microbial biotechnology

Microbial Biotechnology (2010) **3**(3), 336–343 doi:10.1111/j.1751-7915.2009.00158.x

Isolation and characterization of *Cupriavidus basilensis* HMF14 for biological removal of inhibitors from lignocellulosic hydrolysate

Nick Wierckx,[1,2,3*†] Frank Koopman,[2,3,4†] Luaine Bandounas,[2,3,4] Johannes H. de Winde[2,3,4] and Harald J. Ruijssenaars[1,2,3]

[1]*TNO Quality of Life, Department of Bioconversion, Julianalaan 67, 2628 BC, Delft, the Netherlands.*

[2]*Bio-based sustainable industrial chemistry (B-Basic), Julianalaan 67, 2628 BC, Delft, the Netherlands.*

[3]*Kluyver Centre for Genomics of Industrial Fermentation. PO box 5057, 2600 GB, Delft, the Netherlands.*

[4]*Delft University of Technology, Department of Biotechnology. Julianalaan 67, 2628 BC, Delft, the Netherlands.*

Summary

The formation of toxic fermentation inhibitors such as furfural and 5-hydroxy-2-methylfurfural (HMF) during acid (pre-)treatment of lignocellulose, calls for the efficient removal of these compounds. Lignocellulosic hydrolysates can be efficiently detoxified biologically with microorganisms that specifically metabolize the fermentation inhibitors while preserving the sugars for subsequent use by the fermentation host. The bacterium *Cupriavidus basilensis* HMF14 was isolated from enrichment cultures with HMF as the sole carbon source and was found to metabolize many of the toxic constituents of lignocellulosic hydrolysate including furfural, HMF, acetate, formate and a host of aromatic compounds. Remarkably, this microorganism does not grow on the most abundant sugars in lignocellulosic hydrolysates: glucose, xylose and arabinose. In addition, *C. basilensis* HMF14 can produce polyhydroxyalkanoates. Cultivation of *C. basilensis* HMF14 on wheat straw hydrolysate resulted in the complete removal of furfural, HMF, acetate and formate, leaving the sugar fraction intact. This unique substrate profile makes *C. basilensis* HMF14 extremely well suited for biological removal of inhibitors from lignocellulosic hydrolysates prior to their use as fermentation feedstock.

Received 8 September, 2009; revised 16 October, 2009; accepted 21 October, 2009. *For correspondence. E-mail N.J.P.Wierckx@tudelft.nl; Tel. (+31) 15 2787905; Fax (+31) 15 2782355. †These authors contributed equally to this study.

Introduction

Lignocellulosic materials provide a potential source of renewable feedstock for the sustainable production of biofuels and other biochemicals. This concept has been heralded as a viable alternative for traditional oil-based fuel and chemicals production with widespread socio-economical and environmental benefit (Olsson and Hahn-Hagerdal, 1996; Lee, 1997; Thomsen and Haugaard-Nielsen, 2008). For use as feedstock in fermentative production processes, the sugars within the lignocellulosic matrix are commonly released by acid pretreatment followed by either chemical or enzymatic hydrolysis. A major drawback of this procedure is the formation of toxic by-products (Palmqvist and Hahn-Hagerdal, 2000a; Klinke *et al.*, 2004; Mussatto and Roberto, 2004). In general, three groups of fermentation inhibitors can be distinguished: furan derivatives, organic acids and aromatics. These compounds severely inhibit the growth of microorganisms, leading to a marked decrease of overall productivity in fermentation processes (Taherzadeh *et al.*, 1997; Palmqvist and Hahn-Hagerdal, 2000b; Mussatto and Roberto, 2004). The toxic effect of individual inhibitors is exacerbated by synergistic effects with other toxic feedstock components as well as fermentation products (Palmqvist and Hahn-Hagerdal, 2000a; Zaldivar *et al.*, 2000; Nigam, 2001).

Several methods to remove inhibitors from lignocellulosic hydrolysate have been described, such as ether extraction, alkaline precipitation or enzymatic treatment using laccases (Palmqvist and Hahn-Hagerdal, 2000b; Mussatto and Roberto, 2004). Biological detoxification or bio-abatement relies on microorganisms that specifically remove fermentation inhibitors. This presents an attractive alternative to physico-chemical detoxification, because it does not require the addition of chemicals and produces mostly biodegradable waste.

Efficient biological detoxification requires microorganisms with an unusual substrate utilization profile. Ideally all inhibitors should be metabolized while the sugar fraction should be preserved. Several microorganisms, both fungi and bacteria, have been isolated that either

Table 1. Identification of bacteria isolated from enrichment cultures on HMF as the sole carbon source by 16S rDNA sequencing.

Strains	Best blast hit of 16S rDNA	Sequence % identities	Reference
HMF 1, 2, 3, 4, 7, 9, 10, 11, 12	*Telluria mixta* (DQ005909.2)	98%	Bowman *et al.* (1993)
HMF 5, 6, 8	*Arthrobacter nicotianae* (EU857420.1)	99%	Gelsomino *et al.* (2004)
HMF 13, 14	*Cupriavidus basilensis* (AM048887.1)	99%	Steinle *et al.* (1998); Goris *et al.* (2001); Vandamme and Coenye (2004)

completely or partially degrade inhibitors such as furfural, 5-hydroxy-2-methylfurfural (HMF) and acetate, but also sugars (Boopathy *et al*., 1993; Lopez *et al*., 2004; Nichols and Mertens, 2008; Okuda *et al*., 2008; Yu and Stahl, 2008; Hou-Rui *et al*., 2009). These organisms may be suited for fermentation of lignocellulosic feedstock, but for bioabatement purposes, organisms unable to utilize sugars would be greatly preferred.

In this study, we isolated several bacterial species from soil and water that utilize HMF as the sole carbon source. One of the isolates, *Cupriavidus basilensis* HMF14, utilized HMF, furfural and a wide variety of organic acids and aromatics as a sole carbon source. Remarkably, *C. basilensis* HMF14 was unable to metabolize sugars. When cultured in wheat straw hydrolysate, fermentation inhibitors were removed while retaining the sugar fraction. Furthermore, this bacterium is capable of producing polyhydroxyalkanoates (PHA). The combination of these traits makes *C. basilensis* HMF14 a promising microorganism for cost-effective biological removal of inhibitors from lignocellulosic hydrolysate.

Results

Enrichment and characterization of HMF-degrading bacteria

In search for (prokaryotic) microorganisms that can utilize HMF as a sole carbon source, we inoculated enrichment cultures on HMF-supplemented minimal medium with soil and water samples. After two transfers into fresh medium, the cultures were plated on solid HMF medium to isolate individual bacteria capable of degrading HMF. Fourteen individual colonies were selected and initial identification was performed by partial 16S rDNA sequencing. The isolates were found to belong to three distinct genera (Table 1): *Telluria*, *Arthrobacter* and *Cupriavidus* [formerly known as *Ralstonia* (Vandamme and Coenye, 2004)]. Phenotypic characterization confirmed that all isolates utilized HMF as a sole carbon source. In addition, all isolates were capable of utilizing furfural. Interestingly, isolates HMF13 and HMF14 were the only isolates not capable of utilizing glucose. Moreover, HMF13 and HMF14 could be easily cultured and genome sequences of related strains were available (Schwartz *et al*., 2003; Pohlmann *et al*., 2006; Fricke *et al*., 2009). Strain HMF14 was selected for further study.

Phenotypic characterization of HMF-degrading strain HMF14

Isolate HMF14 was able to grow on gluconate, succinate, citrate, acetate, benzene, toluene and phenol. No growth was observed on glucose, xylose, arabinose and mannose. Cells were short rods, either single, in pairs or in short chains. On LB agar plates, round colonies were formed that had a mucous appearance. Formation of a mucous extracellular matrix was also observed in liquid cultures. Strain HMF14 could be cultured at temperatures up to 41°C and did not show anaerobic nitrate respiration. As both the 16S rDNA sequencing and the phenotypic characteristics best matched the type species of *C. basilensis* (DSMZ 11853^T) (Steinle *et al*., 1998; Goris *et al*., 2001; Vandamme and Coenye, 2004), strain HMF14 was designated *C. basilensis* HMF14 (DSM 22875).

The genus *Cupriavidus* is well known for its ability to efficiently produce PHA (Yu and Stahl, 2008; Reinecke and Steinbuchel, 2009). In order to verify PHA production by the newly isolated *C. basilensis* HMF14, this strain was cultivated in minimal medium with acetate as a carbon source. Fluorescence microscopic analysis showed PHA granules within the cells of *C. basilensis* (Fig. 1).

Degradation of furan derivatives by C. basilensis *HMF14*

In addition to HMF, other furan derivatives are present in lignocellulosic hydrolysates. In order to demonstrate whether *C. basilensis* HMF14 was capable of utilizing furan derivatives other than HMF, growth was assessed on minimal medium with 3.5 mM HMF, furfural, furfuryl alcohol or furoic acid as sole carbon source. Growth was observed on all tested furan derivatives, with slightly different growth characteristics (Table 2). Cultures on furfural rapidly converted the substrate to furfuryl alcohol during the lag phase, while a small amount of furoic acid accumulated (Fig. 2). Conversion of furfural to its alco-

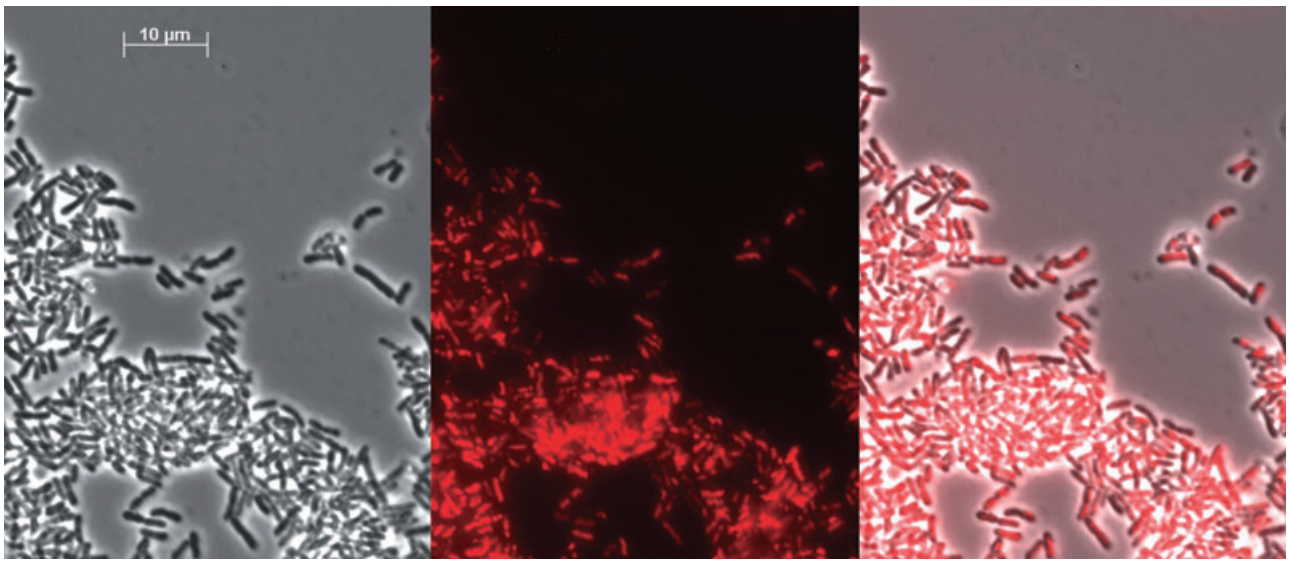

Fig. 1. Detection of PHA in cultures of *C. basilensis* HMF14 in minimal medium with 120 mM acetate. Left: Phase contrast image. Middle: Fluorescence microscopic image of the same slide stained with Nile Blue A. Right: Overlay of the two previous images.

holic and/or acid form is a common mechanism of furfural detoxification (Boopathy *et al*., 1993; Liu *et al*., 2004; Okuda *et al*., 2008). At the onset of logarithmic growth, furfuryl alcohol production decreased in favour of biomass formation, which likely proceeds via furoic acid (Trudgill, 1969; Koenig and Andreesen, 1990). Similarly, HMF-acid and -alcohol were formed in cultures with HMF as the carbon source. In addition, trace amounts of 2,5-furandicarboxylic acid and furoic acid were found in the HMF cultures (not shown).

Cupriavidus basilensis HMF14 grew in the presence of 5 mM of furfural or HMF (0.48 g l^{-1}, and 0.63 g l^{-1} respectively). However, the concentration of these toxic compounds is often higher in lignocellulosic hydrolysates, ranging from 0 to 3.5 g l^{-1} for furfural, and from 0 to 5.9 g l^{-1} for HMF (Klinke *et al*., 2004; Almeida *et al*., 2009). Therefore, the tolerance of *C. basilensis* HMF14 towards furfural and HMF was determined in shake-flask cultures with 3–15 mM furfural or HMF (Fig. 3). The time to double the starting optical density was found to increase with increasing concentrations of HMF or furfural, likely as a result of substrate toxicity. Nevertheless, after 24 h of cultivation growth was observed at all concentrations tested (not shown). No stable exponential growth phase was reached at furfural concentrations above 6 mM because the apparent growth rates increased continuously. Also in the HMF cultures, no stable exponential growth was obtained; however, the apparent growth rate decreased rather than increased with increasing HMF concentrations.

Table 2. Growth characteristics of *C. basilensis* HMF14 on furan derivatives.

Carbon source	μ_{max} (h^{-1})	Max. OD_{600}
HMF	0.25[a]	0.95
Furfural	0.22	1.09
Furfuryl alcohol	0.22	1.08
Furoic acid	0.29	0.99

a. This culture did not reach a stable exponential growth phase, since the growth rate increased continuously.

Removal of inhibitors from lignocellulosic hydrolysate using C. basilensis *HMF14*

In addition to furanic compounds, lignocellulosic hydrolysate contains many other components that can inhibit fermentation. While *C. basilensis* HMF14 was unable to

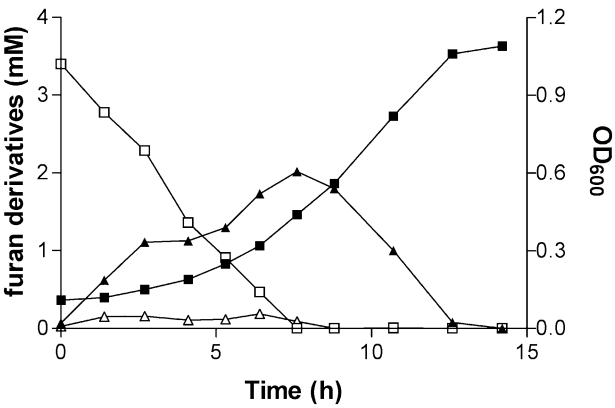

Fig. 2. Growth of *C. basilensis* HMF14 on minimal medium with furfural as the sole carbon source. □, furfural; ▲, furfuryl alcohol; △, furoic acid; ■, OD_{600}. Cultures were performed in triplicate and the variation between replicate data points was less than 10%.

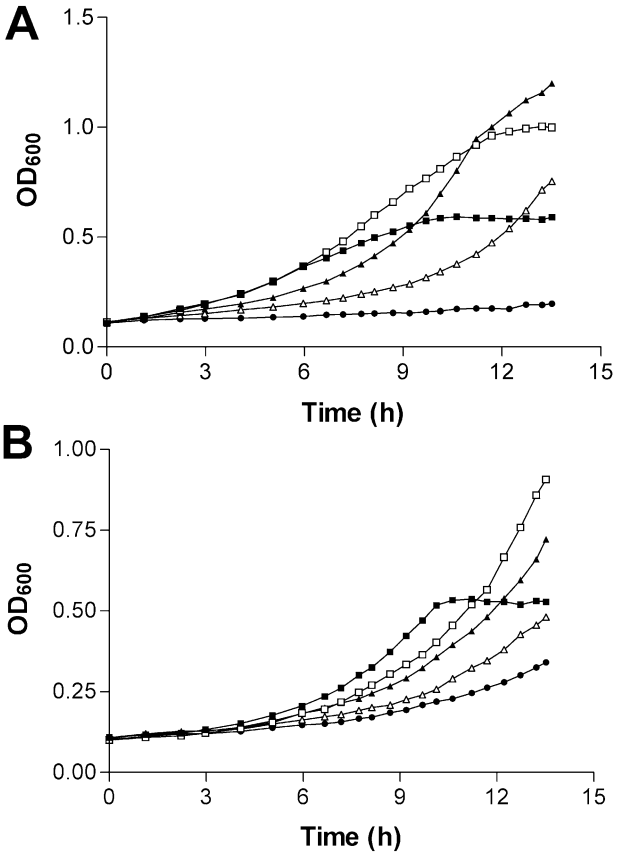

Fig. 3. Growth of *C. basilensis* HMF14 on different concentrations of furfural (A) or HMF (B). The concentrations used were ■, 3 mM; □, 6 mM; ▲, 9 mM; △, 12 mM; ●, 15 mM. The data points are the averages of duplicate experiments. The maximum variation between duplicates was less than 10%.

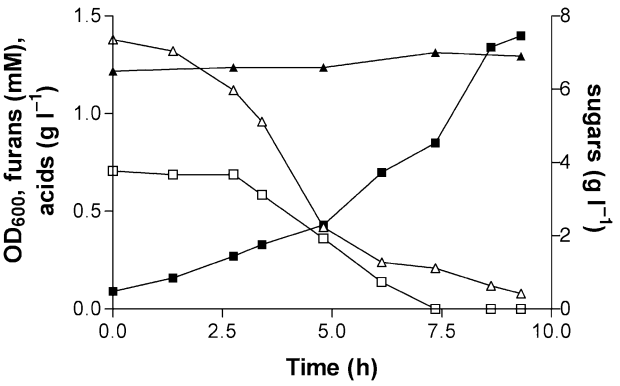

Fig. 4. Inhibitor removal from threefold diluted lignocellulosic hydrolysate WSH1 by *C. basilensis* HMF14. The sugars concentration (▲) is the sum of the concentrations of glucose, xylose and arabinose. The furans concentration (△) is the sum of the concentrations of the alcohol, aldehyde and acid derivatives of furfural and HMF. The acids concentration (□) is the sum of the concentrations of formic and acetic acid; ■; the optical density at 600 nm, corrected for the hydrolysate background.

Table 3. Growth of *C. basilensis* HMF14 in shake-flask cultures on minimal medium with lignocellulosic hydrolysate constituents as the sole source of carbon and energy.

Compound	Growth
Sugars	
Glucose	–
Xylose	–
Arabinose	–
Mannose	–
Furans	
Furfural	+
Furfuryl alcohol	+
Hydroxymethylfurfural	+
Furoic acid	+
Organic acids	
Acetic acid	+
Formic acid	+[a]
Levulinic acid	+
Ferulic acid	+
Aromatics	
4-Hydroxybenzoic acid	+
Vanillic acid	+
Syringic acid	–
Phenol	+
4-Hydroxybenzaldehyde	+
4-Hydroxybenzyl alcohol	+
Guaiacol	+
Vanillin	+
Vanillyl alcohol	+
Syringol	–
Syringaldehyde	–

a. Formic acid was only co-utilized with another primary carbon source, e.g. acetate.

degrade the sugars present in lignocellulosic hydrolysate, it was shown to metabolize many toxic hydrolysate constituents (Table 3). This further confirmed that C. *basilensis* HMF14 has a unique substrate specificity that makes this strain ideally suited for the removal of inhibitors from lignocellulosic hydrolysate.

The inhibitory effect of lignocellulosic hydrolysate on fermentation is brought about by a concerted effect of many toxic constituents (Palmqvist and Hahn-Hagerdal, 2000b; Zaldivar *et al*., 2000). In order to evaluate the capacity of *C. basilensis* HMF14 to selectively remove a mix of inhibitors from lignocellulosic hydrolysate, the strain was cultured in minimal medium with threefold diluted wheat straw hydrolysate WSH1 (MMhyd). Figure 4 shows that the furan derivatives, acetate and formate were completely removed after only 10 h of cultivation. During this period, the glucose, xylose and arabinose concentrations were unchanged. When the incubation was prolonged after the inhibitors were consumed, the sugar concentration decreased by approximately 11% within 15 h. The content of glucose, xylose and arabinose decreased simultaneously and to the same relative extent.

In order to assess the ability of *C. basilensis* HMF14 to remove inhibitors from a more concentrated and therefore toxic lignocellulose hydrolysate, it was cultured in WSH2. As expected, this hydrolysate was found to be more toxic

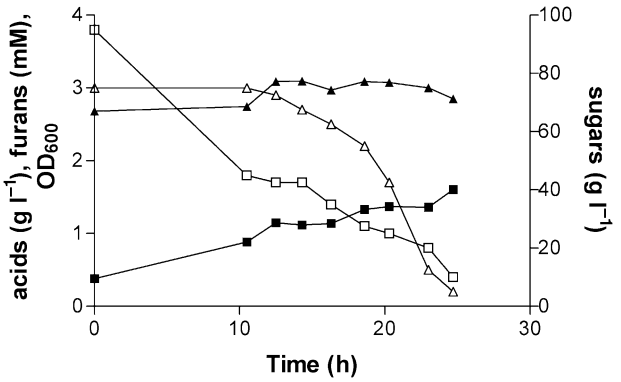

Fig. 5. Inhibitor removal from lignocellulosic hydrolysate WSH2 by *C. basilensis* HMF14. ▲, total sugars concentration; △, total furans concentration; □, total acids concentration; ■, optical density at 600 nm, corrected for the hydrolysate background.

to *C. basilensis* HMF14, which was reflected by the four-fold increased inoculum density required to achieve growth compared with the WSH1 cultures. Nevertheless, C. *basilensis* HMF14 was capable of removing the inhibitors from WSH2 within 25 h without affecting the total sugars concentration as shown in Fig. 5.

Discussion

The presence of inhibitory compounds in lignocellulosic hydrolysate puts a serious constraint on the effective use of biomass as feedstock for the fermentative production of biofuels and bio-chemicals. (Palmqvist and Hahn-Hagerdal, 2000a,b; Klinke *et al.*, 2004; Mussatto and Roberto, 2004; Almeida *et al.*, 2007). Biological detoxification (bioabatement) of lignocellulosic hydrolysates has been shown to be an effective method of inhibitor removal and many microorganisms have been identified over the past few years that either degrade inhibitors or convert them into less toxic forms (Boopathy *et al.*, 1993; Lopez *et al.*, 2004; Nichols *et al.*, 2005, 2008; Okuda *et al.*, 2008; Yu and Stahl, 2008; Hou-Rui *et al.*, 2009). All of these microorganisms, however, also metabolize sugars which potentially compromise the fermentable sugar content of the bioabated lignocellulosic hydrolysate. By contrast, *C. basilensis* strain HMF14 isolated in the present study does not utilize sugars while metabolizing the majority of inhibitory compounds in lignocellulosic hydrolysate.

Cupriavidus basilensis HMF14 was isolated on HMF, but also utilized furfural. The pathways by which these furanic aldehydes are metabolized are largely unknown, although a degradation route for furfural has been proposed based on enzyme activities (Trudgill, 1969; Koenig and Andreesen, 1990; Nichols and Mertens, 2008). In the proposed pathway, furfural is oxidized to furoic acid which enters the cellular metabolism as the actual substrate for growth. Also *C. basilensis* HMF14 appeared to consume furfural via furoic acid because this compound was transiently observed in furfural-grown cultures. The steady state levels of furoic acid in growing cultures were very low, suggesting that furoic acid is metabolized with such efficiency that no appreciable accumulation occurs. During the initial growth phase, however, *C. basilensis* HMF14 rapidly reduced furfural to its alcoholic form. This may appear inefficient because furfuryl alcohol must be re-oxidized to furfural prior to the final oxidation to furoic acid. Considering the high toxicity of furanic aldehydes, however, the initial preference of furfural reduction over oxidation may be interpreted as a classical, non-specific detoxification mechanism to ensure that furfural concentrations are kept below inhibitory levels. The accumulated furfuryl alcohol subsequently serves as a relatively non-toxic substrate pool for generating furoic acid.

The assumption that furoic acid is the actual growth substrate is in agreement with the observation that no stable exponential growth phase was observed on furfural or furfuryl alcohol. The growth rate on furoic acid was higher than on furfural or furfuryl alcohol, which suggests that the apparent growth rate is determined by the interconversions between furfural, furfuryl alcohol and furoic acid. This may also explain the higher apparent growth rates in cultures with higher initial concentrations of furfural. These probably affect the alcohol–aldehyde–carboxylic acid equilibrium such that a higher rate of furoic acid formation occurs, resulting in an apparent growth rate that approaches the maximum growth rate on furoic acid.

Considering the structural similarity between HMF and furfural, it may be expected that these compounds are metabolized at least partly via a shared pathway. This is supported by the observation that all HMF degraders isolated in this study were capable of utilizing furfural. In addition, transient accumulation of furoic acid was observed in cultures of *C. basilensis* HMF14 growing on HMF. The even more complex interconversions that determine the lag phase and growth rate on this substrate will be addressed in a separate paper (F. Koopman, manuscript in preparation).

In addition to individual compounds, complex mixtures of toxic inhibitors were also efficiently metabolized by *C. basilensis* HMF14 as exemplified by the removal of inhibitors from two batches of hydrolysate. The fact that *C. basilensis* HMF14 was able to grow in the more toxic batch of hydrolysate by increasing the inoculum size is in good agreement with results described previously (Yu and Stahl, 2008). In both batches, the treatment resulted in a solution of sugars while the concentration of furanic compounds, acetate and formate was reduced to less than 10% of the starting concentration. *Cupriavidus basilensis* HMF14 did not utilize individual sugars, but prolonged incubation with dilute wheat straw hydrolysate appeared

to result in slightly decreased soluble sugar concentrations. It was considered unlikely that the sugars were actually metabolized, because all three sugars disappeared simultaneously and to the same relative extent. In addition, no polyol (sorbitol, xylitol, arabitol) formation was observed, indicating that the sugars were not converted in its corresponding polyols. Rather, the sugar content may have decreased as a result of adsorption to the mucous extracellular matrix of *C. basilensis* HMF14.

Based on the present study, it is concluded that the unique substrate profile of *C. basilensis* HMF14 makes this bacterium promising for biological removal of inhibitors from lignocellulosic hydrolysate under aerobic conditions. An aerobic treatment of lignocellulosic hydrolysate prior to a fermentation process may be advantageous because *C. basilensis* HMF14 would not be able to grow under the (anaerobic) fermentation conditions. Its capability of producing PHA may furthermore contribute to cost-effectiveness because the biomass generated in the bioabatement treatment may be employed for the production of bioplastics (Yu and Stahl, 2008). Further studies on the feasibility of this process are currently ongoing as well as studies to demonstrate the effect of bioabatement on the fermentability of toxic lignocellulosic hydrolysates for industrially relevant microorganisms, such as *Saccharomyces cerevisiae*, *Zymomonas mobilis*, *Clostridium acetobutylicum* and *Escherichia coli*.

Experimental procedures

Culture conditions

Cultures were performed either in Luria broth (LB) or in a minimal medium (MM) described previously (Hartmans *et al*., 1989). Enrichment cultures were performed in liquid MM medium with 20 mM HMF as the carbon source (MMH_{20}), supplemented as indicated with 0.1 g l^{-1} yeast extract as vitamin source (MM_yH_{20}). Soil and water samples were collected from the botanical garden of the Delft University of Technology and from a peat lake named 'het Kootwijkerveen' near Apeldoorn, the Netherlands. The samples were mixed and approximately 1 g was used to inoculate 50 ml MMH_{20} or MM_yH_{20} in 500 ml Erlenmeyer flasks that were incubated at 30°C for 2 days in a rotary shaker. One millilitre samples were transferred twice to fresh medium and incubated until bacterial growth was apparent ($OD_{600} > 1$). The final enrichment cultures were streaked onto MM medium solidified with 1.5% (w/v) agar and containing 10 mM HMF (MMH_{10}) or the same medium supplemented with 0.1 g l^{-1} yeast extract (MM_yH_{10}). The plates were incubated at 30°C until colonies appeared.

Wheat straw hydrolysate batch 1 (WSH1), made by acid hydrolysis, was obtained from Johan van Groenestijn (TNO Quality of Life, Zeist, the Netherlands). The hydrolysate contained 14.2 g l^{-1} glucose, 7.9 g l^{-1} xylose, 0.8 g l^{-1} arabinose, 42.2 mg l^{-1} HMF, 346 mg l^{-1} furfural, 60.5 mg l^{-1} formate and 197.1 mg l^{-1} acetate. A more concentrated batch of wheat straw hydrolysate (WSH2), was obtained from an industrial source. This hydrolysate contained 67 g l^{-1} total sugars, 3 mM total furans, 3.8 g l^{-1} formate and acetate. The hydrolysates were neutralized by adding 37 mM phosphate buffer (pH 7) and adjusting the pH to 7.0 with 10 M NaOH, resulting in a brown precipitate. After addition of minimal medium components, the solution was centrifuged at 10 000 *g* for 5 min. The supernatant was filter sterilized through a sterile PTFE filter with a pore size of 0.22 μm. The resulting medium (MMhyd) was inoculated with an overnight pre-culture of *C. basilensis* HMF14 in minimal medium with 3 mM HMF, 3 mM furfural and 12 mM sodium succinate.

Bacterial identification

Partial sequence analysis of the 16S rDNA gene was performed for preliminary identification of the bacteria isolated from the enrichment cultures. Total DNA was isolated with a FastDNA kit (QBioGene/MP Biomedicals) and the partial 16S gene was amplified by PCR using primers FD1/2, AGAGTTTGATCMTG2CAG and RP1/2, ACGGYTACCTTGTTACGACTT (Weisburg *et al*., 1991). PCR products were purified with a Qiaquick PCR purification kit (Qiagen) and sequenced by MWG Biotech AG with the same primers used for amplification. The resulting sequences were assigned GenBank accession numbers GU220488, GU220489, GU220490. Results of 16S rDNA sequencing were confirmed as indicated, by phenotypic characterization tests based on Bergey's Manual of Systematic Bacteriology (Holt, 1984) and Steinle and colleagues (1998).

Analytical methods

Bacterial growth was determined by measuring optical density at 600 nm (OD_{600}) using a Biowave Cell Density Meter (WPA Ltd) or a μQuant MQX200 universal microplate spectrophotometer (Bio-tek), using flat-bottom 96-well microplates (Greiner). For OD_{600} measurements in hydrolysate cultures, lignocellulosic hydrolysate without cells was used as a blank. Furan derivatives were analysed on an Agilent 1100 system equipped with a diode array detector set at 230 nm. The column used was a Zorbax Eclipse XDB-C8 (length, 150 mm; internal diameter, 4.6 mm; particle size, 5 μm; Agilent) operated at 25°C. As eluent, a gradient of acetonitrile in 20 mM KH_2PO_4 (pH 2) with 1% acetonitrile was used at a flow of 1.2 ml min^{-1}, increasing from 0% to 5% in 3.5 min and from 5% to 40% in 2.5 min, set as smooth gradients.

Glucose, xylose and arabinose were analysed by ion chromatography (Dionex ICS3000 system), using a CarboPac PA20 column (length, 150 mm; internal diameter, 3 mm) with 10 mM NaOH at a flow rate of 0.5 ml min^{-1} as the eluent.

For production of PHA, *C. basilensis* HMF14 was cultured in minimal medium with 120 mM acetate as a carbon source and 6 mM $(NH_4)_2SO_4$ as a nitrogen source. Polyhydroxyalkanoate was visualized by fluorescence microscopy using Nile Blue A staining, basically as described by Johnson and colleagues (2009).

Chemicals

The analytical standard of 2,5-furandicarboxylic acid was purchased from Immunosource B.V. (Halle-Zoersel, Belgium).

5-Hydroxymethyl-furoic acid (HMF acid) was purchased from Matrix Scientific (Columbia SC, United States). This compound was found to be highly esterified. Therefore, immediately prior to use, a 10 mM solution of the esterified HMF acid was boiled for 2 h in 2 M H_2SO_4, cooled, and adjusted to pH 7.0 with NaOH after addition of 50 mM of phosphate buffer (pH 7). All other chemicals were purchased from Sigma-Aldrich Chemie B.V. (Zwijndrecht, the Netherlands). 5-Hydroxy-2-methylfurfuryl alcohol was identified based on its UV-VIS spectrum (Boopathy *et al*., 1993).

Acknowledgements

The authors would like to thank Johan van Groenestijn (TNO Quality of Life, Zeist, the Netherlands) for providing hydrolysate WSH1. This project is financially supported by the Netherlands Ministry of Economic Affairs and the B-Basic partner organizations (http://www.b-basic.nl) through B-Basic, a public-private NWO-ACTS programme (ACTS = Advanced Chemical Technologies for Sustainability). This project was co-financed by the Kluyver Centre for Genomics of Industrial Fermentation, which is part of the Netherlands genomics Initiative/Netherlands Organisation for scientific Research.

References

Almeida, J.R.M., Modig, T., Petersson, A., Hahn-Hagerdal, B., Liden, G., and Gorwa-Grauslund, M.F. (2007) Increased tolerance and conversion of inhibitors in lignocellulosic hydrolysates by *Saccharomyces cerevisiae*. *J Chem Technol Biotechnol* **82:** 340–349.

Almeida, J.R.M., Bertilsson, M., Gorwa-Grauslund, M.F., Gorsich, S., and Liden, G. (2009) Metabolic effects of furaldehydes and impacts on biotechnological processes. *Appl Microbiol Biotechnol* **82:** 625–638.

Boopathy, R., Bokang, H., and Daniels, L. (1993) Biotransformation of furfural and 5-hydroxymethyl furfural by enteric bacteria. *J Ind Microbiol Biotechnol* **11:** 147–150.

Bowman, J.P., Sly, L.I., Hayward, A.C., Spiegel, Y., and Stackebrandt, E. (1993) *Telluria mixta* (*Pseudomonas mixta* Bowman, Sly, and Hayward 1988) gen. nov., comb. nov., and *Telluria chitinolytica* sp. nov., soil-dwelling organisms which actively degrade polysaccharides. *Int J Syst Bacteriol* **43:** 120–124.

Fricke, W.F., Kusian, B., and Bowien, B. (2009) The genome organization of *Ralstonia eutropha* strain H16 and related species of the *Burkholderiaceae*. *J Mol Biol* **16:** 124–135.

Gelsomino, R., Vancanneyt, M., and Swings, J. (2004) Reclassification of *Brevibacterium liquefaciens* Okabayashi and Masuo 1960 as *Arthrobacter nicotianae* Giovannozzi-Sermanni 1959. *Int J Syst Evol Microbiol* **54:** 615–616.

Goris, J., De Vos, P., Coenye, T., Hoste, B., Janssens, D., Brim, H., *et al.* (2001) Classification of metal-resistant bacteria from industrial biotopes as *Ralstonia campinensis sp.* nov., *Ralstonia metallidurans sp.* nov. and *Ralstonia basilensis* Steinle *et al.* 1998 emend. *Int J Syst Evol Microbiol* **51:** 1773–1782.

Hartmans, S., Smits, J.P., van der Werf, M.J., Volkering, F., and de Bont, J.A. (1989) Metabolism of styrene oxide and 2-phenylethanol in the styrene-degrading *Xanthobacter* strain 124X. *Appl Environ Microbiol* **55:** 2850–2855.

Holt, J.G. (1984) *Bergey's Manual of Systematic Bacteriology*. Baltimore, MD, USA: Williams & Wilkins.

Hou-Rui, Z., Xiang-Xiang, Q., Silva, S.S., Sarrouh, B.F., Ai-Hua, C., Yu-Heng, Z., *et al.* (2009) Novel isolates for biological detoxification of lignocellulosic hydrolysate. *Appl Biochem Biotechnol* **152:** 199–212.

Johnson, K., Jiang, Y., Kleerebezem, R., Muyzer, G., and van Loosdrecht, M.C. (2009) Enrichment of a mixed bacterial culture with a high polyhydroxyalkanoate storage capacity. *Biomacromolecules* **10:** 670–676.

Klinke, H.B., Thomsen, A.B., and Ahring, B.K. (2004) Inhibition of ethanol-producing yeast and bacteria by degradation products produced during pre-treatment of biomass. *Appl Microbiol Biotechnol* **66:** 10–26.

Koenig, K., and Andreesen, J.R. (1990) Xanthine dehydrogenase and 2-furoyl-coenzyme A dehydrogenase from *Pseudomonas putida* Fu1: two molybdenum-containing dehydrogenases of novel structural composition. *J Bacteriol* **172:** 5999–6009.

Lee, J. (1997) Biological conversion of lignocellulosic biomass to ethanol. *J Biotechnol* **56:** 1–24.

Liu, Z.L., Slininger, P.J., Dien, B.S., Berhow, M.A., Kurtzman, C.P., and Gorsich, S.W. (2004) Adaptive response of yeasts to furfural and 5-hydroxymethylfurfural and new chemical evidence for HMF conversion to 2,5-bis-hydroxymethlfuran. *J Ind Microbiol Biotechnol* **31:** 345–352.

Lopez, M.J., Nichols, N.N., Dien, B.S., Moreno, J., and Bothast, R.J. (2004) Isolation of microorganisms for biological detoxification of lignocellulosic hydrolysates. *Appl Microbiol Biotechnol* **64:** 125–131.

Mussatto, S.I., and Roberto, I.C. (2004) Alternatives for detoxification of diluted-acid lignocellulosic hydrolyzates for use in fermentative processes: a review. *Bioresour Technol* **93:** 1–10.

Nichols, N.N., and Mertens, J.A. (2008) Identification and transcriptional profiling of *Pseudomonas putida* genes involved in furoic acid metabolism. *FEMS Microbiol Lett* **284:** 52–57.

Nichols, N.N., Dien, B.S., Guisado, G.M., and Lopez, M.J. (2005) Bioabatement to remove inhibitors from biomass-derived sugar hydrolysates. *Appl Biochem Biotechnol* **121–124:** 379–390.

Nichols, N.N., Sharma, L.N., Mowery, R.A., Chambliss, C.K., van Walsum, G.P., Dien, B.S., and Iten, L.B. (2008) Fungal metabolism of fermentation inhibitors present in corn stover dilute acid hydrolysate. *Enzyme Microb Technol* **42:** 624–630.

Nigam, J.N. (2001) Ethanol production from wheat straw hemicellulose hydrolysate by *Pichia stipitis*. *J Biotechnol* **87:** 17–27.

Okuda, N., Soneura, M., Ninomiya, K., Katakura, Y., and Shioya, S. (2008) Biological detoxification of waste house wood hydrolysate using *Ureibacillus thermosphaericus* for bioethanol production. *J Biosci Bioeng* **106:** 128–133.

Olsson, L., and Hahn-Hagerdal, B. (1996) Fermentation of lignocellulosic hydrolysates for ethanol production. *Enzyme Microb Technol* **18:** 312–331.

Palmqvist, E., and Hahn-Hagerdal, B. (2000a) Fermentation of lignocellulosic hydrolysates. II: inhibitors and mechanisms of inhibition. *Bioresour Technol* **74:** 25–33.

Palmqvist, E., and Hahn-Hagerdal, B. (2000b) Fermentation of lignocellulosic hydrolysates. I: inhibition and detoxification. *Bioresour Technol* **74:** 17–24.

Pohlmann, A., Fricke, W.F., Reinecke, F., Kusian, B., Liesegang, H., Cramm, R., *et al.* (2006) Genome sequence of the bioplastic-producing 'Knallgas' bacterium *Ralstonia eutropha* H16. *Nat Biotechnol* **24:** 1257–1262.

Reinecke, F., and Steinbuchel, A. (2009) *Ralstonia eutropha* strain H16 as model organism for PHA metabolism and for biotechnological production of technically interesting biopolymers. *J Mol Microbiol Biotechnol* **16:** 91–108.

Schwartz, E., Henne, A., Cramm, R., Eitinger, T., Friedrich, B., and Gottschalk, G. (2003) Complete nucleotide sequence of pHG1: A *Ralstonia eutropha* H16 megaplasmid encoding key enzymes of H-2-based lithoautotrophy and anaerobiosis. *J Mol Biol* **332:** 369–383.

Steinle, P., Stucki, G., Stettler, R., and Hanselmann, K.W. (1998) Aerobic mineralization of 2,6-dichlorophenol by *Ralstonia sp.* strain RK1. *Appl Environ Microbiol* **64:** 2566–2571.

Taherzadeh, M.J., Eklund, R., Gustafsson, L., Niklasson, C., and Liden, G. (1997) Characterization and fermentation of dilute-acid hydrolysates from wood. *Ind Eng Chem Res* **36:** 4659–4665.

Thomsen, M.H., and Haugaard-Nielsen, H. (2008) Sustainable bioethanol production combining biorefinery principles using combined raw materials from wheat undersown with clover-grass. *J Ind Microbiol Biotechnol* **35:** 303–311.

Trudgill, P.W. (1969) Metabolism of 2-furoic acid by *Pseudomonas* F2. *Biochem J* **113:** 577–587.

Vandamme, P., and Coenye, T. (2004) Taxonomy of the genus *Cupriavidus*: a tale of lost and found. *Int J Syst Evol Microbiol* **54:** 2285–2289.

Weisburg, W.G., Barns, S.M., Pelletier, D.A., and Lane, D.J. (1991) 16s ribosomal DNA amplification for phylogenetic study. *J Bacteriol* **173:** 697–703.

Yu, J., and Stahl, H. (2008) Microbial utilization and biopolyester synthesis of bagasse hydrolysates. *Bioresour Technol* **99**: 8042–8048.

Zaldivar, J., Martinez, A., and Ingram, L.O. (2000) Effect of alcohol compounds found in hemicellulose hydrolysate on the growth and fermentation of ethanologenic *Escherichia coli*. *Biotechnol Bioeng* **68:** 524–530.

