J Ind Microbiol Biotechnol (2008) 35:1117–1122
DOI 10.1007/s10295-008-0390-1

ORIGINAL PAPER

A strategy to prevent the occurrence of *Lactobacillus* strains using lactate-tolerant yeast *Candida glabrata* in bioethanol production

Itsuki Watanabe · Toshihide Nakamura · Jun Shima

Received: 3 April 2008 / Accepted: 23 June 2008 / Published online: 3 July 2008
© Society for Industrial Microbiology 2008

Abstract Contamination of *Lactobacillus* sp. in the fermentation broth of bioethanol production decreases ethanol production efficiency. Although the addition of lactate to the broth can effectively inhibit the growth of *Lactobacillus* sp., it also greatly reduces the fermentation ability of *Saccharomyces cerevisiae*. To overcome this conflict, lactate-tolerant yeast strains were screened. *Candida glabrata* strain NFRI 3164 was found to exhibit both higher levels of lactate tolerance and fermentation ability. Co-cultivation of *C. glabrata* was performed with *Lactobacillus brevis* and *Lb. fermentum*, which were reported as major contaminating bacteria during bioethanol production, in culture medium containing 2% lactate. Under these culture conditions, the growth of *Lactobacillus* strains was greatly inhibited, but the ethanol production of *C. glabrata* was not significantly affected. Our data show the possibility of designing an effective fuel ethanol production process that eliminates contamination by *Lactobacillus* strains through the combined use of lactate addition and *C. glabrata*.

Keywords Bacterial contamination · Ethanol fermentation · Lactate-tolerant yeast · Lactic acid bacteria

Introduction

The production and utilization of bioethanol as an alternative fossil fuel have attracted attention in the effort to combat global warming and improve energy reserves [1, 8, 13, 41]. Bioethanol production generally utilizes derivatives from food crops such as corn grain and sugar cane [6, 12, 29, 31, 36, 38, 42]. Bacterial contamination is known as a major cause of the reduction in ethanol yield during ethanol production from such feedstock by yeast, *Saccharomyces cerevisiae* [26, 35]. These bacterial contaminants grow under conditions suitable for the growth of yeast and reduce ethanol yields by consuming sugars such as glucose. Among bacteria that contaminate corn mash and cane juice, lactic acid bacteria (LAB) may be the most serious because of their rapid growth [37]. It is reported that *Lactobacillus* strains, such as *Lb. brevis* and *Lb. fermentum*, among LAB are the most abundant isolates from commercial plants [35]. Lactate produced by contaminated LAB has been reported as a strong inhibitor of ethanol production of yeast strains [19].

To avoid the reduction of ethanol yields by LAB contamination, various agents have been examined to control LAB. It has been reported that hydrogen peroxide, potassium metabisulfite, 3,4,4′-trichlorocarbanilide, and antibiotics effectively inhibit LAB [2, 4, 5, 11, 23, 27, 43]. In fact, antibiotics such as penicillin and virginiamycin are used in commercial bioethanol production today [4, 11]. However, the addition of antibiotics to the broth may not be preferable from an ecological viewpoint, because the waste generated during bioethanol production should be recycled as useful products including forage or fertilizer. The remaining antibiotics in the waste can lead to the emergence and spread of mutants resistant to antibiotics, which would threaten the safety of food and human health. Therefore, it is important to develop a method to control LAB during bioethanol production without the use of antibiotics.

To design a bioethanol production process that eliminates LAB contamination, in this study we focused on lactate as an agent to control LAB strains. It is known that elevated lactate levels strongly inhibit LAB growth and viability, although LAB stoichiometrically produces lactate

I. Watanabe · T. Nakamura · J. Shima (✉)
National Food Research Institute, 2-1-12 Kannondai, Tsukuba, Ibaraki 305-8642, Japan
e-mail: shimaj@affrc.go.jp

from sugars. In general, the ability of *Lactobacillus* strains to grow was inhibited in culture medium containing elevated lactate levels [39]. However, the addition of high concentrations of lactate may also reduce the fermentation ability of *S. cerevisiae* [18]. To overcome the conflict arising from the addition of lactate, we attempted to screen lactate-tolerant yeast strains whose fermentation abilities were not affected by the addition of lactate. In this study, we show the results of the screening of lactate-tolerant yeast belonging to *Candida glabrata*. It is known that several strains of *Candida* are not able to sufficiently grow and produce ethanol under anaerobic condition [40]. However, *C. glabrata* show higher abilities of ethanol production and growth under anaerobic conditions and the characteristics of *C. glabrata* in ethanol production were similar with those of *S. cerevisiae* [20]. It is considered that the condition of conventional ethanol production using *S. cerevisiae* can be applied to ethanol production using *C. glabrata*. We also show the yeast strain's ability to produce ethanol under conditions including artificially infection by LAB. Our study shows the possibility of designing a novel bioethanol production system using lactate-tolerant yeast.

Materials and methods

Strains and media

Yeast strains (383 strains) used for screening lactate-tolerant yeasts were obtained from the Microbiological Bank of the National Food Research Institute (NFRI). These strains consisted of strains isolated from fermented foods, products of baker's yeast, and strains purchased from other culture collections. *S. cerevisiae* NBRC 0224, reportedly suitable for bioethanol production [15, 32], was used as a control strain. *Lb. brevis* IFO 3960 and *Lb. fermentum* IAM 1083 were used as models of contaminated LAB.

Synthetic dextrose complete (SC) medium [33] was used for cultivation of yeast strains. MRS medium (Difco Laboratory, Detroit, MI, USA) was used for the cultivation of LAB and the co-cultivation of yeast and LAB strains. The pH of culture medium containing lactate was determined using a pH meter. The concentration of undissociated forms of lactate in the medium was calculated by using the Henderson–Hasselbach equation [22].

Screening of lactate-tolerant yeast

Yeast strains from the NFRI Microbiological Bank were inoculated into 100 µl of SC medium in microtiter plates (Corning Inc., Corning, NY, USA) and incubated at 30 °C for 48 h (pre-culture). Portions (1.2 µl) of the pre-culture were transferred into 100 µl of SC media containing 0–5% (v/v) of lactate, and then the cultures were incubated for 48 h at 30 °C. The optical density at 630 nm (OD_{630}) of the cultures was measured using a microtiter plate reader (Elx800; BioTek Instruments, Winooski, VT, USA).

Taxonomic identification of screened yeast strain

The screened yeast strain was taxonomically identified by 26S rDNA sequencing and the assimilation ability of sugars. The partial 26S rDNA of the strain was amplified by PCR and directly sequenced using the method described by Kurtzman and Robnett [16]. The homology of the sequence was determined using the BLAST system in the DNA Data Bank of Japan (DDBJ). The assimilation abilities of sugars were assessed by the method described previously [25].

Characteristics of growth and ethanol production of screened yeast strain

The lactate tolerance of the screened yeast strain was assessed based on the growth and ethanol production abilities in SC medium containing lactate. The yeast strain was inoculated into SC medium containing 1% (v/v) lactate at the cell density of 4×10^5 and cultivated for 48 h at 30 °C without shaking. The optical density at 600 nm (OD_{600}) and the amounts of ethanol production in culture medium were monitored during cultivation.

Lactate sensitivity of *Lactobacillus* strains

To assess the lactate sensitivity of *Lactobacillus* strains, the growth in MRS medium containing lactate was assessed. *Lb. brevis* or *Lb. fermentum* was inoculated into MRS medium containing either no lactate or 1% lactate at a cell density of 1×10^6 cells ml^{-1}. The growth of the strains was monitored as OD_{600}.

Co-cultivation of yeast and LAB strains

To assess the effects of bacterial contamination on ethanol production, yeast and LAB strains were co-cultivated. The yeast and LAB strains were inoculated into MRS medium containing 2% (v/v) lactate at cell densities of 4×10^5 and $1.6–2.3 \times 10^6$ cells ml^{-1}, respectively, and cultivated for 48 h at 30 °C without shaking. The amounts of ethanol in the supernatants during co-cultivation were monitored.

Measurement of ethanol, glucose, and undissociated lactate

Amounts of ethanol and glucose in the culture medium were measured using an HPLC system containing a refractive index detector (Prominence series, Shimadzu, Kyoto, Japan) equipped with a fermentation monitoring column

Table 1 Genetic and phenotypic characteristics of strain NFRI 3164

Parameter or test	Characteristic
Homology of 26S rDNA partial sequence	99.8% Identity with *C. glabrata* NRRL Y-65^T
Spore formation	Not detected
Assimilable sugars	D-glucose and D-trehalose
Non-assimilable sugars	Glycerol, calcium 2-keto-gluconate, L-arabinose, D-xylose, adonitol, xylitol, D-galactose, inositol, D-sorbitol, D-lactose, methyl-α-D-glucopyranoside, *N*-acetyl-glucosamine, D-maltose, D-cellobiose, D-saccharose, D-melezitose and D-raffinose

NFRI National Food Research Institute, Tsukuba, Japan; *NRRL* National Center for Agricultural Utilization Research, Peoria, IL, USA

(Bio-Rad Laboratories, Hercules, CA, USA). The analysis was carried out under the conditions described by Narendranath et al. [22]. The ethanol yield (g g^{-1}) was calculated as grams of ethanol produced per grams of total glucose consumed, and the ethanol production rate (g^{-1} lh^{-1}) was calculated as grams of ethanol produced per liter of medium per fermentation hour.

Results and discussion

Screening of lactate-tolerant yeast

To obtain lactate-tolerant yeasts that could grow in medium containing elevated levels of lactate, we assessed the growth and ethanol production abilities of 383 yeast strains. We monitored the growth of each strain in SC medium containing 5% lactate (initial pH: 2.13) and found that four strains (NFRI 3163, NFRI 3164, NFRI 3165, and NFRI 3309), which were taxonomically unidentified, grew rapidly in the medium and that their turbidities exceeded OD_{630} = 0.3 at 48 h cultivation (data not shown).

Among the screened strains, NFRI 3164 produced the most ethanol in the medium containing 5% lactate for 48 h. Based on these results, we decided to use strain NFRI 3164 for further analyses in this study. Strain NFRI 3164 was first reported by Nikkuni [24] as an isolate from fermented rice in Southeast Asia.

Taxonomic identification of strain NFRI 3164

Strain NFRI 3164 was taxonomically identified. Identification involved 26S rDNA sequencing and the assimilation potential of various sugars. Table 1 summarizes the identification results. The sequence of 26S rDNA of strain NFRI 3164 showed 99.8% identity with *C. glabrata* NRRL Y-65^T. The sequence was deposited in DDBJ under accession no. AB366746. The pattern of the assimilation ability of sugars in strain NFRI 3164 agreed well with the metabolic characteristics of *C. glabrata* described by Barnett et al. [3] and Kreger and Jack [14]. These results strongly suggest that strain NFRI 3164 belongs to *C. glabrata*.

Kinetic study of growth and ethanol production of strain NFRI 3164

To compare the characteristics of NFRI 3164 with those of *S. cerevisiae* NBRC 0224 under lactate-containing conditions, we monitored time-dependent changes in ethanol concentrations and growth in media containing 0 or 1% (initial pH values of 4.79 and 2.52, respectively) lactate (Fig. 1). In the lactate-free medium, both strains grew rapidly, although the maximum optical density of strain NFRI 3164 was much higher than that of NBRC 0224 (Fig. 1a), and the ethanol yields of NFRI 3164 and NBRC 0224 were nearly equal (Fig. 1b). Although the growth ability and ethanol yield of NBRC 0224 drastically decreased when 1% lactate was added, such parameters of NFRI 3164 were not strongly affected (Fig. 1c, d). It is reported that the inhibitory effects of lactate on yeast growth depend on the concentration of undissociated lactate in the culture medium [17, 22, 37]. The addition of 1% lactate to SC medium (pH 2.52) gave 105.2 mM of undissociated forms of lactate. These data clearly showed that neither the growth nor the ethanol production of NFRI 3164 was affected by approximately 100 mM of undissociated forms of lactate, and that the strain's lactate tolerance was much higher than *S. cerevisiae*'s.

Candida glabrata is considered a close relative of *S. cerevisiae* based on genome evolution [7, 10, 21, 28]. However, the mechanisms of stress response in *C. glabrata* may be different from those of *S. cerevisiae*. In fact, Gregori et al. [9] pointed out the differences in the high-osmolarity glycerol pathway, which is important for tolerance to high osmolarity, between *C. glabrata* and *S. cerevisiae*. We speculated that *C. glabrata* may have specific mechanisms for tolerance to acids, because our results indicated that strain NFRI 3164 was also tolerant to other acids such as sulfate and acetate (data not shown).

Lactate sensitivity of *Lactobacillus* strains

To determine the effects of lactate addition on the growth of *Lactobacillus* strains including *Lb. brevis* and *Lb. fermentum,*

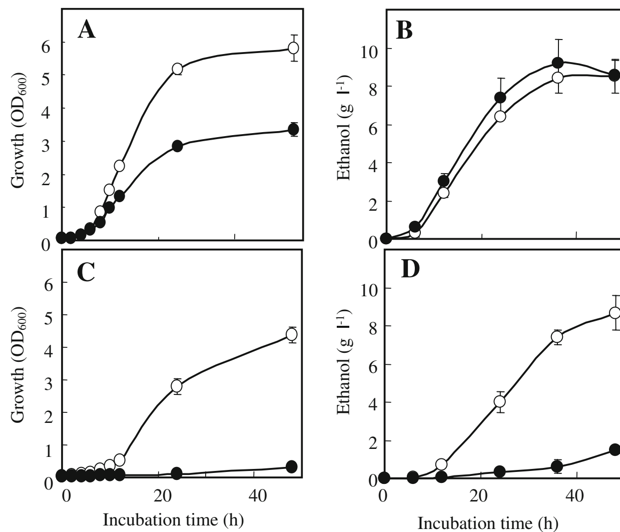

Fig. 1 The effects of lactate addition on the growth and ethanol production of *S. cerevisiae* NBRC 0224 (*closed circle*) and *C. glabrata* NFRI 3164 (*open circle*). The growth and ethanol contents in the lactate-free SC medium (**a**, **b**) or SC medium containing 1% lactate (**c**, **d**) were monitored. Data shown are mean ± SD

the changes in cell densities were monitored. In this analysis, we selected *Lb. brevis* and *Lb. fermentum* as model strains of LAB because they were reported as major contaminants in commercial ethanol production processes [30, 34, 35]. As shown in Fig. 2, the growth of both *Lb. brevis* and *Lb. fermentum* was greatly inhibited by the addition of 1% lactate (equivalent to 20.9 mM of undissociated forms of lactate), although growth was not completely inhibited. These data indicated that lactate addition above 1% may be effective for preventing the occurrence of *Lactobacillus* strains.

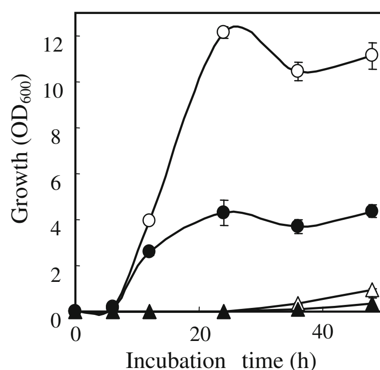

Fig. 2 The effects of lactate addition on the growth of *Lactobacillus* strains. Symbols used: *open circle Lb. brevis* in MRS medium, *closed circle Lb. fermentum* in MRS medium, *open triangle Lb. brevis* in MRS medium containing 1% lactate, *closed triangle Lb. fermentum* in MRS medium containing 1% lactate. Data shown are mean ± SD

Co-cultivation of strain NFRI 3164 with *Lactobacillus* strains

To determine the possibility of designing an ethanol production process that prevents LAB contamination through the combined use of lactate and strain NFRI 3164, we analyzed ethanol production in medium artificially infected by LAB (co-cultivation). Figure 3 shows the changes in ethanol production during co-cultivation of yeast strains (NFRI 3164 and *S. cerevisiae* NBRC 0224) and *Lb. brevis*. When the lactate concentration was 0%, the yields of ethanol produced by NFRI 3164 and NBRC 0224 were drastically decreased (Fig. 3a). The loss rates of ethanol yields were 59.4 and 49.2% compared with the cases free of artificial infection, in NFRI 3164 and NBRC 0224, respectively. The competition of the glucose between yeasts and LAB may be a main reason for the decrease in ethanol yields, because the decrease rate of glucose in co-cultivation is much higher than that in cultivation without artificial infection (Fig. 3a). We also observed similar patterns in which ethanol production decreased under the 0% lactate-added condition even if *Lb. fermentum* was used as a model contaminant (data not shown).

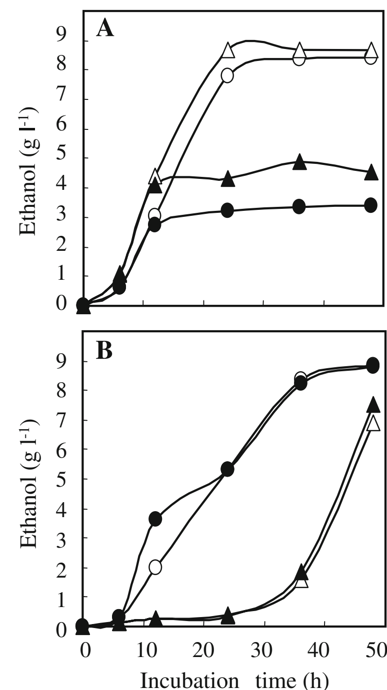

Fig. 3 The effects of co-cultivation with *Lactobacillus* strains on ethanol production by yeast strains in MRS medium (**a**) or MRS medium containing 2% lactate (**b**). Symbols used in each panel: *open circle*, *C. glabrata* NFRI 3164 cultivated with no *Lactobacillus* strains; *open triangle*, *S. cerevisiae* NBRC 0224 cultivated with no *Lactobacillus* strain; *closed circle*, *C. glabrata* NFRI 3164 co-cultivated with *Lb. brevis*; *closed triangle*, *S. cerevisiae* NBRC 0224 co-cultivated with *Lb. brevis*. Data shown are mean ± SD

To assess the effects of lactate addition under co-cultivation conditions, we monitored ethanol production in MRS medium containing 2% lactate (equivalent to 113.8 mM of undissociated lactate). The molar concentration of the undissociated lactic acid in this experiment was adjusted to be approximately equal to previous experiments using SC medium. If the lactate concentration was 2%, the loss of ethanol yield and the ethanol production rate during co-cultivation of NFRI 3164 with *Lb. brevis* was completely suppressed by the addition of 2% lactate (Fig. 3b). However, the ethanol production rate of NBRC 0224 showed a 1.5-fold decrease by the addition of lactate, although the final ethanol yield was suppressed. These data indicated that lactate addition at approximately 2% suppresses the loss of ethanol yields without decreasing the ethanol production rate if strain NFRI 3164 is used. This strain's higher production rate under lactate addition may depend on its lactate tolerance. Our data show the possibility of designing an effective fuel ethanol production process that eliminates contamination by *Lactobacillus* strains through the combined use of lactate addition and strain NFRI 3164.

Strain NFRI 3164 exhibited lactate tolerance as well as tolerance to other acids, including acetate and sulfate (data not shown). Interestingly, our preliminary data showed that this strain exhibited higher tolerance to high temperature and NaCl than did *S. cerevisiae*. These characteristics are great advantages for bioethanol production. These results, taken together with the data above, indicate that *C. glabrata* should be a useful strain for bioethanol production based on its tolerance of multiple stresses. To the best of our knowledge, this is the first report to show the usefulness of *C. glabrata* for bioethanol production.

In conclusion, we have shown the importance of lactate-tolerant yeast and the possibility of designing novel bioethanol production systems that prevent contamination of LAB strains. It has also been clarified that the screened strain NFRI 3164 belongs to *C. glabrata*. Although we do not yet have any data on the mechanisms underlying lactate tolerance, we consider that this phenotype may be useful for commercial applications. Kinetic analysis indicates that ethanol yields and production rates of NFRI 3164 were much higher than those of *S. cerevisiae* NBRC 0224, which is known to be suitable for bioethanol production, under conditions containing 1–2% lactate. Co-cultivation analysis of NFRI 3164 with *Lactobacillus* strains known as contaminants suggests that the addition of 2% lactate effectively prevents LAB contamination without a loss of ethanol yield or ethanol production. Since antibiotics are unnecessary, this ethanol production system offers several advantages. Among these is that the waste generated during antibiotic-free bioethanol could be recycled as forage or fertilizer.

To verify strain NFRI 3164's effectiveness in an industrial process, we are planning to test ethanol production under simulated industrial conditions. In particular, we will examine in detail this strain's practical usefulness in currently used cell-recycled continuous fermentation systems. Furthermore, inexpensive methods for preparing lactate, such as recycling from the resultant culture broth, should be investigated for industrial application. Further molecular-biological study of the strain's lactate tolerance will be worthwhile.

Acknowledgments This work was supported by the project for the development of biomass utilization technologies for revitalizing rural areas from the Ministry of Agriculture, Forestry, and Fisheries (MAFF) of Japan. We thank S. Mizukami-Murata and Y. Sakayori (National Food Research Institute) for their technical assistance in this study.

References

1. Antoni D, Zverlov VV, Schwarz WH (2007) Biofuels from microbes. Appl Microbiol Biotechnol 77:23–35
2. Aquarone E (1960) Penicillin and tetracycline as contamination control agents in alcoholic fermentation of sugar cane molasses. Appl Microbiol 8:263–268
3. Barnett JA, Payne RW, Yaroww D (2000) Yeasts: characteristics and identification. Cambridge University Press, Cambridge
4. Bayrock DP, Thomas KC, Ingledew WM (2003) Control of *Lactobacillus* contaminants in continuous fuel ethanol fermentations by constant or pulsed addition of penicillin G. Appl Microbiol Biotechnol 62:498–502
5. Chang IS, Kim BH, Shin PK (1997) Use of sulfite and hydrogen peroxide to control bacterial contamination in ethanol fermentation. Appl Environ Microbiol 63:1–6
6. Dawson L, Boopathy R (2007) Use of post-harvest sugarcane residue for ethanol production. Bioresour Technol 98:1695–1699
7. Dujon B, Sherman D, Fischer G, Durrens P, Casaregola S, Lafontaine I, De Montigny J, Marck C, Neuveglise C, Talla E, Goffard N, Frangeul L, Aigle M, Anthouard V, Babour A, Barbe V, Barnay S, Blanchin S, Beckerich JM, Beyne E, Bleykasten C, Boisrame A, Boyer J, Cattolico L, Confanioleri F, De Daruvar A, Despons L, Fabre E, Fairhead C, Ferry-Dumazet H, Groppi A, Hantraye F, Hennequin C, Jauniaux N, Joyet P, Kachouri R, Kerrest A, Koszul R, Lemaire M, Lesur I, Ma L, Muller H, Nicaud JM, Nikolski M, Oztas S, Ozier-Kalogeropoulos O, Pellenz S, Potier S, Richard GF, Straub ML, Suleau A, Swennen D, Tekaia F, Wesolowski-Louvel M, Westhof E, Wirth B, Zeniou-Meyer M, Zivanovic I, Bolotin-Fukuhara M, Thierry A, Bouchier C, Caudron B, Scarpelli C, Gaillardin C, Weissenbach J, Wincker P, Souciet JL (2004) Genome evolution in yeasts. Nature 430:35–44
8. Gray KA, Zhao L, Emptage M (2006) Bioethanol. Curr Opin Chem Biol 10:141–146
9. Gregori C, Schuller C, Roetzer A, Schwarzmuller T, Ammerer G, Kuchler K (2007) The high-osmolarity glycerol response pathway in the human fungal pathogen *Candida glabrata* strain ATCC 2001 lacks a signaling branch that operates in baker's yeast. Eukaryot Cell 6:1635–1645
10. Herrero E (2005) Evolutionary relationships between *Saccharomyces cerevisiae* and other fungal species as determined from genome comparisons. Rev Iberoam Micol 22:217–222
11. Hynes SH, Kjarsgaard DM, Thomas KC, Ingledew WM (1997) Use of virginiamycin to control the growth of lactic acid bacteria during alcohol fermentation. J Ind Microbiol Biotechnol 18:284–291

12. Kadam KL, McMillan JD (2003) Availability of corn stover as a sustainable feedstock for bioethanol production. Bioresour Technol 88:17–25
13. Kim S, Dale B (2005) Life cycle assessment of various cropping systems utilized for producing biofuels: bioethanol and biodiesel. Biomass Bioenerg 29:426–439
14. Kreger-van Rij NJW, Jack WF (1998) The yeasts: a taxonomic study. Elsevier Science Ltd, Amsterdam
15. Kuriyama H, Seiko Y, Murakami T, Kobayashi H, Sonoda Y, Silva H (1985) Selection of yeast strains for effective ethanol fermentation. Rep Ferment Res Inst 64:43–50
16. Kurtzman CP, Robnett CJ (1998) Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. Antonie Van Leeuwenhoek 73:331–371
17. Levine AS, Fellers CR (1940) Action of acetic acid on food spoilage microorganisms. J Bacteriol 39:499–515
18. Maiorella B, Blanch HW, Wilke CR (1983) By-product inhibition effects on ethanolic fermentation by *Saccharomyces cerevisiae*. Biotechnol Bioeng 25:103–131
19. Makanjuola DB, Tymon A, Springham DG (1992) Some effects of lactic acid bacteria on laboratory-scale yeast fermentations. Enzyme Microb Technol 14:350–357
20. Merico A, Sulo P, Piskur J, Compagno C (2007) Fermentative lifestyle in yeasts belonging to the *Saccharomyces* complex. FEBS J 274:976–989
21. Nakagawa Y, Mizuguchi I (2005) The nucleotide sequence determination of catalases of three medically important yeasts using newly designed degenerated primers. Nihon Ishinkin Gakkai Zasshi/Jpn J Med Mycol 46:35–42
22. Narendranath NV, Thomas KC, Ingledew WM (2001) Effects of acetic acid and lactic acid on the growth of *Saccharomyces cerevisiae* in a minimal medium. J Ind Microbiol Biotechnol 26:171–177
23. Narendranath NV, Thomas KC, Ingledew WM (2000) Urea hydrogen peroxide reduces the numbers of lactobacilli, nourishes yeast, and leaves no residues in the ethanol fermentation. Appl Environ Microbiol 66:4187–4192
24. Nikkuni S (1990) Annual report on exploration and introduction of microbial genetic resources. Annu Rep Natl Inst Agrobiol Resour 2:59–71
25. Nishida O, Kuwazaki S, Suzuki C, Shima J (2004) Superior molasses assimilation, stress tolerance, and trehalose accumulation of baker's yeast isolated from dried sweet potatoes (hoshi-imo). Biosci Biotechnol Biochem 68:1442–1448
26. Nishino N, Hattori H, Kishida Y (2007) Alcoholic fermentation and its prevention by *Lactobacillus buchneri* in whole crop rice silage. Lett Appl Microbiol 44:538–543
27. Oliva-Neto PD, Yokoya F (1998) Effect of 3,4,4′-trichlorocarbanilide on growth of lactic acid bacteria contaminants in alcoholic fermentation. Bioresour Technol 63:17–21
28. Pikur J, Langkjr RB (2004) Yeast genome sequencing: the power of comparative genomics. Mol Microbiol 53:381–389
29. Rudolf A, Baudel H, Zacchi G, Hahn-Hagerdal B, Liden G (2008) Simultaneous saccharification and fermentation of steam-pretreated bagasse using *Saccharomyces cerevisiae* TMB3400 and *Pichia stipitis* CBS6054. Biotechnol Bioeng 99(4):783–790
30. Schell DJ, Dowe N, Ibsen KN, Riley CJ, Ruth MF, Lumpkin RE (2007) Contaminant occurrence, identification and control in a pilot-scale corn fiber to ethanol conversion process. Bioresour Technol 98:2942–2948
31. Schell DJ, Riley CJ, Dowe N, Farmer J, Ibsen KN, Ruth MF, Toon ST, Lumpkin RE (2004) A bioethanol process development unit: initial operating experiences and results with a corn fiber feedstock. Bioresour Technol 91:179–188
32. Seiko Y, Murakami T, Kuriyama H, Sonoda Y (1985) Selection of yeast strains having tolerance to inhibitive conditions. Rep Ferment Res Inst 63:55–63
33. Sherman F, Fink G, Lawrence C (eds) (1979) Methods in yeast genetics. Cold Spring Harbor Laboratory, New York
34. Simpson KL, Pettersson B, Priest FG (2001) Characterization of lactobacilli from Scotch malt whisky distilleries and description of *Lactobacillus ferintoshensis* sp. nov., a new species isolated from malt whisky fermentations. Microbiology 147:1007–1016
35. Skinner KA, Leathers TD (2004) Bacterial contaminants of fuel ethanol production. J Ind Microbiol Biotechnol 31:401–408
36. Solomon BD, Barnes JR, Halvorsen KE (2007) Grain and cellulosic ethanol: history, economics, and energy policy. Biomass Bioenerg 31:416–425
37. Thomas KC, Hynes SH, Ingledew WM (2001) Effect of lactobacilli on yeast growth, viability and batch and semi-continuous alcoholic fermentation of corn mash. J Appl Microbiol 90:819–828
38. Torney F, Moeller L, Scarpa A, Wang K (2007) Genetic engineering approaches to improve bioethanol production from maize. Curr Opin Biotechnol 18:193–199
39. Vinderola CG, Reinheimer JA (2003) Lactic acid starter and probiotic bacteria: a comparative "in vitro" study of probiotic characteristics and biological barrier resistance. Food Res Int 36:895–904
40. Visser W, Scheffers WA, Batenburg-van der Vegte WH, van Dijken JP (1990) Oxygen requirements of yeasts. Appl Environ Microbiol 56:3785–3792
41. von Blottnitz H, Curran MA (2007) A review of assessments conducted on bio-ethanol as a transportation fuel from a net energy, greenhouse gas, and environmental life cycle perspective. J Cean Prod 15:607–619
42. Wheals AE, Basso LC, Alves DM, Amorim HV (1999) Fuel ethanol after 25 years. Trends Biotechnol 17:482–487
43. William RG, Carl AW (1986) Effects of sodium meta bisulfite on diffusion fermentation of fodder beets for fuel ethanol production. Biotechnol Bioeng 30:909–916