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Utilisation of wheat bran as a substrate for bioethanol production using recombinant cellulases and amylolytic yeast

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HIGHLIGHTS

- A cocktail of recombinant cellulases was proposed for wheat bran hydrolysis.
- Optimal conditions for enzymatic hydrolysis of wheat bran were determined.
- Recombinant amylolytic strains completely hydrolysed the starch in wheat bran.
- Addition of cellulases to SSF with amylolytic strains enhanced ethanol yield.

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ABSTRACT

Wheat bran, generated from the milling of wheat, represents a promising feedstock for the production of bioethanol. This substrate consists of three main components: starch, hemicellulose and cellulose. The optimal conditions for wheat bran hydrolysis have been determined using a recombinant cellulase cocktail (RCC), which contains two cellobiohydrolases, an endoglucanase and a β-glucosidase. The 10% (w/v, expressed in terms of dry matter) substrate loading yielded the most glucose, while the 2% loading gave the best hydrolysis efficiency (degree of saccharification) using unmilled wheat bran. The ethanol production of two industrial amylolytic *Saccharomyces cerevisiae* strains, MEL2[TLG1-SFA1] and M2n [TLG1-SFA1], were compared in a simultaneous saccharification and fermentation (SSF) for 10% wheat bran loading with or without the supplementation of optimised RCC. The recombinant yeast *S. cerevisiae* MEL2[TLG1-SFA1] and M2n[TLG1-SFA1] completely hydrolysed wheat bran's starch producing similar amounts of ethanol (5.3 ± 0.14 g/L and 5.0 ± 0.09 g/L, respectively). Supplementing SSF with RCC resulted in additional ethanol production of about 2.0 g/L. Scanning electron microscopy confirmed the effectiveness of both RCC and engineered amylolytic strains in terms of cellulose and starch depolymerisation.

This study demonstrated that untreated wheat bran could be a promising ready-to-use substrate for ethanol production. The addition of crude recombinant cellulases improved ethanol yields in the SSF process and *S. cerevisiae* MEL2[TLG1-SFA1] and M2n[TLG1-SFA1] strains can efficiently convert wheat bran's starch to ethanol.

1. Introduction

Lignocellulosic biomass is the preferred substrate for bioethanol as it is more abundant and less expensive than sucrose and starch substrates [1]. However, the limitations associated with lignocellulosic ethanol production include the slow rate of enzymatic degradation, high enzyme cost and the requirement of inhibitor-tolerant industrial yeast strains [2–4]. Consequently, starch is still the most commonly used feedstock for ethanol production, with a relatively mature technology developed for corn in the USA [5] that produced about 52.5 billion litres of bioethanol in 2012, an increase from 49.2 billion litres in 2010 [6].

Current starch-to-ethanol processes require an energy-intensive liquefaction step, as well as substantial amounts of exogenous amylases for enzymatic hydrolysis of raw starch; both these significantly impact the economic viability of starch as feedstock [7]. In order to implement the large scale ethanol production from raw starch, the development of an industrial yeast

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that converts starch to ethanol in one step (called consolidated bioprocessing – CBP) is needed [8–11].

Recently, few studies reported the use of *Saccharomyces cerevisiae* strains for the fermentation of natural starchy substrates at a bioreactor scale. Favaro and colleagues described the direct ethanol production from natural starchy substrates (corn, sorghum and triticale), using industrial yeast strains co-secreting glucoamylase and α-amylase enzymes [12]. Yamada et al. [13] achieved the CBP of brown rice by the amylolytic laboratory strain MNIV/δGS producing almost 80 g/L of alcohol from 200 g/L of brown rice after 120 h. Although the above reports pave the way for the industrial CBP of raw starch to ethanol, their focus was on substrates composed only of starch, meanwhile many industrial starch-rich by-products are available in great quantities with different compositions in terms of cellulose and hemicellulose. These polysaccharides first have to be converted into sugars, in order to achieve high ethanol efficiencies and make the overall process economically viable. This is the case with wasted crop, cereal bran, cassava pulp, sago pith residues and brewery-spent grains, which have been proposed as low-cost materials for bioethanol, mainly by means of chemical pre-treatment, commercial cellulases, xylanase and amylases addition and subsequent fermentation [14–19]. The previously mentioned studies, though achieving promising results, demonstrate that the total exploitation of such substrates still needs to be addressed and that there is an opportunity to further increase the hydrolysis and fermentation yields from agricultural by-products containing different polysaccharides. Cheap and plentiful residual biomass has been investigated as renewable material to be converted into fuels, polymers, enzymes and bulk chemicals [20–23].

This research focused on wheat bran as an abundant and inexpensive starchy substrate, with a high potential for bioethanol due to its low pre-treatment cost [14,15]. In addition to the starch content (15–30% dry matter), the hemicellulose and cellulose fractions can also be used for bioethanol production [24]. Although wheat bran does not require costly pre-treatments for hydrolysis [15,25], not many studies have used this substrate for ethanol production [26]. Therefore, there is scope to optimise current technologies.

The hydrolysis of cellulose, starch and hemicellulose requires commercial enzymes that are very costly and not feedstock specific. Banerjee and colleagues [27] have developed a core set of recombinant enzymes for the hydrolysis of ammonia fibre expansion (AFEX) treated corn stover, using *Trichoderma reesei* enzymes produced in *Pichia pastoris*. However, there is still limited information available on the use of feedstock specific recombinant enzyme cocktails. An advantage of recombinant cocktails over commercial cocktails is that they are defined mixtures and do not contain unnecessary proteins.

In this present study, we examine the use of recombinant cellulolytic enzymes and engineered amylase-secreting strains for the hydrolysis and saccharification of wheat bran's cellulose and starch. The first objective was to investigate the simultaneous hydrolysis of cellulose using a recombinant cellulase cocktail (RCC) produced by engineered yeast and fungal strains. For the first time, the crude enzymes secreted in the supernatant were directly used to optimise the hydrolysis of wheat bran in terms of glucose yield. Once the optimisation of hydrolysis was achieved, the industrial *S. cerevisiae* MEL2[TLG1-SFA1] and M2n[TLG1-SFA1] strains (both secreting the *Thermomyces lanuginosus* glucoamylase, TLG1, and the *Saccharomycopsis fibuligera* α-amylase, SFA1) were utilised for the simultaneous saccharification and fermentation (SSF) process in the presence of RCC resulting in high ethanol yields. This is the first report describing the conversion of starchy and cellulosic substrate into ethanol using crude recombinant enzymes and engineered amylolytic strains.

2. Material and methods

2.1. Strains, media and cultivations

The genotype and origin of strains used in this work are summarised in Table 1. The wild type *S. cerevisiae* MEL2 and M2n, with their respective recombinant strains MEL2[TLG1-SFA1] and M2n[TLG1-SFA1], were utilised for wheat bran fermentation. The engineered strains contained the *TLG1* gene (glucoamylase from *T. lanuginosus*) expressed under the control of the *ENO1* promoter and the *SFA1* gene (α–amylase from *S. fibuligera*) expressed under the control of the *PGK1* promoter sequences [12]. Both genes were codon optimised for expression in *S. cerevisiae* and integrated into the delta sequences on the genomes of the industrial *S. cerevisiae* MEL2 and M2n strains [12].

Unless stated otherwise, all chemicals were of analytical grade and were obtained from Merck (Darmstadt, Germany).

The recombinant *S. cerevisiae* strains were maintained on either solid SC^{-URA} agar plates (containing 6.7 g/L yeast nitrogen base without amino acids [Difco Laboratories], 20 g/L glucose and yeast synthetic drop-out medium supplements (Sigma–Aldrich (Germany) or solid YPD (Yeast Peptone Dextrose) medium (10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose and 20 g/L agar).

Culture medium (6.7 g/L yeast nitrogen base, 20 g/L peptone and 20 g/L glucose, 0.05 mM citric acid buffer, pH5) was used to prepare the yeast inocula for the fermentation studies. Fermentation medium is similar to the cultivation medium, but contained 0.5 g/L glucose and 10% (w/v) unmilled wheat bran. The *Aspergillus niger* D15[EgA] strain was maintained on spore plates and

Table 1
Strains and recombinant enzymes used in this study.

Strains	Relevant enzyme[a]	Source organism	Reference
RCC[b]			
S. cerevisiae Y294[CbhI]	Cellobiohydrolase I (CbhI)	*Talaromyces emersonii*	[28]
S. cerevisiae Y294[CbhII]	Cellobiohydrolase II (CbhII)	*Chrysosporium lucknowense*	[28]
Aspergillus niger D15[EgA]	Endoglucanase I (EgA)[c]	*Aspergillus niger*	[29]
S. cerevisiae Y294[Pcbgl1B]	β-glucosidase (Bgl)	*Phanerochaete chrysosporium*	[30]
SSF			
S. cerevisiae MEL2	–	Industrial strain for bioethanol	[15]
S. cerevisiae M2n	–	Semi-industrial strain	[31]
S. cerevisiae MEL2[TLG1-SFA1]	Glucoamylase (TLG1) α-Amylase (SFA1)	*T. lanuginosus* *S. fibuligera*	[12]
S. cerevisiae M2n[TLG1-SFA1]	Glucoamylase (TLG1) α-Amylase (SFA1)	*T. lanuginosus* *S. fibuligera*	[12]

[a] All enzymes were secreted using their native secretion signal, with the exception of Pcbgl1B (using the *T. reesei* Xyn2 secretion signal).
[b] RCC (recombinant cellulase cocktail) [31].
[c] EgA was expressed using the native DNA sequence, whereas all other genes were codon optimised for expression in *S. cerevisiae*.

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