·综 述·

酵母表面展示技术应用的最新进展

赵黎丽，苏炳恺，杜姝姝，丁文婷，刘熔增*

河南科技大学基础医学院 免疫学教研室，河南 洛阳 471023

赵黎丽，苏炳恺，杜姝姝，丁文婷，刘熔增. 酵母表面展示技术应用的最新进展[J]. 生物工程学报，2023，39(11): 4358-4375.
ZHAO Lili, SU Bingkai, DU Shushu, DING Wenting, LIU Rongzeng. Advances in the application of yeast surface display technology[J]. Chinese Journal of Biotechnology, 2023, 39(11): 4358-4375.

摘 要：酵母表面展示(yeast surface display, YSD)技术是一种将外源靶蛋白基因序列与特定的载体基因序列融合后导入酵母细胞，利用酵母细胞内蛋白转运机制将靶蛋白表达并定位于酵母细胞表面的技术，最常用的是 α-凝集素表达系统。酵母细胞具有真核细胞翻译后修饰机制，能够帮助目的蛋白正确折叠，可以用来展示各种真核蛋白，包括抗体、受体、酶和抗原肽等。酵母表面展示技术已成为生物技术和生物医学领域的强大蛋白质工程工具，结合流式细胞分选可用于改善蛋白质性质，包括亲和力、特异性、酶功能和稳定性等。本文从文库构建与筛选、抗体工程、蛋白质工程、酶工程和疫苗开发等方面对酵母表面展示技术应用最新进展进行了综述。

关键词：酵母表面展示技术；文库构建；抗体工程；蛋白质工程；酶工程；口服疫苗

Advances in the application of yeast surface display technology

ZHAO Lili, SU Bingkai, DU Shushu, DING Wenting, LIU Rongzeng*

Department of Immunology, School of Basic Medical Sciences, Henan University of Science and Technology, Luoyang 471023, Henan, China

Abstract: Yeast surface display (YSD) is a technology that fuses the exogenous target protein gene sequence with a specific vector gene sequence, followed by introduction into yeast cells. Subsequently, the target protein is expressed and localized on the yeast cell surface by using the intracellular protein transport mechanism of yeast cells, whereas the most widely used YSD

资助项目：国家自然科学基金(82271851，81901663)；河南科技大学大学生研究训练计划项目(2022400)
This work was supported by the National Natural Science Foundation of China (82271851, 81901663) and the Student Research Training Program of Henan University of Science and Technology (2022400).
*Corresponding author. E-mail: liurz@haust.edu.cn
Received: 2023-02-08; Accepted: 2023-05-31

system is the α-agglutinin expression system. Yeast cells possess the eukaryotic post-translational modification mechanism, which helps the target protein fold correctly. This mechanism could be used to display various eukaryotic proteins, including antibodies, receptors, enzymes, and antigenic peptides. YSD has become a powerful protein engineering tool in biotechnology and biomedicine, and has been used to improve a broad range of protein properties including affinity, specificity, enzymatic function, and stability. This review summarized recent advances in the application of YSD technology from the aspects of library construction and screening, antibody engineering, protein engineering, enzyme engineering and vaccine development.
Keywords: yeast surface display technology; library construction; antibody engineering; protein engineering; enzyme engineering; oral vaccine

酵母表面展示(yeast surface display, YSD)是一种广泛使用的细胞表面展示技术。酵母表面展示技术将目的蛋白展示在细胞表面，并赋予酵母宿主新的能力，与流式细胞分选相结合，该技术既可用于定量检测目的蛋白的平衡结合常数、解离动力学性质，也可用于提高目的蛋白稳定性和特异性，节省了烦琐的纯化步骤[1]。酵母展示技术在生物技术、工业以及农业领域得到广泛应用，有助于实现人类社会可持续发展。

1 酵母展示系统概述

酵母细胞是最成熟的真核表达宿主之一，作为一种优良的细胞表面展示微生物，酿酒酵母(*Saccharomyces cerevisiae*)、巴斯德毕赤酵母(*Pichia pastoris*)、解脂耶氏酵母(*Yarrowia lipolytica*)和多形汉逊酵母(*Hansenula polymorpha*)已经在酵母表面展示技术中应用，它们分别利用特定的锚定蛋白将目的蛋白靶向并定位到酵母细胞表面[2]，本文对现有酵母展示系统进行总结，包括所使用的酵母菌株、锚定蛋白、启动子和其构建策略等(表 1)。目前，*S. cerevisiae*、*P. pastoris* 和 *Y. lipolytica* 已获得美国食品和药品管理局(US Food and Drug Administration, FDA)安全认证[1]。

1.1 凝集素系统

酵母展示凝集素系统包括 a-凝集素系统和 α-凝集素系统。a-凝集素系统由 Aga1p 和 Aga2p 两个亚单位组成，通常将目的蛋白与 Aga2p 的羧基端融合，Aga2p 与 Aga1p 通过二硫键形成复合物，同时 Aga1p 通过 β-1,6-葡聚糖共价键锚定在 *S. cerevisiae* 酵母细胞壁上，从而使目的蛋白展示在酵母表面；构建表达载体时通常包含 2 个表位标签，位于目的蛋白的 N 端和 C 端，如图 1A 所示，分别是血凝素(hemagglutinin, HA)标签和 C-myc 标签，允许同时检测配体结合和酵母表面目的蛋白表达水平(图 1B)[21]。而 α-凝集素系统只含有一个锚定单位 Agα1，通常将目的蛋白与 Agα1 的氨基端融合[22]。

1.2 絮凝素系统

酵母絮凝素蛋白 Flo1p 是酵母细胞壁上的凝集素样蛋白，由 *FLO1* 基因编码。Flo1p 由 4 个结构域组成，包括分泌信号结构域、絮凝功能结构域(flocculation functional domain, FFD)、糖基磷脂酰肌醇(glycosylphosphatidylinositol, GPI)锚定附着信号结构域和膜锚定结构域，其中 FFD 可以识别和非共价结合细胞壁成分。絮凝素系统可以通过 2 种方式锚定目的蛋白，既可以通过 Flo1p 羧基端的 GPI 附着信号与细胞壁结合，在氨基端展示目的蛋白(图 2A)，又可以通过 Flo1p 氨基端的 FFD 与细胞壁结合，在羧基端展示目的蛋白(图 2B)[22]。

表 1 酵母表面展示系统

Table 1 Yeast surface display systems

Yeast strain	Anchor	Promoter	Target protein	Construction strategy
S. cerevisiae BY4741	SED1 or SAG1	SED1	BGL and EG	The target protein was anchored on *S. cerevisiae* through SED1p and SAG1p, both were C-terminal domain of α-agglutinin[3]
S. cerevisiae BY4741 VMY4567	CCW12 or PIR4	GAL1 or PHO5	Xylose reductase	The target gene GRE3 was fused with two parts of the CCW12 gene, and linked with dextran through its N-terminal[4]
S. cerevisiae BY4741	SAG1 or SED1	TDH3or SED1	BGL and EG II	The target gene was integrated into the HIS3 locus of the *S. cerevisiae* genome[5]
S. cerevisiae BY4741	Flo428	PGK1	ZZ domain	The α-factor signal peptide was fused to the N-terminal of the ZZ protein, while Flo428 fused to the C-terminal[6]
S. cerevisiae BY4741	α-agglutinin or 649-stalk	GAP or GAL1	Nanobody cAbLys3	The anchor proteins were fused with HA tag to the C-terminal of the nanobody sequence[7]
S. cerevisiae BY4741 *spi1* Δ	Spi1	SPI1 or PGK1	Luciferase	The whole coding region, promoter and terminator of Spi1 gene were cloned into the *Bam*H I and *Hin*d III sites of plasmid pRS316[8]
S. cerevisiae W303	GPI	GAPDH	Pediocin PA-1	Pediocin PA-1 was genetically grafted onto α-agglutinin and GPI anchor to be expressed on the cell surface of *S. cerevisiae* W303 cells[9]
S. cerevisiae BY4741	SED1 SAG1 CWP2	GPD or SED1	BGL	The display system of BGL incorporated the GPD and SED1 promoters and the GPI anchoring regions of Sag1, Sed1, and Cwp2[10]
S. cerevisiae BY4741	Flo1	GAPDH	BGL and EG	The plasmid pMUF-GFP contained the fusion gene of prepro-α-factor, GFP and the Flo1p[11]
S. cerevisiae ATCC 60715	Flo1	UPR-ICL	rProROL	The N terminus of the target protein was fused to the flocculation functional region of Flo1p[12]
S. cerevisiae EBY100	Aga2 and aScafs	GAL1	EG, CBHI, CBHII, BGL	aScaf was fused to the C-terminal of Aga2, and it was also fused with a C-terminal V5 tag for detection[13]
S. cerevisiae CEN.PK102-5B	Aga1 without Aga2	TEF1	Exoglucanase BGL	The α-Gal gene with signal peptide SUC2 and a V5 tag were ligated into the yeast plasmid pJFE3 with the TEF1 promoter and PGK1 terminator[1]
P. pastoris GS115	Im7-SED1	AOX1	EG, CBHI, BGL and CBM	The target genes were fused with N-terminal CL7 tags, IM7 was anchored on the yeast by fusion with SED1[14]
P. pastoris GS115	Im7-SED1	AOX1	sfGFP and human arginase I	Anchoring Im7 protein on the surface of *P. pastoris* with a CL7 fusion tag[15]
P. pastoris GS115	GPI-anchored proteins	AOX1	CALB	The GPI-anchored proteins were fused to an alpha-factor secretion signal and tagged with FLAG tag and mature Candida antarctica lipase B (CALB)[16]
Y. lipolytica CGMCC7326	Pir1	hp4d	β-amylase	The target gene was fused and anchored on the surface of *Y. lipolytica* yeast cell through Pir1[17]
Y. lipolytica Po1h	YALI0F24255p	hp4d	SBP	The target gene was anchored on the surface of *Y. lipolytica* Po1h by fusing with YALI0F24255p[18]
Y. lipolytica	YlCwp1p and YlYwp1p	pTEF1	α-1,2-mannosidase	The target gene was fused with a secretion signal sequence (ss) tagged with cMyc at its N-terminus and fused with a FLAG tag at the C-terminus[19]
Y. lipolytica Po1h	CWP110	hp4d	*p*SIase	The gene encoding *p*SIase was fused with the vector pINA1317-CWP110[20]

BGL: β-glucosidase; EG: Endoglucanase; CBHI: Reducing-end-cleaving cellobiohydrolase; rProROL: Recombinant lipase with a pro sequence from *Rhizopus oryzae*; CBHII: Non-reducing-end-cleaving cellobiohydrolase; CBM: Carbohydrate-binding module; CALB: *Candida antarctica* lipase B; SBP: Soybean seed coat peroxidase; *p*SIase: Sucrose isomerase from *Pantoea dispersa*.

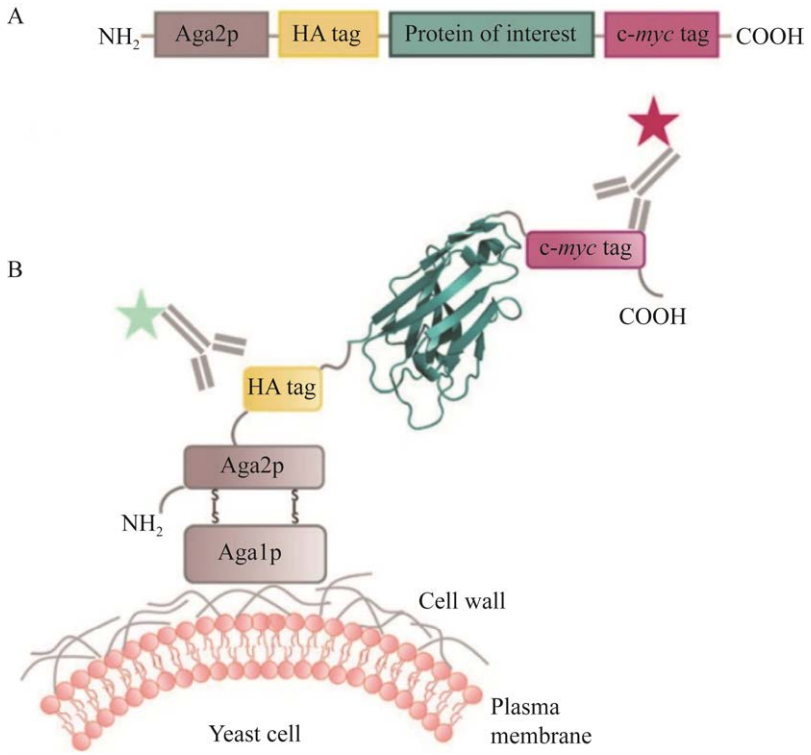

图 1　基于 α-凝集素的 *Saccharomyces cerevisiae* 酵母表面展示系统示意图[21]

Figure 1　Schematic diagram of a surface display system for *Saccharomyces cerevisiae* based on α-agglutinin[21]. A: The protein of interest (POI) is fused to C-terminal of the Aga2p with two epitope tags, HA tag and c-myc tag. B: The POI displaying on the surface of *S. cerevisiae* could be detected by anti-HA and anti-c-myc antibodies.

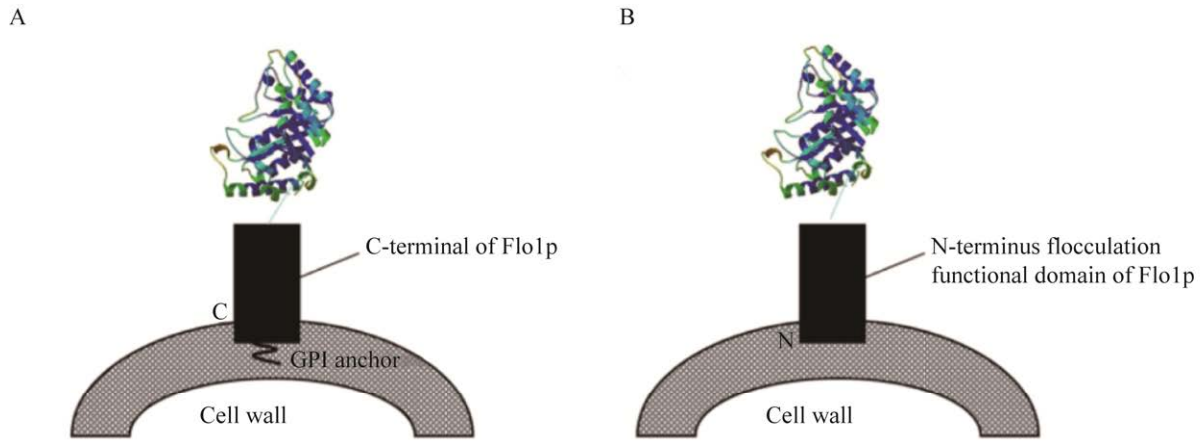

图 2　基于絮凝素系统的酵母表面展示目的蛋白示意图[22]

Figure 2　Schematic diagram of the yeast cell surface display of target protein based on flocculation system[22]. A: C-terminus fusion. B: N-terminus fusion.

2 酵母展示文库的构建及应用

酵母展示文库的产生通常依赖于前体蛋白的随机突变，突变一般通过易错PCR获得，将产生的目的基因突变文库与载体酶切产物同时转化感受态酵母细胞，细胞内突变基因和载体序列发生同源重组，从而制备大小约为10^7–10^9的酵母展示蛋白质突变文库[23]。

2.1 cDNA文库

Boder等[24]首先发现单链抗体片段能够展示在*S. cerevisiae*表面，易于被大分子识别，并发现酵母展示技术非常适合用于构建抗体文库和文库筛选。酵母展示系统可在体外实现抗体亲和力成熟，弥补了噬菌体展示、细菌表面展示和酵母双杂交等技术的不足。酵母双杂交系统局限于依赖“诱饵”和“猎物”融合蛋白在内部的共同表达，不能用于鉴定外部合成或修饰的蛋白质或化合物的配体[25]。Bidlingmaier等[25-26]构建了一个更大的酵母表面展示人类cDNA文库，筛选得到可与磷酸酪氨酸肽特异性结合并且含有SH2结构域的蛋白质配体；同时发现人类cDNA文库可以在*S. cerevisiae*表面正确折叠且行使正常功能，证实功能性人类蛋白质片段的大型文库可以成功展示在酵母表面，可用于鉴定药物靶点和寻找小分子或药物交叉反应蛋白；结合荧光激活细胞分选(fluorescence-activated cell sorting, FACS)，使用荧光标记的可溶性分子筛选其配体；也可用于筛选新的具有酶活性的细胞蛋白。

此外，酵母表面展示的人类cDNA文库可以用于鉴定药物作用靶点，例如Bidlingmaier等[27]证实*S. cerevisiae*酵母表面展示人类cDNA文库结合荧光激活细胞分选，可以筛选出能够与小分子相互作用的蛋白质，后来又成功用于快速鉴定间皮瘤的一种靶抗原黑色素瘤细胞粘附分子(melanoma cell adhesion molecule, MCAM)，为鉴定肿瘤细胞表面靶点提供了一种新的方法。最近，有研究人员构建了一种定量的*S. cerevisiae*酵母双杂交系统(quantiative yeast-yeast two hybrid, qYY2H)，既可以通过筛选cDNA文库以鉴定蛋白质-蛋白质相互作用(protein-protein interaction, PPIs)，还可以对诱饵-猎物结合的亲和力进行定量[28]。

通过构建酵母展示SARS-CoV-2刺突蛋白受体结合结构域(receptor-binding domain, RBD)突变文库，结合FACS进行深度抗原突变筛选，可快速鉴定所有影响中和性抗体结合RBD的单氨基酸突变[29]。考虑到FACS筛选突变文库受到低通量的限制，Cao等[30]基于酵母展示技术建立一种中和性抗体表位高通量鉴定的方法，该方法利用磁珠激活细胞分选(magnetic-activated cell sorting, MACS)将通量提高了2个数量级。该研究在RBD中鉴定出247种免疫逃逸突变谱，这些突变将影响中和性抗体结合RBD，并发现感染SARS-CoV-2 B.1.1.529 (Omicron)将导致强烈的体液免疫逃逸，证实针对沙贝病毒(Sarbecvirus)保守区的中和性抗体是最有效的，该研究为Omicron和新冠变异株的抗体药物以及疫苗的开发提供了依据[30]。

2.2 pMHC文库

抗原提呈细胞(antigen presenting cell, APC)摄取抗原并对抗原进行加工处理，产生的抗原肽与主要组织相容性复合体(major histocompatibility complex, MHC) I类和II类分子结合，递呈在APC表面，供T细胞受体(T cell receptor, TCR)识别，其中抗原肽-MHC (peptide-major histocompatibility complex, pMHC)与TCR之间相互作用对于启动适应性免疫应答至关重要，在移植、感染、疫苗接种和自身免疫过程中发挥重要作用[31]。Sibener等[32]利用*S. cerevisiae*酵母展示pMHC文库成功鉴定无反应TCR的肽激动剂，阐明了

体内发生的非激动性高亲和力 TCR-肽-MHC 相互作用的分子机制，同时发现酵母展示 pMHC 文库中 TCR 配体的生物物理特性，其中激动剂和非激动剂之间最小差异仅为一个亚甲基。Gee 等[33]使用 *S. cerevisiae* 酵母展示 pHLA 文库，在人类大肠腺癌中筛选肿瘤浸润淋巴细胞(tumor-infiltrating lymphocyte, TIL)表面表达的“孤儿” TCR 的抗原，发现 TCR 可以特异性识别具有同源性的抗原，此方法有助于无偏倚筛选肿瘤抗原。

本课题组近期开发一种在蛋白质抗原中快速鉴定 MHC-II 配体(rapid identification of peptide ligands from protein antigens, RIPPA)的方法，将人类 MHC-II等位基因(HLA-DR4)以天然非共价 α 链、β 链二聚体的形式展示在 *S. cerevisiae* 细胞表面，使用 GAL1-10 双向启动子引导 α 链和 β 链的表达，而 Fos/Jun 亮氨酸拉链(leucine zipper, LZ)二聚化基序可以促进 α 链与 β 链配对，有助于提高酵母表面 HLA-DR4 表达水平；如图 3 所示，融合抗原肽或者空载的 DR4 通过 Aga2p 和 Aga1p 锚定在酵母细胞表面，通过流式细胞术可检测目的蛋白表达水平，箭头所示是可用于抗体染色的蛋白质或表位标签，酵母细胞表面展示 HLA-DQ6 采用与此相似的方式；本课题组基于酵母表面展示技术，利用空载的 MHC-II建立一种快速抗原表位鉴定方法，结合流式细胞术从发作性睡病自身抗原下丘脑泌素(hypocretin, HCRT)中鉴定 DQ6 配体，并使用 SARS-CoV-2 刺突蛋白作为模型抗原，进一步验证 RIPPA 可以快速鉴定 MHC-II 抗原肽配体，RIPPA 为 MHC-肽-TCR 相互作用的深入研究奠定了基础[34-35]。

Rappazzo 等[36]基于 *S. cerevisiae* 酵母展示技术建立一种高通量鉴定 MHC 结合肽的方法，然后构建包含 SARS-CoV-2 蛋白质组的抗原肽文库，在蛋白组中鉴定 MHC 结合肽；为了验证

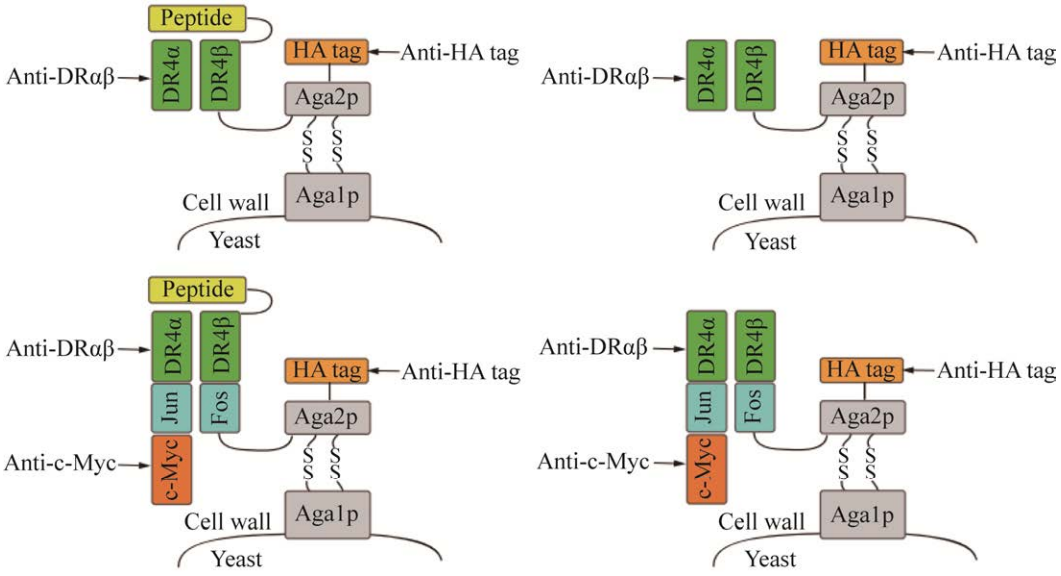

图 3　适当组装的抗原肽/DR4 或空载 DR4 与酵母表面蛋白凝集素融合的示意图[34]

Figure 3　Schematic illustration of appropriately assembled peptide/DR4 or “empty” DR4 as a fusion to the yeast surface protein agglutinin, composed of the Aga1p and Aga2p subunits[34]. The arrows indicate protein or epitope tags for antibody staining and detection by flow cytometry.

该方法，该研究随后在 4 种登革热病毒血清型组成的抗原肽文库中鉴定 MHC-II结合的抗原肽；进一步证实该方法可以从病毒蛋白组中鉴定潜在的抗原肽，在确定肽-MHC 结合相互作用方面发挥重要作用[37]。

3 酵母展示技术在抗体工程中的应用

抗体广泛应用于基础研究、诊断、治疗和公共卫生等领域，其亲和力对于特异性识别生物分子非常重要[38]。早期利用动物免疫制备抗体，存在很多缺陷，时间长、成本高且不易获取、对自身抗原的耐受性不高以及涉及科学和伦理问题等[38-39]。相比噬菌体展示技术，酵母展示技术更适合抗体文库的构建以及抗体片段的筛选，它可以区分亲和力相差 2 倍的蛋白质，说明酵母展示技术具有较高的敏感性[40]。

3.1 抗体亲和力成熟

特定抗体制备通常需要使用抗体工程化改造，YSD 可以满足这一要求[41-42]。Sun 等[43]从 *S. cerevisiae* 酵母表面展示 IL-17A 抗体的随机突变文库中筛选出高亲和力的抗体 8D3，然后将其互补决定区移植至 HuFv4D5 的稳定框架上，最终得到的杂交抗体 9NT/S 具有更高的稳定性和亲和力，并可有效抑制致炎细胞因子诱导的 IL-17A 分泌。Keck 等[44]利用简化的单链抗体组装方法，创建改良的 YSD 载体，减少 PCR 错配引起的碱基交换，通过中和试验筛选活化 B 细胞上清，富集分泌丙型肝炎病毒(hepatitis C virus, HCV)单抗的 B 细胞，最终成功从丙型肝炎病毒感染者中分离得到高亲和力的 HCV 中和性单抗。以鸡作为宿主制备高亲和力单抗可以克服很多哺乳动物对保守表位产生免疫的缺陷，Bogen 等[45]基于 FACS 建立一种 *S. cerevisiae* 酵母展示技术平台，用于分离高亲和力抗体，并利用该平台分离和鉴定一种结合表皮生长因子受体(epidermal growth factor receptor, EGFR)的特异性抗体 FEB4，进一步利用基于表位结合的新筛选方法分离出更高亲和力的抗体 SEB7，其亲和力比 FEB4 高近 30 倍[46]。Elter 等[46]从免疫鸡中分离出 EGFR 胞外结构域的高亲和力抗体，将其互补决定区移植到人源抗体框架上，构建 *S. cerevisiae* 酵母展示抗体文库，筛选获得 scFv 和 Fab 类的高亲和力抗体，经过人源化改造，与亲代鸡抗体相比，使人源化抗体变体具有更高的亲和力和改善的蛋白质特性。近年来单链可变区片段(single-chain fragment variable, scFv)抗体筛选已成为开发疾病有效疗法的重要工具[47]，Lajoie 等[48]用功能性 *S. cerevisiae* 酵母展示免疫沉淀(functional yeast display immunoprecipitation, fYDIP)方法在血脑屏障(blood-brain barrier, BBB)细胞膜裂解液中筛选 scFv，成功筛选出能够与含衔接蛋白 2 (adaptin 2, AP-2)的蛋白复合物结合的抗体分子。

3.2 酵母展示抗体 Fab 片段

van den Beucken 等[49]使用 pYD1 酵母展示载体构建抗体 Fab 片段表达质粒 pTQ3，该重组质粒允许可溶性轻链和重链串联表达，并与锚定蛋白 Aga2p 融合表达在 *S. cerevisiae* 表面，结合随机突变构建 Fab 抗体文库，经过一轮随机突变和 FACS 筛选，多价抗原链霉亲和素的抗体特异性提高了 10.7 倍。酵母展示技术是人抗体片段亲和力成熟的强大工具，但受到酵母展示文库大小的限制，Blaise 等[50]分别构建了重链表达质粒 pTQ5-HC 和轻链表达质粒 pTQ6-LC，转化 *S. cerevisiae* EBY100 和 BJ5457，2 种菌株在一定条件下交配得到酵母展示人源 Fab 抗体库，文库大小达到 10^9，进一步使用易错 PCR 可将文库大小提高到 5×10^9。Rosowski

等[51]将抗体重链和轻链编码基因构建在一个表达载体上，获得 *S. cerevisiae* 酵母表面展示 Fab 抗体库，相比酵母交配，该方法可以减少构建文库所需要的时间和成本，并且易于构建文库大小 10^8 以上的大型抗体展示文库，便于筛选获得高亲和力的抗体[52]。

3.3 酵母展示 VHH 单域抗体

酵母表面展示文库也可用于筛选单域抗体，例如 Roth 等[53]将 *S. cerevisiae* 酵母表面展示重链单域抗体(variable domain of the heavy chain of heavy-chain antibody, VHH)文库与 FACS 相结合筛选出具有抗原特异性的 VHH 单域抗体。纳米抗体是一种无轻链的功能性抗体，已在临床诊断中得到广泛应用，VHH 结构域有显著的抗体特异性，能够作为治疗药物[54]。一些研究者将纳米抗体文库编码序列与 *S. cerevisiae* 酵母 α-凝集素基因的 C 端结合，并在 *P. pastoris* 表面表达[2]。直到 2018 年 McMahon 等[55]基于 *S. cerevisiae* 酵母展示技术开发一种体外纳米抗体文库平台，该平台使用结构已经解析的纳米抗体序列设计文库，结合 FACS 可以直接、快速地筛选能够特异性结合人类 G 蛋白偶联受体的纳米抗体，现有的纳米抗体几乎都是免疫动物获得的，成为限制纳米抗体应用的瓶颈。不同于以往，该平台将纳米抗体基因融合到 Aga2p 的氨基端，除用于纳米抗体免疫文库筛选，还能通过正交标记检测酵母展示水平，并便于对配体进行初步的生物物理和生化性质鉴定[54]。

4 酵母展示技术在蛋白质工程中的应用

4.1 提高蛋白质的亲和力

酵母表面展示是一种强大的蛋白质工程技术，广泛用于提高蛋白质的各种特性，包括亲和力、特异性和稳定性[21]。酵母展示技术可以区分亲和力只有 2 倍差异的蛋白质，具有较高的敏感性，已经成为蛋白质亲和力成熟的重要工具。如图 4 所示，一般通过随机突变创建大小为 10^7–10^9 的蛋白质突变文库，转化 *S. cerevisiae*

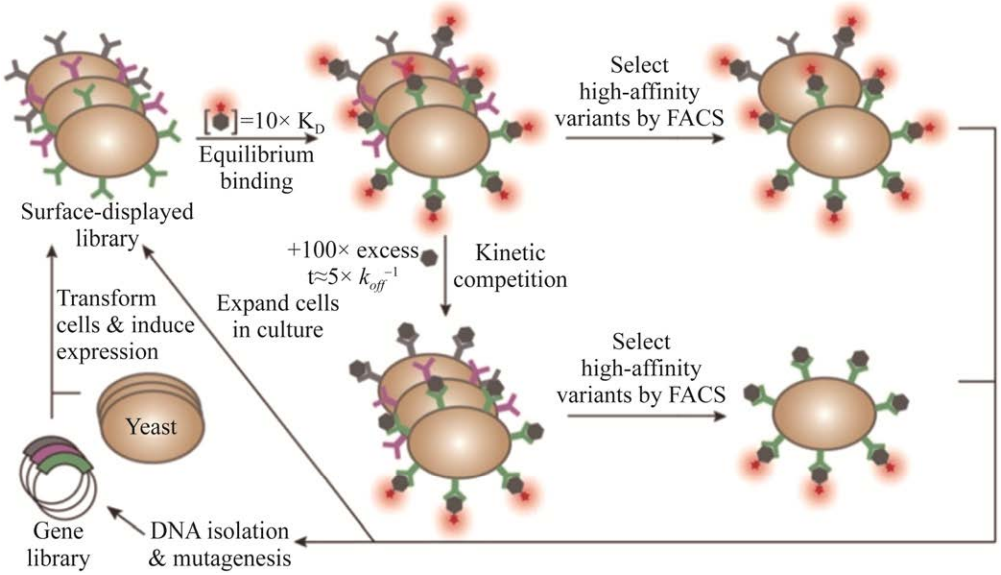

图 4 通过 FACS 从酵母展示文库中筛选高亲和力蛋白突变体[23]

Figure 4 Isolating high-affinity protein variants from a yeast-displayed library by FACS[23].

EBY100,这些突变体与 Aga2p 融合展示在酵母表面；在 FACS 分选之前，将突变文库与饱和浓度的荧光标记配体一起孵育，通过洗涤去除未结合的配体，然后将文库与 100 倍未标记配体孵育，或者在足够体积的缓冲液中孵育，从而避免解离后荧光标记配体再结合；进一步使用 FACS 分选高亲和力突变体，每一轮分选的细胞可以扩增培养参与再次分选，从而降低文库多样性，最终获得少量的高亲和力酵母细胞克隆；也可以提取所选酵母细胞 DNA，进行新一轮随机突变，构建新的展示文库，参与新一轮分选，直到筛选出所需亲和力的蛋白质[23]。

基于酵母展示技术提高蛋白质亲和力应用非常广泛。FK506 结合蛋白 51 (FK506-binding protein 51, FKBP51)是应激相关障碍的潜在靶点，Lerma Romero 等[56]对 FKBP51 编码序列的 FK1 结构域进行随机诱变和位点饱和诱变，构建 *S. cerevisiae* 酵母表面展示 FKBP51 突变文库，使用构象特异性荧光标记配体染色，利用 FACS 分选高亲和力 FKBP51 变体，鉴定出 15 种不同亲和力蛋白突变体，与野生型相比亲和力提高了 34 倍。van Rosmalen 等[57]通过 *S. cerevisiae* 酵母展示技术和深度突变扫描，将中间位(meditope)肽对西妥昔单抗的亲和力提高了 10 倍，该方法广泛应用于开发对治疗性抗体具有低亲和力的中间位肽。Jeong 等[58]将单链胰岛素类似物 SCI-57 展示在 *S. cerevisiae* DY1632 表面，SCI-57 能够正确折叠，并且仍保持对胰岛素受体的亲和力，此方法可以用于构建胰岛素样肽文库，以便筛选化学探针或治疗分子。

消旋胰蛋白酶是一种参与肿瘤进展的蛋白酶，与肿瘤的生长和许多癌症的进展及不良预后密切相关，有效的消旋胰蛋白酶抑制剂可以为侵袭转移性癌症患者的治疗提供希望，但是，消旋胰蛋白酶作为底物，往往裂解和灭活许多蛋白酶抑制剂，成为制备抑制剂的巨大挑战。研究发现，在天然丝氨酸蛋白酶抑制剂中，Kunitz 结构域家族有助于抵抗消旋胰蛋白酶裂解。Cohen 等[59]使用 *S. cerevisiae* 酵母展示平台，对人淀粉样前体蛋白(amyloid precursor proteins, APPI) Kunitz 结构域进行定向进化，最终筛选得到三位点突变体 $APPI_{M17G/I18F/F34V}$，相比野生型 APPI，该突变体与消旋胰蛋白酶的亲和力提高 1 459 倍，蛋白水解稳定性提高 83 倍，从而获得更好的抗癌蛋白抑制剂。之后 Sananes 等[60]基于 *S. cerevisiae* 酵母展示技术构建 APPI 突变体文库,通过流式细胞术筛选工程化 KLK6 抑制剂，得到一种 APPI 突变体，即 $APPI_{M17L、I18F、S19F、F34V}$ (APPI-4M)，与野生型 APPI 相比，其亲和力提高了 146 倍，蛋白水解稳定性提高了 13 倍，APPI-4M 未来有望用于临床显像和肿瘤治疗。

4.2 提高蛋白质稳定性

蛋白质稳定性通常是指其抵抗热变性、化学变性以及蛋白质降解的能力。酵母展示技术筛选高稳定性的蛋白质可以采取 2 种策略，第一种策略基于酵母表面适当折叠蛋白质表达水平与其热稳定性之间的相关性，酵母表面高表达的蛋白突变体，其稳定性也高；第二种策略是在细胞分选之前，选择合适的温度和时间对酵母展示文库进行热激处理，结合 FACS 筛选出耐热变性的突变体，既可以参与新一轮的筛选，也可以定向进化构建新一轮的酵母展示文库,以此循环筛选出具有热稳定性的突变体[61]。

高稳定性对于蛋白质的广泛应用非常重要。Laurent 等[62]使用野生型的可溶性 CD19 胞外结构域(extracellular domain, ECD)基因通过 PCR 错配构建 *S. cerevisiae* 酵母展示 CD19-ECD 文库，然后分别使用 CD19-ECD 构象特异性抗体 FMC63 和 c-myc 抗体染色，通过 FACS 筛选 CD19-ECD 突变文库，成功获得高稳定性的

CD19-ECD突变体SF05，有望用于B细胞免疫学检测和靶向CD-19肿瘤免疫治疗。木质素过氧化物酶(lignin peroxidase, LiP)是一种含血红素的氧化还原酶，具有氧化多种污染物的能力，为了提高其氧化稳定性，Ilić Durdic 等[63]基于*S. cerevisiae*酵母展示技术构建LiP随机突变文库，结合流式细胞术筛选高活性LiP突变体，通过2轮分选，得到具有较高氧化稳定性的LiP突变体，其活性不随时间延长而显著降低，非常适合生物修复和工业应用。

4.3 T细胞受体工程化改造

此外，Lee 等[64]基于*S. cerevisiae*酵母展示技术对T细胞受体样抗体H9进行工程化改造，提高抗体亲和力。H9特异性识别人类巨细胞病毒(cytomegalovirus, CMV) pp65蛋白衍生肽与HLA-A*02:01 (CMVpp65$_{495\text{-}503}$/HLA-A*02:01)复合物，通过酵母表面展示技术对H9的重链(variable region of heavy chain, VH)和轻链(variable region of light chain, VL)可变区的第三互补决定区(VH-CDR3/VL-CDR3)进行随机突变得到酵母展示H9文库，经过3轮筛选得到较高亲和力的C1 (scFab)；为了进一步提高抗体C1的亲和力和特异性，随后再次通过酵母展示技术对C1的VH-CDR2/VL-CDR2进行随机突变构建酵母展示C1文库，经过4轮筛选最终得到高亲和力抗体C1-17，其亲和力是亲本H9的67倍，可用于检测和治疗CMV感染。

5 酵母展示技术在酶工程中的应用

5.1 酶的定向进化及其工业应用

人工定向进化是改变酶原有性质、产生新型高效酶的重要手段，已被用于生产高活性和强特异性的酶[65]。酵母展示技术结合同源重组和文库筛选已经实现酶的人工定向进化，目前有多种酶成功展示在酵母细胞表面(表2)。米黑根毛霉脂肪酶(*Rhizomucor Miehei* Lipase, RML)是一种生物催化剂，可用于食品工业、精细化工和生物柴油生产，将经过定点突变的米黑根毛霉脂肪酶与Flo1p结合并展示在*P. pastoris*

表2 酵母表面展示在酶工程中的应用

Table 2 Application of yeast surface display technology in enzyme engineering

Anchor	Enzyme	Yeast strain	Property	Main observations
Flo1p	RML	*P. pastoris*	Higher activity	The synthetic activity of the variant was 1.1–5.0 times that of natural lipase[66]
pPink LC/HC	RML	Protease-deficient *P. pastoris*	Higher activity	The lipase activity reached 121.72 U/g in proteinase-A-deficient *P. pastoris* harboring high-copy plasmid[67]
YALI0F24255p	SBP	*Y. lipolytica*	Higher activity	The activity was 4 432.5 U/mL, which was the highest recombinant SBP activity reported up to now[18]
GCW21 GCW51 GCW61	PETase	*P. pastoris*	Higher and stabler degradation efficiency	The turnover rate dramatically increased about 36-fold compared with that of purified PETase[68]
Pir1p	Trehalose synthase	*P. pastoris*	Higher catalytic activity after recycled three times	The conversion rate of trehalose reached about 63.2% of total maltose at about 20 h and the enzyme activity retained more than 85% of its initial activity[69]
a-agglutinin	GA and GOx	*S. cerevisiae*	Higher catalytic activity and stability	The starch/O2 EBFC registered the largest OCV, the highest Pmax, and the most prominent stability[70]

RML: *Rhizomucor miehei* lipase; SBP: Soybean seed coat peroxidase; PETase: PET-hydrolyzing enzyme; GA: Glucoamylase; Gox: Glucose oxidase.

表面，得到的突变体的酯化活性是原始脂肪酶活性的 1–5 倍，酵母展示技术允许高效快速筛选高酯化活性脂肪酶突变体[66]，同时避免纯化和固定化过程，但宿主细胞自身蛋白酶可能影响酵母表面展示的脂肪酶活性。最近有研究使用自身蛋白酶缺陷的 *P. pastoris* 表达系统，其表面展示的 RML 活性比野生型酵母高出 46.7%，更利于工业应用[67]。抗坏血酸过氧化物酶(engineered ascorbate peroxidase, APEX)是一种工程过氧化物酶，参与多种底物的氧化过程，Han 等[71]开发了一种进化的分裂 APEX2 (sAPEX)系统，通过 *S. cerevisiae* 酵母展示系统进行定向进化，使 APEX2 在重组过程中保持较高过氧化物酶活性，未来可应用到生物学研究的新领域。研究发现，在不影响发酵性能的情况下，*S. cerevisiae* 酵母表面展示的 α-乙酰乳酸脱羧酶(α-acetolactate decarboxylase, ALDC)可以生产出更有味道的酒精饮料；酵母表面展示 β-葡萄糖苷酶可以增加饮料的香气和风味[10,72]。

5.2 酵母全细胞生物催化系统

有机磷水解酶(organophosphorus hydrolase, OPH)能够有效水解多种有机磷酸酯，减少污染，将 OPH 锚定在 *S. cerevisiae* MT8-1 表面，其水解活性增高，不仅可以成功水解神经毒药物，还可快速检测出有机磷中毒[73]。为了进一步快速准确地检测有机磷农药，Liang 等[74]基于 *S. cerevisiae* 酵母展示技术构建了乙酰胆碱酯酶的全细胞酵母生物催化剂。对羟基苯甲酸酯是新兴的环境污染物，Zhu 等[75]将茄皮镰刀菌角质酶(*Fusarium solani pisi* cutinase, FsC)展示在 *S. cerevisiae* 表面，开发了一种全新的生物催化剂 SDFsC，该研究首次使用酶法去除对羟基苯甲酸酯。聚对苯二甲酸乙二醇酯(polyethylene terephthalate, PET)也是广泛使用的塑料品之一，将聚对苯二甲酸乙二醇酯降解酶(PETase)展示在 *P. pastoris* 表面，可以提高此酶对 PET 的降解效率，而且酵母全细胞生物催化剂转化率比可溶性的 PETase 高约 36 倍，在重复使用 7 次之后，得到的全细胞生物催化剂拥有稳定的周转率[68]。*β*-烟酰胺单核苷酸(*β*-nicotinamide mononucleotide, *β*-NMN)是一种天然存在的生物活性核苷酸，在新陈代谢、衰老、细胞死亡、DNA 修复和基因表达等生命过程中发挥重要作用[76]。烟酰胺核糖激酶 2 (nicotinamide riboside kinase 2, NRK-2)可以一步法催化烟酰胺核糖(nicotinamide riboside, NR)转化为 *β*-NMN，He 等[77]将 NRK-2 展示在 *S. cerevisiae* EBY100 细胞表面，成功获得高 pH 稳定性和热稳定性的全细胞 NRK-2 催化剂，以此催化 NR 合成 *β*-NMN，其转化率高达 98.2%，远高于原有合成工艺，在工业生产中有巨大的应用价值。

5.3 酵母多酶共展示体系

在生物转化和污染物降解的过程中，一般有连续的级联反应，通常需要多种酶的参与，因此开发工程酵母生物催化剂，可以同时展示多种酶，将多个反应整合到一个体系中，在乙醇的生产、生物降解或污染物的生物处理中得到广泛应用[78]。Bae 等[79]发现，*S. cerevisiae* 表面可以同时展示 3 种不同的纤维素酶，其降解活性是单一酶的 2 倍。在此基础上，Chen 等[80]建立了糖化与发酵联合生物处理(consolidated bioprocessing, CBP)体系，该体系由 2 种 *S. cerevisiae* 工程菌株 Y5/XynII XylA (同时展示 2 种木聚糖酶)和 Y5/EG-CBH-BGL (同时展示 3 种纤维素酶)组成，木聚糖酶(xylanase, XYN)作为一种辅助酶，可以改变纤维素纤维的物理特性，帮助纤维素酶更有效地接近纤维素底物，从而加速底物水解。Guirimand 等[81]通过在 *S. cerevisiae* 工程菌株(YPH499-XR41-BGL-XYL-XYN)表面展示 3 种酶，包括细胞溶质木糖还原酶(xylose reductase,

XR)、β-D-葡萄糖苷酶(β-D-glucosidase, BGL)、木糖酶(xylosidase, XYL)和木聚糖酶(XYN)，显著提高木糖还原酶的活性。此外，Liang 等[82]通过 *S. cerevisiae* 酵母表面展示技术同时展示 2 种木聚糖酶，获得较高酶活性，可作为诱导玉米防御反应的有力工具。

6 酵母展示技术在疫苗开发中的应用

酵母表面展示技术作为开发口服疫苗的工具，在人和动物传染性疾病预防中已经得到广泛应用。Lei 等[83]基于酵母展示技术开发一种 H7N9 流感病毒口服疫苗，可对同源流感病毒提供有效的免疫保护作用。Shibasaki 等[84]将白色念珠菌(*Candida albicans*)的烯醇化酶 1 (enolase 1, Eno1p)展示在 *S. cerevisiae* 表面，成功制备一种新型的抗念珠菌感染的口服疫苗，接种疫苗的小鼠抗 Eno1p 抗体的滴度明显增加，表明酵母展示系统可能成为预防传染病的有力工具。Gao 等[85]将 SARS-CoV-2 刺突蛋白 RBD 结构域展示在 *S. cerevisiae* 表面，作为候选疫苗，在小鼠中诱发强烈的免疫应答。Xing 等[86]将 SARS-CoV-2 及其突变株 B.1.1.7、B.1.351、B.1.617.1 的刺突蛋白 RBD 融合到 *S. cerevisiae* 酵母 Aga2 锚定蛋白的 C 末端，接种口服疫苗的小鼠产生强烈的体液免疫和细胞免疫应答。研究发现，基于酵母展示技术开发口服疫苗可以实现低成本、快速、大规模制备有潜力的 SARS-CoV-2 候选疫苗。

疫苗在动物传染病的预防和控制中发挥着重要的作用。Le Linh 等[87]在 *S. cerevisiae* 表面展示 VP28 抗原，将酵母细胞提取物与益生菌结合，获得针对白斑综合征病毒的口服疫苗，可用于预防虾口腔癌。传染性造血坏死病毒(infectious hematopoietic necrosis virus, IHNV)感染养殖虹鳟和各种鲑鱼[88]，利用改进的酵母表面展示技术制备一种预防 IHNV 的口服疫苗，虽然效果不及 DNA 疫苗，但作为候选疫苗可以保护鱼类免受 IHNV 的感染[89]。鲤疱疹病毒 2 型(cyprinid herpesvirus 2, CyHV-2)引起的疱疹病毒性造血坏死(herpesviral hematopoietic necrosis, HVHN)导致鲫鱼的急性群体性死亡，严重影响了鲫鱼养殖产业的发展[90]。Dong 等[91]将 CyHV-2 的亚单位 ORF25 展示在 *S. cerevisiae* 细胞表面，制备出一种口服疫苗，可以引发黏膜和全身组织发生强烈的先天和适应性免疫应答，用于控制吉贝鲤鱼养殖中的 CyHV-2 感染；之后 Wang 等[92]在 *S. cerevisiae* 表面展示 CyHV-2 包膜蛋白 ORF132，口服 EBY100/pYD1-ORF132 疫苗后，也能对鲫鱼感染 CyHV-2 提供保护。Zhang 等[93]在 *S. cerevisiae* 表面展示大口黑鲈蛙虹彩病毒(largemouth bass ranavirus, LMBV)主衣壳蛋白(main capsid protein, MCP)，以大肠杆菌热敏肠毒素(*Escherichia coli* heat-labile enterotoxin, LTB)的 B 亚基作为佐剂，开发口服疫苗 EBY100-OMCP 和 EBY100-LTB-OMCP，二者均能诱导大口黑鲈的全身免疫，且有效促进肠黏膜免疫的激活，使用 LMBV 感染时，疫苗接种组死亡率明显降低。

7 结论及展望

YSD 是极有应用价值的一项技术，尤其是在生物技术领域，将异源蛋白表达在酵母表面，结合流式细胞分选，可以筛选出具有理想特性的蛋白质，已经广泛应用于抗体工程、酶工程、蛋白质工程和疫苗开发等领域。此外，荧光蛋白和酶已经被展示在酵母表面作为生物传感器[94]。Zhao 等[95]将葡萄糖脱氢酶和胆固醇氧化酶表达在 *S. cerevisiae* 的表面，构建了全细胞生物传感器，用来检测葡萄糖和胆固醇。酵母表面展

示系统的现有应用如此广泛，同时也在不断发展和完善，已经有研究开发出一个全新的酵母展示系统，仅使用 Aga1p 亚基，相比 a-凝集素可以提高异源蛋白的展示效率[1]。而 SpyTag/SpyCatcher 系统可将展示效率提高到 90%以上[65]。将来可能会有更多优良的酵母表面展示系统出现，为体外展示各种异源蛋白提供更多的选择。

REFERENCES

[1] YANG XY, TANG HT, SONG MH, SHEN Y, HOU J, BAO XM. Development of novel surface display platforms for anchoring heterologous proteins in *Saccharomyces cerevisiae*[J]. Microbial Cell Factories, 2019, 18(1): 85.

[2] RYCKAERT S, PARDON E, STEYAERT J, CALLEWAERT N. Isolation of antigen-binding camelid heavy chain antibody fragments (nanobodies) from an immune library displayed on the surface of *Pichia pastoris*[J]. Journal of Biotechnology, 2010, 145(2): 93-98.

[3] INOKUMA K, KITADA Y, BAMBA T, KOBAYASHI Y, YUKAWA T, den HAAN R, van ZYL WH, KONDO A, HASUNUMA T. Improving the functionality of surface-engineered yeast cells by altering the cell wall morphology of the host strain[J]. Applied Microbiology and Biotechnology, 2021, 105(14/15): 5895-5904.

[4] HOSSAIN AS, TEPARIĆ R, MRŠA V. Comparison of two models of surface display of xylose reductase in the *Saccharomyces cerevisiae* cell wall[J]. Enzyme and Microbial Technology, 2019, 123: 8-14.

[5] INOKUMA K, HASUNUMA T, KONDO A. Efficient yeast cell-surface display of exo- and endo-cellulase using the SED1 anchoring region and its original promoter[J]. Biotechnology for Biofuels, 2014, 7(1): 8.

[6] KATSURADA K, TOMINAGA M, KAISHIMA M, KATO H, MATSUNO T, OGINO C, KONDO A, ISHII J, TAKAYAMA K. Constitutive cell surface expression of ZZ domain for the easy preparation of yeast-based immunosorbents[J]. The Journal of General and Applied Microbiology, 2021, 67(6): 265-268.

[7] KAJIWARA K, AOKI W, UEDA M. Evaluation of the yeast surface display system for screening of functional nanobodies[J]. AMB Express, 2020, 10(1): 51.

[8] ANDREU C, DEL OLMO ML. Development of a new yeast surface display system based on Spi1 as an anchor protein[J]. Applied Microbiology and Biotechnology, 2017, 101(1): 287-299.

[9] NGUYEN TPA, NGUYEN TTM, NGUYEN NH, NGUYEN TN, DANG TTP. Application of yeast surface display system in expression of recombinant pediocin PA-1 in *Saccharomyces cerevisiae*[J]. Folia Microbiologica, 2020, 65(6): 955-961.

[10] ZHANG Y, MIN Z, QIN Y, YE DQ, SONG YY, LIU YL. Efficient display of *Aspergillus niger* β-glucosidase on *Saccharomyces cerevisiae* cell wall for aroma enhancement in wine[J]. Journal of Agricultural and Food Chemistry, 2019, 67(18): 5169-5176.

[11] MATSUOKA H, HASHIMOTO K, SAIJO A, TAKADA Y, KONDO A, UEDA M, OOSHIMA H, TACHIBANA T, AZUMA M. Cell wall structure suitable for surface display of proteins in *Saccharomyces cerevisiae*[J]. Yeast, 2014, 31(2): 67-76.

[12] MATSUMOTO T, FUKUDA H, UEDA M, TANAKA A, KONDO A. Construction of yeast strains with high cell surface lipase activity by using novel display systems based on the Flo1p flocculation functional domain[J]. Applied and Environmental Microbiology, 2002, 68(9): 4517-4522.

[13] SMITH MR, GAO H, PRABHU P, BUGADA LF, ROTH C, MUTUKURI D, YEE CM, LEE L, ZIFF RM, LEE JK, WEN F. Elucidating structure-performance relationships in whole-cell cooperative enzyme catalysis[J]. Nature Catalysis, 2019, 2(9): 809-819.

[14] DONG C, QIAO J, WANG XP, SUN WL, CHEN LX, LI ST, WU K, MA LX, LIU Y. Engineering *Pichia pastoris* with surface-display minicellulosomes for carboxymethyl cellulose hydrolysis and ethanol production[J]. Biotechnology for Biofuels, 2020, 13(1): 1-9.

[15] LI ST, QIAO J, LIN SY, LIU Y, MA LX. A highly efficient indirect *P. pastoris* surface display method based on the CL7/Im7 ultra-high-affinity system[J]. Molecules, 2019, 24(8): 1483.

[16] ZHANG L, LIANG SL, ZHOU XY, JIN Z, JIANG FC, HAN SY, ZHENG SP, LIN Y. Screening for glycosylphosphatidylinositol-modified cell wall proteins in *Pichia pastoris* and their recombinant expression on the cell surface[J]. Applied and Environmental Microbiology, 2013, 79(18):

5519-5526.
[17] 刘大文, 程海荣, 邓子新. 解脂耶氏酵母表面展示 β-淀粉酶与 α-葡萄糖转苷酶及一步法由淀粉合成低聚异麦芽糖[J]. 生物工程学报, 2019, 35(1): 121-132.
LIU DW, CHENG HR, DENG ZX. One step production of isomalto-oligosaccharides by engineered *Yarrowia lipolytica* yeast co-displayed β-amylase and α-transglucosidase[J]. Chinese Journal of Biotechnology, 2019, 35(1): 121-132 (in Chinese).
[18] WANG JM, WANG CM, MEN X, YUE TQ, MADZAK C, XIANG XH, XIANG HY, ZHANG HB. Construction of arming *Yarrowia lipolytica* surface-displaying soybean seed coat peroxidase for use as whole-cell biocatalyst[J]. Enzyme and Microbial Technology, 2020, 135: 109498.
[19] MOON HY, VAN TL, CHEON SA, CHOO J, KIM JY, KANG HA. Cell-surface expression of *Aspergillus saitoi*-derived functional α-1,2-mannosidase on *Yarrowia lipolytica* for glycan remodeling[J]. Journal of Microbiology, 2013, 51(4): 506-514.
[20] ZHENG Y, WANG ZP, JI XF, SHENG J. Display of a sucrose isomerase on the cell surface of *Yarrowia lipolytica* for synthesis of isomaltulose from sugar cane by-products[J]. 3 Biotech, 2019, 9(5): 179.
[21] ZAJC CU, TEUFL M, TRAXLMAYR MW. Affinity and stability analysis of yeast displayed proteins[M]//Methods in Molecular Biology. New York, NY: Springer US, 2022: 155-173.
[22] YE MQ, YE YQ, DU ZJ, CHEN GJ. Cell-surface engineering of yeasts for whole-cell biocatalysts[J]. Bioprocess and Biosystems Engineering, 2021, 44(6): 1003-1019.
[23] CHERF GM, COCHRAN JR. Applications of yeast surface display for protein engineering[M]//Methods in Molecular Biology. New York, NY: Springer New York, 2015: 155-175.
[24] BODER ET, WITTRUP KD. Yeast surface display for screening combinatorial polypeptide libraries[J]. Nature Biotechnology, 1997, 15(6): 553-557.
[25] BIDLINGMAIER S, LIU B. Construction of yeast surface-displayed cDNA libraries[M]//Methods in Molecular Biology. Totowa, NJ: Humana Press, 2011: 199-210.
[26] BIDLINGMAIER S, LIU B. Construction and application of a yeast surface-displayed human cDNA library to identify post-translational modification-dependent protein-protein interactions[J]. Molecular & Cellular Proteomics, 2006, 5(3): 533-540.
[27] BIDLINGMAIER S, HE J, WANG Y, AN F, FENG JJ, BARBONE D, GAO DW, FRANC B, BROADDUS VC, LIU B. Identification of MCAM/CD146 as the target antigen of a human monoclonal antibody that recognizes both epithelioid and sarcomatoid types of mesothelioma[J]. Cancer Research, 2009, 69(4): 1570-1577.
[28] BACON K, BLAIN A, BOWEN J, BURROUGHS M, MCARTHUR N, MENEGATTI S, RAO BM. Quantitative yeast-yeast two hybrid for the discovery and binding affinity estimation of protein-protein interactions[J]. ACS Synthetic Biology, 2021, 10(3): 505-514.
[29] GREANEY AJ, STARR TN, GILCHUK P, ZOST SJ, BINSHTEIN E, LOES AN, HILTON SK, HUDDLESTON J, EGUIA R, CRAWFORD KHD, DINGENS AS, NARGI RS, SUTTON RE, SURYADEVARA N, ROTHLAUF PW, LIU ZM, WHELAN SPJ, CARNAHAN RH, CROWE JE JR, BLOOM JD. Complete mapping of mutations to the SARS-CoV-2 spike receptor-binding domain that escape antibody recognition[J]. Cell Host & Microbe, 2021, 29(1): 44-57.e9.
[30] CAO YL, WANG J, JIAN FC, XIAO TH, SONG WL, YISIMAYI A, HUANG WJ, LI QQ, WANG P, AN R, WANG J, WANG Y, NIU X, YANG SJ, LIANG H, SUN HY, LI T, YU YL, CUI QQ, LIU S, et al. Omicron escapes the majority of existing SARS-CoV-2 neutralizing antibodies[J]. Nature, 2022, 602(7898): 657-663.
[31] WIECZOREK M, ABUALROUS ET, STICHT J, ÁLVARO-BENITO M, STOLZENBERG S, NOÉ F, FREUND C. Major histocompatibility complex (MHC) class I and MHC class II proteins: conformational plasticity in antigen presentation[J]. Frontiers in Immunology, 2017, 8: 292.
[32] SIBENER LV, FERNANDES RA, KOLAWOLE EM, CARBONE CB, LIU F, MCAFFEE D, BIRNBAUM ME, YANG XB, SU LF, YU W, DONG S, GEE MH, JUDE KM, DAVIS MM, GROVES JT, GODDARD WA III, HEATH JR, EVAVOLD BD, VALE RD, GARCIA KC. Isolation of a structural mechanism for uncoupling T cell receptor signaling from peptide-MHC binding[J]. Cell, 2018, 174(3): 672-687.e27.
[33] GEE MH, HAN A, LOFGREN SM, BEAUSANG JF, MENDOZA JL, BIRNBAUM ME, BETHUNE MT, FISCHER S, YANG XB, GOMEZ-EERLAND R,

BINGHAM DB, SIBENER LV, FERNANDES RA, VELASCO A, BALTIMORE D, SCHUMACHER TN, KHATRI P, QUAKE SR, DAVIS MM, GARCIA KC. Antigen identification for orphan T cell receptors expressed on tumor-infiltrating lymphocytes[J]. Cell, 2018, 172(3): 549-563.e16.

[34] LIU RZ, JIANG W, MELLINS ED. Yeast display of MHC-II enables rapid identification of peptide ligands from protein antigens (RIPPA)[J]. Cellular & Molecular Immunology, 2021, 18(8): 1847-1860.

[35] LIU RZ, JIANG W, LI Y, MELLINS ED. RIPPA: identification of MHC-II binding peptides from antigen using a yeast display-based approach[J]. Current Protocols, 2022, 2(1): e350.

[36] RAPPAZZO CG, HUISMAN BD, BIRNBAUM ME. Repertoire-scale determination of class II MHC peptide binding *via* yeast display improves antigen prediction[J]. Nature Communications, 2020, 11: 4414.

[37] HUISMAN BD, DAI Z, GIFFORD DK, BIRNBAUM ME. A high-throughput yeast display approach to profile pathogen proteomes for MHC-II binding[J]. eLife, 2022, 11: 78589.

[38] WELLNER A, MCMAHON C, GILMAN MSA, CLEMENTS JR, CLARK S, NGUYEN KM, HO MH, HU VJ, SHIN JE, FELDMAN J, HAUSER BM, CARADONNA TM, WINGLER LM, SCHMIDT AG, MARKS DS, ABRAHAM J, KRUSE AC, LIU CC. Rapid generation of potent antibodies by autonomous hypermutation in yeast[J]. Nature Chemical Biology, 2021, 17(10): 1057-1064.

[39] GRAY A, BRADBURY ARM, KNAPPIK A, PLÜCKTHUN A, BORREBAECK CAK, DÜBEL S. Animal-free alternatives and the antibody iceberg[J]. Nature Biotechnology, 2020, 38(11): 1234-1239.

[40] BOWLEY DR, LABRIJN AF, ZWICK MB, BURTON DR. Antigen selection from an HIV-1 immune antibody library displayed on yeast yields many novel antibodies compared to selection from the same library displayed on phage[J]. Protein Engineering, Design and Selection, 2007, 20(2): 81-90.

[41] SCHOLLER N. Selection of antibody fragments by yeast display[M]//Antibody Engineering. New York, NY: Springer New York, 2018: 211-233.

[42] SCHOLLER N. Selection of antibody fragments by yeast display[M]//Antibody Engineering. Totowa, NJ: Humana Press, 2012: 259-280.

[43] SUN W, YANG ZN, LIN H, LIU M, ZHAO CX, HOU XY, HU ZW, CUI B. Improvement in affinity and thermostability of a fully human antibody against interleukin-17A by yeast-display technology and CDR grafting[J]. Acta Pharmaceutica Sinica B, 2019, 9(5): 960-972.

[44] KECK ZY, WANG Y, LAU P, FOUNG SKH. Isolation of HCV neutralizing antibodies by yeast display[M]// Methods in Molecular Biology. New York, NY: Springer New York, 2018: 395-419.

[45] BOGEN JP, CARRARA SC, FIEBIG D, GRZESCHIK J, HOCK B, KOLMAR H. Expeditious generation of biparatopic common light chain antibodies *via* chicken immunization and yeast display screening[J]. Frontiers in Immunology, 2020, 11: 606878.

[46] ELTER A, BOGEN JP, HINZ SC, FIEBIG D, MACARRÓN PALACIOS A, GRZESCHIK J, HOCK B, KOLMAR H. Humanization of chicken-derived scFv using yeast surface display and NGS data mining[J]. Biotechnology Journal, 2021, 16(3): 2000231.

[47] JIA YR, REN P, DUAN SX, ZENG P, XIE DB, ZENG FL. An optimized yeast display strategy for efficient scFv antibody selection using ribosomal skipping system and thermo resistant yeast[J]. Biotechnology Letters, 2019, 41(8/9): 1067-1076.

[48] LAJOIE JM, CHO YK, FROST D, BREMNER S, LILJ, SHUSTA EV. A yeast display immunoprecipitation screen for targeted discovery of antibodies against membrane protein complexes[J]. Protein Engineering, Design and Selection, 2019, 32(5): 219-230.

[49] van den BEUCKEN T, PIETERS H, STEUKERS M, van der VAART M, LADNER RC, HOOGENBOOM HR, HUFTON SE. Affinity maturation of Fab antibody fragments by fluorescent-activated cell sorting of yeast-displayed libraries[J]. FEBS Letters, 2003, 546(2/3): 288-294.

[50] BLAISE L, WEHNERT A, STEUKERS MPG, van den BEUCKEN T, HOOGENBOOM HR, HUFTON SE. Construction and diversification of yeast cell surface displayed libraries by yeast mating: application to the affinity maturation of Fab antibody fragments[J]. Gene, 2004, 342(2): 211-218.

[51] ROSOWSKI S, BECKER S, TOLEIKIS L, VALLDORF B, GRZESCHIK J, DEMIR D, WILLENBÜCHER I, GAA R, KOLMAR H, ZIELONKA S, KRAH S. A novel one-step approach for the construction of yeast surface display Fab antibody libraries[J]. Microbial Cell Factories, 2018, 17(1): 1-11.

[52] KRAH S, GRZESCHIK J, ROSOWSKI S, GAA R, WILLENBUECHER I, DEMIR D, TOLEIKIS L, KOLMAR H, BECKER S, ZIELONKA S. A streamlined approach for the construction of large yeast surface display fab antibody libraries[M]//Antibody Engineering. New York, NY: Springer New York, 2018: 145-161.

[53] ROTH L, KRAH S, KLEMM J, GÜNTHER R, TOLEIKIS L, BUSCH M, BECKER S, ZIELONKA S. Isolation of antigen-specific VHH single-domain antibodies by combining animal immunization with yeast surface display[M]//Methods in Molecular Biology. New York, NY: Springer US, 2019: 173-189.

[54] UCHAŃSKI T, ZÖGG T, YIN J, YUAN DP, WOHLKÖNIG A, FISCHER B, ROSENBAUM DM, KOBILKA BK, PARDON E, STEYAERT J. An improved yeast surface display platform for the screening of nanobody immune libraries[J]. Scientific Reports, 2019, 9: 382.

[55] MCMAHON C, BAIER AS, PASCOLUTTI R, WEGRECKI M, ZHENG SD, ONG JX, ERLANDSON SC, HILGER D, RASMUSSEN SGF, RING AM, MANGLIK A, KRUSE AC. Yeast surface display platform for rapid discovery of conformationally selective nanobodies[J]. Nature Structural & Molecular Biology, 2018, 25(3): 289-296.

[56] LERMA ROMERO JA, MEYNERS C, CHRISTMANN A, REINBOLD LM, CHARALAMPIDOU A, HAUSCH F, KOLMAR H. Binding pocket stabilization by high-throughput screening of yeast display libraries[J]. Frontiers in Molecular Biosciences, 2022, 9: 1023131.

[57] van ROSMALEN M, JANSSEN BMG, HENDRIKSE NM, van der LINDEN AJ, PIETERS PA, WANDERS D, de GREEF TFA, MERKX M. Affinity maturation of a cyclic peptide handle for therapeutic antibodies using deep mutational scanning[J]. Journal of Biological Chemistry, 2017, 292(4): 1477-1489.

[58] JEONG MY, RUTTER J, CHOU DHC. Display of single-chain insulin-like peptides on a yeast surface[J]. Biochemistry, 2019, 58(3): 182-188.

[59] COHEN I, KAYODE O, HOCKLA A, SANKARAN B, RADISKY DC, RADISKY ES, PAPO N. Combinatorial protein engineering of proteolytically resistant mesotrypsin inhibitors as candidates for cancer therapy[J]. Biochemical Journal, 2016, 473(10): 1329-1341.

[60] SANANES A, COHEN I, SHAHAR A, HOCKLA A, de VITA E, MILLER AK, RADISKY ES, PAPO N. A potent, proteolysis-resistant inhibitor of kallikrein-related peptidase 6 (KLK6) for cancer therapy, developed by combinatorial engineering[J]. Journal of Biological Chemistry, 2018, 293(33): 12663-12680.

[61] TRAXLMAYR MW, SHUSTA EV. Directed evolution of protein thermal stability using yeast surface display[M]//Methods in Molecular Biology. New York, NY: Springer New York, 2017: 45-65.

[62] LAURENT E, SIEBER A, SALZER B, WACHERNIG A, SEIGNER J, LEHNER M, GEYEREGGER R, KRATZER B, JÄGER U, KUNERT R, PICKL WF, TRAXLMAYR MW. Directed evolution of stabilized monomeric CD19 for monovalent CAR interaction studies and monitoring of CAR-T cell patients[J]. ACS Synthetic Biology, 2021, 10(5): 1184-1198.

[63] ILIĆ ĐURĐIĆ K, ECE SL, OSTAFE R, VOGEL S, BALAŽ AM, SCHILLBERG S, FISCHER R, PRODANOVIĆ R. Flow cytometry-based system for screening of lignin peroxidase mutants with higher oxidative stability[J]. Journal of Bioscience and Bioengineering, 2020, 129(6): 664-671.

[64] LEE SY, KO DH, SON MJ, KIM JA, JUNG K, KIM YS. Affinity maturation of a T-cell receptor-like antibody specific for a *Cytomegalovirus* pp65-derived peptide presented by HLA-A*02:01[J]. International Journal of Molecular Sciences, 2021, 22(5): 2349.

[65] KAJIWARA K, AOKI W, KOIKE N, UEDA M. Development of a yeast cell surface display method using the SpyTag/SpyCatcher system[J]. Scientific Reports, 2021, 11: 11059.

[66] HAN SY, ZHANG JH, HAN ZL, ZHENG SP, LIN Y. Combination of site-directed mutagenesis and yeast surface display enhances *Rhizomucor miehei* lipase esterification activity in organic solvent[J]. Biotechnology Letters, 2011, 33(12): 2431-2438.

[67] LI ZS, MIAO YL, YANG JM, ZHAO FG, LIN Y, HAN SY. Efficient improvement of surface displayed lipase from *Rhizomucor miehei* in PichiaPink™ protease-deficient system[J]. Protein Expression and Purification, 2021, 180: 105804.

[68] CHEN ZZ, WANG YY, CHENG YY, WANG X, TONG SW, YANG HT, WANG ZF. Efficient biodegradation of highly crystallized polyethylene terephthalate through cell surface display of bacterial PETase[J]. Science of the Total Environment, 2020, 709: 136138.

[69] YANG SJ, LV X, WANG XH, WANG JQ, WANG RM, WANG TF. Cell-surface displayed expression of trehalose synthase from *Pseudomonas putida* ATCC 47054 in *Pichia pastoris* using Pir1p as an anchor protein[J]. Frontiers in Microbiology, 2017, 8: 2583.

[70] FAN SQ, LIANG B, XIAO XX, BAI L, TANG XJ, LOJOU E, COSNIER S, LIU AH. Controllable display of sequential enzymes on yeast surface with enhanced biocatalytic activity toward efficient enzymatic biofuel cells[J]. Journal of the American Chemical Society, 2020, 142(6): 3222-3230.

[71] HAN YS, BRANON TC, MARTELL JD, BOASSA D, SHECHNER D, ELLISMAN MH, TING A. Directed evolution of split APEX2 peroxidase[J]. ACS Chemical Biology, 2019, 14(4): 619-635.

[72] CEJNAR R, HLOŽKOVÁ K, KOTRBA P, DOSTÁLEK P. Surface-engineered *Saccharomyces cerevisiae* displaying α-acetolactate decarboxylase from *Acetobacter aceti* ssp. *xylinum*[J]. Biotechnology Letters, 2016, 38(12): 2145-2151.

[73] TAKAYAMA K, SUYE SI, KURODA K, UEDA M, KITAGUCHI T, TSUCHIYAMA K, FUKUDA T, CHEN W, MULCHANDANI A. Surface display of organophosphorus hydrolase on *Saccharomyces cerevisiae*[J]. Biotechnology Progress, 2006, 22(4): 939-943.

[74] LIANG B, LU XQ, WANG G, YAN L, REN H, FENG RR, XIONG Z, LIU AH. Functional cell surface displaying of acetylcholinesterase for spectrophotometric sensing organophosphate pesticide[J]. Sensors and Actuators B: Chemical, 2019, 279: 483-489.

[75] ZHU BT, WEI N. Biocatalytic degradation of parabens mediated by cell surface displayed cutinase[J]. Environmental Science & Technology, 2019, 53(1): 354-364.

[76] RAJMAN L, CHWALEK K, SINCLAIR DA. Therapeutic potential of NAD-boosting molecules: the *in vivo* evidence[J]. Cell Metabolism, 2018, 27(3): 529-547.

[77] HE ZH, YANG XS, TIAN X, LI LJ, LIU MY. Yeast cell surface engineering of a nicotinamide riboside kinase for the production of β-nicotinamide mononucleotide *via* whole-cell catalysis[J]. ACS Synthetic Biology, 2022, 11(10): 3451-3459.

[78] LYND LR, van ZYL WH, MCBRIDE JE, LASER M. Consolidated bioprocessing of cellulosic biomass: an update[J]. Current Opinion in Biotechnology, 2005, 16(5): 577-583.

[79] BAE JG, KURODA K, UEDA M. Proximity effect among cellulose-degrading enzymes displayed on the *Saccharomyces cerevisiae* cell surface[J]. Applied and Environmental Microbiology, 2015, 81(1): 59-66.

[80] CHEN L, DU JL, ZHAN YJ, LI JA, ZUO RR, TIAN S. Consolidated bioprocessing for cellulosic ethanol conversion by cellulase-xylanase cell-surfaced yeast consortium[J]. Preparative Biochemistry & Biotechnology, 2018, 48(7): 653-661.

[81] GUIRIMAND G, INOKUMA K, BAMBA T, MATSUDA M, MORITA K, SASAKI K, OGINO C, BERRIN JG, HASUNUMA T, KONDO A. Cell-surface display technology and metabolic engineering of *Saccharomyces cerevisiae* for enhancing xylitol production from woody biomass[J]. Green Chemistry, 2019, 21(7): 1795-1808.

[82] LIANG HL, WANG Q, SHEN S, JIA YR, LI P, ZENG FL, HAO ZM, DONG JG. Yeast surface displayed xylanase is an efficient strategy to induce corn defence response[J]. Biotechnology & Biotechnological Equipment, 2020, 34(1): 665-672.

[83] LEI H, XIE BW, GAO T, CEN QH, REN Y. Yeast display platform technology to prepare oral vaccine against lethal H7N9 virus challenge in mice[J]. Microbial Cell Factories, 2020, 19(1): 1-9.

[84] SHIBASAKI S, AOKI W, NOMURA T, MIYOSHI A, TAFUKU S, SEWAKI T, UEDA M. An oral vaccine against candidiasis generated by a yeast molecular display system[J]. Pathogens and Disease, 2013, 69(3): 262-268.

[85] GAO T, REN Y, LI SQ, LU X, LEI H. Immune response induced by oral administration with a *Saccharomyces cerevisiae*-based SARS-CoV-2 vaccine in mice[J]. Microbial Cell Factories, 2021, 20(1): 1-10.

[86] XING HG, ZHU LY, WANG PP, ZHAO GP, ZHOU ZH, YANG Y, ZOU H, YAN X. Display of receptor-binding domain of SARS-CoV-2 spike protein variants on the *Saccharomyces cerevisiae* cell surface[J]. Frontiers in Immunology, 2022, 13: 935573.

[87] LE LINH H, THU NPA, DUNG TTX, van HAU N, NGHIA NH, THAO DTP. Yeast cell surface displaying VP28 antigen and its potential application for shrimp farming[J]. Applied Microbiology and Biotechnology, 2021, 105(16/17): 6345-6354.

[88] VENDRAMIN N, ALENCAR ALF, IBURG TM, DAHLE MK, WESSELØ, OLSEN AB, RIMSTAD E, OLESEN NJ. Piscine orthoreovirus infection in

Atlantic salmon (*Salmo salar*) protects against subsequent challenge with infectious hematopoietic necrosis virus (IHNV)[J]. Veterinary Research, 2018, 49(1): 1-12.

[89] ZHAO JZ, XU LM, LIU M, CAO YS, LAPATRA SE, YIN JS, LIU HB, LU TY. Preliminary study of an oral vaccine against infectious hematopoietic necrosis virus using improved yeast surface display technology[J]. Molecular Immunology, 2017, 85: 196-204.

[90] THANGARAJ RS, NITHIANANTHAM SR, DHARMARATNAM A, KUMAR R, PRADHAN PK, THANGALAZHY GOPAKUMAR S, SOOD N. Cyprinid herpesvirus-2 (CyHV-2): a comprehensive review[J]. Reviews in Aquaculture, 2021, 13(2): 796-821.

[91] DONG ZR, MU QJ, KONG WG, QIN DC, ZHOU Y, WANG XY, CHENG GF, LUO YZ, AI TS, XU Z. Gut mucosal immune responses and protective efficacy of oral yeast cyprinid herpesvirus 2 (CyHV-2) vaccine in *Carassius auratus* gibelio[J]. Frontiers in Immunology, 2022, 13: 932722.

[92] WANG LC, YANG MX, LUO S, YANG GJ, LU XJ, LU JF, CHEN J. Oral vaccination of recombinant *Saccharomyces cerevisiae* expressing ORF132 induces protective immunity against cyprinid herpesvirus-2[J]. Vaccines, 2023, 11(1): 186.

[93] ZHANG MJ, CHEN XY, XUE MY, JIANG N, LI YQ, FAN YD, ZHANG P, LIU NC, XIAO ZD, ZHANG QH, ZHOU Y. Oral vaccination of largemouth bass (*Micropterus salmoides*) against largemouth bass *Ranavirus* (LMBV) using yeast surface display technology[J]. Animals, 2023, 13(7): 1183.

[94] PARK M. Surface display technology for biosensor applications: a review[J]. Sensors, 2020, 20(10): 2775.

[95] ZHAO SY, GUO D, ZHU QC, DOU WW, GUAN WJ. Display of microbial glucose dehydrogenase and cholesterol oxidase on the yeast cell surface for the detection of blood biochemical parameters[J]. Biosensors, 2020, 11(1): 13.

(本文责编　陈宏宇)