Dynamic strategies of hemicellulases production for an efficient lignocellulosic biomass fractionation

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INTRODUCTION

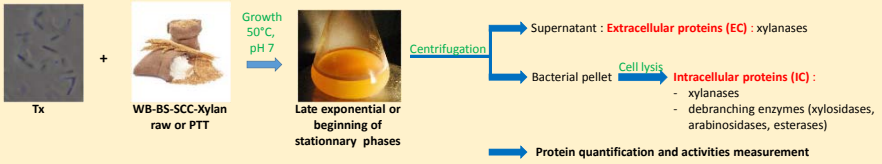
In the aim to improve the valorization of lignocellulosic biomasses, it is necessary to use all part of plant cell walls. The objective of the BABET-REAL5 project is to develop an alternative solution to produce 2G ethanol at small industrial scale by using the deconstruction of lignocellulosic biomasses (LCB) such as barley straw (BS) and sweet corn cob (SCC). LCB are known to be recalcitrant to enzymatic fractionation [1]. One challenge is to develop enzymatic processes for the deconstruction of hemicellulose to favor the liberation of sugars (hexoses and pentoses) and to improve the access of cellulases to cellulose (development of 2G ethanol).

Thermobacillus xylanilyticus (Tx) is a thermophilic and hemicellulolytic bacterium able to grow on various LCB and to produce hemicellulolytic enzymes for the deconstruction of LCB [2, 3, 4]. To improve the liberation of pentoses from LCB, we studied the behavior and the enzymatic strategies used by the bacterium on several LCB with contrasted chemicals and architectures (wheat bran (WB), BS, SCC hydrothermally pretreated (PTT) or not) and on xylan. Multiple approaches combining growth studies, hemicellulasic activities productions and proteomic analyses were performed to identify and quantify the expression level of hemicellulases implicated on the deconstruction of the various LCB used by the bacterium.

1 – Varnai A. *et al.*, Enzyme Microb. Technol., 2010 ; 2 – Rakotoarivonina H. *et al.*, Appl. Microbiol. and Biotechnol., 2011 ; 3 – Rakotoarivonina H. *et al.*, Microb. Cell Fact., 2012 ; 4 – Rakotoarivonina H. *et al.*, Bioresource Technol., 2014.

METHOD AND STRATEGY

T. xylanilyticus growth and enzymes production on LCB



Proteomic approaches

EC proteins : concentration and precipitation
IC proteins

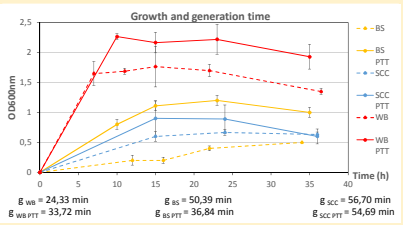
→ sample preparation for mass spectrometry analyses :
- protein in gel reduction and alkylation,
- trypsin digestion.

Mass spectrometry analysis were performed at the **plateform « *Exploration du métabolisme* », INRA, Clermont-Ferrand, France :**

- Proteins identification was performed with Mascot v.2.5.1 and Proteome discoverer 1.4. and by interrogating Tx genome. Protein abundances in each condition were calculated based on the exponentially modified protein abundance index (emPAI).
- Quantification of proteins expression was calculated with the sum of peptide normalized abundances for each proteins within each condition used. Expression comparison was done with QI Progenesis software.

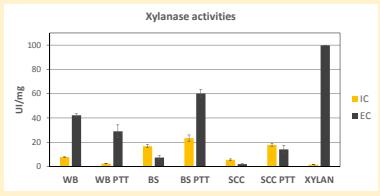
RESULTS

1 – *T. XYLANILYTICUS* GROWTH ON VARIOUS LCB

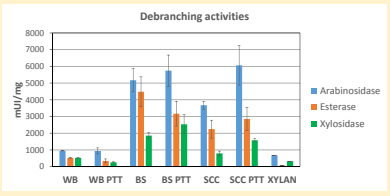


- Tx was able to grow on the various LCB used. Growth is approximately 2-fold faster on WB compared to BS and SCC.
- LCB PTT increased by 2-fold the generation time on BS. For WB and SCC, pretreatment did not show significant effect on the growth.

2 – HEMICELLULASIC ENZYMES ACTIVITIES PRODUCTION



- Xylanase activities were the main activities detected whatever the substrates used. Low xylanase activities were produced on raw BS and SCC compared to WB and xylan.
- The maximal EC xylanase activities were detected on xylan (2,4, 13,4 and 50-fold higher compared to WB, BS and SCC respectively.
- Results showed high IC xylanase activities produced on BS and SCC compared to WB and xylan.
- PTT improved xylanase activities on BS and SCC especially for EC activities (8 and 7-fold higher compared with raw BS and SCC respectively).



- Low debranching activities were detected on xylan compared to LCB.
- High debranching activities were obtained with BS compared to WB and SCC. Arabinosidase and esterase activities were 5,5 and 1,5-fold and 8,5 and 2-fold lower respectively on WB and SCC.
- PTT did not show significant effect on debranching enzymes activities except for SCC for which arabinosidase and xylosidase activities increased by 1,8 and 2,0-fold respectively.

3 – PROTEOMIC ANALYSES REVEAL THE ENZYMATIC STRATEGIES USED BY Tx FOR THE DECONSTRUCTION OF LCB

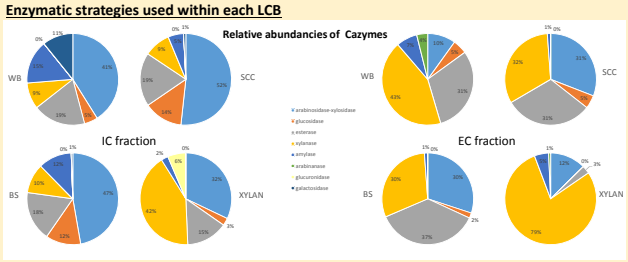
Global proteins identifications

	Xylan		WB				BS				SCC			
			raw		PTT		raw		PTT		raw		PTT	
Numbers	total	Cazymes	total	Cazymes	total	Cazymes	total	Cazymes	total	Cazymes	total	Cazymes	total	Cazymes
IC fraction	362	10GH - 2CE	334	25GH - 3CE	327	23GH - 3CE	240	16GH - 3CE	306	21GH - 6CE	233	17GH - 4CE	293	19GH - 4CE
% abundance		1,5		6,5		5,9		5,5		6,7		5,5		5,8
EC fraction	228	9GH - 1CE	227	13GH - 4CE	231	7GH - 3CE	207	13GH - 4CE	178	12GH - 3CE	227	13GH - 5CE	239	14GH - 4CE
EC proteins	59		65		63		53		39		66		59	
% abundance		3,2		7,5		5,1		4,8		8,4		4,7		6,5

GH : glycoside hydrolase ; CE : Carbohydrate esterase

- The global number of identified IC and EC proteins was rather similar on the tested substrates. In the EC fraction, approximately 1/4 are predicted as EC proteins.
- The number of Cazymes identified was stimulated on LCB compared to xylan. The Cazymes abundances were 1,5 and 4-fold higher on LCB compared to xylan.
- The effect of PTT on the Cazymes detected, varied within the LCB : SCC, similar Cazymes number ; BS, increase of IC Cazymes number ; WB, decrease of EC Cazymes number.

Enzymatic strategies used within each LCB



- In IC fractions, results showed that the ratio of endo-enzymes and exo-enzymes was 1/9 on LCB and 2/3 on xylan. Exo-enzymes identities and abundances depended on the substrate used. For example, galactosidase and glucuronidase were expressed only on WB and xylan respectively. TXYL_16210, a GH10 xylanase, was the most abundant endo-enzyme in all IC fractions.
- In EC fractions, xylanase ratio of endo/exo-activities was 4/1, 3/7, 3/7 and 5/5 on xylan, BS, SCC and WB respectively. Arabinanases were expressed only on WB. Esterases were mainly expressed on LCB.
- Although no significant PTT effect was observed on IC fractions, a significant increase of xylanase abundances was observed on EC fractions.

Expression of core enzymes identified on the different substrates

IC FRACTION

Names	Total	Elements	Description	WB	BS	SCC	XYLAN
BS SCC WB Xylan	9	TXYL_16175	Reducing end xylose-releasing exo-oligoxylanase GH8	20018	18982	10471	19404
		TXYL_00800	Cephalosporin C deacetylase - acetyl-xylan esterase CE7	49454	74636	66585	32779
		TXYL_19775	Glycoside hydrolase family 3 domain protein	35399	39519	22623	25497
		TXYL_11180	1,4- beta xylosidase GH43	150879	209616	106001	125954
		TXYL_16220	Alpha-L-arabinofuranosidase GH51*	153592	161412	292234	65821
		TXYL_14165	Glucoamylase TGA	38809	24480	13901	8329
		TXYL_18060	Cyclomaltodextrinase, alpha-amylase with CBM13	148670	99903	65237	31650
		TXYL_16210	Endo-1,4-beta-xylanase GH10*	33238	50467	63608	66589
		TXYL_10005	Beta glucosidase	10851	16465	8991	6392
BS SCC WB	9	TXYL_00645	Alpha-N-arabinofuranosidase 1 GH familly 51	148970	160579	112310	
		TXYL_10200	Alpha-galactosidase AgaN	31595	32685	35716	
		TXYL_00785	Endoglucanase D	12537	20434	10722	
		TXYL_07365	Betaglucosidase	29768	74978	78451	
		TXYL_15880	Carboxylesterase	51643	22189	18633	
		TXYL_09595	Xylosidase/arabinosidase GH43	18282	14503	9512	
		TXYL_14875	Alpha-N-arabinofuranosidase 1 GH51	67889	100250	75127	
		TXYL_00055	Endo-1,4-beta-xylanase A with CBM22, CBM9 and SLH domain GH10	186710	147935	113417	
		TXYL_09630	Carbohydrate esterase 1 with CBM 9	69936	73956	49994	

* Characterized proteins

- In IC fractions, 9 GH are expressed whatever the substrates and 9 others GH are observed on LCB only.
- Protein expression levels differed according to the substrate. Within enzyme classes, identities and expression level varied with the substrat used.

Ex : Xylanases expression : TXYL_16210 GH10 Xylan=SCC >BS >WB ; TXYL_16175 GH8 WB=BS=xylan >SCC and TXYL_00055 WB >BS >SCC.
Esterases expression : TXYL_00800 CE7 BS >SCC >WB >Xylan ; TXYL_09630 CE1 BS=WB >SCC.

EC FRACTION

Names	Total	Elements	Description	WB	BS	SCC	XYLAN
BS SCC WB Xylan	7	TXYL_00800**	Cephalosporin C deacetylase - acetyl-xylan esterase CE7	11779	28614	57012	8526
		TXYL_04370	Endo-1,4-beta-xylanase GH11	240437	10156	11691	80582
		TXYL_04380	Endo-1,4-beta-xylanase GH11*	3220608	136584	103014	1603891
		TXYL_16220**	Alpha-L-arabinofuranosidase *	10267	13110	42167	4240
		TXYL_18060**	Cyclomaltodextrinase, alpha-amylase with CBM13	14529	9618	16133	1407
		TXYL_16210**	Endo-1,4-beta-xylanase Xyn10	7712	10700	24823	14035
		TXYL_00055	Endo-1,4-beta-xylanase A with CBM22, CBM9 and SLH domain GH10	111686	39948	63830	21578
BS SCC WB	7	TXYL_19775**	Glycoside hydrolase family 3 domain protein	4334	7857	15081	
		TXYL_09635	Carbohydrate esterase 1 with CBM 9	26889	9849	8927	
		TXYL_00645**	Alpha-N-arabinofuranosidase 1 GH familly 51	6658	12045	16888	
		TXYL_11180**	1,4- beta xylosidase	6560	26316	39349	
		TXYL_14165	Glucoamylase TGA	22105	23221	38407	
		TXYL_00785	Endoglucanase D	35125	9986	15348	
		TXYL_09630	Carbohydrate esterase 1 with CBM 9	231130	73266	79120	

* Characterized proteins; ** IC proteins

- In EC fractions, 7 GH were expressed on all substrates used and 7 others GH were expressed only on LCB mainly xylanases and esterases.
- Enzymes expression level varied with the substrates. For the two EC GH11 xylanases (TXYL_04370 and TXYL_04380), expression levels were significatively higher on xylan and WB compared to BS and SCC. For xylan, ratio of these 2 enzymes (TXYL_04380/TXYL_04370) was approximately 20 while it was lower on LCB (9 on SCC and 13 on WB and BS).
- The two CE1 (TXYL_09630 and TXYL_09635) were only expressed on LCB. Level expressions on WB >BS=SCC.

CONCLUSION

The ability of *T. xylanilyticus* to grow and use complex substrates as primary C source was studied in the aim to identify the enzymatic strategies used by the bacterium to deconstruct LCB. The global enzymatic activities produced and quantified resulted from the activity of several types of enzymes with variable abundances and expression levels depending on the LCB used. These results will allow to develop performant enzymatic cocktails with the necessary keys enzymes, ratio of activities,... for an efficient and specific LCB fractionation according to their compositions.

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