



Optimal Fermentation of *Saccharomyces cerevisiae* Expressing a Dextranase from *Chaetomium gracile*

Jidong Liu^{1,2} · Qinju Sun¹ · Hang Yin¹ · Leyi Wang¹ · Hongbei Wei¹ · Kai Li^{1,2} · Fangxue Hang^{1,2}

Received: 28 January 2019 / Accepted: 6 June 2019 / Published online: 29 July 2019
© Society for Sugar Research & Promotion 2019

Abstract Dextranase from *Chaetomium gracile* is generally considered safe for use in the sugarcane industry. Herein, a truncated and codon-optimised α -dextranase gene from *C. gracile* was successfully cloned and expressed in *Saccharomyces cerevisiae* for the first time. The optimum conditions of fermentation was achieved when the maximum dextranase activity reached to 58.45 U/mL after 48 h in shake flasks. The optimal pH and temperature were 5.5 and 60 °C, respectively. The recombinant dextranase remained stable between pH 4 and 6 and temperature between 55 and 60 °C. The findings in the present study could facilitate large-scale production of food-grade recombinant dextranase for use in the sugar industry.

Keywords Dextranase · *Chaetomium gracile* · *Saccharomyces cerevisiae* · Heterologous expression · Fermentation optimisation

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s12355-019-00746-5>) contains supplementary material, which is available to authorized users.

✉ Kai Li
gxlikai@126.com

✉ Fangxue Hang
liuan6126@126.com

¹ School of Light Industry and Food Engineering, Guangxi University, 100 Daxue Road, Nanning 530004, Guangxi, China

² Sugar Industry Collaborative Innovation Center, Guangxi University, 100 Daxue Road, Nanning 530004, Guangxi, China

Introduction

Dextrans are the collective name for high molecular weight polymers composed of D-glucose units connected via α -1,2, α -1,3 and α -1,4 main-chain linkages (Zhang et al. 2017). Dextrans are synthesised from sucrose by the enzyme dextranase secreted by bacteria such as *Leuconostoc mesenteroides* (Fraga Vidal et al. 2011). The presence of dextrans in syrup enhances the viscosity and thereby affects filtration, clarification and crystal formation, leading to economic losses (Eggleston and Monge 2005; Purushe et al. 2012). Physical methods such as ultrafiltration, dialysis and reverse osmosis have proven useful for removing dextrans from sugarcane juice yet are not implemented in the sugarcane industry due to high costs (Mao et al. 2018).

Commercial dextranase has been used in sugarcane factories to degrade dextrans into smaller molecules by endogenously hydrolysing the α -(1,6) linkages, as exemplified in the extraction of sugar from sugar beet (Li et al. 2017). Dextranases are present in a wide variety of fungi and bacteria, and fungi are the main commercial sources (Khalikova et al. 2005). Most commercial dextranases in the USA are produced from either *Penicillium* spp. or *Chaetomium* spp. (Eggleston 2009). However, there are safety concerns, and relatively low productivity restricts their application in the sugar-making process (Zhang et al. 2018). To solve these problems, researchers have focused on recombinant enzymes to meet the immense demand for dextranases in the in food, medicine and chemical industries. Although various dextranases of bacterial and fungal origin have been previously reported, few are currently employed in the sugar industry (Khalikova et al. 2005; Bertrand et al. 2014) due to high enzyme cost, cumbersome

in dextranase purification and toxic by-products from fungi (Rerngsamran et al. 2014).

A solution is the deployment of engineered strains to produce dextranases, among which *Escherichia coli*, *Pichia pastoris* and *Saccharomyces cerevisiae* are the three most commonly used heterologous expression systems. Dextranase genes from bacteria and fungi have been heterologously expressed in *E. coli*, often as insoluble inclusion bodies (Purushe et al. 2012; Khalikova et al. 2005). Although the methylotrophic yeast *P. pastoris* is widely used for the heterologous production of recombinant proteins (Macauley-Patrick et al. 2005), including dextranases (Kang et al. 2009; Chen et al. 2008), volatile toxic compounds such as methanol can impact food safety of products from this organism. *S. cerevisiae* is easily cultivated, has been used successfully in industrial applications for many years and has Generally Recognised as Safe (GRAS) status (Liu et al. 2013). Kang et al. (2005) reported the cloning and characterisation of a dextranase gene from *Lipomyces starkeyi* and its heterologous expression in *S. cerevisiae*, but the activity of the recombinant enzyme was not given, and the optimal temperature of the recombinant enzyme was 37 °C, which is too low for use in the sugarcane industry.

China is the fourth largest producer of sugarcane in the world, producing ~ 10 million tons of sugarcane per year, but residual dextrans in syrup result in large economic losses. Also, the sugar-making process usually requires high temperatures, and hence, thermostable enzymes are needed. In the present study, an optimised dextranase-encoding gene from *Chaetomium gracile* was expressed in *S. cerevisiae*, and various enzymatic properties of the recombinant enzyme, including optimal temperature, pH, thermal and pH stabilities, were studied. We also optimised the fermentation conditions to maximise dextranase production in engineered *S. cerevisiae* strains.

Materials and Methods

Microorganisms, Plasmids and Culture Medium

Escherichia coli JM109 was used as the recipient strain for cloning manipulation and plasmid amplification and was cultivated in Luria–Bertani (LB) medium containing tryptone (10 g/L), yeast extract (5 g/L) and NaCl (10 g/L) with or without 100 µg/L of ampicillin. *S. cerevisiae* CEN.PK2-1B (*MAT α* ; *his3 Δ 1*, *trp1-289*, *leu2-3, 112*, *ura3-52*, *MAL2-8^c*, *SUC2*) was obtained from EUROSCARF (Frankfurt, Germany) and used for genetic manipulation. Engineered *S. cerevisiae* strains were grown in yeast extract–peptone–dextrose (YPD) medium (2% glucose, 2% tryptone, 1% yeast extract) or yeast nitrogen base (YNB)

medium (0.17% yeast nitrogen base without amino acids, 0.5% ammonium sulphate, 2% glucose, supplemented with 50 µg/mL of each of the required amino acids). All chemicals were of reagent grade and were obtained from commercial sources.

Construction and Transformation of Recombinant Plasmids

The plasmid T-vector pMD19 (Simple) was purchased from TaKaRa (Dalian, China), and the yeast expression plasmid pY26-TEF1-GPD1 was kindly provided by Li et al. (2007). Plasmid pRS306 was kindly provided by Sikorski and Hieter (1989).

SignalP 4.1 (<http://www.cbs.dtu.dk/services/SignalP/>) was used to predict the signal peptide sequence of the α -dextranase gene from *C. gracile* (GenBank: KC707808.1), suggesting the first 54 bp encodes a signal peptide, and the 4–54 bp region was truncated in the codon-optimised *dex* gene synthesised by Sangon (Shanghai, China). The sequence of the *dex* gene is provided in Supplementary data.

The synthesised *dex* gene was cloned into the pY26 and pRS306 vectors to obtain the expression constructs pY26-*dex* and pRS306-*dex*, which were verified and transformed into *S. cerevisiae* CEN.PK2-1B using Fast-Yeast Transformation reagent (G-Biosciences, St. Louis, MO) according to the manufacturer's instructions. Strains harbouring integrated plasmids containing auxotrophic selection markers were screened using corresponding auxotrophic YNB plates and verified by PCR analysis.

Enzyme Assays and Sodium Dodecyl Sulphate–Polyacrylamide Gel Electrophoresis (SDS–PAGE)

In this study, dextranase was produced by *S. cerevisiae* as an intracellular hydrolase, and snailase was used to destroy the yeast cell wall. Yeast cells were harvested by centrifugation (1500g at 25 °C for 5 min), and cell pellets were resuspended in 5 mL of solution containing 1.0 M sorbitol, 250 µL of 20 × phosphate-buffered saline (PBS) and 1% snailase (Sigma, Shanghai) and incubated at 37 °C for 1 h. Then, the cells were further treated with glass beads (0.4–0.6 mm, OMEGA) by vortex. The supernatant of cell-broken cells was used as crude enzyme.

Dextranase activity was measured using the dinitrosalicylic acid (DNS) method by incubating 100 µL of enzyme with 900 µL of 2% dextran T2000 (Sigma) as previously reported (Li et al. 2006). One unit of dextranase (U/mL) is defined as the amount of enzyme that degrades dextran T2000 to produce reducing sugar per min under assay conditions at 37 °C. Crude dextranase was analysed by

SDS–PAGE, and gels were stained with Coomassie Brilliant Blue R-250 (Kang et al. 2009).

Optimisation of Fermentation Conditions

To determine the initial culture pH, we varied the substrate pH range from pH 4.0 to 7.0, and the enzyme activity of the recombinant dextranase was calculated as described above. To investigate the optimum temperature for enzyme production, culturing was carried out at different temperatures between 26 and 34 °C.

In this experiment, we tested the effects of different substrates and substrate concentration on dextranase production. Glucose (1% w/v) present in the basal medium was replaced by sucrose, glycerol, fructose, lactose or starch. Meanwhile, tryptone (2% w/v) present in the basal medium was substituted by organic and inorganic nitrogen sources including casein, ammonium chloride and urea.

Properties of Recombinant Dextranase

To determine the optimum pH of the recombinant dextranase, dextran T-2000 (2%) was dissolved in different substrate solutions at pH 3.0–8.0 using citrate–phosphate buffers. The pH stability was assessed by treating recombinant dextranase at pH 3.0–8.0 for 1 h, and the residual activity at each pH was expressed relative to the highest activity (100%). All experiments were performed in triplicate.

To determine the optimum temperature for dextranase catalysis, enzyme was incubated with dextran T-2000 (2%) at different temperatures between 30 and 80 °C, and thermostability was assessed by incubating dextranase at 30–80 °C for an h. The activity at each temperature was expressed relative to the highest activity (100%). All experiments were performed in triplicate.

Results

Expression of Codon-Optimised Dextranase in *S. cerevisiae*

To evaluate the expression of dextranase from *C. gracile* in *S. cerevisiae*, the codon-optimised *dex* gene encoding the synthase was expressed in *S. cerevisiae* CEN. PK2-1B using the episome plasmid pY26-TEF-GPD, resulting in the engineered *S. cerevisiae* strain XD02. The SDS–PAGE results verified the secretion of the 63 kDa dextranase by the XD02 cells, and no comparable band was visible with the XD01 control strain carrying empty pY26-TEF-GPD (Fig. 1). The DNS results indicated that dextranase production by XD02 in YNB medium reached a maximum of 17.86 U/mL after 72 h of cultivation.

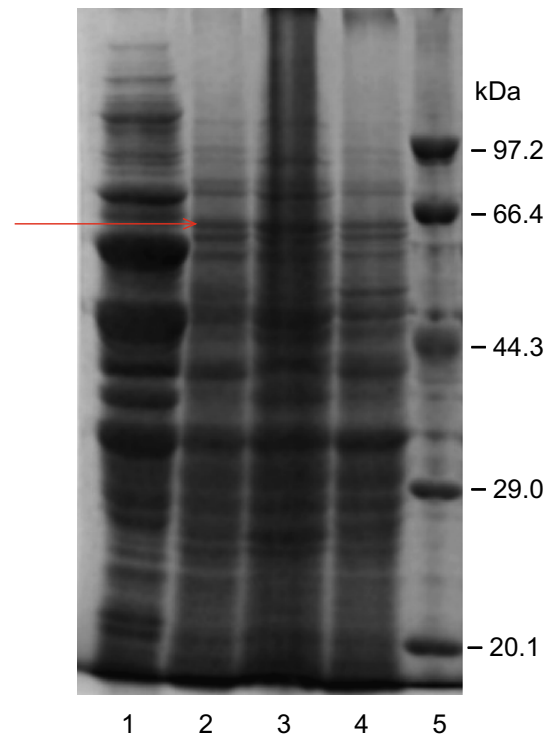


Fig. 1 SDS–PAGE analysis of recombinant dextranase expressed in *S. cerevisiae*. Lane 1, *S. cerevisiae* CEN. PK-1B (negative control); lane 2, supernatant of *S. cerevisiae* XD02 wall-broken cells; lane 3, cell suspension of *S. cerevisiae* XD02 wall-broken cells; lane 4, supernatant of *S. cerevisiae* XD03 wall-broken cells

We also evaluated the expression of *dex* following incorporation into the chromosome of *S. cerevisiae* CEN. PK2-1B using the integrant plasmid pRS306, resulting in engineered *S. cerevisiae* strain XD03. When cultured in YPD medium, the cell density of *S. cerevisiae* strain XD03 increased rapidly during the first 36 h and then remained consistent after 48 h. Despite the slower cell growth after 24 h of incubation indicating stationary phase, more dextranase was produced between 24 and 48 h, suggesting cells at stationary phase produced more target enzyme. The maximum dextranase activity of strain XD03 was obtained after 60 h (40.42 U/mL), slightly higher than that after 48 h (40.26 U/mL; Fig. 2). Afterwards, dextranase production declined gradually to the end of the incubation, as cell growth further declined (data not shown). Previous research reported that production of *C. gracile* dextranase was maximal after incubation for 96 h (Li et al. 2017). Thus, our results indicate that heterologous gene expression in *S. cerevisiae* is more rapid than the time-consuming fermentation of *C. gracile*. Since the engineered *S. cerevisiae* strain XD03 exhibited superior dextranase production compared with strain XD02, XD03 was employed for dextranase production in subsequent experiments.

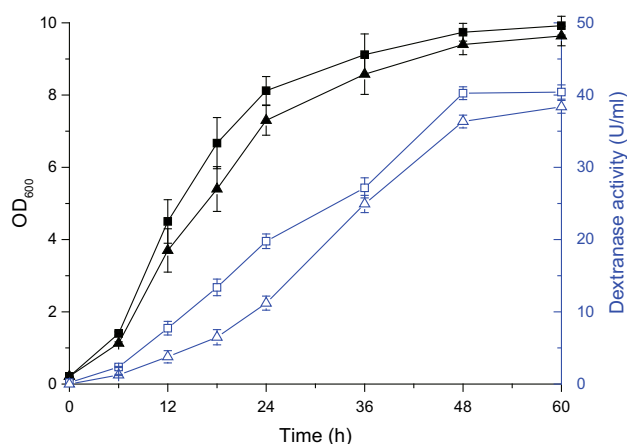


Fig. 2 Cell growth and dextranase production in the engineered *S. cerevisiae* strain. The solid black and open blue squares represent the absorbance at 600 nm and dextranase activity of strain XD03, respectively. The solid black and blue open triangles represent the absorbance at 600 nm and dextranase activity of strain XD02, respectively. The results are presented as means and standard deviations of at least three independent experiments

Effects of Medium pH and Incubation Temperature on Dextranase Production

Relatively few studies have explored the influence of initial pH and temperature of the medium on yeast intracellular recombinant protein production, although both parameters are known to affect fungal growth. In the present study, the influence of initial pH and culture temperature on dextranase production in engineered *S. cerevisiae* was examined. Strain XD03 cells were incubated in YPD medium in 500 mL flasks with a working volume of 50 mL and incubated at 30 °C for 60 h. Maximum growth was observed at pH 6.0, at which the absorbance at 600 nm (OD₆₀₀) reached 10.3. Maximum dextranase production was achieved at pH 5.5, at which dextranase activity reached 42.1 U/mL. These results indicate dextranase production over a broad pH range (pH 4.0–7.0; Fig. 3a). Thus, pH 5.5 was considered the most suitable pH for achieving the highest yield of dextranase in engineered yeast strain XD03.

To address the effect of temperature on dextranase production, XD03 cells were cultured in YPD medium in a 500-mL flask with a working volume of 50 mL at various temperatures from 26 to 34 °C for up to 60 h. Cell density and dextranase production were analysed, and the results are summarised in Fig. 3b. Maximum cell growth rate was achieved at 28 °C, and cell growth decreased gradually thereafter with increasing temperature. The optimal temperature for dextranase production was 30 °C, at which activity reached 42.13 U/mL. Thus, to maximise dextranase production, we selected 30 °C as the optimum temperature in subsequent experiments.

Effects of Nitrogen and Carbon Source on Dextranase Production

We also studied the effects of different carbon and nitrogen sources on dextranase production at different concentrations. While keeping all other culture conditions unchanged, the sole carbon source was sequentially replaced by sucrose, glucose, glycerol, fructose, lactose and starch. As shown in Fig. 4a, dextranase production and cell growth of strain XD03 on glycerol, fructose and glucose were clearly higher than on sucrose, lactose or starch. Maximal growth of XD03 cells occurred using glycerol, and the highest dextranase yield was achieved using glucose. Thus, glucose was regarded as the best alternative carbon source for dextranase production. With increasing glucose concentration, the growth of yeast cells increased almost proportionately, suggesting the carbon source was growth limiting. We established that the optimal glucose concentration was 25 g/L (Fig. 4b). Previous reports also emphasised that obtaining the maximum biomass does not guarantee successful fermentation and production of target molecules (Martinez-Moreno et al. 2012).

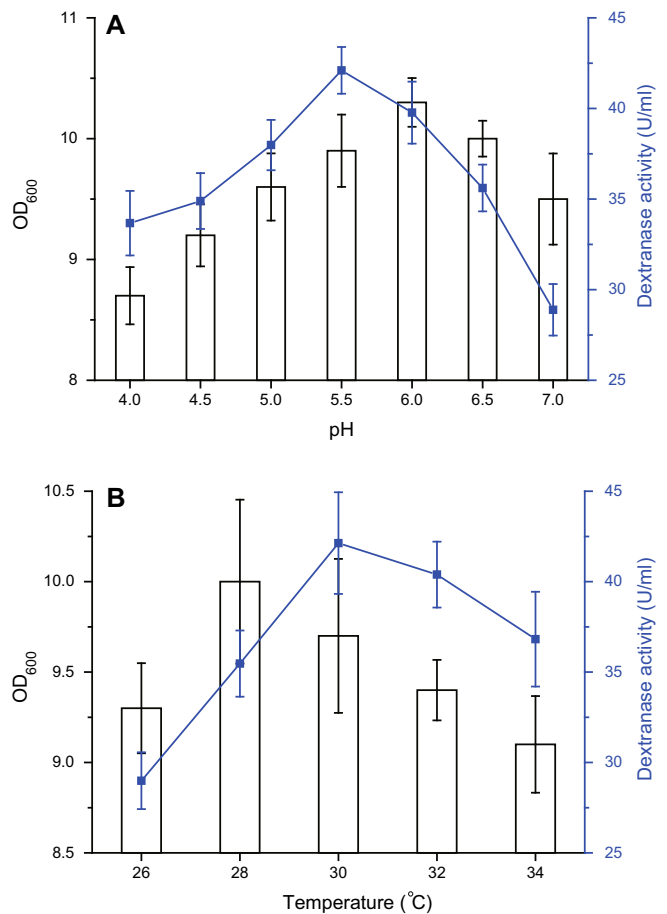
We also investigated effect of nitrogen source at different concentrations and found that tryptone and casein stimulated greater enzyme production than other nitrogen sources such as ammonium chloride and urea. Furthermore, the optimal casein concentration was 25 g/L, the same as the optimal glucose concentration (Fig. 4c, d). Consequently, 25 g/L casein was regarded as the best nitrogen source for dextranase production. Using the optimal fermentation conditions, we achieved dextranase production of 58.45 U/mL in 48 h, ~ 1.45-fold higher than the initial fermentation conditions.

Characterisation of Recombinant Dextranase

Temperature and pH are two fundamental factors involved in enzyme catalytic activity. By subjecting recombinant dextranase to different pH conditions ranging from pH 3.0 to 8.0, maximum dextranase activity was observed at pH 5.5 (Fig. 5a). To assess the pH stability of recombinant dextranase, dextranase activity was measured after a 1-h incubation at pH 3.0–8.0. The results showed that the enzyme maintained more than 90% activity at pH 4–6 (Fig. 5b).

Temperature and thermal stability are generally regarded as the primary factors determining whether an enzyme is suitable for industrial applications. To determine the optimal temperature, dextranase activity was measured at temperatures ranging from 30 to 80 °C. As shown in Fig. 5c, enzymatic activity increased with increasing temperature and exhibited maximal activity at 60 °C, but activity decreased sharply thereafter at higher

Fig. 3 Effects of initial pH (a) and temperature (b) on dextranase production by *S. cerevisiae* XD03. Columns represent the absorbance at 600 nm, and closed blue squares represent dextranase activity. The results are presented as means and standard deviations of at least three independent experiments



temperatures. Recombinant enzyme retained > 80% of initial activity after incubation at 50–60 °C for 1 h (Fig. 5d). These results were similar to those of previous characterisations of thermostable dextranases from *Talaromyces pinophilus* (Zhang et al. 2017) and *Chaetomium erraticum* (Virgen-Ortíz et al. 2015).

Discussion

Given the importance of dextranases in the sugar industry for alleviating the viscosity of syrups and cleaning blocked machines (Chen et al. 2009), much effort has gone into screening high-level dextranase-producing strains, including expressing dextranase genes from *Streptococcus rattus* and *P. minioluteum* in *E. coli* or *P. pastoris* (Roca et al. 1996; Igarashi et al. 2004). At present, commercial sources of dextranase from *Chaetomium* spp. require long fermentation times, and enzymes from *Penicillium* spp. can suffer from food safety issues (Eggleston 2009). Additionally, the main two dextranase producers mentioned above usually utilise expensive dextran as a carbon source. Thus, efficient production of food-grade dextranase from cheap substrates could benefit the sugar-making process.

To our knowledge, this is the first report of the production of recombinant dextranase from *C. gracile* in *S. cerevisiae*.

S. cerevisiae is a well-characterised eukaryotic model organism for the production of heterologous proteins (Liu et al. 2012), and this organism has been used safely for food production for thousands of years. *S. cerevisiae* strains have many advantages including a well-understood genetic background, easy cultivation, a cheap substrate spectrum, and many years of use in industrial-scale fermentation processes (Lian et al. 2018). The food safety of dextranase from *C. gracile* was confirmed in the USA in 1986, it has Generally Recognised as Safe (GRAS) status, and it has been applied industrially (Eggleston and Monge 2005; Virgen-Ortíz et al. 2015). To achieve food-grade dextranase production, we chose *S. cerevisiae* as host and the *C. gracile* dextranase gene, and no resistance markers were introduced in the present work. Heterologous gene expression can suffer from low productivity due to the presence of a signal peptide, which has an adverse impact on protein folding (Ljungdahl and Daignan-Fornier 2012). In the present study, we removed the region of the dextranase gene predicted to encode a signal peptide, and the rest of the gene encoding the remaining residues was synthesised using codon optimisation. Following cloning

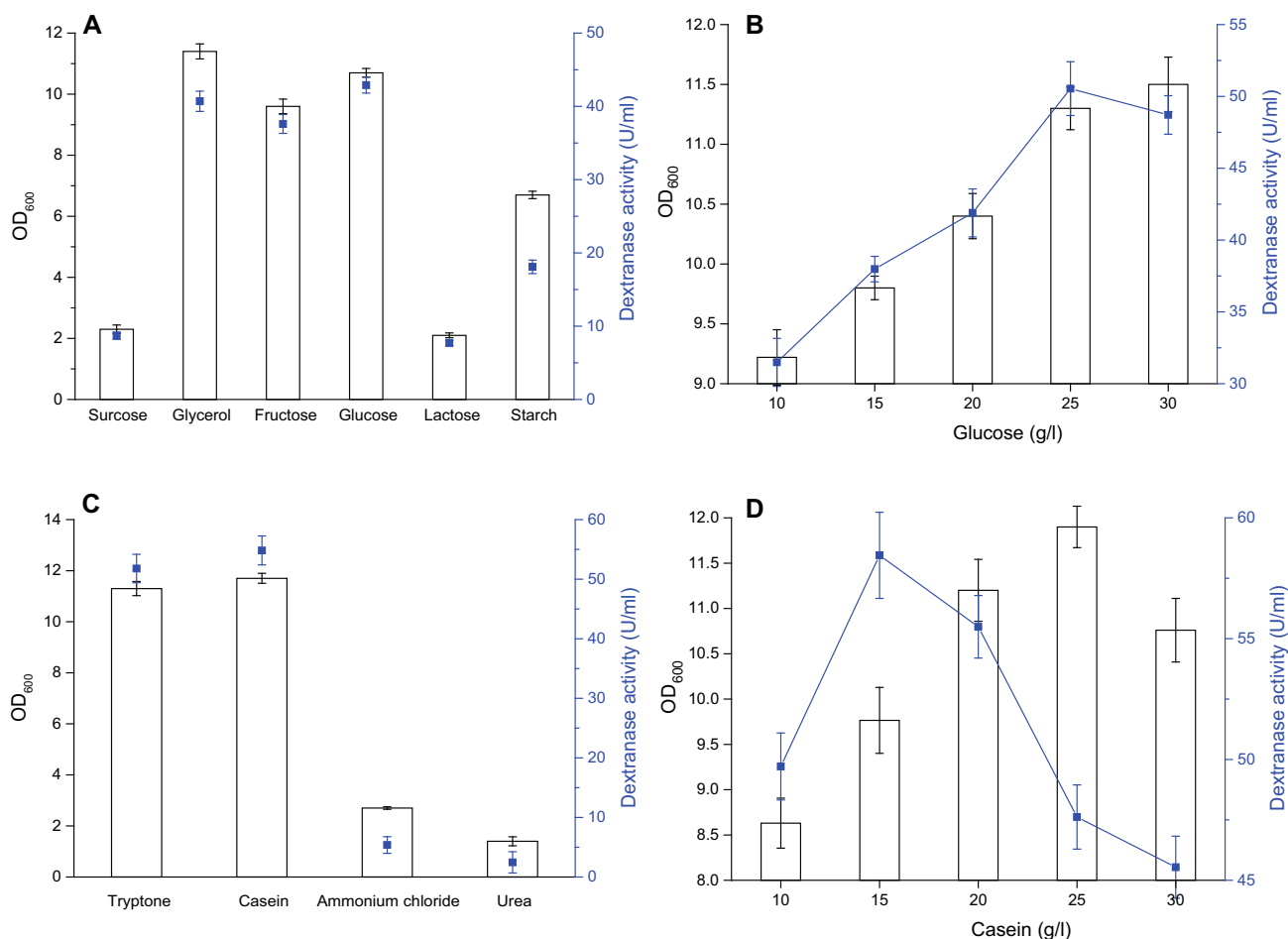


Fig. 4 Effects of carbon and nitrogen source on dextranase production and cell growth. **a** Different carbon sources; **b** different glucose concentrations; **c** different nitrogen sources; **d** different casein

of the resulting gene into appropriate expression vectors, culturing resulted in detectable dextranase production in the engineered *S. cerevisiae* strains (Figs. 1, 2).

Cheap substrates can reduce the cost of fermentation processes and thereby decrease product price. Much research has been conducted on optimising the production of valuable products using low-cost substrates in fermentation processes in *S. cerevisiae* (Kwak and Jin 2017). Carbon sources are indispensable nutrients for microbial growth and metabolite accumulation and can impact on microbial growth and metabolism, and thus enzyme production (Luo et al. 2018). Nitrogen sources are also vital for the synthesis of microbial cell proteins and nucleic acids and can have an important influence on cell growth and the accumulation of metabolites (Armando et al. 2013). In the present study, we evaluated the influence of different carbon and nitrogen sources on dextranase production in *S. cerevisiae*, and optimum concentrations were also determined (Fig. 4). Optimising the culture medium in this way resulted in a 44.6% increase compared with the initial

concentrations. Columns represent the absorbance variation at 600 nm, and filled blue squares represent dextranase activity changes

medium. Moreover, the fermentation time was shortened from more than 96 h in *Chaetomium* spp. to 48 h in engineered *S. cerevisiae* cells.

In general, weak acidic conditions can decrease non-specific reactions and the accumulation of undesirable by-products that may be accrued under alkaline and high-temperature conditions (Chen et al. 2018). In the present work, the recombinant dextranase exhibited high activity and stability under weak acid conditions (Fig. 5). Also, industrial production is often carried out at high temperatures; in the sugar-making process, the temperature is generally > 60 °C (Eggleston and Monge 2005). The recombinant dextranase produced in the present work exhibited high enzyme activity and thermal stability at high temperatures (Fig. 5).

In summary, a truncated dextranase from *C. gracile* was recombinantly expressed in *S. cerevisiae*, and the enzyme exhibited high pH and thermal stability, making it suitable for use in industrial applications. Dextranase production by the engineered *S. cerevisiae* strain XD03 was

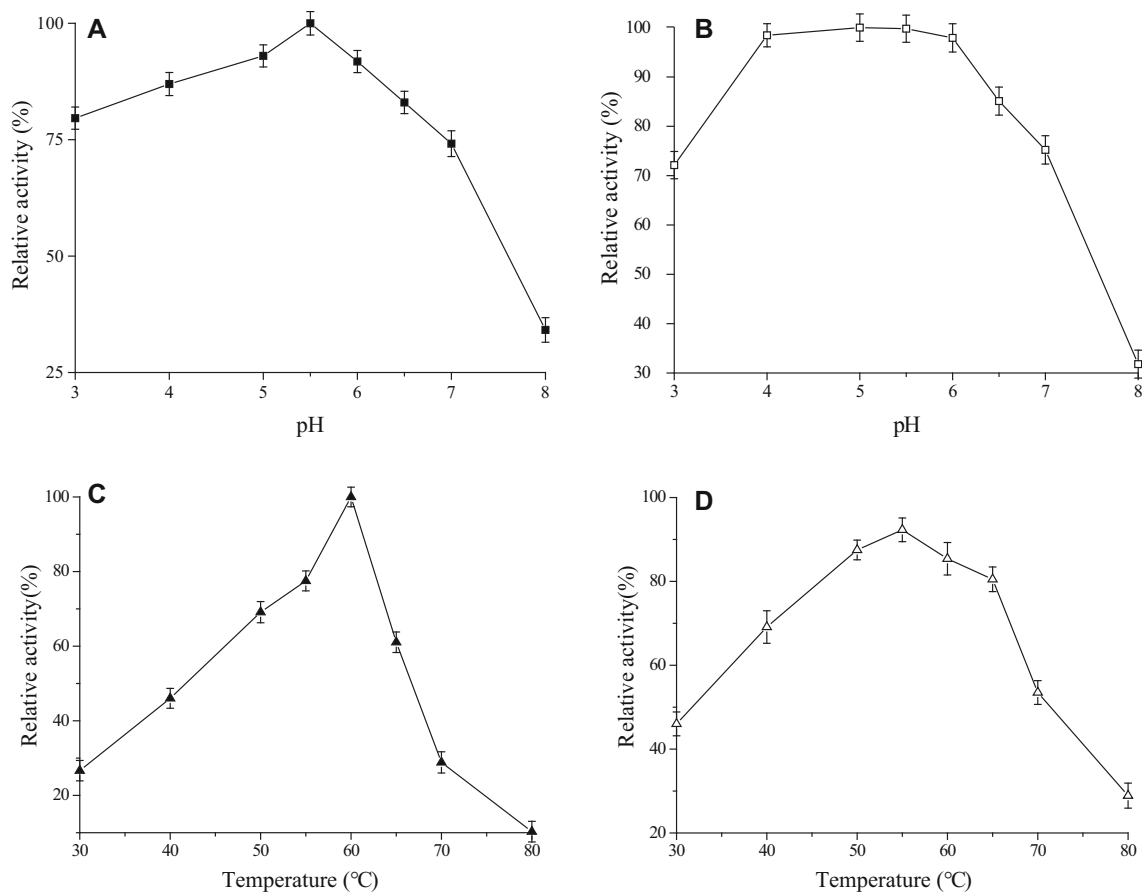


Fig. 5 Properties of the recombinant dextranase. **a** Effect of pH on dextranase activity; **b** pH stability; **c** effect of temperature on dextranase activity; **d** temperature stability

increased significantly following optimisation of the fermentation conditions. This work provides a strategy for expression dextranase genes from *C. gracile* in recombinant *S. cerevisiae* for dextranase production. Although the engineered dextranase displayed good food safety and thermostability credentials, further studies are needed to assess the large-scale application of this enzyme.

Acknowledgements This work was supported by the National Natural Science Foundation of China (31460026), the National Natural Science Foundation of Guangxi Province (2018GXNSFAA050126), the Bosch Young Teachers Innovation Training Project (BRP180215) and the Fundamental Research Fund of Guangxi University (XJZ140293).

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Statement This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Informed consent was obtained from all individual participants included in the study.

References

- Armando, M.R., M.A. Galvagno, C.A. Dogi, P. Cerrutti, A.M. Dalcerro, and L.R. Cavaglieri. 2013. Statistical optimization of culture conditions for biomass production of probiotic gut-borne *Saccharomyces cerevisiae* strain able to reduce fumonisin B1. *Journal of Applied Microbiology* 114(5): 1338–1346. <https://doi.org/10.1111/jam.12144>.
- Bertrand, E., G. Pierre, C. Delattre, C. Gardarin, N. Bridiau, T. Maugard, A. Strancar, and P. Michaud. 2014. Dextranase immobilization on epoxy CIM (R) disk for the production of isomaltooligosaccharides from dextran. *Carbohydrate Polymers* 111: 707–713. <https://doi.org/10.1016/j.carbpol.2014.04.100>.
- Chen, Z.W., W. Xu, W.L. Zhang, T. Zhang, B. Jiang, and W.M. Mu. 2018. Characterization of a thermostable recombinant L-rhamnose isomerase from *Caldicellulosiruptor obsidiansis* OB47 and its application for the production of L-fructose and L-rhamnulose. *Journal of the Science of Food and Agriculture* 98(6): 2184–2193. <https://doi.org/10.1002/jsfa.8703>.
- Chen, L., C. Yu, X.S. Zhou, and Y.X. Zhang. 2009. Rational introduction of disulfide bond to enhance optimal temperature of *Lipomyces starkeyi* alpha-dextranase expressed in *Pichia pastoris*. *Journal of Microbiology and Biotechnology* 19(12): 1506–1513. <https://doi.org/10.4014/jmb.0902.0096>.
- Chen, L., X.S. Zhou, W.M. Fan, and Y.X. Zhang. 2008. Expression, purification and characterization of a recombinant *Lipomyces starkeyi* dextranase in *Pichia pastoris*. *Protein Expression and*

- Purification* 58(1): 87–93. <https://doi.org/10.1016/j.pep.2007.10.021>.
- Eggleston, Gillian. 2009. Application of dextranases in sugarcane factory: Overcoming practical problems. *Sugar Tech* 11(2): 135–141. <https://doi.org/10.1007/s12355-009-0020-x>.
- Eggleston, G., and A. Monge. 2005. Optimization of sugarcane factory application of commercial dextranases. *Process Biochemistry* 40(5): 1881–1894. <https://doi.org/10.1016/j.procbio.2004.06.025>.
- Fraga Vidal, Reinaldo, Aidin Martinez, Claire Moulis, Pierre Escalier, Sandrine Morel, Magali Remaud-Simeon, and Pierre Monsan. 2011. A novel dextranase is produced by *Leuconostoc citreum* strain B/110-1-2: An isolate used for the industrial production of dextran and dextran derivatives. *Journal of Industrial Microbiology and Biotechnology* 38(9): 1499–1506. <https://doi.org/10.1007/s10295-010-0936-x>.
- Igarashi, T., H. Morisaki, and N. Goto. 2004. Molecular characterization of dextranase from *Streptococcus rattus*. *Microbiology and Immunology* 48(3): 155–162. <https://doi.org/10.1111/j.1348-0421.2004.tb03501.x>.
- Kang, H.K., S.H. Kim, J.Y. Park, X.J. Jin, D.K. Oh, S.S. Kang, and K. Doman. 2005. Cloning and characterization of a dextranase gene from *Lipomyces starkeyi* and its expression in *Saccharomyces cerevisiae*. *Yeast* 22(15): 1239–1248. <https://doi.org/10.1002/yea.1311>.
- Kang, H.K., J.Y. Park, J.S. Ahn, S.H. Kim, and D. Kim. 2009. Cloning of a gene encoding dextranase from *Lipomyces starkeyi* and its expression in *Pichia pastoris*. *Journal of Microbiology and Biotechnology* 19(2): 172–177. <https://doi.org/10.4014/jmb.0802.100>.
- Khalikova, E., P. Susi, and T. Korpela. 2005. Microbial dextran-hydrolyzing enzymes: Fundamentals and applications. *Microbiology and Molecular Biology Reviews* 69(2): 306–325. <https://doi.org/10.1128/Jmrb.69.2.306-325.2005>.
- Kwak, Suryang, and Yong-Su Jin. 2017. Production of fuels and chemicals from xylose by engineered *Saccharomyces cerevisiae*: A review and perspective. *Microbial Cell Factories* 16: 82. <https://doi.org/10.1186/s12934-017-0694-9>.
- Li, X., S.H. Millson, R.D. Coker, and I.H. Evans. 2006. Cloning and expression of *Penicillium minioluteum* dextranase in *Saccharomyces cerevisiae* and its exploitation as a reporter in the detection of mycotoxins. *Biotechnology Letters* 28(23): 1955–1964. <https://doi.org/10.1007/s10529-006-9183-7>.
- Li, Aimin, Zengshan Liu, LuYu. Qianxue Li, Dacheng Wang, and Xuming Deng. 2007. Construction and characterization of bidirectional expression vectors in *Saccharomyces cerevisiae*. *FEMS Yeast Research* 8(1): 6–9. <https://doi.org/10.1111/j.1567-1364.2007.00335.x>.
- Li, K., H.Q. Lu, F.X. Hang, S.B. Li, and J.D. Liu. 2017. Improved dextranase production by *Chaetomium gracile* through optimization of carbon source and fermentation parameters. *Sugar Tech* 19(4): 432–437. <https://doi.org/10.1007/s12355-016-0476-4>.
- Lian, Jiazhang, Shekhar Mishra, and Huimin Zhao. 2018. Recent advances in metabolic engineering of *Saccharomyces cerevisiae*: New tools and their applications. *Metabolic Engineering*. <https://doi.org/10.1016/j.mben.2018.04.011>.
- Liu, Z.H., K.E.J. Tyo, J.L. Martinez, D. Petranovic, and J. Nielsen. 2012. Different expression systems for production of recombinant proteins in *Saccharomyces cerevisiae*. *Biotechnology and Bioengineering* 109(5): 1259–1268. <https://doi.org/10.1002/bit.24409>.
- Liu, J.D., W.P. Zhang, G.C. Du, J. Chen, and J.W. Zhou. 2013. Overproduction of geraniol by enhanced precursor supply in *Saccharomyces cerevisiae*. *Journal of Biotechnology* 168(4): 446–451. <https://doi.org/10.1016/j.jbiotec.2013.10.017>.
- Ljungdahl, Per O., and Bertrand Daignan-Fornier. 2012. Regulation of amino acid, nucleotide, and phosphate metabolism in *Saccharomyces cerevisiae*. *Genetics* 190(3): 885–929. <https://doi.org/10.1534/genetics.111.133306>.
- Luo, G., J.H. Tian, H.Q. Huang, and L. An. 2018. Improving heterologous expression of porcine follicle-stimulating hormone in *Pichia pastoris* by integrating molecular strategies and culture condition optimization. *Applied Microbiology and Biotechnology* 102(20): 8867–8882. <https://doi.org/10.1007/s00253-018-9260-6>.
- Macauley-Patrick, S., M.L. Fazenda, B. McNeil, and L.M. Harvey. 2005. Heterologous protein production using the *Pichia pastoris* expression system. *Yeast* 22(4): 249–270. <https://doi.org/10.1002/yea.1208>.
- Mao, H.Y., M.H. Qiu, X.F. Chen, H. Verweij, and Y.Q. Fan. 2018. Fabrication and in situ fouling mitigation of a supported carbon nanotube/gamma-alumina ultrafiltration membrane. *Journal of Membrane Science* 550: 26–35. <https://doi.org/10.1016/j.memsci.2017.12.050>.
- Martinez-Moreno, R., P. Morales, R. Gonzalez, A. Mas, and G. Beltran. 2012. Biomass production and alcoholic fermentation performance of *Saccharomyces cerevisiae* as a function of nitrogen source. *FEMS Yeast Research* 12(4): 477–485. <https://doi.org/10.1111/j.1567-1364.2012.00802.x>.
- Purushe, Shweta, Divya Prakash, Neelu N. Nawani, Prashant Dhakephalkar, and Balasaheb Kapadnis. 2012. Biocatalytic potential of an alkalophilic and thermophilic dextranase as a remedial measure for dextran removal during sugar manufacture. *Bioresource Technology* 115: 2–7. <https://doi.org/10.1016/j.biortech.2012.01.002>.
- Rerngsamran, P., P. Temjitpukdee, N. Assavasirijinda, S. Chareonpornwattana, and S. Thaniyavarn. 2014. Cloning, characterization, and heterologous expression of a dextranase gene from *Penicillium pinophilum* SMCU3-14. *Scienceasia* 40(6): 405–413. <https://doi.org/10.2306/scienceasia1513-1874.2014.40.405>.
- Roca, H., B. Garcia, E. Rodriguez, D. Mateu, L. Coroas, J. Cremata, R. Garcia, T. Pons, and J. Delgado. 1996. Cloning of the *Penicillium minioluteum* gene encoding dextranase and its expression in *Pichia pastoris*. *Yeast* 12(12): 1187–1200. [https://doi.org/10.1002/\(Sici\)1097-0061\(19960930\)12:12%3c1187:Aid-Yea986%3e3.0.Co;2-U](https://doi.org/10.1002/(Sici)1097-0061(19960930)12:12%3c1187:Aid-Yea986%3e3.0.Co;2-U).
- Sikorski, Robert S., and Philip Hieter. 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics* 122: 19–27.
- Virgen-Ortiz, J.J., V. Ibarra-Junquera, P. Escalante-Minakata, J. de Ornelas-Paz, J.A. Osuna-Castro, and A. González-Potes. 2015. Kinetics and thermodynamic of the purified dextranase from *Chaetomium erraticum*. *Journal of Molecular Catalysis B: Enzymatic* 122: 80–86. <https://doi.org/10.1016/j.molcatb.2015.08.020>.
- Zhang, Yu-Qi, Ruo-Han Li, Hong-Bin Zhang, Wu Min, and Hu Xue-Qin. 2017. Purification, characterization, and application of a thermostable dextranase from *Talaromyces pinophilus*. *Journal of Industrial Microbiology and Biotechnology* 44(2): 317–327. <https://doi.org/10.1007/s10295-016-1886-8>.
- Zhang, Z.D., J.D. Liu, S.Y. Ma, H.Q. Lu, F.X. Hang, P. Huang, and K. Li. 2018. Enhancement of catalytic performance of alpha-dextranase from *Chaetomium gracile* through optimization and suitable shear force. *Sugar Tech* 20(1): 78–87. <https://doi.org/10.1007/s12355-017-0540-8>.