www.ias.is
http://dx.doi.org/10.16886/IAS.2015.02 Icel. Agric. Sci. 28 (2015), 15 - 24

Ethanol production by a *Paenibacillus* species isolated from an Icelandic hot spring – Production yields from complex biomass

Jan Eric Jessen[1], Thoroddur Sveinsson[2], Sean Michael Scully[1] and Johann Orlygsson[1*]

*[1]University of Akureyri, Faculty of Natural Resource Sciences, Borgir, Nordurslod 2, 600 Akureyri, Iceland *E-mail: jorlygs@unak.is (corresponding author)*
[2]The Agricultural University of Iceland, Mödruvellir Experimental Station, IS-603, Akureyri, Iceland.

ABSTRACT
Ethanol production using *Paenibacillus* strain J2 was studied on carbohydrates and lignocellulosic biomass hydrolysates including grass (*Phleum pratense*) and barley straw (*Hordeum vulgare*). The strain has a broad substrate spectrum; fermentation of glucose yielded ethanol (major product), acetate, butyrate (minor), hydrogen, and carbon dioxide. At glucose concentrations below 30 mM fermentation was not inhibited. Higher substrate loadings resulted in decreased glucose utilization and a shift of end products towards butyrate. The maximum yields of ethanol were 1.45 mol ethanol mol glucose^{-1}. The end products from lignocellulosic (4.5 g L^{-1} dw) biomass hydrolysates pretreated with 0.5% HCl or NaOH (control was unpretreated) prior to cellulase treatment were investigated. Ethanol production from cellulose hydrolysates without chemical pre-treatment yielded 5.5 mM ethanol g^{-1} with lower yields from paper and lignocellulosic biomasses (1.2-1.7 mM g^{-1}). Ethanol production was enhanced by dilute acid or base pre-treatment combined with enzymatic treatment with the highest yields from grass (3.2 mM ethanol g^{-1}).

Key words: Bioethanol, Lignocellulosic biomass, *Paenibacillus*, Thermophiles

YFIRLIT
Etanól framleiðsla hjá *Paenibacillus*, stofni J2 var rannsakaður á sykrum og hýdrólýstötum úr flóknum lífmassa, m.a. vallarfoxgrasi (*Phleum pratense*) og hveitihálmi (*Hordeum vulgare*). Stofninn var með breytt hvarfefnasvið; gerjun á glúkósa leiddi til myndunar á etanóli (meginafurð), ediksýru, smjörsýru, vetnis og koltvísýrings. Of hár upphafsstyrkur (> 30 mM) glúkósa leiddi til hindrunar á niðurbroti hans og aukinnar framleiðslu á smjörsýru. Hámarksframleiðsla á etanóli var 1.45 mól etanól mól glúkósa^{-1}. Lokaafurðir úr hýdrólýsötum úr flóknum lífmassa (4.5 g L^{-1} þurrvigt) sem var formeðhöndlaður með annað hvort 0.5% HCl eða NaOH auk sellulasa meðhöndlunar var rannsökuð. Etanól framleiðsla úr sellulósa án efnafræðilegrar formeðhöndlunar gaf af sér 5.5 mM etanól g^{-1} en minni nýting var á pappír og flóknum lífmassa (1.2-1.7 mM g^{-1}). Etanólframleiðsla á grasi jókst með sýru- og basameðhöndlun ásamt ensímformeðhöndlun, en hámarksnýting var 3.2 mM etanól g^{-1}.

INTRODUCTION

Paenibacillus is a large genus currently containing 148 species and 4 subspecies according to the Euzeby List of Prokaryotes with standing in Nomenclature (LPSN); many species are notable for their potential applications in biotechnology. Species belonging to the genus are typically gram-positive, mesophilic, endospore-forming flagellated rods that are facultative anaerobes or strict aerobes (Priest, 2009). Several moderate thermophiles within the genus have been described including *P. thermophilus*, *P. thermoaerophilus*, *P. marinum,* and *P. tezpurensis* (Zhou et al. 2012, Ueda et al. 2013, Bouraoui et al. 2013, Rai et al. 2010). The genus was originally established in 1993 (Ash et al. 1993) when *Bacillus* was shown to consist of at least five phyletic lines and one of them was reclassified to form the separate genus of *Paenibacillus*; *Paenibacillus polymyxa* was designated as the type species. In 1996, the genus *Paenibacillus* was reassessed on the basis of polyphasic taxonomic results (Heyndrickx et al. 1996). Paenibacilli can be isolated from a wide range of habitats including plant materials, soil, and geothermal areas (Ash et al. 1993, Logan et al. 2004, Khianngam et al. 2009, Behrendt et al. 2010, Zhou et al. 2012).

Ethanol production from complex biomass such as lignocellulose by using thermophilic bacteria has received increased interest in the past ten years (Taylor et al. 2009, Scully & Orlygsson 2015). The primary reasons include the ability of many thermophiles to tolerate extremes of both temperature and pH, their increased doubling times as compared to mesophiles, simplified end product profiles, and decreased susceptibility to mesophilic contamination. Additionally, many thermophiles have broad substrate spectra making them well suited to degrading the monomers present in complex biomass. Strict anaerobes within the genera of *Clostridium, Thermoanaerobacter,* and *Thermoanaerobacterium* have been extensively studied for their ethanol production from sugars and biomass (Wiegel et al. 1983, Lacis & Lawford, 1988, Lynd et al. 1989, Almarsdottir et al. 2012) while little attention has been paid to other Firmicutes such as members of the genus *Paenibacillus*. Few reports on the fermentative metabolism of members of *Paenibacillus* have been reported despite some strains being able to degrade cellulose and other polymeric substrates (Dasman et al. 2002, Daane et al. 2002). *Paenibacillus macerans* (formerly *Bacillus macerans*) has been reported to produce 1.3 mole of ethanol from one mole of glucose (Weimer, 1984a); this species is very versatile even degrading glycerol and deoxyhexoses to 1,2-propanediol (Weimer, 1984b, Gupta et al. 2009).

The present investigation was towards ethanol production by a *Paenibacillus* strain J2, isolated from an Icelandic hot spring, using both simple sugars and various lignocellulosic biomasses. Special attention was given to the effect of various culture variables on ethanol yields.

MATERIALS AND METHODS

Isolation of strain and medium

Strain J2 was isolated on glucose (20 mM) in a basal medium (BM) from a hot spring (50°C, pH 7.4) in Grensdalur in SW Iceland, as described earlier (Sveinsdottir et al. 2009). The BM medium was prepared anaerobically by using the Hungate technique (Hungate, 1969); briefly the medium was boiled for 5-10 min and cooled while flushing with nitrogen. The mixture was then transferred to cultivation bottles closed with butyl rubber stoppers and aluminium caps and autoclaved for 60 minutes. All other components of the medium were added separately through filter-sterilized (Whatman, PES 0.45 µm) solutions. The gas phase in all experiments consisted of nitrogen (99.999% purity, <5 ppm O_2). All carbon sources used were from filter sterilized stock solutions except for polymeric substrates (cellulose, avicel, CMC, xylan, and starch) which were added prior to autoclaving. All experiments were performed at 50°C and pH 7.0 without agitation. The inoculum volume was 2% (v/v) taken from the exponential growth phase of a culture cultivated on 20 mM glucose. All experiments were performed in duplicate.

Phylogenetic analysis
Partial 16S rRNA analysis of a 692 nucleotide long sequence was done according to Vesteinsdottir et al. (2011) and the phylogenetic relationship has been described previously (Sveinsdottir et al. 2009).

Determination of temperature and pH optimum
To determine the pH optimum of the strain, it was cultivated at 50°C with the initial pH set between pH 4.0 and 9.0 with increments of 1.0 pH unit. The pH values of the experimental bottles were adjusted with HCl or NaOH. The temperature optimum was determined at pH 7.0 using seven different temperature ranges (22, 37, 40, 45, 50, 55, 60, 65°C). Experiments were done in 117.5 mL bottles with 50 mL BM medium using glucose (20 mM) as the carbon source. The maximum growth rate (μ_{max}) was obtained from absorbance data as previously described (Orlygsson, 2012).

Substrate utilization spectrum
The ability of strain J2 to utilize different carbohydrates was tested in 24.5 mL serum bottles using a 10 mL BM medium supplemented with various substrates. The substrates tested included xylose, arabinose, glucose, mannose, galactose, fructose, rhamnose, maltose, cellobiose, sucrose, lactose, trehalose, raffinose, starch, cellulose, CMC, avicel, xylan (oat spelt), glycerol, pyruvate, serine, and threonine. Final concentration of all substrates was 20 mM or 0.2% (w/v) for xylan, starch, CMC, cellulose, and avicel. Growth was measured after 5 days using an increase in optical density (600 nm) and hydrogen production as an indicator of positive growth. Samples with positive growth were then further analysed for, hydrogen, volatile fatty acids, and ethanol.

Effect of initial glucose concentrations on end product formation
The effect of initial glucose concentration on the growth behaviour of strain J2 was done by varying the concentration from 5 to 200 mM. Control bottles only contained yeast extract (2 g L^{-1}). Glucose, hydrogen, acetate, butyrate, and ethanol concentrations were measured at the beginning and at the end of the incubation period (5 days). Experiments were done in 117.5 mL serum bottles with 60 mL liquid medium.

Effect of liquid gas phases
The strain was cultivated in 117.5 mL serum bottles containing different amounts of liquid (2 mL, 5 mL, 10 mL, 30 mL, 60 mL, and 90 mL), thus yielding different liquid-to-gas (L/G) phase ratios (0.02 to 3.27).

Pretreatment of biomass and hydrolysate preparation
Four different materials were used for the preparation of hydrolysates: Whatman filter paper (cellulose), newspaper, barley straw (*Hordeum vulgare*), and timothy grass (*Phleum pratense*). The biomass (22.5 g L^{-1}) was either non-chemically treated or chemically (0.5% HCl or NaOH) in addition to enzymatic treatment with cellulases (Celluclast and Novozyme 188) as earlier described (Sveinsdottir et al. 2009, Almarsdottir et al. 2012). After pretreatment, the hydrolysates were adjusted with NaOH or HCl to pH 7.0 since the strain has a pH optimum between pH 7.0 and 8.0 (Sveinsdottir et al. 2009). The hydrolysates were then filtered through 0.45 µm syringe filters into sterile nitrogen-flushed serum bottles.

Fermentation of hydrolysates
Fermentation of carbohydrates present in hydrolysates was performed in 24.5 mL serum bottles with 8.0 mL of BM medium and 2.0 mL of hydrolysate for a final hydrolysate concentration of 4.5 g·L^{-1}. Control samples did not contain hydrolysate, only yeast extract (2 g L^{-1}).

Analytical methods
Hydrogen, ethanol, and volatile fatty acids were measured by gas chromatography as previously described (Orlygsson & Baldursson, 2007). Glucose was determined by slight modification of the anthrone method of Laurentin and Edwards (2003); briefly, centrifuged sample (400 µL) was mixed with 2 mL of anthrone solution (0.2%

[w/v] anthrone in 72% [v/v] sulfuric acid). The sample was boiled for 11 minutes and then cooled on ice. Absorbance was then measured at 600 nm by using a Perkin-Elmer Lambda-25 UV-Vis spectrophotometer.

RESULTS AND DISCUSSION

Physiology

Strain J2 was isolated from a hot spring (50 °C, pH 7.4) in Iceland as described earlier (Sveinsdottir et al. 2009). The strain grows both aerobically (results not shown) and anaerobically; strain J2 is thus a facultative anaerobe as are many members within the genus (Priest, 2009). All data in the present investigation are on its fermentative metabolism. The strain grows at a relatively narrow temperature spectra, from 37°C to 55°C with T_{opt} between 45 and 50°C (generation time 1.76 h for both temperatures). The strain grows between pH 5.0 and 8.0 with a pH_{opt} of 7.0 to 8.0 (generation time 1.99 to 2.05 h).

Phylogeny

The partial 16S rDNA (692 bases) sequence of strain J2 revealed that the strain belongs to the genus *Paenibacillus*. It has 97.9 % similarity with *P. phoenicis*, 97.8% with *P. barengoltzii*, and 96.6% with *P. taiwanensis*. All these strains are mesophilic, either strict aerobes or facultative anaerobes, and do not originate from geothermal areas (Benardini et al. 2011, Osman et al. 2006, Lee et al. 2007). Among thermophilic species, strain J2 has the closest similarity to *P. thermophiles* (95.4%), *P. tezpurensis* (91.6%), *P. marinum* (91.2%), and *P. thermoaerophilus* (90.6%). The most closely related thermophile, *P. thermophiles*, is a strict aerobe. Despite its close relationship to several mesophilic strains, strain J2 may represent a new species based on temperature growth range and temperature optimum. This will be studied in the near future by fully sequencing the strain's 16S gene, more detailed investigation of phenotypic traits, and by DNA-DNA hybridization experiments performed with the closest related species.

Substrate spectrum

Figure 1 shows the fermentative substrate spectrum of strain J2. The strain showed moderately broad substrate spectrum producing a mixture of mainly ethanol and acetate from most of the sugars tested. Starch and xylan were also converted to ethanol and acetate. The ratio between ethanol and acetate was lowest during growth on xylose (1.08) but highest on trehalose (1.75). The highest ethanol concentration was observed on glucose, 25.9 mm (controls subtracted), thus producing 1.29 mol ethanol per mol glucose (64.5% of theoretical yield). More oxidized substrates, such as pyruvate and serine, were predominantly converted to acetate. This is not surprising as the oxidation states of these substrates is greater compared to monosaccharides and thus there is less need to direct the electrons produced to NAD^+ via ethanol production. Ethanol generation by *Paenibacillus* species has received relatively

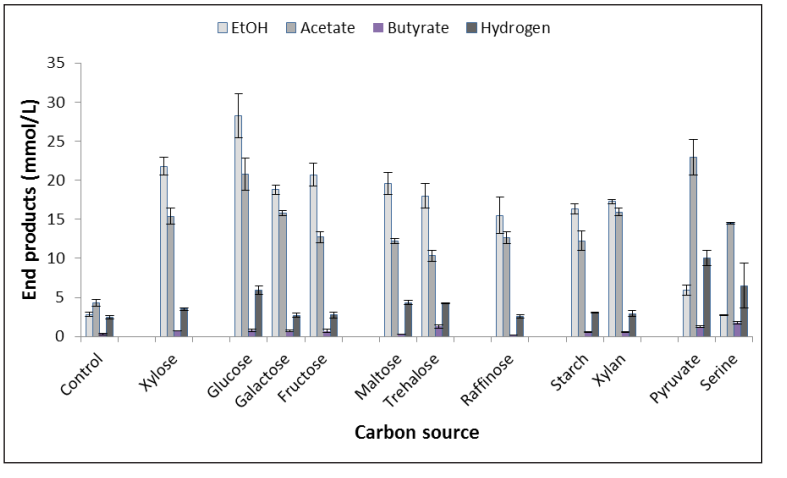

Figure 1. End product formation from various substrates by strain J2. Data represent the average of two replicate experiments. Standard deviations are shown as error bars. Hydrogen values are corrected to volume units.

little attention although *Paenibaillus macerans* has been shown to produce 1.30 mM ethanol per mol of glucose (Weimer, 1984a). Strain J2 did not utilize arabinose, mannose, rhamnose, cellobiose, sucrose, lactose, cellulose, CMC, avicel, glycerol, and threonine.

Effect of different initial glucose concentrations

One of the main obstacles of using thermophilic bacteria for ethanol production is their relatively low tolerance of high initial glucose concentrations, although this can be overcome through the use of continuous culture techniques. Low sensitivity to initial substrate concentrations has been demonstrated with thermophilic members of various genera including *Clostridium* (Orlygsson, 2012), *Thermoanaerobacterium* (Almarsdottir et al. 2012), and *Thermoanaerobacter* (Lacis & Lawford 1988, Sommer et al. 2004). The main reason for this is due to the low buffer capacity of batch cultures these experiments have been performed in, end product inhibition (either acids or hydrogen), or substrate inhibition (Lacis & Lawford, 1988). At low initial glucose concentrations (≤30 mM) strain J2 effectively degraded most of the glucose, with ethanol as the primary end product accompanied by smaller concentrations of acetate, butyrate, and hydrogen (Figure 2A). At higher glucose concentrations, less glucose was degraded; at an initial glucose concentration of 200 mM only 17% was degraded. The most likely reasons for this inhibition are due to lower pH values observed in the experimental bottles with high glucose loadings. *Paenibacillus macerans*, for example, has been shown to stop carbohydrate fermentation when the pH drops below pH 4.9 (Weimer, 1984a). To look at the relationship between pH and acetate formation these data were plotted together (Figure 2B) showing that increased acetate formation leads to lower pH at the end of incubation. Ethanol yields per mole of glucose consumed ranged from 1.18 to 1.45. However, there was a shift in the end product formation with increasing

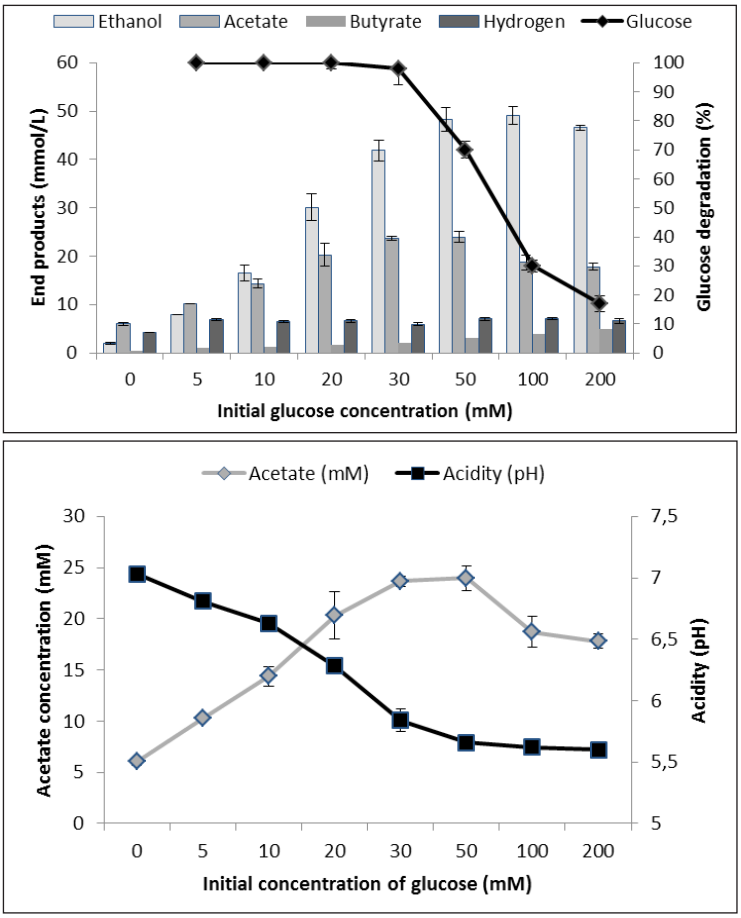

Figure 2. A) End product formation at different initial glucose concentrations. Also shown are the residual glucose concentrations (in percent) after fermentation. Data represent the average of two replicate experiments ± standard deviations. Hydrogen values are corrected to volume units. B) Relationship between acetate and pH after fermentation.

substrate loading. The strain did not produce more ethanol when the substrate concentration was increased; increased glucose loadings shifted end product formation towards butyrate rather than ethanol and acetate. The observed acetate/butyrate ratio was between 13:1 and 10:1 at concentrations of less than 30 mM but to 3.6:1 at the highest glucose concentrations.

Effect of different liquid-gas ratios on strain J2

The partial pressure of hydrogen (pH_2) has been shown to be important as an inhibitor for substrate utilization in strict anaerobes (Hawkes et al. 2002, van Niel et al. 2003). To investigate the effect of pH_2 on end product formation, strain J2 was cultivated with six different ratios of liquid to gas (L/G) phase on 20 mM glucose. Figure 3 shows that the ethanol formation for strain J2 was rather stable over all the different L/G ratios applied. Ethanol production was highest at the lowest L/G ratio (0.02) but acetate, butyrate, and hydrogen production decreased with higher L/G ratios. In all the cultures the amount of ethanol observed was between 23.1 and 31.2 mM, and the amount of acetate between 10.0 and 15.6 mM. It is a well-known phenomenon that the partial pressure of hydrogen (pH_2) strongly influences strict anaerobic bacteria that possess the pyruvate ferredoxin oxidoreductases for hydrogen production (Ben-Bassat et al. 1981, Jones, 2008, Hawkes et al. 2002). Thus, high pH_2 inhibits hydrogenases and directs the electron flow to more reduced end products like ethanol, butyrate and lactate (Levin et al. 2004, Nath & Das, 2004). Indeed, for strain J2, hydrogen together with acetate and butyrate production decreased with increasing L/G phases but ethanol production did not increase (Figure 3). The main reason for this could be that the strain may not possess pyruvate ferredoxin oxidoreductase but pyruvate formate lyase as demonstrated in strains of *P. macerans* (Weimer, 1984a). *P. macerans* produces 1.3 mol ethanol mol glucose^{-1} but during kinetic study of the strain a late appearance of hydrogen and disappearance of formate suggest that formate is cleaved to hydrogen and carbon dioxide (Weimer, 1984a). Also, results indicated that the reducing equivalents for ethanol production were not only derived from the oxidation of glucose to pyruvate but also from pyruvate degradation to formate and hydrogen. Unfortunately, formate was not analysed in the present study but our results suggest a similar fermentation pattern could be present in strain J2. It has been shown that *Paenibacillus macerans* also produces more ethanol when exogenous hydrogen is added to closed batch fermentation on glucose (Weimer, 1984a) but this strain is also known to convert acetate to acetone at low pH.

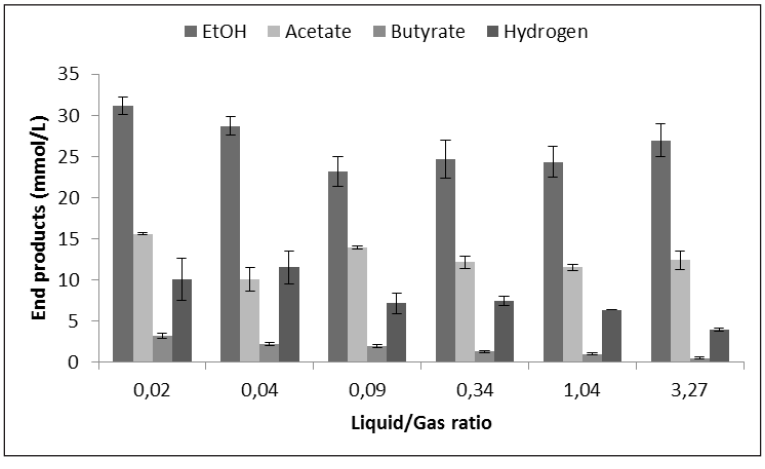

Figure 3. Effect of liquid/gas phase ratio on the end product formation for strain J2. Results are expressed as the average of two replicates ± standard deviation.

Hydrolysates

A large focus of the present investigation was on the production of ethanol from lignocellulosic biomass. Production of biofuels from lignocellulosic biomass using thermophilic organisms has gained increased interest in recent years and almost exclusively with "true thermophiles" which have temperature optima above 60°C (Ahring et al. 1999, Georgieva et al. 2008,

Table 1. Production of end products from hydrolysates (4.5 g L-1) from different biomasses. The biomass was either pretreated with 0.5% HCl or NaOH or received no chemical pretreatment prior to enzymatic treatment. Data represent average ± standard deviation of two replicate experiments. Ethanol yields in mM g-1 dw are given within brackets with control (yeast extract) subtracted. Nd = below detection limit (< 0.1 mM).

	Concentration (mmol L^{-1})					
Substrate (pretreatment)	**Ethanol**	**Acetate**	**Butyrate**	**Hydrogen**	**Glucose** (t = 0)	**Glucose** (t = final)#
Control	2.7 ± 0.1	2.7 ± 0.1	0.2 ± 0.0	2.4 ± 0.1	Nd	Nd
Whatman	27.5 ± 0.5	9.5 ± 0.4	1.1 ± 0.1	5.1 ± 0.6	30.3 ± 1.4	9.1 ± 0.3
Whatman (acid)	27.1 ± 0.8	10.9 ± 0.2	1.1 ± 0.1	7.8 ± 0.4	31.3 ± 1.8	7.2 ± 0.2
Whatman (alkali)	20.4 ± 0.7	11.5 ± 0.1	4.8 ± 0.3	15.3 ± 1.4	29.5 ± 0.4	6.5 ± 0.1
Paper	9.6 ± 0.7	8.5 ± 0.6	0.8 ± 0.1	6.6 ± 0.3	8.6 ± 0.1	0.0
Paper (acid)	11.5 ± 0.7	8.9 ± 0.2	0.8 ± 0.6	6.9 ± 0.1	10.2 ± 0.1	0.0
Paper (alkali)	5.1 ± 0.5	7.1 ± 0.5	1.7 ± 0.1	8.1 ± 0.2	11.3 ± 0.1	0.0
Grass	10.6 ± 0.5	9.1 ± 0.1	1.7 ± 0.0	10.3 ± 0.6	9.4 ± 0.1	1.2
Grass (acid)	19.7 ± 1.9	12.1 ± 0.0	2.3 ± 0.8	9.9 ± 0.1	13.9 ± 0.1	1.3
Grass (alkali)	10.0 ± 0.3	10.6 ± 0.4	3.7 ± 0.2	14.4 ± 0.5	14.2 ± 0.1	2.1
Straw	8.0 ± 0.1	6.9 ± 0.2	0.6 ± 0.1	6.8 ± 0.1	4.5 ± 0.1	3.0
Straw (acid)	14.5 ± 0.5	10.2 ± 0.0	1.2 ± 0.4	6.6 ± 0.0	6.1 ± 0.1	5.7
Straw (alkali)	14.4 ± 0.2	12.2 ± 0.2	3.6 ± 0.5	12.6 ± 0.1	13.5 ± 0.1	1.2

For paper, grass, and straw hydrolysates, glucose was not the only sugar present but results reflected total hexoses and pentoses.

Almarsdottir et al. 2012) while little attention has been allocated to moderate thermophiles. *Clostridium* AK1, a moderate thermophile with a T_{opt} of 50°C, was recently investigated in our laboratory and shown to produce 7.4 mM g^{-1} cellulose (Whatman paper), pretreated in the same way as in the present investigation. The highest yields on grass and straw were 3.1 mM g^{-1} and 3.2 mM g^{-1} when pretreated with acid and alkali, respectively (Orlygsson, 2012). The maximum yield of ethanol from the fermentation of glucose is 2 mol ethanol mol $hexose^{-1}$ or 11.1 mM g^{-1} Whatman paper. Strain J2 produced the highest amounts of ethanol on untreated and acid pretreated Whatman paper, between 24.5 to 25.0 mM (controls subtracted) or 5.6 to 5.8 mM g^{-1} (dw), respectively (Table 1). This was approximately 55% of theoretical yields which may be explained by acetate formation and that some glucose remained unutilized at the end of the incubation period. When cellulose was pretreated with alkali, considerably less ethanol was produced; instead more butyrate and hydrogen were produced. Cultivation on lignocellulosic biomass resulted in much lower yields. The highest ethanol yields of chemically unpretreated biomass were 1.75 mM g^{-1} grass. This was, however, increased to 3.77 mM g^{-1} with acid pretreatment. These yields were in a range similar to those in some recent studies on wheat straw and beet molasses by *Thermoanaerobacter mathranii* and *Thermoanaerobacter ethanolicus* (Ahrin g et al. 1999, Avci & Donmez, 2006) but considerably lower as compared to *Thermoanaerobacterium* AK17 on grass hydrolysates (Almarsdottir et al. 2012). Generally, both butyrate and hydrogen production were highest using an alkali pretreated biomass. The reason for this shift in lower ethanol and higher butyrate and hydrogen production from an alkaline pretreated biomass is difficult to explain but could have been

caused by different substrates available or the production of inhibitory compounds.

CONCLUSION

Ethanol production capacity was studied by *Paenibacillus* strain J2 isolated from an Icelandic hot spring. The strain produced a maximum of 1.45 mol ethanol mol glucose^{-1} and had a moderate substrate spectrum, degrading various hexoses, xylose, disaccharides and polymeric substrates to ethanol, acetate and traces of butyrate. The main drawback of the strain is its relatively low tolerance to substrate (glucose) concentrations. This limitation can however be resolved by cultivating the strain in fed-batch or continuous culture. Finally, the strain produced up to 3.77 mM ethanol g^{-1} on chemically pretreated grass hydrolysates, which corresponds to up to 250 L of ethanol from one ton of this type of biomass.

ACKNOWLEDGEMENTS

This work was sponsored by RANNÍS, (Technology Development Fund, project RAN091016-2376 (BioFuel)), and The Research Fund of the University of Akureyri.

REFERENCES

Ahring BK, Licht D, Schmidt AS, Sommer P & Thomsen AB 1999. Production of ethanol from wet oxidised wheat straw by *Thermoanaerobacter mathranii*. *Bioresource Technology* 68, 3-9. doi**:** http://dx.doi.org/10.1016/S0960-8524(98)00077-7

Almarsdottir AR, Sigurbjornsdottir MA & Orlygsson J 2012. Effects of various factors on ethanol yields from lignocellulosic biomass by *Thermoanaerobacterium* AK17. *Biotechnology and Bioeng*ineering 109, 686-694. doi**:** http://dx.doi.org/10.1002/bit.24346

Ash C, Priest FG & Collins MD 1993. Molecular identification of rRNA group 3 bacilli (Ash, Farrow, Wallbanks and Collins) using a PCR probe test (Proposal for the creation of a new genus *Paenibacillus*). *Antonie van Leeuwenhoek* 64, 253-260.

Avci A & Donmez S 2006. Effect of zinc on ethanol production by two *Thermoanaerobacter* strains. *Process Biochemistry* 41, 984-989**.** doi**:** http://dx.doi.org/10.1016/j.procbio.2005.11.007

Benardini JN, Vaishampayan, Schwendner P, Swanner E, Fukui Y, Osman S, Satomi M & Venkateswaran K 2011. *Paenibacillus phoenicis* sp. nov., isolated from the Phoenix Lander assembly facility and a subsurface molybdenum mine. *International Journal of Systematic and Evolutionary Microbiology* 61, 1338-1343. doi**:** 10.1099/ijs.0.021428-0

Behrendt U, Schumann P, Stieglmeier M, Pukall R, Augustin J, Spröer C, Schwendner P, Moissl-Eichinger C, Ulrich A 2010. Characterization of heterotrophic nitrifying bacteria with respiratory ammonification and dentirification activity – Description of *Paenibacillus uliginis* sp. nov., and inhabitant of fen peat soil and *Paenibacillus purispatii* sp. nov., isolated from a spacecraft assembly clean room. *Systematic and Applied Microbiology* 33, 328-336. doi**:** http://dx.doi.org/10.1016/j.syapm.2010.07.004

Ben-Bassad A, Lamed R & Zeikus JG 1981. Ethanol-production by thermophilic bacteria – Metabolic control of end product formation in *Thermoanaerobium brockii*. *Journal of Bacteriology* 146, 192-199.

Bouraoui H, Rebib H, Aissa MB, Touzel JP, O'donohue M & Manai M 2013. *Paenibacillus marinum* sp. nov., a thermophilic xylanolytic bacterium isolated from a marine hot spring in Tunisia. *Journal of Basic Microbiology* 53, 877-883. doi**:** http://dx.doi.org/10.1002/jobm.201200275

Daane LL, Harjono I, Barns SM, Launen LA, Palleroni NJ & Haggblom MM 2002. PAH-degradation by *Paenibacillus* spp. and description of *Paenibacillus naphthalenovorans* sp. nov., a naphthalene-degrading bacterium from the rhizosphere of salt marsh plants. *International Journal of Systematic and Evolutionary Microbiology* 52, 131-139.

Dasman, Kajiymana S, Kawasaki H, Yagi M, Seki T, Fukusaki E & Kobayshi A 2002. *Paenibacillus glycanilyticus* sp. nov. a novel species that degrades heteropolysaccharide produced by the cyanobacterium *Nostoc commune*. *International Journal of Systematic and Evolutionary Microbiology* 52, 1669-1674**.** doi**:** http://dx.doi.org/10.1099/ijs.0.02171-0

Georgieva TI, Mikkelsen MJ & Ahring BK 2008. Ethanol production from wet-exploded wheat straw hydrolysate by thermophilic anaerobic bacterium *Thermoanaerobacter* BG1L1 in a continuous immobilized reactor. *Applied Biochemistry and Biotechnology* 145, 99-110. doi**:** http://dx.doi.org/10.1007/s12010-007-8014-1

Gupta A, Murarka A, Campbell P & Gonzales R 2009. Anaerobic fermentation of glycerol in *Paenibacillus macerans*: Metabolic pathways and environmental determinants. *Applied and Environmental Microbiology* 75, 5871-5833. doi**:** http://dx.doi.org/10.1128/AEM.01246-09

Hawkes FR, Dinsdale R, Hawkes DL & Hussy I 2002. Sustainable fermentative hydrogen production: Challenges for process optimization. *International Journal of Hydrogen Energy* 27, 1339-1347. doi**:** http://dx.doi.org/10.1016/S0360-3199(02)00090-3

Heyndrickx M, Vandemeulebroecke, K, Scheldman P, Kersters K, De Vos P, Logan NA, Aziz AM, Ali N & Berkely RCW 1996. A polyphasic reassessment of the genus *Paenibacillus*, reclassification of *Bacillus lautus* (Nakamura 1984) as *Paenibacillus lautus* comb. nov. and of *Bacillus peoriae* (Montefusco et al. 1993) as *Paenibacillus peoriae* comb. nov., and emended descriptions of *P. lautus* and of *P. peoriae*. *International Journal of Systematic Bacteriology* 46, 988-1003.

Jones PR 2008. Improving fermentative biomass-derived H_2-production by engineering microbial metabolism. *International Journal of Hydrogen Energy* 33, 5122-5130. doi**:** http://dx.doi.org/10.1016/j.ijhydene.2008.05.004

Khianngam S, Tanasupawat S, Lee JS, Lee KC & Akaracharanya A 2009. *Paenibacillus siamensis* sp. nov., *Paenibacillus septentrionalis* sp. nov. and *Paenibacillus montaniterrae* sp. nov., xylanase-producing bacteria from Thai soils. *International Journal of Systematic and Evolutionary Microbiology* 59, 130-134. doi**:** http://dx.doi.org/10.1099/ijs.0.65851-0

Lacis LS & Lawford HG 1988. Ethanol production from xylose by *Thermoanaerobacter ethanolicus* in batch and continuous culture. *Archives of Microbiology* 150, 48-55. doi**:** http://dx.doi.org/10.1007/BF00409717

Laurentin A & Edwards CA 2003. A microtiter modification of the anthrone-sulfuric acid colorimetric assay for glucose-based carbohydrates. *Analytical Biochemistry* 315, 143-145. doi**:** http://dx.doi.org/10.1016/S0003-2697(02)00704-2

Lee F-L, Kuo H-P, Tai C-J, Yokota A & Lo C-C 2007. *Paenibacillus taiwanensis* sp. nov., isolated from soil in Taiwan. *International Journal of Systematic and Evolutionary Microbiology* 57, 1351-1354. doi**:** http://dx.doi.org/10.1099/ijs.0.64764-0

Logan NA, Clerck ED, Lebbe L, Verhelst A, Goris J, Forsyth G, Rodríguez-Díaz M, Heyndrickx M & De Vos P 2004. *Paenibacillus cineris* sp. nov. and *Paenibacillus cookii* sp. nov., from Antartic volcanic soils and a gelatin-processing plant. *International Journal of Systematic and Evolutionary Microbiology* 54, 1071-1076. doi**:** http://dx.doi.org/10.1099/ijs.0.02967-0

Lynd LR, Grethlein HE & Wolkin RH 1989. Fermentation of cellulosic substrates in batch and continuous culture by *Clostridium thermocellum*. *Applied and Environmental Microbiology* 55, 3131-3139.

Orlygsson J & Baldursson. SRB 2007. Phylogenetic and physiological studies of four hydrogen-producing thermoanaerobes from Icelandic geothermal areas. *Icelandic Agricultural Sciences* 20, 93-106.

Orlygsson J 2012. Ethanol production from biomass by a moderate thermophile, *Clostridium* AK1. *Icelandic Agricultural Sciences* 25, 23-35.

Osman S, Satomi M & Venkateswaran K 2006. *Paenibacillus pasadenensis* sp. nov. and *Paenibacillus barengoltzii* sp. nov., isolated from a spacecraft assembly facility. *International Journal of Systematic and Evolutionary Microbiology* 56, 1509-1514. doi**:** http://dx.doi.org/10.1099/ijs.0.64085-0

Priest, F.G 2009. Family IV. Paenibacillaceae fam. nov. In: De Vos P, Garrity GM, Jones D, Krieg NR, Ludwig W, Rainey FA, Schleifer K-H & Whitman WB (eds) *Bergey's Manual of Systematic Bacteriology. Volume III – The Firmicutes*, 2nd ed. Springer: New York, pp. 269-327.

Rai SK, Roy JK & Mukherjee AK 2010. Characterization of a detergent-stable alkaline protease from a novel thermophilic strain

Paenibacillus tezpurensis sp. nov. AS-S24_II. *Applied Microbiology and Biotechnology* 85, 1437-1450**.** doi**:** http://dx.doi.org/10.1007/s00253-009-2145-y

Scully SM, & Orlygsson J 2015. Recent advantages in second generation ethanol production by thermophilic bacteria. Energies 8, 1-30. doi: http://dx.doi.org/10.3390/en8010001

Sommer P, Georgieva T & Ahring BK 2004. Potential for using thermophilic anaerobic bacteria for bioethanol production from hemicellulose. *Biochemical Society Transactions* 32, 283-289. doi**:** http://dx.doi.org/10.1042/BST0320283

Sveinsdottir M, Beck SRB & Orlygsson J 2009. Ethanol production from monosugars and lignocellulosic biomass by thermophilic bacteria isolated from Icelandic hot springs. *Icelandic Agricultural Sciences* 22, 45-58.

Taylor MP, Eley KL, Martin S, Tuffin MI, Burton SG & Cowan DA 2009. Thermophilic ethanologenesis: future prospects for second-generation bioethanol production. *Trends in Biotechnology* 27, 398-405. doi**:** http://dx.doi.org/10.1016/j.tibtech.2009.03.006

Ueda J, Yamamoto S & Kurosawa N 2013. *Paenibacillus thermoaerophilus* sp. nov., a moderately thermophilic bacterium isolated from compost. *International Journal of Systematic and Evolutionary Microbiology* 63, 3330-3335. doi**:** http://dx.doi.org/10.1099/ijs.0.048090-0

van Niel EW, Claassen PA & Stams AJM 2003. Substrate and product inhibition of hydrogen production by the extreme thermophile, *Caldicellulosiruptor saccharolyticus*. *Biotechnology and Bioengineering* 81, 255-262. doi**:** http://dx.doi.org/10.1002/bit.10463

Vesteinsdottir H, Reynisdottir D & Orlygsson J 2011. *Thiomonas islandica* sp. nov., a moderately thermophilic, hydrogen- and sulfur-oxidizing betaproteobacterium isolated from a hot spring. *International Journal of Systematic and Evolutionary Microbiology* 61, 132-137. doi**:** http://dx.doi.org/10.1099/ijs.0.023572-0

Weimer PJ 1984a. Control product formation during glucose fermentation by *Bacillus macerans*. *Journal of General Microbiology* 130, 103-111.

Weimer PJ 1984b. Fermentation of 6-Deoxyhexoses by *Bacillus macerans*. *Applied and Environmental Microbiology* 47, 263-267.

Wiegel J, Carreira LH, Mothershed CP & Puls J 1983. Production of ethanol from bio-polymers by anaerobic, thermophilic, and extreme thermophilic bacteria. II. *Thermoanaerobacter ethanolicus* JW200 and its mutants in batch cultures and resting cell experiments *Biotechnology and Bioengineering*. 13, 193-205.

Zhou Y, Gao S, Wei D-Q, Yang L-L, Huang X, He J, Zhang Y-J, Tang S-K, Li W-J 2012. *Paenibacillus thermophilus* sp. nov., a novel bacterium isolated from a sediment of hot spring in Fujian providence, China. *Antonie van Leeuwenhoek* 102, 601-609. doi**:** http://dx.doi.org/10.1007/s10482-012-9755-6

Manuscript received 11 April 2014
Accepted 13 November 2014