

Chapter 2

Which Yeast Species Shall I Choose? *Saccharomyces cerevisiae* Versus *Pichia pastoris* (Review)

Richard A.J. Darby, Stephanie P. Cartwright, Marvin V. Dilworth,
and Roslyn M. Bill

Abstract

Having decided on yeast as a production host, the choice of species is often the first question any researcher new to the field will ask. With over 500 known species of yeast to date, this could pose a significant challenge. However, in reality, only very few species of yeast have been employed as host organisms for the production of recombinant proteins. The two most widely used, *Saccharomyces cerevisiae* and *Pichia pastoris*, are compared and contrasted here.

Key words: Yeast, Host cell, Recombinant protein production

1. Introduction

Yeast is a single-celled, eukaryotic microbe that can grow quickly in complex or defined media (doubling times are typically 2.5 h in glucose-containing media) and is easier and less expensive to use for recombinant protein production than insect or mammalian cells (1). These positive attributes make yeast suitable for use in formats ranging from multi-well plates, shake flasks and continuously stirred tank bioreactors to pilot plant and industrial scale reactors.

The most commonly employed species in the laboratory are *Saccharomyces cerevisiae* (also known as Baker's or Brewer's yeast) and some methylotrophic yeasts of the *Pichia* genus. In particular, *S. cerevisiae* and *P. pastoris* have both been genetically characterized (2–4) and shown to perform the posttranslational disulphide bond formation and glycosylation (5–7) that is crucial for the proper functioning of some recombinant proteins. However, it is impor-

tant to note that yeast glycosylation does differ from that in mammalian cells: in *S. cerevisiae*, O-linked oligosaccharides contain only mannose moieties, whereas higher eukaryotic proteins have sialylated O-linked chains. Furthermore *S. cerevisiae* is known to hyperglycosylate N-linked sites, which can result in altered protein binding, activity, and potentially yield an altered immunogenic response in therapeutic applications (8). In *P. pastoris*, oligosaccharides are of much shorter chain length (9) and a strain has been reported that can produce complex, terminally sialylated or “humanized” glycoproteins (10).

Despite these potential limitations, recombinant pharmaceuticals including insulin (11), interferon-alpha-2a (Reiferon Retard®) and vaccines against hepatitis B virus (Hepavax-Gene and Engerix-B®) (12) and Human papilloma virus (Gardasil®) (13, 14) have all been produced in yeasts for commercial use, demonstrating the importance of yeast as a host organism to the pharmaceutical industry. The benefits and limitations of using *S. cerevisiae* and *P. pastoris* on a laboratory scale are addressed here and specific examples of their uses for the production of both soluble and membrane proteins are discussed.

2. *Saccharomyces cerevisiae*

S. cerevisiae is a single-celled, budding yeast, approximately 5–10 µm in size. Whilst it is commonly associated with the brewing and baking industries on account of its ability to produce ethanol and carbon dioxide, it is also the most widely studied eukaryotic organism. The USA’s Food and Drug Administration (FDA) award of “generally recognized as safe” (GRAS) status to *S. cerevisiae* means that it is the most frequently used species of yeast for the production of many functional proteins. These include several soluble antibody fragments and fusions (15–19) as well as membrane protein drug targets such as G protein-coupled receptors (20–25), ABC transporters (9, 26) and drug resistance proteins (27).

2.1. Microbiology

The microbiology of *S. cerevisiae* is well understood and has been extensively reviewed elsewhere (28). In essence, it can grow both aerobically and anaerobically on a variety of carbon sources, is able to use ammonia or urea as a nitrogen source and also requires phosphorus and sulphur in its growth media. Certain metals such as calcium, iron, magnesium and zinc enhance its growth (29, 30): Tables 1 and 2 summarise typical growth media for *S. cerevisiae*. In culture, it has a relatively short generation time, doubling its cell density approximately every 1.5–2.5 h at its preferred growth temperature of 30°C.

The ability of *S. cerevisiae* to produce ethanol hints at its unusual metabolism: in most eukaryotes, oxygen depletion controls

Table 1
Composition of typical media for culturing *S. cerevisiae* and *P. pastoris*

<i>S. cerevisiae</i> medium	Components (L ⁻¹)
YPD (rich medium)	10 g bacto yeast extract 20 g bacto peptone 20 g glucose
YPG (rich medium with non-fermentable carbon source)	10 g bacto yeast extract 20 g bacto peptone 30 mL glycerol
CSM (complete synthetic medium)	1.7 g bacto yeast nitrogen base (without amino acids) 5 g ammonium sulphate 20 g glucose 100 mL 10× amino acid solution (see Table 2)
2× CBS (Centraalbureau voor Schimmelcultures medium)	10 g ammonium sulphate 6 g potassium dihydrogen phosphate 1 g magnesium sulphate heptahydrate 20 g glucose 100 mL 1 M MES, pH 6 200 mL 10× amino acid solution (see Table 2) 2 mL vitamin solution (see Table 2) 2 mL trace element solution (see Table 2)
<i>P. pastoris</i> medium	Components (L ⁻¹)
BMGY (buffered glycerol-complex medium)	10 g bacto yeast extract 20 g bacto peptone 100 mL 10× YNB (see Table 2) 100 mL 1 M potassium phosphate (pH 6) 2 mL 500× biotin (see Table 2) 100 mL 10× glycerol (see Table 2)
BMMY (buffered methanol-complex medium)	10 g bacto yeast extract 20 g bacto peptone 100 mL 10× YNB (see Table 2) 100 mL 1 M potassium phosphate (see Table 2) 2 mL 500× biotin (see Table 2) 100 mL 10× methanol (see Table 2)
BSM (basal salts medium)	26.7 mL phosphoric acid 0.93 g calcium sulphate 18.2 g potassium sulphate 14.9 g magnesium sulphate heptahydrate 4.13 g potassium hydroxide 40 g glycerol 4.35 mL PTM ₁ salts (see Table 2)

Table 2
Composition of stock solutions required to prepare media in
Table 1

<i>S. cerevisiae</i> medium stocks	Components (L ⁻¹)
10× amino acid solution	200 mg L-adenine hemisulphate 200 mg L-arginine hydrochloride 200 mg L-histidine hydrochloride monohydrate 300 mg L-isoleucine 1000 mg L-leucine 300 mg L-lysine hydrochloride 200 mg L-methionine 500 mg L-phenylalanine 2000 mg L-threonine 200 mg L-tryptophan 300 mg L-tyrosine 200 mg L-uracil 1500 mg L-valine
Vitamin solution (filter sterilised)	0.05 g biotin 1 g calcium D-pantothenate 1 g nicotinic acid 25 g myo-inositol 1 g thiamine hydrochloride 1 g pyridoxol hydrochloride 0.2 g D-amino benzoic acid
Trace element solution	15 g EDTA 4.5 g zinc sulphate heptahydrate 1 g magnesium chloride tetrahydrate 0.3 g cobalt (II) chloride hexahydrate 0.3 g copper (II) sulphate pentahydrate 0.4 g sodium molybdate dihydrate 4.5 g calcium chloride dihydrate 3 g iron sulphate heptahydrate 1 g boric acid 0.1 g potassium iodide
<i>P. pastoris</i> medium stocks	Components (L ⁻¹)
10× YNB (filter sterilised)	34 g yeast nitrogen base without ammonium sulphate and amino acids 100 g ammonium sulphate
500× biotin (filter sterilised)	200 mg biotin
1 M potassium phosphate, pH 6	868 mL 1 M KH ₂ PO ₄ ; 132 mL 1 M K ₂ HPO ₄ (adjust to pH 6 with KOH and phosphoric acid)
10× glycerol (10%)	100 mL glycerol

(continued)

Table 2
(continued)

<i>P. pastoris</i> medium stocks	Components (L ⁻¹)
10× methanol (5%; filter sterilised)	50 mL methanol
PTM ₁ salts (filter sterilised)	6 g cupric sulphate pentahydrate 0.08 g sodium iodide 3 g manganese sulfate monohydrate 0.2 g sodium molybdate dihydrate 0.02 g boric acid 0.5 g cobalt chloride 20 g zinc chloride 65 g ferrous sulphate heptahydrate 0.2 g biotin 5 mL sulphuric acid

the switch from a respiratory to a fermentative metabolism, but in *S. cerevisiae* this switch also occurs in response to a change in the external concentration of a fermentable carbon source such as glucose (31). During the type of aerobic batch cultivation on glucose often performed on a laboratory scale, *S. cerevisiae* displays a biphasic growth pattern (Fig. 1). In the first respiro-fermentative phase, most of the glucose is converted to ethanol (32), which is subsequently consumed to produce carbon dioxide and water in the second phase. This has evolutionary advantages for *S. cerevisiae*, as the ethanol production phase is associated with a higher specific growth rate than the respiratory phase, providing a competitive advantage over other non-ethanol-producing organisms. Maximum recombinant protein yields are usually highest before yeast cells reach the end of this respiro-fermentative phase, before the so-called “diauxic shift” (Fig. 1) into the respiratory phase (33). Consequently, *S. cerevisiae* cells are typically harvested just before this diauxic shift in a protein production experiment, which can be readily assessed by monitoring the off-gas profile or the glucose concentration in the culture (24, 33). A respiratory strain of *S. cerevisiae*, TM6*, has been reported to have improved yield properties for both recombinant soluble and membrane proteins on account of its altered metabolism (24, 33). Its improved biomass yields, which are achieved at the expense of ethanol production, result in an increased volumetric yield of recombinant protein (34).

2.2. Genetics

S. cerevisiae was the first eukaryote to have its complete genome sequenced (2). The data are publicly available from the *Saccharomyces* Genome Database (SGD; <http://www.yeastgenome.org>), which is a scientific database of yeast molecular biology and genetics. The SGD provides detailed descriptions of the phenotypes of many mutant *S. cerevisiae* strains, many of which may have potential as

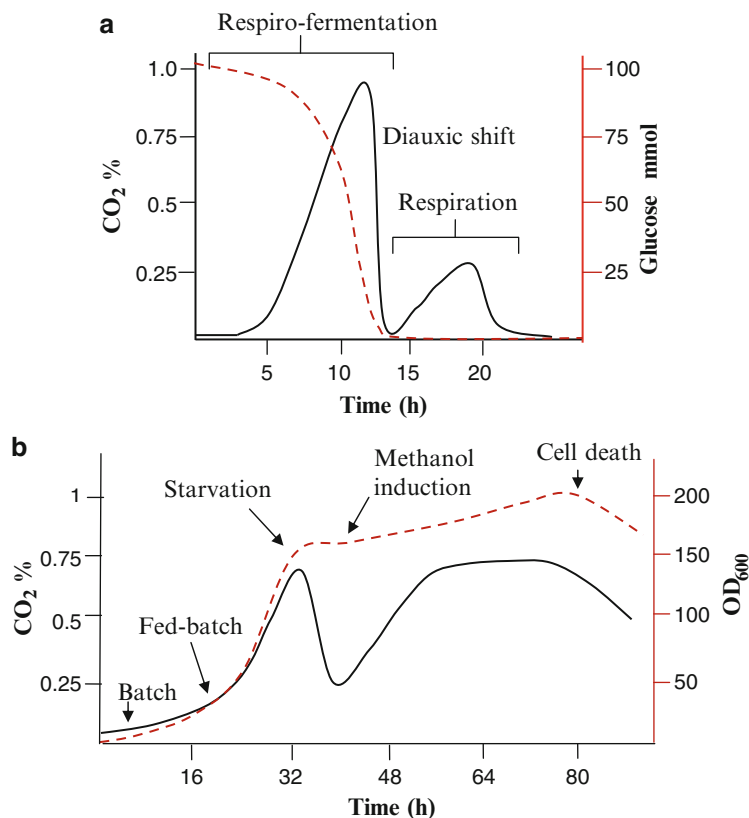


Fig. 1. (a) A representative CO₂ gas profile recorded in our laboratory, using a Tandem gas analyser, for a glucose-limited *S. cerevisiae* culture grown in a 2 L Applikon bioreactor. The solid line shows the production of CO₂ (%) and the dashed line shows the depletion of glucose (mmol). The respiro-fermentation and respiration phases are indicated, as is the diauxic shift between the two phases. (b) A CO₂ gas profile recording for a *P. pastoris* glycerol fed-batch bioreactor culture induced at 28 h with a limiting amount of methanol. The solid line shows the production of CO₂ (%) and the dashed line shows the optical density of the culture.

protein production hosts, with links to supporting literature. Importantly, the SGD is fully annotated and is continually updated.

Resources such as the SGD have facilitated an increased understanding of *S. cerevisiae* in general and, more specifically in the context of this discussion, of recombinant protein production in yeast cells. For example, since the production of high yields of functional recombinant proteins, particularly membrane proteins, remains a significant challenge, we and others (35, 36) have focussed on characterising the cellular response to recombinant protein production. Identifying specific genes that exhibit an altered transcriptional profile, when the cell produces high yields of functional recombinant proteins, has been used to guide subsequent engineering of high-yielding strains (24, 33, 37). This approach is

Table 3
Examples of expression vectors available for *S. cerevisiae* and *P. pastoris*

Vector	Yeast	Selection marker	Promoter	Expression	Episomal or integrative
pYX212	<i>S. cerevisiae</i>	<i>URA3</i>	<i>TPH1</i>	Constitutive	Episomal
pYX222	<i>S. cerevisiae</i>	<i>HIS3</i>	<i>TPH1</i>	Constitutive	Episomal
pYES2	<i>S. cerevisiae</i>	<i>URA3</i>	<i>GAL1</i>	Inducible	Episomal
pVTU260	<i>S. cerevisiae</i>	<i>URA3</i>	<i>ADHI</i>	Constitutive	Episomal
YE _p CTHS	<i>S. cerevisiae</i>	<i>Ampicillin</i>	<i>CUP1</i>	Inducible	Episomal
pPICZ	<i>P. pastoris</i>	<i>Zeocin</i>	<i>AOX1</i>	Inducible	Integrative
pGAPZ	<i>P. pastoris</i>	<i>Zeocin</i>	<i>GAP</i>	Constitutive	Integrative
pBGP1	<i>P. pastoris</i>	<i>Zeocin</i>	<i>GAP</i>	Constitutive	Episomal
pGAPZ-E	<i>P. pastoris</i>	<i>Zeocin</i>	<i>GAP</i>	Constitutive	Episomal

strengthened by the availability of a complete set of single, non-essential gene deletion strains (EUROSCARF) as well as a strain collection of tetracycline-regulated essential genes (Open Biosystems). We demonstrated that increased yields of recombinant proteins can be achieved when specific members of these collections are used as host organisms (37). This permits the production of a recombinant protein to be compared in multiple strains simultaneously whilst gaining an improved knowledge of the molecular pathways involved in producing the protein.

2.3. Molecular Biology

The DNA sequence encoding the target protein of interest is typically amplified via PCR either from genomic DNA or cDNA and then cloned into a suitable expression plasmid with or without signal sequences and fusion partners (see Chapters 3 and 4). Plasmids for *S. cerevisiae* can be sub-divided into three categories: low-copy number replicating plasmids, multi-copy number replicating plasmids and integrative plasmids (38, 39). These can, in turn, contain a range of different promoters of varying strengths that are either inducible or constitutive (Table 3). This means that a variety of different options can be tested in order to optimize the most suitable regime for recombinant protein production. However, it is important to ensure the stability of any transformants generated. For example, autonomous plasmids can be relatively unstable, yielding a heterogeneous population of transformants that routinely require screening for the desired expression level, as well as being prone to genetic loss upon cell division. Furthermore, high-copy number plasmids may also result in expression levels

that can overwhelm the host's post-translational and secretory pathways, yielding misfolded and degraded protein. It may be possible to overcome some of these problems by integrating the expression cassette into the genome, thereby increasing its genetic stability (38, 39).

3. *Pichia pastoris*

P. pastoris is used increasingly as the host cell of choice because of its ability to produce high yields of properly folded proteins in exceptionally high-density cultures. To achieve the highest possible biomass yields, it must be cultured in fully controlled, continuously stirred tank bioreactors (40). Its emergence as an alternative to *S. cerevisiae* is exemplified by the variety of heterologous proteins it has been used to produce in high yields, ranging from tetanus toxin and mouse epidermal growth factor (41–43) to membrane proteins including human ABC transporters, aquaporins and tetraspanins (44–46). A number of different protease-deficient strains are also available (SMD1163, 1165 and 1168) that have been shown to exhibit reduced proteolysis of some recombinant proteins (47–50).

3.1. Microbiology

P. pastoris has a respiratory metabolism (Fig. 1) and can be cultured to exceptionally high cell densities (hundreds of grams per litre) on glycerol-containing media (Tables 1 and 2), often yielding a culture resembling a paste at the end of an experiment (43, 51). The development of a respiratory strain of *S. cerevisiae* (TM6*) (24, 52) has gone some way to permitting similarly high cell density cultures of *S. cerevisiae*. However, while high cell density cultures are very attractive for increasing volumetric yields, productivity does not necessarily increase linearly with increased biomass yields, and in some situations may actually decrease (40, 53). For this reason, the ability to increase “per cell” yields is an area of active research, not only in *P. pastoris* but also in other host cells (34).

P. pastoris is a methylotroph, which has two endogenous copies of the *AOX* gene; *AOX1* expression accounts for more than 90% of the enzyme in the cell whilst *AOX2* expression constitutes less than 10% (see Chapter 15). It is these genes that permit the utilisation of methanol as a carbon source: using the *Pichia* Expression Kit marketed by Invitrogen Corporation, it is possible to use the *AOX* promoter to control heterologous gene expression, which is induced in the presence of methanol and repressed by glucose or glycerol (51). The careful control of the methanol induction regime is central to increasing yields per cell (40). Methanol metabolism is highly dependent on oxygen availability within the culture and it is widely accepted that the dissolved oxygen concentration (DO) should be maintained above 20% (54). This regime has been

successfully employed to produce many different soluble and membrane proteins (40, 44, 46, 53, 55–57).

Despite methanol induction being robust and tightly regulated, the potential risks associated with methanol use, such as its toxicity to cells at concentrations above 5 g/L and its volatility, have led researchers to investigate alternative promoters that do not require the use of methanol (58). Constitutive expression of heterologous genes can be achieved when cloned downstream of the glyceraldehyde 3-phosphate dehydrogenase (*GAP*) promoter (59, 60), whilst strong induction via the formaldehyde dehydrogenase (*FLDI*) promoter has also been reported in the presence of methylamine as well as methanol (61).

Like *S. cerevisiae*, *P. pastoris* is capable of producing disulphide-bonded and glycosylated proteins (62). However, the glycosylation pattern is different in *P. pastoris* compared with *S. cerevisiae* (63): in *P. pastoris* N-linked oligosaccharides are usually no more than 20 residues in length compared with 50–150 residues in *S. cerevisiae*. In addition, *P. pastoris* lacks the mannosyl transferase which yields immunogenic α -1, 3-linked mannosyl terminal linkages in *S. cerevisiae* (64).

3.2. Genetics

P. pastoris is not considered to be as genetically amenable as *S. cerevisiae*, despite the fact that a genomic sequence of the GS115 strain has been commercially available since the mid-2000s. This is partly on account of a restrictive user contract that required all sequence information to be confidentially maintained. Despite this, genetic advances have been made as highlighted by the development of a “humanized” *P. pastoris* strain (10) capable of replicating the most essential glycosylation pathways found in mammalian cells and permitting the production of active recombinant erythropoietin. The open-access publication of the GS115 (3) and DSMZ 70382 (4) genomes in 2009 and their respective annotation at <http://bioinformatics.psb.ugent.be/webtools/bogas/> and <http://www.pichiagenome.org> should make a significant impact in this area, as both sites permit free access to view the genomic sequences and use sequence resource software (65).

3.3. Molecular Biology

As for *S. cerevisiae*, the target protein’s DNA sequence is often PCR amplified from genomic DNA or cDNA and cloned into an expression plasmid with or without signal sequences and fusion partners. The most widely used *P. pastoris* expression vectors are designed to be maintained as stable, integrative elements in its genome (see Chapter 3). Examples include Invitrogen’s pPIC and pGAPZ series of vectors (Table 3). However, transformants often exhibit heterogeneous expression levels, and this necessitates the screening of many colonies to isolate high-yielding clones (see Chapter 7). The limited number of episomal plasmids for *P. pastoris* to date has been predominantly due to plasmid instability during

replication (66). Those that are available (Table 3) often utilize the constitutive *GAP* promoter (67, 68) and require the addition of selective antibiotic to maintain the vector. With the advent of open access genomic data it is hoped that there will be an increase in the number of episomal vectors that contain different auxotrophic markers for selection.

4. Which Species Should I Choose?

There are benefits and drawbacks to using both *S. cerevisiae* and *P. pastoris* as hosts for recombinant protein production. For the production of secreted proteins, *P. pastoris* may be the best choice on account of its limited endogenous protein secretion and the number of different protease-deficient strains available. However, the full benefit of *P. pastoris* is achievable only if it is cultured under strictly defined conditions, usually only possible in continuously stirred tank bioreactors. Therefore the optimal use of *P. pastoris* may require a more long-term investment of time and equipment resources than for *S. cerevisiae*. In contrast *S. cerevisiae* provides a much wider range of resources (both strains and expression vectors) and is supported by a much more extensive literature than *P. pastoris*. Consequently, projects requiring a range of strains may benefit from using *S. cerevisiae* as the host. In our laboratory, we often start with *P. pastoris* and if the production is not straightforward, turn to *S. cerevisiae* to troubleshoot, thereby benefitting from the best attributes of the two hosts.

References

1. Bill RM (2001) Yeast – a panacea for the structure-function analysis of membrane proteins? *Curr Genet* 40:157–171
2. Goffeau A, Barrell BG, Bussey H, Davis RW, Dujon B, Feldmann H, Galibert F, Hoheisel JD, Jacq C, Johnston M, Louis EJ, Mewes HW, Murakami Y, Philippsen P, Tettelin H, Oliver SG (1996) Life with 6000 genes. *Science* 274:546–567
3. De Schutter K, Lin YC, Tiels P, Van Hecke A, Glinka S, Weber-Lehmann J, Rouze P, de Peer YV, Callewaert N (2009) Genome sequence of the recombinant protein production host *Pichia pastoris*. *Nat Biotechnol* 27:561–566
4. Mattanovich D, Graf A, Stadlmann J, Dragosits M, Redl A, Maurer M, Kleinheinz M, Sauer M, Altmann F, Gasser B (2009) Genome, secretome and glucose transport highlight unique features of the protein production host *Pichia pastoris*. *Microb Cell Fact* 8:29
5. Demain AL, Vaishnav P (2009) Production of recombinant proteins by microbes and higher organisms. *Biotechnol Adv* 27:297–306
6. Ferrer-Miralles N, Domingo-Espin J, Corchero JL, Vazquez E, Villaverde A (2009) Microbial factories for recombinant pharmaceuticals. *Microb Cell Fact* 8:17
7. Jigami Y (2008) Yeast glycobiology and its application. *Biosci Biotechnol Biochem* 72:637–648
8. Gerngross TU (2004) Advances in the production of human therapeutic proteins in yeasts and filamentous fungi. *Nat Biotechnol* 22:1409–1414
9. Bretthauer RK, Castellino FJ (1999) Glycosylation of *Pichia pastoris* – derived proteins. *Biotechnol Appl Biochem* 30:193–200
10. Hamilton SR, Davidson RC, Sethuraman N, Nett JH, Jiang YW, Rios S, Bobrowicz P,

- Stadheim TA, Li HJ, Choi BK, Hopkins D, Wischniewski H, Roser J, Mitchell T, Strawbridge RR, Hoopes J, Wildt S, Gerngross TU (2006) Humanization of yeast to produce complex terminally sialylated glycoproteins. *Science* 313:1441–1443
11. Kjeldsen T, Ludvigsen S, Diers I, Balschmidt P, Sørensen AR, Kaarsholm NC (2002) Engineering-enhanced protein secretory expression in yeast with application to insulin. *J Biol Chem* 277:18245–18248
 12. Andre FE, Safary A (1987) Summary of clinical findings on engerix-b, a genetically engineered yeast-derived Hepatitis-B vaccine. *Postgrad Med J* 63:169–178
 13. Siddiqui MAA, Perry CM (2006) Human papillomavirus quadrivalent (types 6, 11, 16, 18) recombinant vaccine (Gardasil (R)): profile report. *BioDrugs* 20:313–316
 14. Siddiqui MAA, Perry CM (2006) Human papillomavirus quadrivalent (types 6, 11, 16, 18) recombinant vaccine (Gardasil (R)). *Drugs* 66:1263–1271
 15. Gasser B, Mattanovich D (2007) Antibody production with yeasts and filamentous fungi: on the road to large scale? *Biotechnol Lett* 29:201–212
 16. Hackel BJ, Huang DG, Bubo JC, Wang XX, Shusta EV (2006) Production of soluble and active transferrin receptor-targeting single-chain antibody using *Saccharomyces cerevisiae*. *Pharm Res* 23:790–797
 17. Evans L, Hughes M, Waters J, Cameron J, Dodsworth N, Tooth D, Greenfield A, Sleep D (2010) The production, characterisation and enhanced pharmacokinetics of scFv-albumin fusions expressed in *Saccharomyces cerevisiae*. *Protein Expr Purif* 73:113–124
 18. Frenken LGJ, van der Linden RHJ, Hermans PWJJ, Bos JW, Ruuls RC, de Geus B, Verrips CT (2000) Isolation of antigen specific Llama V-HH antibody fragments and their high level secretion by *Saccharomyces cerevisiae*. *J Biotechnol* 78:11–21
 19. Edqvist J, Keranen S, Penttilä M, Straby KB, Knowles JKC (1991) Production of functional Igm Fab fragments by *Saccharomyces cerevisiae*. *J Biotechnol* 20:291–300
 20. Liitti S, Matikainen MT, Scheinin M, Glumoff T, Goldman A (2001) Immunoaffinity purification and reconstitution of human alpha(2)-adrenergic receptor subtype C2 into phospholipid vesicles. *Protein Expr Purif* 22:1–10
 21. Huang HJ, Liao CF, Yang BC, Kuo TT (1992) Functional expression of rat M5 muscarinic acetylcholine-receptor in yeast. *Biochem Biophys Res Commun* 182:1180–1186
 22. Price LA, Strnad J, Pausch MH, Hadcock JR (1996) Pharmacological characterization of the rat A(2a) adenosine receptor functionally coupled to the yeast pheromone response pathway. *Mol Pharmacol* 50:829–837
 23. Joubert O, Nehme R, Bidet M, Mus-Veteau I (2010) Heterologous expression of human membrane receptors in the yeast *Saccharomyces cerevisiae*, heterologous expression of membrane proteins. *Methods Mol Biol* 601:87–103
 24. Ferndahl C, Bonander N, Logez C, Wagner R, Gustafsson L, Larsson C, Hedfalk K, Darby RAJ, Bill RM (2010) Increasing cell biomass in *Saccharomyces cerevisiae* increases recombinant protein yield: the use of a respiratory strain as a microbial cell factory. *Microb Cell Fact* 9:47
 25. Kapat A, Jaakola VP, Heimo H, Liitti S, Heikinheimo P, Glumoff T, Goldman A (2000) Production and purification of recombinant human alpha 2C2 adrenergic receptor using *Saccharomyces cerevisiae*. *Bioseparation* 9:167–172
 26. Duman JG, Miele RG, Liang H, Grella DK, Sim KL, Castellino FJ, Bretthauer RK (1998) O-Mannosylation of *Pichia pastoris* cellular and recombinant proteins. *Biotechnol Appl Biochem* 28:39–45
 27. Miele RG, Castellino FJ, Bretthauer RK (1997) Characterization of the acidic oligosaccharides assembled on the *Pichia pastoris*-expressed recombinant kringle 2 domain of human tissue-type plasminogen activator. *Biotechnol Appl Biochem* 26:79–83
 28. Barnett JA, Barnett L (2011) Yeast research: a historical overview. ASM, Herndon, VA
 29. Treco DA, Lundblad V (2001) Preparation of yeast media. *Curr Protoc Mol Biol* Chapter 13, Unit13.1
 30. Curran BP, Bugeja V (2006) Basic investigations in *Saccharomyces cerevisiae*. *Methods Mol Biol* 313:1–13
 31. Bonander N, Ferndahl C, Mostad P, Wilks MD, Chang C, Showe L, Gustafsson L, Larsson C, Bill RM (2008) Transcriptome analysis of a respiratory *Saccharomyces cerevisiae* strain suggests the expression of its phenotype is glucose insensitive and predominantly controlled by *Hap4*, *Cat8* and *Mig1*. *BMC Genomics* 9:365
 32. Verduyn C, Zomerdijsk TPL, Dijken JP, Scheffers WA (1984) Continuous measurement of ethanol production by aerobic yeast suspensions with an enzyme electrode. *Appl Microbiol Biotechnol* 19:181–185
 33. Bonander N, Hedfalk K, Larsson C, Mostad P, Chang C, Gustafsson L, Bill RM (2005) Design of improved membrane protein production

- experiments: quantitation of the host response. *Protein Sci* 14:1729–1740
34. Bawa Z, Bland CE, Bonander N, Bora N, Cartwright SP, Clare M, Conner MT, Darby RA, Dilworth MV, Holmes WJ, Jamshad M, Routledge SJ, Gross SR, Bill RM (2011) Understanding the yeast host cell response to recombinant membrane protein production. *Biochem Soc Trans* 39:719–723
 35. Wang H, Prorok M, Bretthauer RK, Castellino FJ (1997) Serine-578 is a major phosphorylation locus in human plasma plasminogen. *Biochemistry* 36:8100–8106
 36. Ren J, Castellino FJ, Bretthauer RK (1997) Purification and properties of alpha-mannosidase II from Golgi-like membranes of baculovirus-infected *Spodoptera frugiperda* (IPLB-SF-21AE) cells. *Biochem J* 324:951–956
 37. Bonander N, Darby RAJ, Grgic L, Bora N, Wen J, Brogna S, Poyner DR, O'Neill MAA, Bill RM (2009) Altering the ribosomal subunit ratio in yeast maximizes recombinant protein yield. *Microb Cell Fact* 8:10
 38. Schneider JC, Guarente L (1991) Vectors for expression of cloned genes in yeast: regulation, overproduction and underproduction. *Methods Enzymol* 194:373–388
 39. Zhang Z, Moo-Young M, Chisti Y (1996) Plasmid stability in recombinant *Saccharomyces cerevisiae*. *Biotechnol Adv* 14:401–435
 40. Holmes WJ, Darby RAJ, Wilks MDB, Smith R, Bill RM (2009) Developing a scalable model of recombinant protein yield from *Pichia pastoris*: the influence of culture conditions, biomass and induction regime. *Microb Cell Fact* 8:35
 41. Clare JJ, Rayment FB, Ballantine SP, Sreekrishna K, Romanos MA (1991) High-level expression of tetanus toxin fragment C in *Pichia pastoris* strains containing multiple tandem integrations of the gene. *Biotechnology* 9:455–460
 42. Clare JJ, Romanos MA, Rayment FB, Rowedder JE, Smith MA, Payne MM, Sreekrishna K, Henwood CA (1991) Production of mouse epidermal growth-factor in yeast – high-level secretion using *Pichia pastoris* strains containing multiple gene copies. *Gene* 105:205–212
 43. Macauley-Patrick S, Fazenda ML, McNeil B, Harvey LM (2005) Heterologous protein production using the *Pichia pastoris* expression system. *Yeast* 22:249–270
 44. Rosenberg MF, Bikadi Z, Chan J, Liu XP, Ni ZL, Cai XK, Ford RC, Mao QC (2010) The human breast cancer resistance protein (BCRP/ABCG2) shows conformational changes with mitoxantrone. *Structure* 18:482–493
 45. Urbatsch IL, Wilke-Mounts S, Gimi K, Senior AE (2001) Purification and characterization of n-glycosylation mutant mouse and human p-glycoproteins expressed in *Pichia pastoris* cells. *Arch Biochem Biophys* 388:171–177
 46. Jamshad M, Rajesh S, Stamatakis Z, McKeating JA, Dafforn T, Overduin M, Bill RM (2008) Structural characterization of recombinant human CD81 produced in *Pichia pastoris*. *Protein Expr Purif* 57:206–216
 47. Grunewald S, Haase W, Molsberger E, Michel H, Reilander H (2004) Production of the human D-2S receptor in the methylotrophic yeast *P. pastoris*. *Receptors Channels* 10:37–50
 48. Shi XL, Feng MQ, Shi J, Shi ZHA, Zhong JA, Zhou P (2007) High-level expression and purification of recombinant human catalase in *Pichia pastoris*. *Protein Expr Purif* 54:24–29
 49. Ogunjimi AA, Chandler JM, Gooding CM, Recinos A, Choudary PV (1999) High-level secretory expression of immunologically active intact antibody from the yeast *Pichia pastoris*. *Biotechnol Lett* 21:561–567
 50. Andre N, Cherouati N, Prual C, Steffan T, Zeder-Lutz G, Magnin T, Pattus F, Michel H, Wagner R, Reinhart C (2006) Enhancing functional production of G protein-coupled receptors in *Pichia pastoris* to levels required for structural studies via a single expression screen. *Protein Sci* 15:1115–1126
 51. Cregg JM, Cereghino JL, Shi JY, Higgins DR (2000) Recombinant protein expression in *Pichia pastoris*. *Mol Biotechnol* 16:23–52
 52. Otterstedt K, Larsson C, Bill RM, Stahlberg A, Boles E, Hohmann S, Gustafsson L (2004) Switching the mode of metabolism in the yeast *Saccharomyces cerevisiae*. *EMBO Rep* 5:532–537
 53. Singh S, Hedley D, Kara E, Gras A, Iwata S, Ruprecht J, Strange PG, Byrne B (2010) A purified C-terminally truncated human adenosine A2A receptor construct is functionally stable and degradation resistant. *Protein Expr Purif* 74:80–87
 54. Li PZ, Anumanthan A, Gao XG, Ilangovan K, Suzara VV, Duzgunes N, Renugopalakrishnan V (2007) Expression of recombinant proteins in *Pichia pastoris*. *Appl Biochem Biotechnol* 142:105–124
 55. Yinliang C, Cino J, Hart G, Freedman D, White C, Komives EA (1997) High protein expression in fermentation of recombinant *Pichia pastoris* by a fed-batch process. *Process Biochem* 32:107–111
 56. Jin H, Liu G, Ye X, Duan Z, Li Z, Shi Z (2010) Enhanced porcine interferon- α production by recombinant *Pichia pastoris* with a combinational control strategy of low induction temperature and high dissolved oxygen concentration. *Biochem Eng J* 52:91–98

57. Fraser NJ (2006) Expression and functional purification of a glycosylation deficient version of the human adenosine 2a receptor for structural studies. *Protein Expr Purif* 49:129–137
58. Cos O, Ramon R, Montesinos JL, Valero F (2006) Operational strategies, monitoring and control of heterologous protein production in the methylotrophic yeast *Pichia pastoris* under different promoters: a review. *Microb Cell Fact* 5:17
59. Waterham HR, Digan ME, Koutz PJ, Lair SV, Cregg JM (1997) Isolation of the *Pichia pastoris* glyceraldehyde-3-phosphate dehydrogenase gene and regulation and use of its promoter. *Gene* 186:37–44
60. Kim SJ, Lee JA, Kim YH, Song BK (2009) optimization of the functional expression of coprinus cinereus peroxidase in *Pichia pastoris* by varying the host and promoter. *J Microbiol Biotechnol* 19:966–971
61. Resina D, Cos O, Ferrer P, Valero F (2005) Developing high cell density fed-batch cultivation strategies for heterologous protein production in *Pichia pastoris* using the nitrogen source-regulated FLD1 promoter. *Biotechnol Bioeng* 91:760–767
62. Daly R, Hearn MTW (2005) Expression of heterologous proteins in *Pichia pastoris*: a useful experimental tool in protein engineering and production. *J Mol Recognit* 18:119–138
63. Dale C, Allen A, Fogerty S (1999) *Pichia pastoris*: a eukaryotic system for the large-scale production of biopharmaceuticals. *Biopharm* 12:36–40
64. Cregg JM, Vedvick TS, Raschke WC (1993) Recent advances in the expression of foreign genes in *Pichia pastoris*. *Nat Biotechnol* 11:905–910
65. Mattanovich D, Callewaert N, Rouze P, Lin YC, Graf A, Redl A, Tiels P, Gasser B, De Schutter K (2009) Open access to sequence: browsing the *Pichia pastoris* genome. *Microb Cell Fact* 8:53
66. Cregg JM, Barringer KJ, Hessler AY, Madden KR (1985) *Pichia-pastoris* as a host system for transformations. *Mol Cell Biol* 5:3376–3385
67. Lee CC, Williams TG, Wong DWS, Robertson GH (2005) An episomal expression vector for screening mutant gene libraries in *Pichia pastoris*. *Plasmid* 54:80–85
68. Choi SG, Hong IP, Anderson S (2006) Evaluation of a new episomal vector based on the GAP promoter for structural genomics in *Pichia pastoris*. *J Microbiol Biotechnol* 16:1362–1368

Recombinant Protein Production in Yeast
Methods and Protocols

Bill, R.M. (Ed.)

2012, XII, 248 p. 47 illus., 6 illus. in color., Hardcover

ISBN: 978-1-61779-769-9

A product of Humana Press