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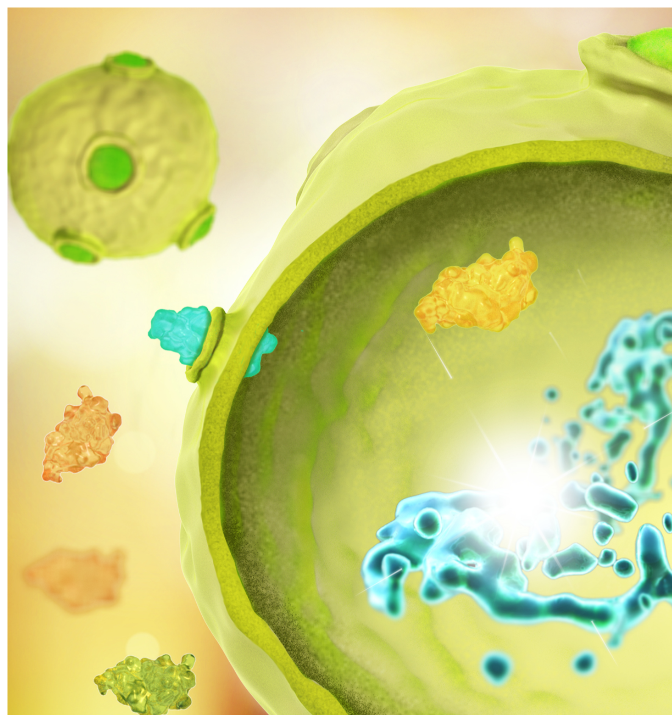
Editorial

Adapting the yeast consolidated bioprocessing paradigm for biorefineries



Abstract

Despite decades long development, no natural or engineered organism has been isolated that can produce commodity products at the rates and yields required by industry via direct microbial conversion. However, new genomic editing tools and systems level knowledge of metabolism provides opportunities to develop yeast strains for second-generation biorefineries.

Biofuel contributions to the global energy supply over the last three decades can inter alia be attributed to (i) energy security, (ii) improved trade balances from limiting oil imports, (iii) socio-economic development of developing nations, (iv) concern over fossil fuel reserves, and (v) the need to mitigate greenhouse gas emissions (Vohra et al., 2014). Bioethanol was rapidly adopted as a liquid transport fuel due to established production technologies and relative compatibility with existing infrastructure. Current bioethanol production is dominated by conversion of cane sugar and grain starch to (1st generation) bioethanol. Given food *vs.* fuel debates, lignocellulosic biomass feedstocks offer the only viable alternative renewable source of (2nd generation) liquid biofuels and green chemicals in the immediate future if technologies can be established to make conversions economically feasible (Den Haan et al., 2013).

Although commercial 2nd generation bioethanol facilities are becoming operative to deliver on the promise of cellulosic ethanol and other green chemicals, biomass conversion to commodity products is far from optimal (Lynd et al., 2017). Factors hampering the growth and sustainability of this industry include the recalcitrance of feedstocks, variation in feedstock composition, high hydrolytic enzyme cost, and the requirement for ethanologens able to thrive in the hostile fermentation environments. The biological conversion of pretreated lignocellulose to ethanol requires depolymerising enzyme production, hydrolysis of biomass polysaccharides, and fermentation of resultant pentoses and hexoses (Olson et al., 2012; van Rensburg et al., 2014). Whereas achieving these four steps through separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) represent the status quo in commercial 2nd generation ethanol production (Lynd et al., 2017), commercial enzymes add cost while productivity is frequently low. On the other hand, consolidated bioprocessing (CBP) envisions one-step lignocellulose conversion to ethanol or other commodity products by organisms that self-produce cellulolytic enzymes in a single unit operation. This technology was suggested as a way to improve process economics almost twenty years ago, yet no organism with the required substrate conversion properties and biofuel productivity parameters has yet been isolated or engineered (Lynd et al., 1999; Lynd et al., 2017).

Challenges facing CBP technology include sufficiently high levels of enzyme production without compromising ethanol productivity, co-fermentation of hexose and pentose sugars, and tolerating harsh fermentation environments (Den Haan et al., 2015). The industry standard ethanologen, *Saccharomyces cerevisiae* was engineered to utilise xylose and secrete cellulases in efforts to develop a fermentative CBP yeast. However, low secretion titres of cellulases lead to poor substrate hydrolysis efficiency and slow conversion rates, preventing their commercial application. Strategies since employed to improve secretion include engineering of peptide leader sequences, optimization of gene copy number, manipulation of promoter strength, and engineering the heterologous protein for codon optimization or to remove inhibition (Kroukamp et al., 2017). Rational strain improvements, including increasing ER-resident chaperones, accelerating vesicle fusion events, altering protein glycosylation, modulating cellular stress. and reducing proteolytic product loss were also investigated (Idiris et al., 2010; Hou et al., 2012; Tang et al., 2016; van Zyl et al., 2016; Kroukamp et al., 2017). In addition, producing the correct ratio of the different cellulases proved critical to enhance enzyme synergies and hence, to decrease the overall amount of cellulase required (Liu et al., 2017).

The need for a suitable strain background that can thrive in hostile fermentation environments is an additional complicating factor. Pretreatment inevitably releases phenolics, furans, and organic acids that inhibit yeast performance (Almeida et al., 2007). Strategies for detoxification of pretreatment liquor must be weighed against economic impacts and evolving or engineering strains amenable to toxic conditions may prove more feasible. Recent reports showed an emerging interest in the application of natural yeast isolates over lab yeast strains or those used in first generation ethanol production as some isolates proved more resistant to inhibitors and may have greater capacity to secrete heterologous enzymes (Davison et al., 2016; Jansen et al., 2017). Such yeasts could provide a superior starting point for engineering the high cellulase secreting, inhibitor-tolerant strains required for CBP.

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Whereas creating strains with such a vast number of heterologous genes or genomic edits in diploid strain backgrounds has proved challenging in the past, CRISPR-Cas9 and other recent technologies now provide the marker-less transformation tools required for such engineering. In addition, synthetic biology technologies such as whole genome engineering and purpose built designer genomes broaden possibilities even further (Richardson et al., 2017).

Considering the genetic malleability of *S. cerevisiae*, commodity products and green chemistry precursors other than ethanol could theoretically be produced from lignocellulose in a biorefinery approach. Interestingly, engineered xylose metabolism in yeasts elicits a respiratory response making it an attractive option in the production of compounds other than ethanol (Lane et al., 2018). Xylose consumption may thus be more amenable to redirection towards production of acetyl-CoA-derived molecules such as 1-hexadecanol, amorphadiene, and squalene. As current xylose-consuming strains of *S. cerevisiae* consume xylose at lower rates than glucose, converting this pentose to higher value products may be advantageous.

In light of the above, a new paradigm for CBP yeasts is suggested. Considering the variation in feedstock content and differences in commodity products sought, there is little chance of engineering one yeast strain for all required conversions. However, a series of yeast strains can be envisaged, based on robust, natural isolates producing ratio-optimised sets of cellulases and engineered for enhanced secretion and inhibitor tolerance, each converting a specific pretreated lignocellulosic substrate to a product of interest. In addition, sets of strains can be engineered to produce several fine chemicals and precursors from lignocellulose or the soluble xylose-containing stream produced during some pretreatment methodologies. Such approaches will offer biorefineries with the flexibility to adapt to economic factors and market requirements for broader product ranges.

References

[1] Almeida, J.R.M., Modig, T., Petersson, A., Hähn-Hägerdal, B., Lidén, G., Gorwa-Grauslund, M.F., 2007. Increased tolerance and conversion of inhibitors in lignocellulosic hydrolysates by *Saccharomyces cerevisiae*. J. Chem. Technol. Biotechnol. 82(4), 340-348.

[2] Davison, S.A., den Haan, R., van Zyl, W.H., 2016. Heterologous expression of cellulase genes in natural *Saccharomyces cerevisiae* strains. Appl. Microbiol. Biotechnol. 100, 8241-8254.

[3] den Haan, R., Kroukamp, H., Mert, M., Bloom, M., Gorgens, J.F., van Zyl, W.H., 2013. Engineering *Saccharomyces cerevisiae* for next generation ethanol production. J. Chem. Technol. Biotechnol. 88(6), 983-991.

[4] den Haan, R., van Rensburg, E., Rose, S.H., Gorgens, J.F., van Zyl, W.H., 2015. Progress and challenges in the engineering of non-cellulolytic microorganisms for consolidated bioprocessing. Curr. Opin. Biotechnol. 33, 32-38.

[5] Hou, J., Tyo, K.E., Liu, Z., Petranovic, D., Nielsen, J., 2012. Metabolic Engineering of Recombinant Protein Secretion by *Saccharomyces cerevisiae.* FEMS Yeast Res. 12, 491-510.

[6] Idiris, A., Tohda, H., Kumagai, H., Takegawa, K., 2010. Engineering of protein secretion in yeast: strategies and impact on protein production. Appl. Microbiol. Biotechnol. 86, 403-417.

[7] Jansen, T., Hoff, J.W., Jolly, N., van Zyl, W.H., 2017. Mating of natural *Saccharomyces cerevisiae* strains for improved glucose fermentation and lignocellulosic inhibitor tolerance. Folia Microbiol. 63, 155-168.

[8] Kroukamp, H., den Haan, R., van Zyl, J.H., van Zyl, W.H., 2017. Rational strain engineering interventions to enhance cellulase secretion by *Saccharomyces cerevisiae.* Biofuels, Bioprod. Biorefin. 12, 108-124.

[9] Lane, S., Dong, J., Jin, Y.S., 2018. Value-added biotransformation of cellulosic sugars by engineered *Saccharomyces cerevisiae.* Bioresour. Technol. 260, 28-394.

[10] Liu, Z., Inokuma, K., Ho, S.H., den Haan, R., van Zyl, W.H., Hasunuma, T., Kondo, A., 2017. Improvement of ethanol production from crystalline cellulose via optimizing cellulase ratios in cellulolytic *Saccharomyces cerevisiae.* Biotechnol. Bioeng. 114(6), 1201-1207.

[11] Lynd, L.R., Wyman, C.E., Gerngross, T.U., 1999. Biocommodity Engineering. Biotechnol. Prog. 15, 777-793.

[12] Lynd, L.R., Liang, X., Biddy, M.J., Allee, A., Cai, H., Foust, T., Himmel, M.E., Laser, M.S., Wang, M., Wyman, C.E., 2017. Cellulosic ethanol: status and innovation. Curr. Opin. Biotechnol. 45, 202-211.

[13] Olson, D.G., McBride, J.E., Shaw, A.J., Lynd, L.R., 2012. Recent progress in consolidated bioprocessing. Curr. Opin. Biotechnol. 23, 1-11.

[14] Richardson, S.M., Mitchell, L.A., Stracquadanio, G., Yang, K., Dymond, J.S., DiCarlo, J.E., Lee, D., Huang, C.L.V., Chandrasegaran, S., Cai, Y., Boeke, J.D., Bader, J.S., 2017. Design of a synthetic yeast genome. Science. 355, 1040-1044.

[15] Tang, H., Wang, S., Wang, J., Song, M., Xu, M., Zhang, M., 2016. N-Hypermannose glycosylation disruption enhances recombinant protein production by regulating secretory pathway and cell wall integrity in *Saccharomyces cerevisiae.* Sci. Rep. 6, 25654.

[16] van Rensburg, E., den Haan, R., La Grange, D.C., Volschenk, H., van Zyl, W.H., Gorgens, J.F., 2014. Engineering recombinant organisms for next-generation ethanol production, in: Lu, X. (ed.), Biofuels: From Microbes to Molecules. Horizon Scientific Press, pp. 93-135.

[17] van Zyl, J.H., den Haan, R., van Zyl, W.H., 2016. Overexpression of native *Saccharomyces cerevisiae* ER-to-Golgi SNARE genes increased heterologous cellulase secretion. Appl. Microbiol. Biotechnol. 100(1), 505-518.

[18] Vohra, M., Manwar, J., Manmode, R., Padgilwar, S., Patil, S., 2014. Bioethanol production: Feedstock and current technologies. J. Environ. Chem. Eng. 2(1), 573-584.

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