The impact of chromatin remodelling on cellulase expression in Trichoderma reesei

BMC Genomics. 2015 Aug 07;16(1):588
http://dx.doi.org/10.1186/s12864-015-1807-7

Downloaded from: Biblioteca Digital da Produção Intelectual - BDPI, Universidade de São Paulo
The impact of chromatin remodelling on cellulase expression in *Trichoderma reesei*

Thiago M. Mello-de-Sousa¹, Alice Rassinger¹, Marion E. Pucher¹, Lilian dos Santos Castro², Gabriela F. Persinoti³, Rafael Silva-Rocha², Marcio J. Poças-Fonseca³, Robert L. Mach¹, Roberto Nascimento Silva² and Astrid R. Mach-Aigner¹*

**Abstract**

**Background:** *Trichoderma reesei* is used for industry-scale production of plant cell wall-degrading enzymes, in particular cellulases, but also xylanases. The expression of the encoding genes was so far primarily investigated on the level of transcriptional regulation by regulatory proteins. Otherwise, the impact of chromatin remodelling on gene expression received hardly any attention. In this study we aimed to learn if the chromatin status changes in context to the applied conditions (repressing/inducing), and if the presence or absence of the essential transactivator, the Xylanase regulator 1 (Xyr1), influences the chromatin packaging.

**Results:** Comparing the results of chromatin accessibility real-time PCR analyses and gene expression studies of the two prominent cellulase-encoding genes, *cbh1* and *cbh2*, we found that the chromatin opens during sophorose-mediated induction compared to D-glucose-conferred repression. In the strain bearing a *xyr1* deletion the sophorose mediated induction of gene expression is lost and the chromatin opening is strongly reduced. In all conditions the chromatin got denser when Xyr1 is absent. In the case of the xylanase-encoding genes, *xyn1* and *xyn2*, the result was similar concerning the condition-specific response of the chromatin compaction. However, the difference in chromatin status provoked by the absence of Xyr1 is less pronounced. A more detailed investigation of the DNA accessibility in the *cbh1* promoter showed that the deletion of *xyr1* changed the in vivo footprinting pattern. In particular, we detected increased hypersensitivity on Xyr1-sites and stronger protection of Cre1-sites. Looking for the players directly causing the observed chromatin remodelling, a whole transcriptome shotgun sequencing revealed that 15 genes encoding putative chromatin remodelers are differentially expressed in response to the applied condition and two amongst them are differentially expressed in the absence of Xyr1.

**Conclusions:** The regulation of xylanase and cellulase expression in *T. reesei* is not only restricted to the action of transcription factors but is clearly related to changes in the chromatin packaging. Both the applied condition and the presence of Xyr1 influence chromatin status.

**Background**

In nature, *Trichoderma reesei* is as a saprophytic fungus an excellent producer of enzymes involved in plant cell wall degradation (PCWD). In industry, these enzymes are used for a number of applications: xylanases are used for example in food industry as a baking agent and for clarification of juice and wine [1] or in the paper industry for de-inking [2]. Cellulases from *T. reesei* are important in textile industry for example for fibre polishing [3] or in the paper industry for recycling processes [2]. In the production of ethanol from cellulosic raw material *T. reesei* enzymes are applied to break down lignocellulose material to release D-glucose. The obtained D-glucose can be used subsequently in the sugar-to-ethanol fermentation (e.g. [4, 5] and citations therein). Due to the multiple applications of these enzymes many research studies have focused on this organism, its PCWD enzyme expression, and finally, the regulation of the encoding genes. Most of these studies were performed in the wild-type strain QM6a [6] or the mutant strain QM9414, which was selected for increased cellulase production [7]. Genome-wide analyses identified
34 cellulolytic and xylanolytic enzyme-encoding genes in *T. reesei* (reviewed in [8]), of which the most prominent cellulases are the cellobiohydrolases CBHI and CBHII (EC 3.2.1.91) [9] and the most studied xylanases are the endo-β-1,4-xylanases XYNI and XYNII (EC 3.2.1.8) [10]. The mentioned research efforts led further to the identification of transcription factors involved in the regulation of the expression of genes coding for PCWD enzymes on the transcriptional level. The most important transactivator is the Xylanase regulator 1 (Xyr1), which is absolutely essential for expression of both, xylanase and cellulase-encoding genes [11]. However, it should be noted that only the cellulase expression strictly follows the induction/repression pattern of the xyr1 gene [12]. The xyr1 gene itself is usually expressed at a low level and can be induced by the disaccharide sophorose formed via transglycosylation [12, 13]. Otherwise, the xylanase expression depends on Xyr1, but the transcript levels of these genes do not strictly reflect xyr1 transcript levels [11, 12]. The most important repressor is the Carbon catabolite repressor 1 (Cre1) [14], which mediates carbon catabolite repression (CCR) in presence of high amounts of easily usable carbon sources, such as D-glucose or D-xylose. Cre1 exerts its repressing function on both, the genes coding for the PCWD enzymes and the gene coding for their activator, xyr1 (e.g. [13, 15]). The different response of *T. reesei*'s transcriptome and secretome to cellulose, sophorose, and D-glucose was just recently investigated in a comparative high-throughput genomic and proteomic study [16]. While a lot is known about the transcriptional regulation of *T. reesei*'s PCWD enzyme-encoding genes by regulatory proteins (reviewed in [17]), so far hardly anything was investigated concerning the impact of the chromatin status on their gene expression. Only for Cre1 it was already earlier suggested that it might influence chromatin remodelling [18]. More recently, it was reported that it is involved in nucleosome positioning [19], and that a truncated version of Cre1, which is present in CCR-released, cellulase hyper-producing strains, supports the opening of chromatin in Cre1-target genes [20]. However, taking into account that chromatin status generally is believed to be a crucial factor in gene expression, this topic did not receive much attention in *T. reesei* yet. Therefore, in this study, we aimed to learn if the opponent of Cre1, the transactivator Xyr1, is also involved in chromatin remodelling, and if this happens in a condition (inducing/repressing carbon source)-dependent way. We used chromatin accessibility real-time PCR (CHART-PCR) for determining the chromatin status of the genes encoding the mentioned, four major PCWD enzymes and compared this with their gene expression. The results prompted us to have a more detailed investigation of the cbh1 promoter by in vivo footprinting analyses. Finally, we used whole transcriptome shotgun sequencing (WTSS) to identify genes putatively involved in chromatin remodelling that are differentially expressed with regards to the applied condition and/or the absence or presence of Xyr1.

**Results**

**Decreased cellulase gene expression in the absence of Xyr1 goes along with denser chromatin**

It is well known that Xyr1 is an essential activator of cellulase gene expression [11]. However, so far it has not been investigated if the deletion of Xyr1 additionally influences the chromatin status in the fungus. In order to study this, the wild-type strain and the xyr1 deletion strain were pre-grown and transferred to sophorose (inducing condition), D-glucose (repressing condition) or no carbon source-containing medium (reference condition) and were incubated for 3 h. By applying CHART-PCR analysis we investigated the chromatin packaging of the core promoter region (bearing the TATA-box) and one upstream regulatory region (URR) bearing Xyr1-binding sites (5′-GCG(T/A)3-3′; [21]) and/or Cre1-binding sites (5′-SYGGRG-3′; [14]) of the cbh1 and cbh2 genes each. For overviews on the investigated regions see Fig. 1a, b. Supplementary, we investigated the transcript levels of these genes by reverse transcription, quantitative PCR (qPCR) to see if the expression is related to chromatin accessibility. The expression of cbh1 and cbh2 is repressed on D-glucose in both strains and induced by sophorose in the wild-type strain (Fig. 2a, b). The induction is lost in the xyr1 deletion strain aside from a small increase in gene expression on sophorose compared to D-glucose. Altogether, we observed in both strains a condition-dependent change (i.e. sophorose-mediated opening) of chromatin that went along with a change (i.e. sophorose-mediated increase) in gene expression. However, comparing the strains under the same condition, the chromatin was always more closed in the xyr1 deletion strain compared to the wild-type strain (Fig. 2a, b) indicating a contribution of Xyr1 to a general (i.e. condition-independent) opening of chromatin in upstream regions of the cellulase-encoding genes.

**Xylanase gene repression in the absence of Xyr1 is not strictly related to chromatin compaction**

In an analogous analysis we investigated the chromatin status of the core promoter and an URR of the xyn1 and xyn2 genes each and compared this to the expression of the respective genes. For overviews on the regions investigated by CHART-PCR see Fig. 1c, d. In the wild-type strain the repression on D-glucose, the basal expression on D-xylose, and the induction on sophorose coincided with the increasing opening of chromatin (Fig. 3a, b). Otherwise, in the xyr1 deletion strain the gene
expression was at a similar low level (repressed) independent from the tested condition, while the chromatin packaging differed between the conditions. Interestingly, the chromatin accessibility on sophorose was even similar between the $\Delta xyr1$-strain and the wild-type strain (except the URR of $xyn1$) but the sophorose-mediated induction was completely lost in the $\Delta xyr1$-strain (Fig. 3a, b). Summarizing, we detected - similar to the case of the cellulase-encoding genes - an induction-specific opening of chromatin together with increasing gene expression in the wild-type strain. However, different from the cellulases, xylanase expression was repressed in the $\Delta xyr1$-strain although the chromatin status differed condition-dependently.

**Contribution of Xyr1 to chromatin opening**

To understand in detail the contribution of Xyr1 to changes in chromatin packing, the relation to induction of gene expression, and its putative impact on transcription initiation, we used CHART analysis again. We compared samples from the *T. reesei* wild-type and the xyr1 deletion strain exposed to sophorose (inducing condition) and to
non carbon source (non inducing condition). In the wild-type strain chromatin opens specifically on sophorose in case of all tested genes, namely xyn1, xyn2, cbh1, and cbh2 (Fig. 4). This is lost for all genes in the Δxyr1-strain (Fig. 4). However, the induction specific opening of chromatin is more pronounced in case of the cellulase-encoding genes. Altogether, the comparison of the chromatin accessibility under induced and non-induced conditions in the wild-type and the Δxyr1-strain even suggested that the open status is a consequence of induction. Xyr1 is required for the chromatin loosening, but this action is not essential for the initiation of transcription because transcripts can also be detected at low levels in a xyr1 deletion strain (compare Figs. 2 and 3).

The absence of Xyr1 changes DNA accessibility in the cbh1 promoter
Since we observed a pronounced induction-specific opening of chromatin that went along with increase of gene expression in presence of Xyr1 and a closing of chromatin together with gene repression in the absence of Xyr1 in the case of the cellulase-encoding genes, we aimed to have a more detailed investigation on the DNA accessibility of the promoter. Therefore, we performed in vivo footprinting analyses of the cbh1 promoter. Two URRs bearing Xyr1-binding sites and/or Cre1-binding sites and the core promoter bearing Xyr1-binding sites close to the TATA-box were investigated (Fig. 5a). The wild-type strain and the Δxyr1-strain were pre-grown on
glycerol and then incubated on D-glucose or sophorose for 3 h followed by dimethyl sulphate (DMS)-induced in vivo methylation. From Fig. 5b–d the footprinting pattern of the \( \Delta xyrl \) deletion strain compared to the wild-type strain for the three investigated regions can be inferred. The first investigated URR bears next to a single Cre1-site and a single Xyr1-site, also two Xyr1-sites arranged as inverted repeat with a spacing of 11 bp, which was reported to be the functional binding motif in vivo [22]. Under both, repressing and inducing conditions we could detect strong differences in the footprinting pattern of the two strains (Fig. 5b). In particular on sophorose, we observed an increased hypersensitivity towards DNA methylation on the Xyr1-sites in the \( \Delta xyrl \)-strain compared to the wild-type strain, whereas the Cre1-site was stronger protected (Fig. 5b). The second investigated URR bears a functional Cre1 double site [23]. Here, we detected strong hypermethylation signals in the \( \Delta xyrl \)-strain compared to the wild-type strain on D-glucose, but none on sophorose (Fig. 5c). The third investigated URR bears three Xyr1-binding sites arranged in tandem. In this case, we detected just a few differences between the two strains, however, most of them on or close to the Xyr1-sites (Fig. 5d).

Identification of differentially expressed genes potentially involved in chromatin remodelling

To learn more about the mechanisms responsible for the chromatin remodelling in context to both, the applied condition and the presence or absence of Xyr1, we used WTSS. Therefore, a \( xyrl \) deletion strain and its parental strain QM9414 were again exposed to repressing conditions (growth on D-glucose) and inducing conditions (incubation on sophorose). Please note that the full data set can be obtained from GEO database (GSE66982). Based on the results obtained by the WTSS, we analyzed the gene expression profiles of 136 candidate genes involved in chromatin structure and dynamics according to the eukaryotic orthologous groups (KOG) in the \( T. reesei \) genome database (http://genome.jgi-psf.org/Trire2/Trire2.home.html). An overview on these 136 genes is provided here. For the differential expression analysis, a two-fold change cut-off, i.e. \( \log_2 \) fold change \( \geq 1 \) or \( \leq -1 \) and an adjusted \( p \)-value \( \leq 0.05 \), was used as threshold. Concerning the first part of our question, i.e. the observed differences in chromatin status dependent on the applied condition, we identified 15 genes differentially expressed on sophorose as compared to D-glucose in the wild-type strain (listed in Table 1).
Concerning the second part of our question, i.e. the influence of Xyr1 on the expression profiles of these genes we examined which ones were differentially expressed in \( \Delta xyr1 \)-strain compared to the wild-type strain under sophorose induction. Out of the 15 genes responding to the applied condition, two genes are additionally differentially expressed in the \( \Delta xyr1 \)-strain (transcript ID 53947 and 73708). Notably, the gene with transcript ID 73708, encoding a putative heterochromatin-associated protein, was down-regulated on sophorose compared to D-glucose and up-regulated in the absence of Xyr1.

Discussion

The aim of this study was to learn more about the contribution of the chromatin compaction to the regulation of gene expression of PCWD enzymes in \( T. reesei \). Altogether, we found for all investigated genes that their induced expression is accompanied by an opening of chromatin and that Xyr1 is required for the open chromatin status.

However, we observed differences between cellulase- and xylanase-encoding genes concerning the involvement of Xyr1 in chromatin remodelling: the chromatin of the upstream regions of the cellulase-encoding genes was more compact under all tested conditions when Xyr1 was missing. This finding is supported by the \emph{in vivo} footprinting results of the \( cbh1 \) URR, which revealed an increased sensitivity towards methylation on the Xyr1-binding sites in the absence of Xyr1, in particular on sophorose (compare Fig. 4b). On the other hand, the accessibility of the functional Cre1-sites was changed only on D-glucose. They were found to be stronger methylated in the \( xyr1 \) deletion strain than in the parent strain (compare Fig. 4c).

We assume that hypersensitivity to DNA methylation can be caused by both, non-occupancy leading to better access for the methylation agent, but also by DNA occupancy and a following increased disposition to be methylated. Considering this, we would suggest that on D-glucose repression the Cre1 DNA-binding affinity to the \( cbh1 \) promoter is higher in the absence of Xyr1. This could explain the less accessible chromatin in the \( \Delta xyr1 \)-strain.
on D-glucose (compare Fig. 2a). In summary, the presence of Xyr1 supported chromatin opening under all investigated conditions in the case of the cellulase-encoding genes. Perhaps this finding is one explanation for the previously reported, condition-dependent transcript level pattern of the cbhl1 and cbhl2 genes that exactly follow the one of the xyr1 gene [12]. For example, if under non-inducing conditions less Xyr1 is present, the positive influence of Xyr1 on chromatin opening might be reduced and this would cause a decrease in cellulase-encoding gene expression. However, the earlier observation that transcript levels of cbhl1 and cbhl2 correlate with those of xyr1 [12], and the result from this study indicating the involvement of Xyr1 in chromatin opening suggest a regulation of the cellulase-encoding genes being dominated by Xyr1.

It is currently thought that transcription factors must induce the reorganization of the local chromatin (reviewed by [24]). One proposed mechanism is the recruitment of nucleosome remodelers by the initiating factor leading to local chromatin conformations [25]. Our current model on the function of the Xyr1 is the following: as shown in previous studies xyr1 transcription is induced on sophorose [12]. This allows the assumption that under this condition Xyr1-sites are occupied, which is supported by in vivo footprinting results obtained during this study. We would suggest that Xyr1 recruits chromatin remodelers leading to the observed, more open chromatin status. This would suggest that Xyr1 recruits chromatin remodelers leading to increased induction of the target gene (i.e. the cellulase-encoding gene) under this condition.

In the case of the xylanase-encoding genes we also detected a condition-dependent induction of gene expression, which was accompanied by chromatin opening in the wild-type strain. However, the involvement of Xyr1 is different in this case as compared to the cellulase-encoding genes. In the absence of Xyr1 gene expression decreased under all conditions, but the chromatin in the upstream regions of the xylanase-encoding genes did not always became more compact. For example, in the case of xyrm2, the URR had a similar chromatin accessibility under non-repressing conditions (sophorose, D-xylose) in the Δxyr1-strain as in the wild-type strain but the gene expression was strongly repressed in the absence of Xyr1. The fact that gene expression can be repressed simultaneously with enhanced chromatin accessibility might be explained by a generally better access for all kinds of regulatory proteins including repressor proteins. Another possible explanation would be that the absence of Xyr1 simply overrules the level of regulation by chromatin opening. Anyway, during this study it became obvious that the activating function of Xyr1 on xylanase-encoding gene expression is not mainly exerted on the chromatin level. There are earlier reports on generally different, condition-dependent transcript level patterns of the xyn1 and xyn2 genes as compared to the xyr1 gene [12]. One example is the low basal xyn2 gene expression on D-glucose (e.g. [26, 27]) that is not detectable for the xyr1 gene [13]. All these findings together strongly indicate that additional regulatory factors (for example the suggested xylanase repressor Xpp1 [28]) and mechanisms, which are responsible for chromatin opening under inducing conditions, need to be involved.

A whole transcriptome analysis was used to identify genes classified as chromatin remodelers in T. reesei, which are differentially expressed dependent on the

| Transcript ID | Annotation | SO/G | p-value | Δxyr1/WT | p-value |
|--------------|------------|------|---------|----------|---------|
| 2648         | Predicted component of NuA3 histone acetyltransferase complex | -1.154 | 0.000 | 0.279 | 0.653 |
| 34402        | Histone H1 | 1.300 | 0.000 | -0.457 | 0.075 |
| 36727        | SWM-SNF chromatin-remodeling complex protein | 1.493 | 0.000 | -0.843 | 0.000 |
| 53947        | SWM-SNF chromatin-remodeling complex protein | 1.196 | 0.000 | 1.332 | 0.000 |
| 56077        | SWM-SNF chromatin-remodeling complex protein | 3.050 | 0.000 | -0.359 | 0.158 |
| 65533        | Histone deacetylase complex, catalytic component HDA1 | 1.237 | 0.000 | -0.347 | 0.170 |
| 73708        | Heterochromatin-associated protein HP1 and related CHROMO domain proteins | -3.012 | 0.002 | 1.253 | 0.000 |
| 76872        | SWM-SNF chromatin-remodeling complex protein | 1.070 | 0.000 | -0.708 | 0.004 |
| 81517        | Sirtuin 5 and related class III sirtuins (SIR2 family) | 1.600 | 0.034 | -0.160 | 0.615 |
| 108909       | Nucleosome-binding factor SPN, POB3 subunit | 1.050 | 0.000 | 0.041 | 0.998 |
| 110409       | Possible homologue of S. cerevisiae SAS10 | -1.298 | 0.000 | -0.001 | 1.000 |
| 110418       | SWM-SNF chromatin-remodeling complex protein | 1.180 | 0.000 | -0.566 | 0.049 |
| 110507       | Histone acetyltransferase (MYST family) | 1.064 | 0.000 | -0.562 | 0.024 |
| 122943       | SWM-SNF chromatin-remodeling complex protein | 1.876 | 0.000 | -0.127 | 0.621 |
| 123327       | SWM-SNF chromatin-remodeling complex protein | 1.852 | 0.002 | 0.508 | 0.036 |

Differential gene expression according to WTSS analysis comparing either sophorose induction (SO) to glucose repression (G) in the wild-type strain or the xyr1 deletion strain (Δxyr1) to the wild-type strain (WT) under sophorose induction.
applied condition (inducing/repressing). Notably, 15 genes are differentially expressed in the wild-type strain (compare Table 1), whereas only ten genes responded in a condition-dependent manner in the Δxyr1-strain (data not shown). This again supports the assumption that Xyr1 is generally involved in chromatin remodelling mechanisms. The identification of two putative chromatin remodelers, which are under the control of Xyr1 (directly or via expression of other regulatory proteins), point to an indirect role of Xyr1 in chromatin remodelling. Moreover, it can be speculated that Xyr1 additionally recruits chromatin-remodelling proteins in a differential manner towards the promoters of the cellulase- and xylanase-encoding genes. This would be a further explanation for the observed differences concerning the influence of Xyr1 on their chromatin status. However, at this stage it remains to be investigated if the open chromatin is indeed the result of chromatin remodelling (as the loss or movement of nucleosomes) or if the loss of the identified putative chromatin remodelers overrules the action of Xyr1.

Conclusions
Investigations on the level of chromatin packaging revealed that the transcription factor Xyr1 does exert its activating function—in addition to other possible mechanisms—by an induction-specific opening of chromatin. The impact of Xyr1 in chromatin opening was more pronounced in the case of cellulase-encoding genes than in the case of the xylanase-encoding genes. The application of WTSS identified one chromatin remodeler that is down-regulated under inducing conditions and up-regulated if Xyr1 is missing. According to the results of the present study, this is a target in engineering strains with enhanced cellulase expression.

Methods
Fungal strains
The following T. reesei strains were used throughout this study: the wild-type strain QM6a (ATCC 13631), and a corresponding xyr1 deletion strain (this study), QM9414 (ATCC 26921), and a QM9414 strain bearing a xyr1 deletion [11]. All strains were maintained on malt extract agar.

Growth conditions
For carbon source replacement experiments mycelia were pre-cultured in 1-L-Erlenmeyer flasks on a rotary shaker (180 rpm) at 30 °C for 24 h in 250 mL of Mandels-Andreotti (MA) medium [29] supplemented with 1 % (w/v) glycerol as sole carbon source. A total of 10⁸ conidia per litre (final concentration) were used as inoculum. Pre-grown mycelia were washed and equal amounts were resuspended in 20 ml MA media containing 1 % (w/v) D-glucose or 2 mM sophorose as sole carbon source or no carbon source and were incubated for 3 h.

For direct cultivation experiments conidia were incubated in 200 mL MA medium containing 2 % (w/v) glucose as the sole carbon source for 24 and 48 h. Samples were derived from three biological replicates.

Deletion of xyr1 from the genome of the T. reesei wild-type strain
The deletion of the xyr1 gene was essentially performed as described in [11]. The plasmid pD2xlr1 was modified by shortening the promoter of the A. nidulans amds gene, which was used as a marker [30]. The obtained plasmid pD5 was applied in a fungal protoplast transformation using QM6aΔtmus53 [31] as a recipient strain and was performed by following the protocol described in [32].

CHART-PCR
DNase I digestion of chromatin and DNA extraction were carried out as described before [20]. qPCR analysis of the DNase I-treated samples was performed to measure the relative abundance of target regions. PCRs were performed in triplicates in a Rotor-Gene Q system (Qiagen, Hilden, Germany) using the amplification mixture (final volume 20 μL) and cycling conditions described before [20]. Primer sequences are provided in Table 2. The amount of intact input DNA of each sample was calculated by comparing the threshold values of the PCR amplification plots with a standard curve generated for each primer set using serial dilutions of genomic, undigested DNA. The chromatin accessibility index (CAI) was defined as: CAI = 1/(Ds/(Dc1 + Dc2)/2)), where Ds is the amount of intact DNA detected for each target region and Dc1 and Dc2 are the amounts of intact DNA detect for the promoter regions of sar1 and act respectively, used as reference genes for normalization.

Analysis of transcript levels
Fungal mycelia were homogenized in 1 mL of peqGOLD-TriFast DNA/RNA/protein purification system reagent (PEQLAB Biotechnologie, Erlangen, Germany) using a FastPrep(R)-24 cell disrupter (MP Biomedicals, Santa Ana, CA, USA). RNA was isolated according to the manufacturer's instructions, and the concentration was measured using the NanoDrop 1000 (Thermo Scientific, Waltham, US). Synthesis of cDNA from mRNA was carried out using the RevertAidTM H Minus First Strand cDNA Synthesis Kit (Thermo Fisher Scientific) according to the manufacturer's instructions. Quantitative PCRs were performed in triplicates in a Rotor-Gene Q system (Qiagen). The amplification mixture (final volume 15 μL) contained 7.5 μL 2 × iQ SYBR Green Mix (Bio-Rad, Hercules, USA),
100 nM forward and reverse primer and 2.5 μL cDNA (diluted 1:20). Primer sequences are provided in Table 2. Cycling conditions and control reactions were performed as described previously [33]. Data normalization using sar1 and act as reference genes and calculations were performed as published previously [33].

**Table 2: Oligonucleotides used in this study**

| Name       | Sequence (5' - 3') | Usage               |
|------------|-------------------|---------------------|
| RG53       | GAATTCAGATC       | ivFP, oligo-short   |
| RG54       | GGGTGTAGCCCGGAGATCTGAATTG | ivFP, oligo-long |
| RG83       | [6-FAM]GCCGCTGGTACATGTGTGTGCCGG | ivFP, cbh1oligo3fw |
| RG84       | [6-FAM]GAGAGTGAGCAGCGACTGAGC | ivFP, cbh1oligo3rev |
| RG89       | [6-FAM]GTATAGGCTATGGTGACTGTGGG | ivFP, cbh1oligo3fw |
| RG90       | [6-FAM]GTATAGGCTATGGTGACTGTGGG | ivFP, cbh1oligo3fw |
| actfw      | TGGATGTCAACTGGTCTACGA | qPCR                |
| actrev     | TTGATGTCAACTGGTCTACGA | qPCR                |
| sar1fw     | GCATGATGACAGCGGAGAGAGAATC | qPCR                |
| sar1rev    | TTGATGTCAACTGGTCTACGA | qPCR                |
| cbh1f      | GATGATGACAGCGGAGAGAATC | qPCR                |
| cbh1r      | ACGCCGCGGGTGGG       | qPCR                |
| cbh2f      | CGCCGCGGGGCACTGGTCTACGA | qPCR                |
| cbh2r      | CGCCGCGGGGCACTGGTCTACGA | qPCR                |
| xyn1f      | CAGCTATGGCGCTTCTCACAACAC | qPCR                |
| xyn1r      | CAGCTATGGCGCTTCTCACAACAC | qPCR                |
| taqpxyn2f  | GGCACACTGCGGGAACCTTT | qPCR                |
| taqpxyn2r  | GGCACACTGCGGGAACCTTT | qPCR                |
| episar1Tr_f| CTCTCCTCCTCTCTCTCTCTC | act CHART, region −226 to +24 |
| episar1Tr_r| CTCTCCTCCTCTCTCTCTC | sar1 CHART, region −490 to −224 |
| episar2Tr_f| GTCGACACACACTGGTCTACGA | qPCR                |
| episar2Tr_r| GTCGACACACACTGGTCTACGA | qPCR                |
| epicbh1_1Tr_f| AAGGGAAACCCGCGATACGCTGTC | cbh1 CHART, region −902 to −610 |
| epicbh1_1Tr_r| AAGGGAAACCCGCGATACGCTGTC | cbh1 CHART, region −902 to −610 |
| epicbh1_2Tr_f| TTTCACCACAGGCAACAAGAAC | cbh1 CHART, region −301 to −27 |
| epicbh1_2Tr_r| TTTCACCACAGGCAACAAGAAC | cbh1 CHART, region −301 to −27 |
| epicbh2_1Tr_f| CGGATCTAGGGCGACATGGGAGATC | cbh2 CHART, region −587 to −338 |
| epicbh2_1Tr_r| CGGATCTAGGGCGACATGGGAGATC | cbh2 CHART, region −587 to −338 |
| epicbh2_2Tr_f| GTGTAGGTGGTGGCTGCACACCGTGAC | cbh2 CHART, region −355 to −62 |
| epicbh2_2Tr_r| GTGTAGGTGGTGGCTGCACACCGTGAC | cbh2 CHART, region −355 to −62 |
| epicxyn1_1Tr_f| GCACACTGCAAGCGGCTTCTCGTTACT | xyn1 CHART, region −577 to −278 |
| epicxyn1_1Tr_r| GCACACTGCAAGCGGCTTCTCGTTACT | xyn1 CHART, region −577 to −278 |
| epicxyn1_3Tr_f| TAGATGAGCGGACGGCGAATACTC | xyn1 CHART, region −306 to −10 |
| epicxyn1_3Tr_r| TAGATGAGCGGACGGCGAATACTC | xyn1 CHART, region −306 to −10 |
| epicxyn2_1Tr_f| GTGGCAGCTGACAGCGGCTGAGA | xyn2 CHART, region −527 to −252 |
| epicxyn2_1Tr_r| GTGGCAGCTGACAGCGGCTGAGA | xyn2 CHART, region −527 to −252 |
| epicxyn2_2Tr_f| CTCGAGACGGCTGAGAAGCGAGCATCAG | xyn2 CHART, region −311 to −38 |
| epicxyn2_2Tr_r| CTCGAGACGGCTGAGAAGCGAGCATCAG | xyn2 CHART, region −311 to −38 |

**In vivo footprinting**

_In vivo_ methylation using DMS followed by ligation mediated PCR was performed as described previously [34]. FAM-labelled fragments were generated by a PCR reaction using RG89 and RG90 or RG83 and RG84 for an URR or a TATA-box containing core region within the
promoter, respectively. Primer sequences are provided in Table 2. FAM-labelled fragments were analyzed by capillary gel electrophoresis (Microsynth, Balgach, Switzerland) and results were analyzed using the program ivFAST [34].

Whole transcriptome shotgun sequencing

The mRNA was extracted from fungal mycelia using TRIzol® RNA Kit (Life Technologies, part of Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer’s instructions. RNA concentration was determined by spectrophotometry at 260/280 nm and RNA integrity was tested by the 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) and gel electrophoresis (1% agarose). The RNA of the biological replicates was pooled, lyophilized, and stored using the RNAStable® Tube Kit (Biomatrica, San Diego, CA, USA). Barcoded libraries were prepared using the TruSeq RNA Sample Prep kit (Illumina, San Diego, CA, USA) and sequenced by LGC Genomics GmbH (Berlin, Germany) using the Illumina HiSeq 2000 platform.

WTSS data analysis

Sequences from approximately 144 million 100 bp paired-end reads were quality-filtered and mapped to the Trichoderma reesei 2.0 reference genome, (http://genom.jgi-psf.org/Trire2/Trire2.home.html) using the Bowtie aligner version 0.12.8 [35] allowing two mismatches and only unique alignments. The SAMtools version 0.1.18 [36] was used to process the alignments files, which were visualized using the Integrative Genomics Viewer [37]. Bioconductor DESeq package version 1.10.1 [38] was utilized for normalization, using the median log deviation, and for the differential expression analysis, applying a two-fold change cut-off, i.e. log₂-fold change ≥ 1 or ≤ −1 and an adjusted p-value ≤ 0.05 were used as thresholds. The log₂-fold change was calculated according to the equation:

\[
\text{log}_2\text{-fold change} = \log_2 \frac{\text{baseMeanB}}{\text{baseMeanA}}
\]

baseMeanB is the mean normalized counts from condition B and baseMeanA is the mean normalized counts from condition A.

Abbreviations

CAI: Chromatin accessibility index; CCR: Carbon catabolite repression; CHART-PCR: Chromatin accessibility real-time PCR; Cre1: Carbon catabolite repressor 1; DMS: Dimethyl sulphate; ivFP: in vivo footprinting; KOG: Eukaryotic orthologous groups; MA: Mandels-Andreotti; PCWD: Plant cell wall-degrading/plant cell wall degradation; qPCR: Quantitative PCR; URR: Upstream regulatory region; WTSS: Whole transcriptome shotgun sequencing; Xyr1: Xylanase regulator 1.

Competing interests

The authors declare that they have no competing interests.

Authors’ contributions

TMMS performed CHART-PCR and transcript analyses and participated in analysis of WTSS data. AR carried out the in vivo footprinting analyses. MEP constructed the xyr1 deletion strain in QM6a. LSC, prepared samples for WTSS and participated in analysis of corresponding data. GFP and RSR participated in analysis of WTSS data. RLM and MJPF participated in conception and revision of the manuscript. RNS participated in design of the study and supervision of experiments. ARMA participated in design of study, supervision of experiments, and prepared the manuscript. All authors read and approved the final manuscript.

Acknowledgements

This work was supported by two grants from the Austrian Science Fund (FWF): V232-B20, P24851 given to ARMA, by a doctoral program of Vienna University of Technology (‘CatMat’), and by The State of São Paulo Research Foundation (FAPESP). (proc. 2010/15683-8). We thank Stephanie Steinberger for help with the footprinting analyses.

Author details

1Department of Biotechnology and Microbiology, Institute of Chemical Engineering, TU Wien, Gumpendorfer Str. 1a, A-1060 Wien, Austria. 2Department of Biochemistry and Immunology, Ribeirão Preto Medical School, University of São Paulo, 14049-900, Ribeirão Preto, SP, Brazil. 3Laboratório Nacional de Ciência e Tecnologia do Biotaneto (CTBTe), Centro Nacional de Pesquisa em Energia e Materiais (CNPEM), Campinas, São Paulo, Brazil. 4Department of Genetics and Morphology, Institute of Biological Sciences, University of Brasilia, Brasília, DF, Brazil.

Received: 12 January 2015 Accepted: 3 August 2015

References

1. Galante YM, de Conti A, Monteverdi R. Application of Trichoderma enzymes in the food and feed industries. In: Harman GE, Kubicek CP, editors. Trichoderma & Gliocladium, vol. 2. London: Taylor & Francis; 1998. p. 327–42.
2. Buchert J, Oksanen T, Pere J, Siika-aho M, Suurnäkki A, Viikari L. Applications of Trichoderma reesei enzymes in the pulp and paper industry. In: Harman GE, Kubicek CP, editors. Trichoderma & Gliocladium, vol. 2. London, UK: Taylor & Francis Ltd; 1998. p. 343–57.
3. Galante YM, de Conti A, Monteverdi R. Application of Trichoderma enzymes in the textile industry. In: Kubicek CP, Harman GE, editors. Trichoderma & Gliocladium, vol. 2. London: Taylor & Francis; 1998. p. 311–25.
4. Gusakov AV. Alternatives to Trichoderma reesei in biofuel production. Trends Biotechnol. 2011;29(9):419–25.
5. Wilson DB. Cellulases and biofuels. Curr Opin Biotechnol. 2009;20(3):295–9.
6. Mandels M, Reese ET. Induction of cellulase in Trichoderma viride as influenced by carbon sources and metals. J Bacteriol. 1957;73(2):269–78.
7. Mandels M, Weber J, Parizk B. Enhanced cellulase production by a mutant of Trichoderma viride. Appl Microbiol. 1971;21(1):152–4.
8. Kubicek CP. Systems biological approaches towards understanding cellulase production by Trichoderma reesei. J Biotechnol. 2013;163(2):133–42.
9. Teen T, Salovouri I, Knowles J. The molecular cloning of the major cellulase gene from Trichoderma reesei. Biotechnology. 1983;1:696–9.
10. Törnönen A, Mach RL, Messner R, Gonzalez R, Kalkkén N, Harkki A, et al. The two major xylanases from Trichoderma reesei: characterization of both enzymes and genes. Biotechnology (N Y). 1992;10(11):1461–5.
11. Stricker AR, Grosssetzer-Hain K, Warlether E, Mach RL. Xyr1 (xylanase regulator 1) regulates both the hydrolytic enzyme system and D-xylose metabolism in Hypocrea jecorina. Eukaryot Cell. 2006;5(12):2128–37.
12. Derrit C, Gudaityne-Savitch L, Caliste S, White T, Mach RL, Mach-Aigner AR. Mutation of the Xylanase regulator 1 causes a glucose blind hydrolase expressing phenotype in industrially used Trichoderma strains. Biotechnol Biofuels. 2013;6(1):62.
13. Mach-Aigner AR, Puchner MJ, Steiger MG, Preis SJ, Mach RL. Transcriptional regulation of xyr1, encoding the main regulator of the xylanolytic and cellulolytic enzyme system in Hypocrea jecorina. Appl Environ Microbiol. 2008;74(12):6554–62.
14. Strauss J, Mach RL, Zeilinger S, Hartler G, Stöffler G, Wolschek M, et al. Cre1, the carbon catabolite repressor protein from Trichoderma reesei. FEBS Lett. 1995;376(1–2):103–7.
15. Mach RL, Strauss J, Zeilinger S, Schindler M, Kubicek CP. Carbon catabolite repression of xylanase I (xyn1) gene expression in Trichoderma reesei. Mol Microbiol. 1996;21(6):1273–81.

16. dos Santos Castro L, Pedersoli WR, Antonieto AC, Steindorff AS, Silva-Rocha R, Martinez-Rossi NM, et al. Comparative metabolism of cellulose, sophorose and glucose in Trichoderma reesei using high-throughput genomic and proteomic analyses. Biotechnol Biofuels. 2014;7(1):14.

17. Stecker AR, Mach RL, de Graaff LH. Regulation of transcription of cellulolytic- and hemicellulases-encoding genes in Aspergillus niger and Hypocrea jecorina (Trichoderma reesei). Appl Microbiol Biotechnol. 2008;78(2):211–20.

18. Portnoy T, Margeot A, Linke R, Atanasova L, Fekete E, Sandor E, et al. The CRE1 carbon catabolite repressor of the fungus Trichoderma reesei: a master regulator of carbon assimilation. BMC Genomics. 2011;12:269.

19. Ries L, Belshaw NJ, Illmén M, Penttilä ME, Alapuranen M, Archer DB. The role of CRE1 in nucleosome positioning within the cbh1 promoter and coding regions of Trichoderma reesei. Appl Microbiol Biotechnol. 2014;98(2):749–62.

20. Mello-de-Sousa TM, Gorsche R, Rassinger A, Pocas-Fonseca MJ, Mach RL, Mach-Aigner AR. A truncated form of the Carbon catabolite repressor 1 increases cellulase production in Trichoderma reesei. Biotechnol Biofuels. 2014;7(1):129.

21. Furukawa T, Shiida Y, Kitagami N, Mori K, Kato M, Kobayashi T, et al. Identification of specific binding sites for XYR1, a transcriptional activator of cellulolytic and xylanolytic genes in Trichoderma reesei. Fungal Genet Biol. 2009;46(8):564–74.

22. Rauscher R, Würleitner E, Wacenovsky C, Aro N, Stricker AR, Zeilinger S, et al. Transcriptional regulation of xyn1, encoding xylanase i, in Hypocrea jecorina. Eukaryot Cell. 2006;5(3):447–56.

23. Illmén M, Onnela ML, Klemidal S, Keranen S, Penttilä M. Functional analysis of the cellulohydrolase I promoter in the filamentous fungus Trichoderma reesei. Mol Gen Genet. 1998;257(3):386.

24. Voss TC, Hager GL. Dynamic regulation of transcriptional states by chromatin and transcription factors. Nat Rev Genet. 2014;15(2):59–81.

25. Voss TC, Schiltz RL, Sung MH, Yen PM, Stamatosyanopoulos JA, Biddie SC, et al. Dynamic exchange at regulatory elements during chromatin remodeling underlies assisted loading mechanism. Cell. 2011;146(4):544–54.

26. Würleitner E, Pera L, Wacenovsky C, Cziferszky A, Zeilinger S, Kubicek CP, et al. Transcriptional regulation of xyn2 in Hypocrea jecorina. Eukaryot Cell. 2003;2(1):150–8.

27. Zeilinger S, Mach RL, Schindler M, Herzog P, Kubicek CP. Different inducibility of expression of the two xylanase genes xyn1 and xyn2 in Trichoderma reesei. J Biol Chem. 1996;271(41):25624–9.

28. Mach-Aigner AR, Grosstessner-Hain K, Pocas-Fonseca MJ, Mechtler K, Mach RL. From an electrophoretic mobility shift assay to isolated transcription factors: a fast genomic-proteomic approach. BMC Genomics. 2010;11:644.

29. Mandels M. Applications of cellulases. Biochem Soc Trans. 1985;13(2):414–6.

30. Hynes MJ, Corrick CM, King JA. Isolation of genomic clones containing the xyn1 gene of Aspergillus niger and their use in the analysis of structural and regulatory mutations. Mol Cell Biol. 1983;3(8):1430–9.

31. Stricker AR, Mach RL, de Graaff LH. Regulation of transcription of cellulases- and hemicellulases-encoding genes in Trichoderma reesei. Mol Genet Genomic. 2010;283(5):1001–13.

32. Gruber F, Visser J, Kubicek CP, de Graaff LH. The development of a heterologous transformation system for the cellulolytic fungus Trichoderma reesei based on a pyrG-negative mutant strain. Curr Genet. 1990;18(1):71–6.

33. Steiger MG, Mach RL, Mach-Aigner AR. An accurate normalization strategy for RT-qPCR in Hypocrea jecorina (Trichoderma reesei). J Biotechnol. 2010;145(1):30–7.

34. Gorsche R, Jovanovic B, Gudynaite-Savitch L, Mach RL, Mach-Aigner AR. A highly sensitive in vivo footprinting technique for condition-dependent identification of cis elements. Nucleic Acids Res. 2013;42(1), e1.

35. Li H, Durbin R. Fast and accurate read alignment with Burrows-Wheeler transform. Bioinformatics. 2009;25(14):1754–60.

36. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics. 2009;25(16):2078–9.

37. Thorvaldsdóttir H, Robinson JT, Mesirov JP. Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration. Brief Bioinform. 2013;14(2):178–92.