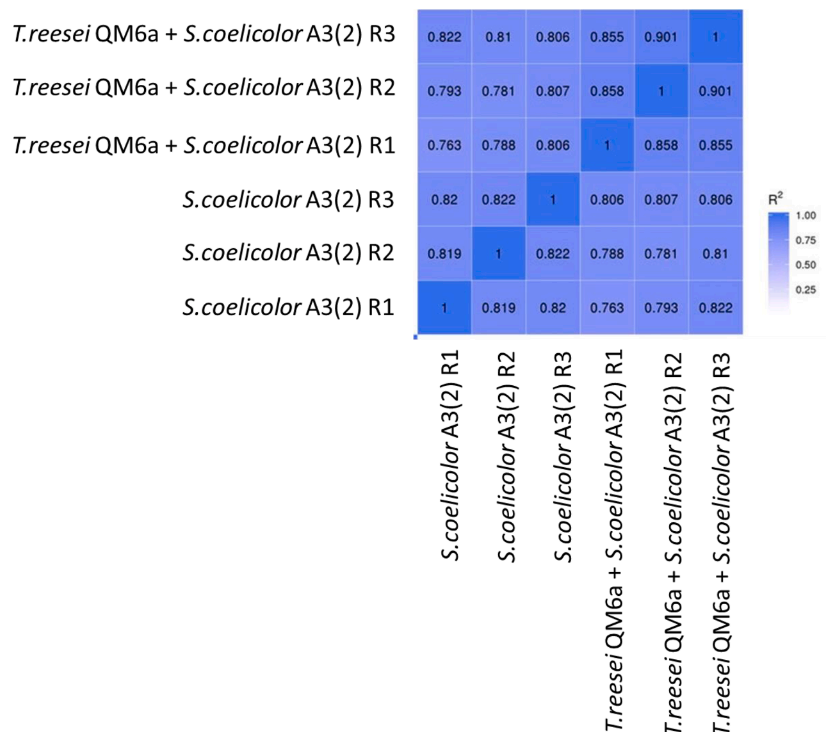

Fig. 5. Correlation coefficient matrix between samples between each biological replicate of the two groups based on all gene expression level (RPKM) of each sample. The legend provides the Square of Pearson correlation coefficient (R^2).

compared to the monoculture such as metabolic, carbohydrate metabolic and oxidation–reduction processes which may contain significantly differentially expressed genes (DEGs).

Differentially expressed genes (DEGs) for S. coelicolor A3(2) when grown in monoculture compared to co-culture

A coexpression Venn diagram was performed in order to which presents the number of DEGs that are uniquely expressed within each condition (mono or co-culture averaged among the 3 biological triplicates) (Fig. 6). The results showed indeed that a large common of transcripts is shared between the mono and co-culture with 652 genes. Among the 652 genes, several encoded for CAZymes such as such as GH11 encoding for endo-1,4-beta-xylanase (SCO2292) and a CE4 secreted acetylxylan esterase (SCO2292) such as (Besaury et al., 2022). Xylanase activity has already been quantified from several *S.* members when lignocellulose residues was used as carbon substrate (Sanjivkumar et al., 2018). The polysaccharide lyase (SCO1880) was also among the CAZymes present in the common core between the 2 conditions which might be involved into a PUL (Polysaccharide Utilization Loci) associated to xylanases and pectinesterase.

No CAZy was detected in the 47 genes expresses only by *S.coelicolor* A3(2) on the opposite to the 64 genes expressed in the *T. reesei* QM6a/ *S. coelicolor* A3(2) co-culture where 4 CAZymes genes were significantly expressed among the 64. Those 4 CAZymes expressed correspond to: a) SCO2833 (p-value $= 1.84 \times 10^{-3}$), encoding for a copper-dependent lytic polysaccharide monooxygenase (AA10), b) SCO2494 (p-value $= 3.17 \times 10^{-3}$), which encodes for a pyruvate phosphate dikinase (GT1), c) SCO3096 (p-value $= 7.23 \times 10^{-3}$) associated to GH13_31 which are CAZymes associated to phosphopyruvate hydratase and d) SCO5373 (p-value $= 7.3 \times 10^{-3}$), identified as transporter of ATP synthase beta chain (GT58).

Among those 4 CAZymes, 3 of them were affiliated to the Glycolysis/ Gluconeogenesis and thus involved into the starch/cellulose metabolism. The corresponding enzymes were SCO2833 which encode for a lytic cellulose monooxygenase, SCO2494 which performs the conversion of pyruvate to phosphoenolpyruvate and SCO3096, a metalloenzyme which catalyzes 2-phospho-d-glycerate into phosphoenolpyruvate. Those results would thus suggest than the co-culture elicits starch degradation and glucose degradation; however, no amylase and glucosidase activities were detected or were below to the limit detection. This would imply that the fungal strain was able to hydrolyze starch into monomers making glucose available for the bacterial strain without any production if hydrolytic enzyme by the bacteria. This transcriptomic analysis of a bacterial strain in mono and co-culture is a great first in the domain of lignocellulose fractionation and gives insights about the mechanical pathways involved.

In this manuscript, the precise mechanism behind the communication and interaction between the two partners has begun to be studied. Although it was inconclusive, the main aim of the experiment was to identify the origin of the enzymatic synergy. The first hypothesis was to study the presence of extracellular molecules that act as a means of communication between the two microorganisms (in the Quorum Sensing (Azimi et al., 2020)). The industrial scope of elicitor molecules is quite significant. The second hypothesis aims to study the physical contact between the bacteria and the fungus (in the same way as mechanisms of membrane recognition of cellular motifs). Although not conclusive, the experiments will be optimized soon. A bioreactor separating the bacteria and the fungus by means of a permeable membrane would be ideal for helping to understand the mechanisms involved in co-cultures (Liu et al., 2017).

Many points can be improved regarding this study in particular the difficulty in quantifying certain inocula and monitoring the growth of microorganisms. One solution could be to use the qPCR technique to quantify the presence of microorganisms during the process. Another point for improvement would be to optimize the culture conditions to achieve better enzyme production and substrate degradation. This manuscript represents a first step to the development of lignocellulolytic enzymatic cocktails which is a key element for a viable bioeconomy to perform an efficient, cheap transformation of lignocellulosic biomass and promote the transition from a fossil carbon-based economy (Ferraz and Pyka, 2023).

Conclusions and future work

The obtained results in that study are interesting with: (1) the setup of microbial cultures between one fungal partner and different bacterial partners affiliated to different phyla, (2) an over expression of several key enzymatic activities and the obtention of efficient cheap lignocellulolytic cocktails, (3) a specific microbial interaction depending on the bacterial partner which is a hot topic given the number of high value-added molecules produced by co-culture, (4) the elicitation of metabolic pathways silent in monoculture but expressed in co-culture.

Future experiments will be performed in our laboratory in order to: (1) decipher the mechanisms elicited by the bacterial and fungal strain in mono and co-culture by Dual RNA-Seq approaches (Tomada et al., 2017), (2) analyze the interactions and elicitations of lignocellulolytic activities between bacterial partners and different fungal partners as in the environment, (3) extend co-cultures to a much larger number of partners.

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Fig. 6. Coexpression venn diagram of the genes expressed between the mono and co-culture.

CRediT authorship contribution statement

Julian Detain: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Ludovic Besaury:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Ludovic Besaury reports was provided by UMR FARE. Ludovic Besaury reports a relationship with UMR FARE that includes: employment.

Data availability

The reference genome of *Rhizobium* sp.XylPr11 was deposited at GenBank under the following accession number (PRJNA1014381). The genome sequences from *T. reesei* QM6a and *S. coelicolor* A3(2) downloaded from GenBank (respective accession numbers: GCF_000167675.1 and GCA_000203835.1). The genome sequences of *S. prati AraPr2* was already available in our laboratory (GCA_013167215.1).The transcriptomic data were deposited at GenBank under the following accession number (PRJNA1014381).

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.crmicr.2024.100271.

References

Akileswaran, L., Brock, B.J., Cereghino, J.L., Gold, M.H., 1999. 1, 4-Benzoquinone reductase from Phanerochaete chrysosporium: cDNA cloning and regulation of expression. Appl. Environ. Microbiol. 65 (2), 415-21.
Alemdar, A., Sain, M., 2008. Biocomposites from wheat straw nanofibers: morphology, thermal and mechanical properties. Compos Sci Technol 68 (2), 557-65.
Almagro Armenteros, J.J., Tsirigos, K.D., Sønderby, C.K., Petersen, T.N., Winther, O., Brunak, S., et al., 2019. SignalP 5.0 improves signal peptide predictions using deep neural networks. Nat. Biotechnol. 37 (4), 420-3.
Anders, S., Pyl, P.T., Huber, W., 2015. HTSeq—a Python framework to work with high-throughput sequencing data. Bioinformatics 31 (2), 166-9.
Andrews, S., 2010. FastQC: a Quality Control Tool For High Throughput Sequence Data. Babraham Bioinformatics, Babraham Institute, Cambridge, United Kingdom.
Armenta, S., Moreno-Mendieta, S., Sánchez-Cuapio, Z., Sánchez, S., Rodríguez-Sanoja, R., 2017. Advances in molecular engineering of carbohydrate-binding modules. Proteins Struct. Funct. Bioinform. 85 (9), 1602-17.
Azimi, S., Klementiev, A.D., Whiteley, M., Diggle, S.P., 2020. Bacterial quorum sensing during infection. Annu. Rev. Microbiol. 74, 201-19.
Bach, C.E., Warnock, D.D., Van Horn, D.J., Weintraub, M.N., Sinsabaugh, R.L., Allison, S. D., et al., 2013. Measuring phenol oxidase and peroxidase activities with pyrogallol, L-DOPA, and ABTS: effect of assay conditions and soil type. Soil Biol. Biochem. 67, 183-91.
Barka, E.A., Vatsa, P., Sanchez, L., Gaveau-Vaillant, N., Jacquard, C., Klenk, H.P., et al., 2016. Taxonomy, physiology, and natural products of Actinobacteria. Microbiol. Molec. Biol. Rev. 80 (1), 1-43.
Besaury, L., Floret, J., Rémond, C., 2021. Sphingobacterium prati sp. nov., isolated from agricultural soil and involved in lignocellulose deconstruction. Int. J. Syst. Evol. Microbiol. 71 (8), 004963.
Besaury, L., Fromentin, J., Detain, J., Rodrigues, C.M., Harakat, D., Rémond, C., 2022. Transcriptomic analysis of lignocellulose degradation by Streptomyces coelicolor A3 (2) and elicitation of secondary metabolites production. FEMS Microbiol. Lett. 369 (1), fnac101.
Besaury, L., Rémond, C., 2022. Culturable and metagenomic approaches of wheat bran and wheat straw phyllosphere's highlight new lignocellulolytic microorganisms. Lett. Appl. Microbiol. 74 (6), 840-50.
Bischof, R.H., Ramoni, J., Seiboth, B., 2016. Cellulases and beyond: the first 70 years of the enzyme producer Trichoderma reesei. Microb. Cell Fact. 15 (1), 106.
Boruta, T., Ścigaczewska, A., Ruda, A., Bizukojć, M., 2023. Effects of the co-culture initiation method on the production of secondary metabolites in bioreactor co-cultures of Penicillium rubens and Streptomyces rimosus. Molecules 28 (16), 6044.
Breton, C., Mucha, J., Jeanneau, C., 2001. Structural and functional features of glycosyltransferases. Biochimie 83 (8), 713-8.
Brown, M.E., Chang, M.C., 2014. Exploring bacterial lignin degradation. Curr. Opin. Chem. Biol. 19, 1-7.
Cantarel, B.L., Coutinho, P.M., Rancurel, C., Bernard, T., Lombard, V., Henrissat, B., 2008. The Carbohydrate-Active EnZymes database (CAZy): an expert resource for glycogenomics. Nucleic Acids Res. 37 (suppl_1), D233-8.
Cassarini, M., Besaury, L., Rémond, C., 2021. Valorisation of wheat bran to produce natural pigments using selected microorganisms. J. Biotechnol. 339, 81-92.
Chandel, A.K., Garlapati, V.K., Singh, A.K., Antunes, F.A.F., da Silva, S.S., 2018. The path forward for lignocellulose biorefineries: bottlenecks, solutions, and perspective on commercialization. Bioresour. Technol. 264, 370-81.
Coluccia, M., Besaury, L., 2023. Acidobacteria members harbour an abundant and diverse carbohydrate-active enzymes (cazyme) and secreted proteasome repertoire, key factors for potential efficient biomass degradation. Molec. Genet. Genom. 1-20.
Condron, L., Stark, C., O'Callaghan, M., Clinton, P., Huang, Z, 2010. The role of microbial communities in the formation and decomposition of soil organic matter. Soil Microbiol. Sustain. Crop Prod. 81-118.
Cortes-Tolalpa, L., Salles, J.F., van Elsas, J.D., 2017. Bacterial synergism in lignocellulose biomass degradation–complementary roles of degraders as influenced by complexity of the carbon source. Front. Microbiol. 8, 1628.
Cortes-Tolalpa, L., Wang, Y., Salles, J.F., Van Elsas, J.D., 2020. Comparative genome analysis of the lignocellulose degrading bacteria Citrobacter freundii so4 and Sphingobacterium multivorum w15. Front. Microbiol. 11, 248.
Detain, J., Rémond, C., Rodrigues, C.M., Harakat, D., Besaury, L., 2022. Co-elicitation of lignocelluoytic enzymatic activities and metabolites production in an Aspergillus-Streptomyces co-culture during lignocellulose fractionation. Curr. Res. Microb. Sci. 3, 100108.
Escarnot, E., Agneessens, R., Wathelet, B., Paquot, M., 2010. Quantitative and qualitative study of spelt and wheat fibres in varying milling fractions. Food Chem. 122 (3), 857-63.
Fackler K., Stevanic J.S., Ters T., Hinterstoisser B., Schwanninger M., Salmén L. FT-IR imaging microscopy to localise and characterise simultaneous and selective white-rot decay within spruce wood cells. 2011.
Feldman, D., Kowbel, D.J., Glass, N.L., Yarden, O., Hadar, Y., 2017. A role for small secreted proteins (SSPs) in a saprophytic fungal lifestyle: ligninolytic enzyme regulation in Pleurotus ostreatus. Sci. Rep. 7 (1), 14553.
Ferraz, D., Pyka, A., 2023. Circular economy, bioeconomy, and sustainable development goals: a systematic literature review. Environ. Sci. Pollut. Res. 1-22.
Fonseca, L.M., Parreiras, L.S., Murakami, M.T., 2020. Rational engineering of the Trichoderma reesei RUT-C30 strain into an industrially relevant platform for cellulase production. Biotechnol. Biofuels 13, 1-15.
Fujimoto, Z., 2013. Structure and function of carbohydrate-binding module families 13 and 42 of glycoside hydrolases, comprising a β-trefoil fold. Biosci. Biotechnol. Biochem. 77 (7), 1363-71.
Goers, L., Freemont, P., Polizzi, K.M., 2014. Co-culture systems and technologies: taking synthetic biology to the next level. J. Royal Soc. Interface 11 (96), 20140065.
Gold, M.H., Alic, M., 1993. Molecular biology of the lignin-degrading basidiomycete Phanerochaete chrysosporium. Microbiol. Rev. 57 (3), 605-22.
Grondin, J.M., Déjean, G., Van Petegem, F., Brumer, H., 2022. Cell surface xyloglucan recognition and hydrolysis by the human gut commensal bacteroides uniformis. Appl. Environ. Microbiol. 88 (1) e01566-21.
Guzmán-Guzmán, P., Porras-Troncoso, M.D., Olmedo-Monfil, V., Herrera-Estrella, A., 2019. Trichoderma species: versatile plant symbionts. Phytopathology 109 (1), 6-16.
Harman, G.E., Howell, C.R., Viterbo, A., Chet, I., Lorito, M., 2004. Trichoderma species—opportunistic, avirulent plant symbionts. Nat. Rev. Microbiol. 2 (1), 43-56.
Hatakka, A., 1994. Lignin-modifying enzymes from selected white-rot fungi: production and role from in lignin degradation. FEMS Microbiol. Rev. 13 (2-3), 125-35.
Huang, L., Zhang, H., Wu, P., Entwistle, S., Li, X., Yohe, T., et al., 2018. dbCAN-seq: a database of carbohydrate-active enzyme (CAZyme) sequence and annotation. Nucleic Acids Res. 46 (D1), D516-21.
Humbird, D., Davis, R., Tao, L., Kinchin, C., Hsu, D., Aden, A., et al., 2011. Process Design and Economics For Biochemical Conversion of Lignocellulosic Biomass to ethanol: Dilute-Acid Pretreatment and Enzymatic Hydrolysis of Corn Stover. National Renewable Energy Lab.(NREL), Golden, CO (United States).
Jackson, C.A., Couger, M.B., Prabhakaran, M., Ramachandriya, K.D., Canaan, P., Fathepure, B.Z., 2017. Isolation and characterization of Rhizobium sp. strain YS-1r that degrades lignin in plant biomass. J. Appl. Microbiol. 122 (4), 940-52.
Ji, X.J., Huang, H., Nie, Z.K., Qu, L., Xu, Q., Tsao, G.T., 2012. Fuels and chemicals from hemicellulose sugars. Biotechnol. China III: Biofuels Bioenergy 199-224.
Jiménez, D.J., de Lima Brossi, M.J., Schückel, J., Kračun, S.K., Willats, W.G.T., Van Elsas, J.D., 2016. Characterization of three plant biomass-degrading microbial consortia by metagenomics-and metasecretomics-based approaches. Appl. Microbiol. Biotechnol. 100, 10463-77.
Jones, J.A., Wang, X., 2018. Use of bacterial co-cultures for the efficient production of chemicals. Curr. Opin. Biotechnol. 53, 33-8.
Jørgensen, H., Pinelo, M., 2017. Enzyme recycling in lignocellulosic biorefineries. Biofuels Bioprod. Biorefin. 11 (1), 150-67.

Krogh, A., Larsson, B., Von Heijne, G., Sonnhammer, E.L., 2001. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J. Mol. Biol. 305 (3), 567-80.
Kumar, M., Kumar, P., Das, P., Solanki, R., Kapur, M.K., 2020. Potential applications of extracellular enzymes from Streptomyces spp. in various industries. Arch. Microbiol. 202, 1597-615.
Kumar, V., Fox, B.G., Takasuka, T.E., 2023. Consolidated bioprocessing of plant biomass to polyhydroxyalkanoate by co-culture of Streptomyces sp. SirexAA-E and Priestia megaterium. Bioresour. Technol. 376, 128934.
Langmead, B., Salzberg, S.L., 2012. Fast gapped-read alignment with Bowtie 2. Nat. Methods 9 (4), 357.
Li, J., Chen, Y., Gao, A., Wei, L., Wei, D., Wang, W, 2023. Simultaneous production of cellulase and β-carotene in the filamentous fungus Trichoderma reesei. J. Agric. Food Chem. 71 (16), 6358-65.
de Lima Brossi, M.J., Jiménez, D.J., Cortes-Tolalpa, L., van Elsas, J.D., 2016. Soil-derived microbial consortia enriched with different plant biomass reveal distinct players acting in lignocellulose degradation. Microb. Ecol. 71 (3), 616-27.
Liu, C., Hao, Y., Jiang, J., Liu, W., 2017. Valorization of untreated rice bran towards bioflocculant using a lignocellulose-degrading strain and its use in microalgal biomass harvest. Biotechnol Biofuels 10 (1), 90.
Llamas, M., Greses, S., Magdalena, J.A., González-Fernández, C., Tomás-Pejó, E., 2023. Microbial co-cultures for biochemicals production from lignocellulosic biomass: a review. Bioresour. Technol. 386, 129499.
Manavalan, T., Manavalan, A., Heese, K., 2015. Characterization of lignocellulolytic enzymes from white-rot fungi. Curr. Microbiol. 70 (4), 485-98.
Mizutani, K., Fernandes, V.O., Karita, S., Luís, A.S., Sakka, M., Kimura, T., et al., 2012. Influence of a mannan binding family 32 carbohydrate binding module on the activity of the appended mannanase. Appl. Environ. Microbiol. 78 (14), 4781-7.
Novy, V., Nielsen, F., Seiboth, B., Nidetzky, B., 2019. The influence of feedstock characteristics on enzyme production in Trichoderma reesei: a review on productivity, gene regulation and secretion profiles. Biotechnol Biofuels 12 (1), 238.
Parks, D.H., Imelfort, M., Skennerton, C.T., Hugenholtz, P., Tyson, G.W., 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res. 25 (7), 1043-55.
Peng, Y., Leung, H.C., Yiu, S.M., Chin, F.Y., 2012. IDBA-UD: a de novo assembler for single-cell and metagenomic sequencing data with highly uneven depth. Bioinformatics 28 (11), 1420-8.
Rana, R., Nanda, S., Meda, V., Dalai, A.K., Kozinski, J.A, 2018. A review of lignin chemistry and its biorefining conversion technologies. J. Biochem. Eng. Bioprocess. Technol. 1 (2).
Rémond, C., Aubry, N., Crônier, D., Noël, S., Martel, F., Roge, B., et al., 2010. Combination of ammonia and xylanase pretreatments: impact on enzymatic xylan and cellulose recovery from wheat straw. Bioresour. Technol. 101 (17), 6712-7.
Ren, D., Madsen, J.S., Sørensen, S.J., Burmølle, M., 2015. High prevalence of biofilm synergy among bacterial soil isolates in co-cultures indicates bacterial interspecific cooperation. ISME J. 9 (1), 81-9.
Sanjivkumar, M., Silambarasan, T., Balagurunathan, R., Immanuel, G., 2018. Biosynthesis, molecular modeling and statistical optimization of xylanase from a mangrove associated actinobacterium Streptomyces variabilis (MAB3) using Box-Behnken design with its bioconversion efficacy. Int. J. Biol. Macromol. 118, 195-208.
Shi, Q., Abdel-Hamid, A.M., Sun, Z., Cheng, Y., Tu, T., Cann, I., et al., 2023. Carbohydrate-binding modules facilitate the enzymatic hydrolysis of lignocellulosic biomass: releasing reducing sugars and dissociative lignin available for producing biofuels and chemicals. Biotechnol. Adv., 108126
Sidar A., Voshol G.P., El-Masoudi A., Vijgenboom E., Punt P.J. Streptomyces small laccase expressed in Aspergillus niger as a new addition for the lignocellulose bioconversion toolbox. 2024.
Sisti, L., Gioia, C., Totaro, G., Verstichel, S., Cartabia, M., Camere, S., et al., 2021. Valorization of wheat bran agro-industrial byproduct as an upgrading filler for mycelium-based composite materials. Ind. Crops Prod. 170, 113742.
Sperandio, G.B., Filho, E.X.F., 2021. An overview of Trichoderma reesei co-cultures for the production of lignocellulolytic enzymes. Appl. Microbiol. Biotechnol. 105, 3019-25.
Sundarraj, A.A., Ranganathan, T.V., 2018. A review on cellulose and its utilization from agro-industrial waste. Drug Invent. Today 10 (1), 89-94.
Taha, M., Shahsavari, E., Al-Hothaly, K., Mouradov, A., Smith, A.T., Ball, A.S., et al., 2015. Enhanced biological straw saccharification through coculturing of lignocellulose-degrading microorganisms. Appl. Biochem. Biotechnol. 175 (8), 3709-28.
Tokunaga, Y., Nagata, T., Suetomi, T., Oshiro, S., Kondo, K., Katahira, M., et al., 2019. NMR analysis on molecular interaction of lignin with amino acid residues of carbohydrate-binding module from Trichoderma reesei Cel7A. Sci. Rep. 9 (1), 1977.
Tomada, S., Sonego, P., Moretto, M., Engelen, K., Pertot, I., Perazzolli, M., et al., 2017. Dual RNA-Seq of Lysobacter capsici AZ78–Phytophthora infestans interaction shows the implementation of attack strategies by the bacterium and unsuccessful oomycete defense responses. Environ. Microbiol. 19 (10), 4113-25.
Torres, L.A.Z., Woiciechowski, A.L., de Andrade Tanobe, V.O., Karp, S.G., Lorenci, L.C. G., Faulds, C., et al., 2020. Lignin as a potential source of high-added value compounds: a review. J. Clean. Prod. 263, 121499.
Ueda, K., Beppu, T., 2017. Antibiotics in microbial co-culture. J. Antibiot. 70 (4), 361-5.
Ueda, K., Kawai, S., Ogawa, H.O., Kiyama, A., Kubota, T., Kawanobe, H., et al., 2000. Wide distribution of interspecific stimulatory events on antibiotic production and sporulation among Streptomyces species. J. Antibiot. 53 (9), 979-82.
Varet, H., Brillet-Guéguen, L., Coppée, J.Y., Dillies, M.A., 2016. SARTools: a DESeq2-and edgeR-based R pipeline for comprehensive differential analysis of RNA-Seq data. PLoS ONE 11 (6), e0157022.
Verardi, A., Lopresto, C.G., Blasi, A., Chakraborty, S., Calabrò, V., 2020. Bioconversion of lignocellulosic biomass to bioethanol and biobutanol. Lignocellulosic Biomass to Liquid Biofuels. Elsevier, p. 67-125.
Vojnovic, S., Aleksic, I., Ilic-Tomic, T., Stevanovic, M., 2024. Nikodinovic-Runic J. Bacillus and Streptomyces spp. as hosts for production of industrially relevant enzymes. Appl. Microbiol. Biotechnol. 108 (1), 1-19.
Wang, Y., Jiménez, D.J., Zhang, Z., van Elsas, J.D., 2023. Functioning of a tripartite lignocellulolytic microbial consortium cultivated under two shaking conditions: a metatranscriptomic study. Biotechnol. Biofuels Bioprod. 16 (1), 54.
Xu, F., Yu, J., Tesso, T., Dowell, F., Wang, D., 2013. Qualitative and quantitative analysis of lignocellulosic biomass using infrared techniques: a mini-review. Appl. Energy 104, 801-9.
Zhang, S., Merino, N., Okamoto, A., Gedalanga, P., 2018. Interkingdom microbial consortia mechanisms to guide biotechnological applications. Microb. Biotechnol. 11 (5), 833-47.
Zhao, X., Zhang, L., Liu, D., 2012. Biomass recalcitrance. Part I: the chemical compositions and physical structures affecting the enzymatic hydrolysis of lignocellulose. Biofuels Bioprod. Biorefin. 6 (4), 465-82.
Zoghlami, A., Paës, G., 2019. Lignocellulosic biomass: understanding recalcitrance and predicting hydrolysis. Front. Chem. 7, 874.
Zuroff, T.R., Xiques, S.B., Curtis, W.R., 2013. Consortia-mediated bioprocessing of cellulose to ethanol with a symbiotic Clostridium phytofermentans/yeast co-culture. Biotechnol. Biofuels 6 (1), 1-12.