Direct microbial conversion of cellulose into ethanol

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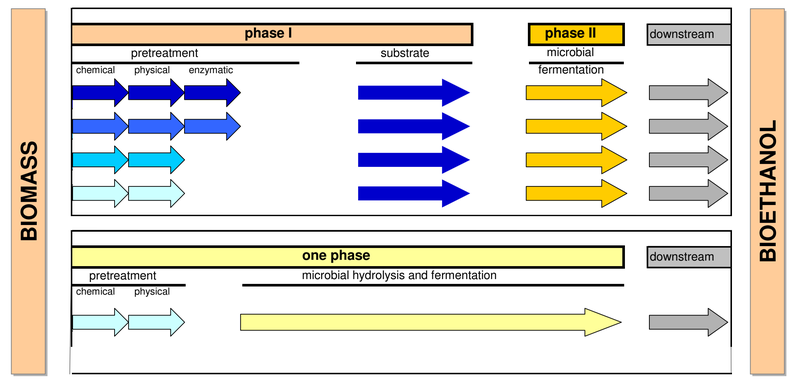
Introduction

Bioethanol is an attractive, sustainable and alternative energy source to conventional fuel. Current ethanol production processes using crops (sugar cane and corn) as a starting substrate are well-established. However, a cheaper feedstock such as lignocellulosic biomass could make bioethanol more competitive with fossil fuel, although its complex structure makes this material more resistant to biological degradation. Recent efforts have focused on the one-step microbial conversion of plant biomass into biofuel since simultaneous microbial hydrolysis and fermentation of lignocellulosic material could represent a strategy to allow cost-effective production of ethanol. This study focused on ethanol production using wheat bran as a model of abundant industrial by-product.

Aim of the project

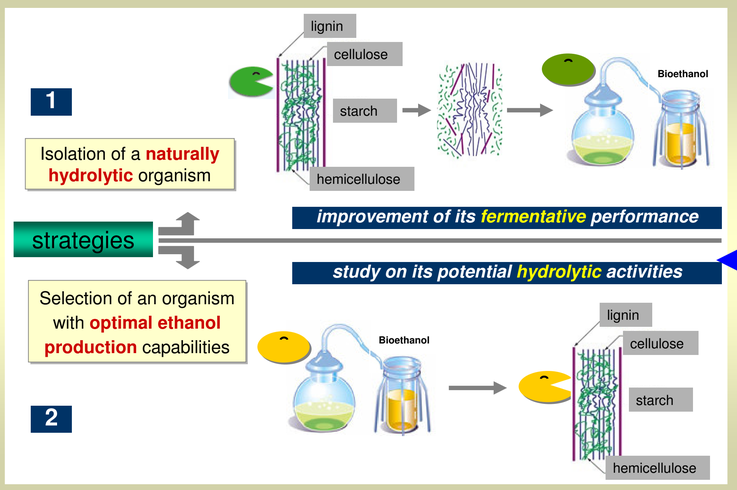
This project aimed to develop a economically feasible process for the one-step bioconversion of wheat bran into ethanol through:

1. the isolation, improvement and possible modification of microrganisms in order to obtain a strain suitable for the industrial ethanol production application.
2. the characterization of wheat bran as feedstock for bioethanol production by analysis of its main components
3. The evaluation of a series of pre-treatments to define the best combination of physical, chemical and enzymatic treatments characterized by low costs, high sugar efficiency and easy industrial applicability.
4. the study of wheat bran hydrolisates fermentability by microbial strains previously selected on the basis of their high ethanol performance (Favaro *et al.*, 2008).



Schematic representation of bioethanol production: double and single step.

Strategies and Methods

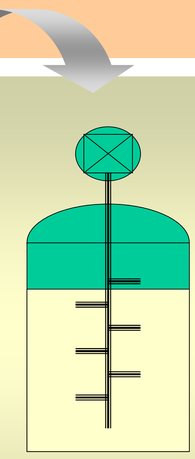


Wild-type microorganisms having both the properties to utilise biomass polysaccharides and to produce ethanol have not been described. The development of such an organisms can be attained by (1) improving ethanol production properties of a naturally hydrolytic organism, (2) endowing hydrolytic abilities to a high ethanol producing organism. During this first project phase, experimental activity focused on the two above perspectives by (1) isolation of hydrolytic organisms and (2) screening of cellulolytic activities on 170 *Saccharomyces cerevisiae* strains.

Pretreatment trails were carried out on wheat bran and milled wheat bran. Deionized water was added to obtain a slurry that was pretreated as indicated in Table 1. The pretreated wheat bran was fermented by *S. cerevisiae* strains previously selected for both high ethanol performance and potential cellulolytic activity.

N. Trials	Thermal treatment	Chemical treatment	Enzymatic treatment
1	100°C, 1h	-	-
2	100°C, 1h	-	MED (single)
3a	121° C, 1h, 1 atm	-	MED (single)
3b		-	MED (complex)
4a	121° C, 1h, 1 atm	-	MIN (single)
4b		-	MED (single)
4c		-	MAX (single)
5a	121° C, 1h, 1 atm	-	-
5b		-	MED (single)
6a	121° C, 1h, 1 atm	H_2SO_4 1%	MIN (single)
6b			MED (single)
6c			MAX (single)
7a	121° C, 1h, 1 atm	-	MAX (single)
7b		H_2SO_4 1%	MAX (single)

Table 1. Experimental scheme of pretreatments on wheat bran.
All enzymes (*Biomass Kit*) were used with operating conditions and dosage (MIN:minimum,MED:medium,MAX: maximum) proposed by Novozymes.



Results and Discussion

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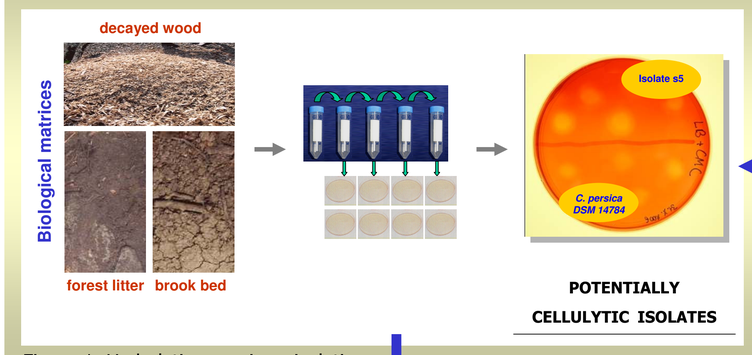
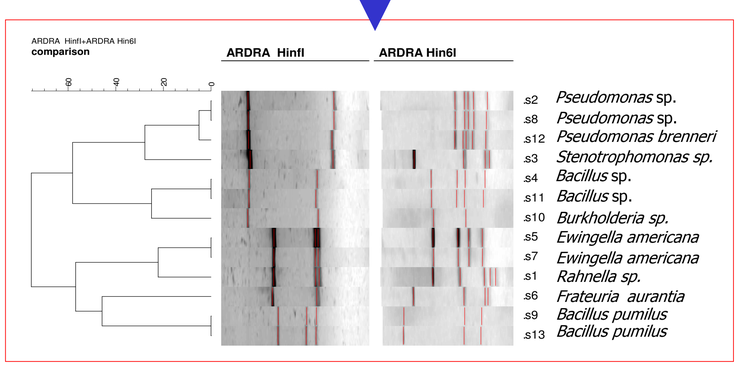


Figure 1. Hydrolytic organisms isolation

Hydrolytic organisms isolation. Samples were collected from various biological matrices. Microbial strains were isolated plating on Tansey medium and Hankin medium (Favaro *et al.*, 2008a). Cellulase activity of the obtained colonies was screened according to Kluepfel method (Kluepfel, 1988). As an example, in Figure 1 cellulose degradation of the isolate named s5 is compared to the activity of the reference strain *Cellulomonas persica* DSM 14784.

***S. cerevisiae*: screening of cellulolytic activities.** One hundred and seventy *S. cerevisiae* strains isolated from grape marc on the basis of their fermentative vigour (Delfini, 1995) were screened for cellulose degradation. Eighteen *S. cerevisiae* strains showed a potential activity, although their growth on media containing cellulose was slow.

12 strains with the capability to degrade cellulose comparable to that of *C. persica* were characterized by ARDRA technique (Amplified Ribosomal DNA Restriction Analysis). Prokaryotic small subunit rDNA were amplified using eubacterial primers 1389r and 63F (Hongoh *et al.*, 2003) and digested with restriction endonucleases. The analyses of their restriction patterns performed by the G*elCompar*II program (Applied Maths) indicated a strict similarity between bacterial strain s2 and s8, s4 and s11, s5 and s7, respectively. These results were confirmed by sequencing 16S rDNA gene of the 12 microbial isolates.



N. trials	Total sugars (g/L)	Glucose (g/L)	T4AC6	EC1118	Ethanol (g/L)	Yield (g Ethanol/ g total sugars)
3a	19.2	17.4	+		7.5	0.39
	19.1	17.5		+	7.4	0.38
3b	16.1	15.6	+		6.8	0.43
	16.2	15.4		+	6.4	0.40
4a	14.5	12.4	+		7.5	0.52
	14,6	12,5		+	6.8	0.47
4b	18.4	16.3	+		8.1	0.44
	18.4	16.5		+	7.4	0.40
4c	19.8	16.5	+		8.5	0.43
	19.9	16.9		+	7.9	0.40

Table 2. Selected experimental data of wheat bran fermentation trials by *S. cerevisiae* T4AC6 and *S. cerevisiae* EC118.
Pretreatments and fermentation studies were conducted in collaboration with the research group of Prof. Cecchi (University of Verona).

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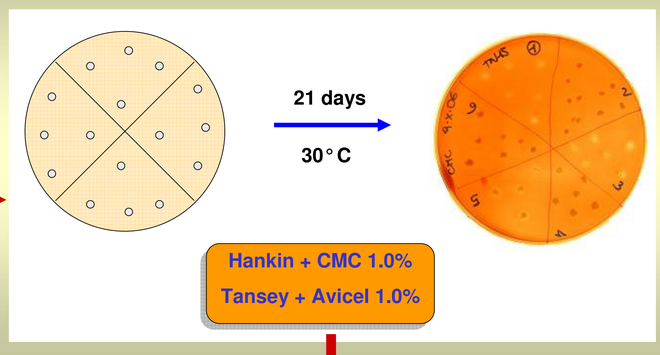

S. cerevisiae T4AC6 showing the highest potential cellulase activity was selected for the bioconversion of wheat bran into ethanol. Wheat bran previously pretreated as indicated in table 1 was fermented by this strain and by the reference strain *S. cerevisiae* EC1118, a non cellulolytic yeast isolated from Champagne wine plants. As shown in table 2, *S. cerevisiae* T4AC6 presented a higher ethanol yield (grams of ethanol/grams total sugars) on every bran slurry tested.

Conclusions

The collection of new cellulolytic strains selected for their high hydrolytic ability is a good platform to achieve the development of a single step microbe for bioethanol production. Further characterization and study on their potential ethanol properties are in progress. Moreover, the preliminary results of fermentation trials suggest that the definition of effective pretreatments is crucial for utilising wheat bran to produce ethanol. The efficiency of the pre-treatments studied could also be further improved by dosage optimization.

However, on the basis of our results, the most promising approach to develop the one-step bioconversion of cellulose into biofuel seems to rely on oenological yeasts with desired properties for both cellulose hydrolysis and fermentation. In addition, these natural yeasts could be further genetically modified for the construction of more efficient recombinant strains using cellulolytic microrganisms isolated as source of genes for hydrolytic activities.

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