2017-12-25 DOI: 10.3724/SP.J.1145.2017.01031 http://www.cibj.com/
应用与环境生物学报 Chin J Appl Environ Biol 2017, 23 (6): 1006-1010

产甘油假丝酵母抗逆转录因子的过表达对酿酒酵母耐酸胁迫性的影响*

李倩倩[1,2] 王 燕[1,2] 诸葛斌[1,2**] 陆信曜[2] 宗 红[2] 方慧英[1,2] 宋 健[3]

[1]江南大学糖化学与生物技术教育部重点实验室 无锡 214122
[2]江南大学工业生物技术教育部重点实验室, 生物工程学院, 工业微生物研究中心 无锡 214122
[3]江南大学化学与材料工程学院 无锡 214122

摘 要 以具有优良环境耐受性的产甘油假丝酵母(*Candida glycerinogenes*)为研究对象, 考察其抗逆转录因子对酿酒酵母(*Saccharomyces cerevisiae*)酸胁迫耐受性的影响. 分别克隆获得*C. glycerinogenes*和*S. cerevisiae*的转录因子基因*haa1*和*asg1*, 在*S. cerevisiae* W303-1A中分别过表达这4个基因, 继而进行摇瓶试验考察重组菌株的酸耐受性. 结果显示, 过表达不同转录因子均能提高细胞酸耐受性, 其中90 mmol/L乙酸时重组菌*S. cerevisiae/Cghaa1*和*S. cerevisiae/Cgasg1*的生物量与*S. cerevisiae/Schaa1*和*S. cerevisiae/Scasg1*相比分别提高了44.3%和18.9%. qRT-PCR发现, 与*Schaa1*和*Scasg1*相比, 过表达*Cghaa1*和*Cgasg1*能够显著上调下游酸耐受相关基因的表达水平. 酸胁迫下乙醇发酵结果显示, 相比对照组, 重组菌*S. cerevisiae/ Cgasg1*的乙醇产量提高11.1%. 上述结果表明转录因子HAA1和ASG1均能提高酿酒酵母酸耐受性和酸胁迫下乙醇产量, 其中*Cghaa1*和*Cgasg1*效果更为明显, 结果可为提高酿酒酵母酸耐受性提供新的基因资源和思路, 为进一步挖掘*C. glycerinogenes*抗逆基因提供借鉴. (图3 表1 参21)

关键词 产甘油假丝酵母; 转录因子; 过表达; 酸耐受性; 乙醇产量
CLC Q78 : TQ923

Effect of overexpressing transcription factors of *Candida glycerinogenes* on acid tolerance of *Saccharomyces cerevisiae**

LI Qianqian[1,2], WANG Yan[1,2], ZHUGE Bin[1,2**], LU Xinyao[2], ZONG Hong[2], FANG Huiying[1,2] & SONG Jian[3]

[1] *Key Laboratory of Carbohydrate Chemistry and Biotechnology, Ministry of Education, School of Biotechnology, Jiangnan University*, Wuxi 214122, China
[2] *Key Laboratory of Industrial Biotechnology, Ministry of Education, Jiangnan University, Laboratory of Industrial Microorganisms, School of Biotechnology, Jiangnan University*, Wuxi 214122, China
[3] *School of Chemical and Material Engineering, Jiangnan University*, Wuxi 214122, China

Abstract The aim of this study was to examine the effect of antiretroviral transcription factors of *Candida glycerinogenes* on the tolerance of *Saccharomyces cerevisiae* to acid stress. The *haa1* and *asg1* genes of *C. glycerinogenes* and *S. cerevisiae* strains were obtained by cloning, and these four genes were subsequently overexpressed in *S. cerevisiae* W303-1A. The acid tolerance of the recombinant strains was examined using shake flask experiments. The results showed that overexpression of different transcription factors increased the acid tolerance. In comparison with *S. cerevisiae*/*Schaa1* and *S. cerevisiae*/*Scasg1*, the biomass of *S. cerevisiae*/*Cghaa1* and *S. cerevisiae*/*Cgasg*1 in 90 mmol/L acetic acid was 44.3% and 18.9% higher, respectively. Strains overexpressing *Cgasg1* and *Cghaa1* exhibited enhanced tolerance to a high concentration of acetic acid. qRT-PCR analysis revealed that overexpression of *Cghaa1* and *Cgasg1* significantly up-regulated the expression of downstream acid-tolerance-related genes. Under conditions of acid stress, the ethanol yield of *S. cerevisiae*/*Cgasg1* was 11.1% higher than that of the control group. These results indicate that the transcription factors *HAA1* and *ASG1* can enhance the tolerance of *S. cerevisiae* to acid stress. This study provides a valuable insight into techniques for improving the acid tolerance of *S. cerevisiae* and for future research into *C. glycerinogenes* resistance genes.

Keywords *Candida glycerinogenes*; transcription factor; overexpression; acid tolerance; ethanol yield

收稿日期 Received: 2017-01-31 接受日期 Accepted: 2017-03-13
*国家自然科学基金项目(31570052, 31601456)、江苏省自然科学基金项目(BK20140138)和111项目(111-2-06)资助 Supported by the National Natural Science Foundation of China (31570052, 31601456), the Natural Science Foundation (BK20140138) and 111 Program (111-2-06) of Jiangsu, China
**通讯作者 Corresponding author (E-mail: bzhuge@163.com)

酸胁迫是微生物发酵过程中普遍存在的环境胁迫，由弱有机酸引起的环境低pH会影响细胞壁结构、改变质膜蛋白质的构型、增加细胞对离子和其它小分子代谢物的渗透性，导致胞内pH降低，最终影响微生物生长和产物合成[1-5]. 因此，提高微生物酸耐受性对细胞生长和产物高效合成至关重要.

已有研究发现，酵母细胞对酸胁迫的响应调控机制涉及基因、转录及蛋白质水平[6]. 在对上述酸耐受机理认识的基础上，前人已从不同水平对酵母酸耐受性进行调控，以强化酸胁迫下细胞生长和产物合成. 其中，转录水平调节能够对细胞生理代谢进行系统性全局调控，进而显著提高菌株酸耐受性. 文献报道，酵母转录因子HAA1调控80%的乙酸耐受性相关基因，对细胞耐受酸胁迫起到重要作用[7-9]；类似地，转录因子ASG1能够调控压力响应基因的表达，从而调节胞内pH、维持细胞稳态和对酸胁迫的耐受[10]. 基于此，Tanaka等[7]及Wu等[10]在*Saccharomyces cerevisiae*或*Candida glabrata*过表达HAA1、ASG1，显著改善了酸胁迫下重组菌的生长和产物合成.

课题组前期研究发现，与模式菌*S. cerevisiae*相比，产甘油假丝酵母（*Candida glycerinogenes*）[11]具有更高的酸胁迫耐受性，显示其可能具有更高效酸胁迫应答系统. 本研究在酿酒酵母中分别表达*S. cerevisiae*和*C. glycerinogenes*的转录因子基因*haa1*及*asg1*，分析比较不同来源转录因子HAA1和ASG1对重组菌株乙酸耐受性及酸胁迫下乙醇发酵的影响，为代谢改造提高细胞酸耐受性提供优良的基因资源和新的思路.

1 材料与方法

1.1 菌株、质粒

本研究中所使用的菌株为*Escherichia coli* JM109、*Saccharomyces cerevisiae* W303-1A、*Candida glycerinogenes*-WL2002-5、*Saccharomyces cerevisiae* W303/YEplac181、*Saccharomyces cerevisiae* W303/YEplac181-P*CgHAA1-Cghaa1*、*Saccharomyces cerevisiae* W303/YEplac181-P*CgASG1-Cgasg1*、*Saccharomyces cerevisiae* W303/YEplac181-P*ScHAA1-Schaa1*、*Saccharomyces cerevisiae* W303/YEplac181-P*ScASG1-Scasg1*. 引物设计序列如表1所示.

1.2 工具酶及试剂

酵母膏和蛋白胨购自OXOID公司；实验用各种限制性内切酶和ExTaq DNA聚合酶购自TaKaRa公司；质粒小量抽提试剂盒购自上海生工生物工程有限公司，胶回收试剂盒购自天根生化科技有限公司. 所用引物由苏州泓迅生物技术有限公司合成.

1.3 培养基与培养方法

LB培养基（g/L）：酵母膏5，胰蛋白胨10，NaCl 10；氨苄青霉素抗性平板需额外添加100 μg/mL Amp.

亮氨酸缺陷培养基（g/L）：葡萄糖20，YNB 6.7，尿嘧啶0.02，腺嘌呤0.02，除亮氨酸外其他氨基酸0.076.

YEPD培养基（g/L）：酵母膏10，胰蛋白胨20，葡萄糖20；酸性培养条件则需额外添加4.2 g/L（70 mmol/L）或5.4 g/L（90 mmol/L）的乙酸.

发酵培养基（g/L）：酵母膏10，胰蛋白胨20，葡萄糖100，乙酸4.2.

发酵培养：250 mL三角瓶50 mL装液量，2%接种量（体积比），30 ℃、100 r/min振荡培养.

1.4 重组载体的构建

*C. glycerinogenes*基因组DNA和*S. cerevisiae*基因组DNA[12]为模板，PCR扩增P*CgHAA1-Cghaa1*、P*CgASG1-Cgasg1*、P*ScHAA1-Schaa1*、P*ScASG1-Scasg1*，双酶切后连接YEplac181载体，构建重组质粒YEplac181-P*CgHAA1-Cghaa1*、YEplac181-P*CgASG1-Cgasg1*、YEplac181-P*ScHAA1-Schaa1*、YEplac181-P*ScASG1-Scasg1*.

1.5 电转化构建重组菌

重组质粒YEplac181-P*CgHAA1-Cghaa1*、YEplac181-P*CgASG1-Cgasg1*、YEplac181-P*ScHAA1-Schaa1*、YEplac181-P*ScASG1-Scasg1*电击转化*S. cerevisiae* W303，在亮氨酸营养缺陷培养基上筛选得到重组菌.

表 1 文中所用到的引物
Table 1 Primers used in this paper

引物 Primer	序列 Sequence (5′ to 3′)	酶切位点 Restriction site
P*CgHAA1-Cghaa1*-F	CGCGGATCCAATGAGGGAAATAATGAAAA	*Bam*HI
P*CgHAA1-Cghaa1*-R	CGAGCTCGAGTTCTAACAACGGGTAAA	*Sac* I
P*CgASG1-Cgasg1*-F	CATGCATGCGAAACGATTAATTGAAAGAAAAAA	*Sph* I
P*CgASG1-Cgasg1*-R	CCCCCGGGTGGAAGTAGGTCACTTTAAAAC	*Xma* I
P*ScHAA1-Schaa1*-F	GCGGATCCCCTTAAAAGCGTATACATCA	*Bam*H I
P*ScHAA1-Schaa1*-R	CGAGCTCTTCGGACTTGCCTTCCTAGT	*Sac* I
P*ScASG1-Scasg1*-F	GCGTCGACCAACTCAGAGAGGAATTGTT	*Sal* I
P*ScASG1-Scasg1*-R	CCCCCGGGTTCATTTAAAACTCATTTGG	*Xma* I
rt*Cghaa1*	GGGTCAAATTTCCAATGGTG & TTGCCGGTGTGAAATCATAA	
rt*Cgasg1*	ACAGATGCCGGAAATTGAAG & GGTGGTGGTTTCCTCTTTGA	
rt*Schaa1*	CGCCGATATCAAACTCTCGT & GGCCAATCGTAGACCAAAGA	
rt*Scasg1*	GTTCTTCTCCGATCCAACCA & TGTCTTGTTCCCTGTTGCTG	
rt*Sctpo2*	CATCCAGTGCTCTGGCTACA & CAACAACCAAGAACCCCAGT	
rt*Sctpo3*	ACGTCCAATACGCATCCAGT & CAAGAACCCCAGTGCAGATT	
rt*Scyro2*	GCCATCTCCAGCTTCTTTCA & CCTTCTTGGCCTTCTTAGCC	
rt*Scygp1*	ATGCCATTCCAGTCGCTAAC & CAGCACCGTAAGAACAAGCA	
rt*Scact1*	AATTGAGAGTTGCCCCAGAA & GAAGGCTGGAACGTTGAAAG	

1.6 重组菌耐受性测定

将重组酿酒酵母摇瓶培养至对数中后期，按照相同的接种量接种含有不同浓度乙酸的YEPD液体培养基，30 ℃下200 r/min摇床培养72 h，每12 h测一次OD_{600}值得到重组菌株耐受乙酸的生长曲线.

1.7 实时荧光定量PCR

摇瓶培养重组菌，用酸性培养基（YEPD + 70 mmol/L乙酸）处理. 酵母总RNA提取方法参见文献[13]. 反转录及实时定量qRT-PCR方法与条件参见试剂盒说明书. 内参基因选用*S. cerevisiae*的*act1*，以70 mmol/L乙酸处理下*S. cerevisiae* W303转录水平作为对照值设为1，将重组菌在此胁迫下的转录水平与之对比.

1.8 乙醇产量测定

通过高效液相色谱法[14]定量测定乙醇发酵产量.

2 结果与分析

2.1 转录因子基因的克隆及生物信息学分析

*C. glycerinogenes*与*Pichia kudriavzevii*在基因水平上具有高度同源性[15]. 基于此，根据*P. kudriavzevii* SD108（GenBank assembly accession GCA_000764455.1）基因组信息设计引物，以*C. glycerinogenes*染色体DNA为模板，克隆得到含有自身启动子序列的*Cghaa1*和*Cgasg1*. 测序结果表明：转录因子CgHAA1与*S. cerevisiae*的ScHAA1氨基酸同源性为27.6%，DNA结合域相似性为63.3%；转录因子CgASG1与*S. cerevisiae*的ScASG1基因氨基酸同源性为38.7%，DNA结合域相似性为44.4%. 同时克隆*S. cerevisiae*来源的转录因子基因*Schaa1*和*Scasg1*，分别构建重组质粒YEplac181-P*CgHAA1-Cghaa1*、YEplac181-P*ScHAA1-Schaa1*、YEplac181-P*CgASG1-Cgasg1*、YEplac181-P*ScASG1-Scasg1*转化*S. cerevisiae*，考察不同转录因子对重组菌酸耐受性的影响.

2.2 过表达不同转录因子对重组菌酸耐受性的影响

酸耐受性研究发现，过表达不同转录因子重组菌在乙酸胁迫下的生长明显改善（图1）. 在较低浓度乙酸条件下（70 mmol/L），*S. cerevisiae/Cghaa1*的生长略好于过表达*Cgasg1*、*Schaa1*、*Scasg1*的重组菌. 增加乙酸浓度到90 mmol/L时，重组菌*S. cerevisiae/Cghaa1*和*S. cerevisiae/Cgasg1*的生物量与*S. cerevisiae/Schaa1*和*S. cerevisiae/Scasg1*相比分别提高了44.3%和18.9%. 上述结果表明，来源于*C. glycerinogenes*的转录因子HAA1、ASG1能够更有效提高重组菌酸耐受性.

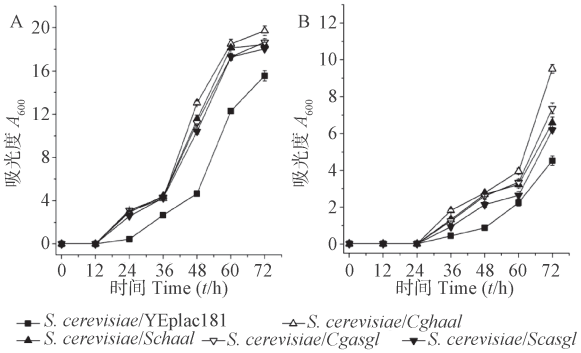

图1 重组酿酒酵母对乙酸的耐受性. A：70 mmol/L乙酸；B：90 mmol/L乙酸.

Fig. 1 Tolerance of recombinant *S. cerevisiae* strains to acetic acid stress. A: 70 mmol/L acetic acid ; B: 90 mmol/L acetic acid.

2.3 过表达不同转录因子对下游酸耐受基因转录水平的影响

已有研究发现，细胞壁、膜结构的变化是酵母酸胁迫响应机制的重要组成部分[16]. 其中，*tpo2*、*tpo3*（编码聚胺转运蛋白的基因）[8]和*yro2*（编码离子通道蛋白基因）[8]、*ygp1*（编码细胞壁分泌蛋白基因）[17]参与质子转运、细胞壁成分重构等，从而减少乙酸的毒性. 考察酸胁迫下不同重组菌中上述基因转录水平的变化，结果如图2，与*S. cerevisiae/Schaa1*相比，*S. cerevisiae/Cghaa1*具有相近的*haa1*表达水平，所调控的上述基因具有更高的转录水平，其中*tpo2*、*tpo3*均提高1.3倍，*yro2*提高1.1倍，*ygp1*表达水平稍低. 与*S. cerevisiae/Scasg1*相比，*S. cerevisiae/Cgasg1*中总*asg1*的表达水平相近，*tpo2*、*tpo3*转录水平提高了0.4和1.3倍，*yro2*提高1.6倍，*ygp1*表达水平相近. 上述结果表明，来源于*C. glycerinogenes*的转录因子HAA1和ASG1对下游酸耐受基因总体表现出更强的调控能力.

2.4 过表达不同转录因子对酸胁迫下乙醇发酵的影响

添加70 mmol/L乙酸以模拟考察过表达不同转录因子对酸胁迫下乙醇发酵的影响，结果如图3所示. 过表达转录因子后，酸胁迫下重组菌葡萄糖消耗加速，并在80 h时几乎全部耗光，而对照菌残余8.6 g/L葡萄糖. 过表达转录因子的重组菌生长速率更快，重组菌*S. cerevisiae/Cghaa1*、*S. cerevisiae/Schaa1*、*S. cerevisiae/Cgasg1*和*S. cerevisiae/Scasg1*最终生物

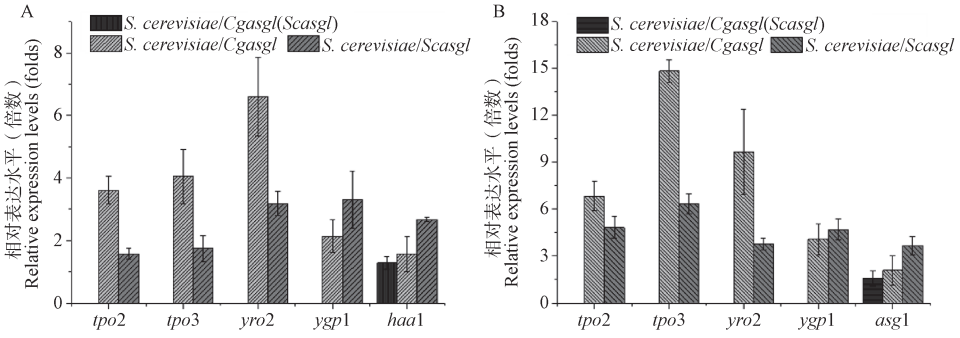

图2 不同重组酵母中压力响应基因表达水平差异. A：*haa1*过表达菌株；B：*asg1*过表达菌株.

Fig. 2 Expression levels of stress-related genes in the *S. cerevisiae* mutants with overexpressing *haa1* (A) and *asg1* (B) from different sources.

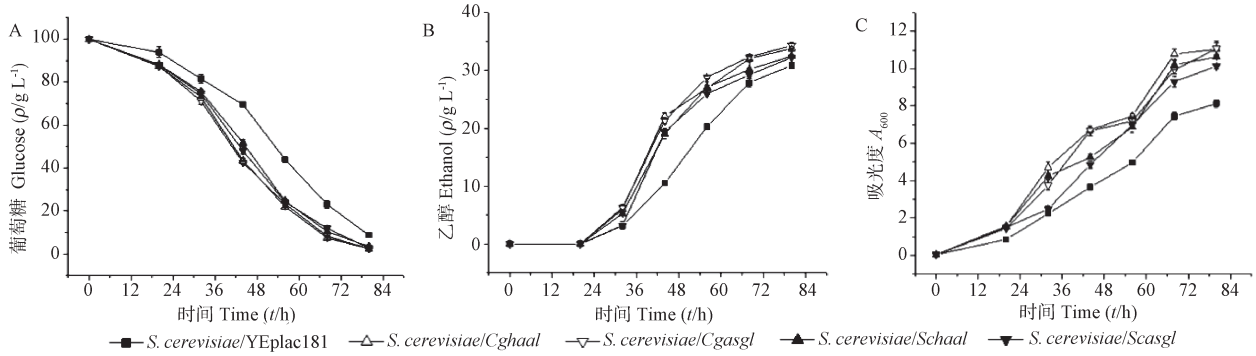

图3 过表达不同转录因子基因*haa1*和*asg1*对乙醇发酵的影响（添加70 mmol/L乙酸）. A：葡萄糖消耗；B：乙醇积累；C：生长曲线.
Fig. 3 Impacts of overexpressing *haa1* and *asg1* on ethanol production with the addition of 70 mmol/L acetic acid. A: Glucose consumption; B: Ethanol accumulation; C: Growth curve.

量分别提高约35%、31%、36%和25%. 耗糖速率的提高和生物量的加速积累同样促进了乙醇的快速合成，其中重组菌*S. cerevisiae*/*Cgasg1*、*S. cerevisiae*/*Scasg1*的乙醇终产量分别达到34.3 g/L和32.3 g/L，相比对照菌提高了约11.1%和4.6%. *S. cerevisiae*/*Cghaa1*、*S. cerevisiae*/*Schaa1*的乙醇终产量分别达到33.8 g/L和32.5 g/L，相比对照菌提高了约9.5%和5.3%. 上述结果表明，酸胁迫下过表达转录因子基因*Cghaa1*或*Cgasg1*的重组菌具有更强的乙醇发酵能力.

3 讨论与结论

本研究考察了来源于*S. cerevisiae*和*C. glycerinogenes*的转录因子HAA1和ASG1对重组菌乙酸耐受性的影响. 结果表明，与*S. cerevisiae*来源的转录因子相比，*Cghaa1*和*Cgasg1*能够更有效提高细胞对乙酸的耐受性，而这种差异在高浓度乙酸下表现得更为显著. 酸胁迫下，氢离子导致的低pH将影响酵母质膜所带的电荷，引起质膜渗透性的变化，从而影响酵母细胞对营养物质的吸收、酶的合成和活性、代谢途径效率[18]，限制细胞生长和产物合成[5]. 研究表明，转录因子HAA1和ASG1通过直接调控细胞壁合成蛋白、酸转运蛋白、转录调节蛋白等对细胞结构、跨膜转运、能量和物质代谢等进行系统调控，从而提高细胞酸耐受性[16]. 其中，精胺转运蛋白TPO2和TPO3转录水平的上调表明过表达转录因子利于胞内精胺积累以促进酸胁迫下细胞增殖[8]. 离子通道蛋白YRO2参与胞内氢离子向胞外的跨膜转运，过表达HAA1和ASG1均能有效提高其转录水平，表明氢离子的跨膜转运得到强化，利于细胞耐受酸胁迫. 此外，转录因子HAA1还能对转录调节蛋白NRG1（调节葡萄糖抑制）、MSN4（压力响应转录因子）、MCM1（调节细胞周期的基因转录）和HRK1（丝氨酸蛋白激酶K）等进行直接调控，涉及的代谢过程包括细胞胁迫反应、糖代谢、细胞周期控制、蛋白合成以及降解等.

qRT-PCR发现，与*Schaa1*和*Scasg1*相比，过表达*Cghaa1*和*Cgasg1*能够显著上调下游酸耐受相关基因的表达水平，从而更有效激活酵母酸耐受响应系统，使得重组菌*S. cerevisiae*/*Cghaa1*、*S. cerevisiae*/*Cgasg1*具有更高的乙酸耐受性. 文献报道，转录因子的DNA结合区对其功能和特性非常重要[19-21]. 氨基酸序列分析发现转录因子ScHAA1、ScASG1的DNA结合域分别是Met^{1}-Ser^{40}和Cys^{21}-Cys^{47}，而CgHAA1、CgASG1的DNA结合域分别是Met^{1}-K^{60}和Cys^{56}-Ser^{109}，推测后者能更有效地与下游靶基因结合，从而起到更强的调控作用. 乙酸胁迫下，过表达转录因子的重组菌的乙醇产量均有不同程度提高. 数据分析发现，所有实验菌株的乙醇耗糖转化率较为接近，表明过表达转录因子并没有影响乙醇途径的碳流分配，而是通过提高发酵过程细胞生物量来强化乙醇合成的.

综上，来源于产甘油假丝酵母的转录因子HAA1和ASG1对下游酸胁迫应答基因具有更强的调控能力，能够显著提高乙酸胁迫（特别是高浓度乙酸胁迫）下菌株生物量，从而强化酸胁迫下的乙醇产量. 本研究为提高酿酒酵母酸耐受性提供了新的基因资源和思路，也为进一步挖掘*C. glycerinogenes*抗逆基因提供了借鉴.

参考文献 [References]

1 Mira NP, Becker JD, Sd-Correia I. Genomic expression program involving the haalp-regulon in *Saccharomyces cerevisiae* response to acetic acid [J]. *OMICS*, 2010, **14** (5): 587-601

2 Ough CS. Fermentation rates of grape juice. II. Effect of initial ethyl alcohol, pH, and fermentation temperature [J]. *Am J Enol Vitic*, 1966, **17** (2): 74-81

3 Buzas Z, Dallmann K, Szajani B. Influence of pH on the growth and ethanol production of free and immobilized *Saccharomyces cerevisiae* cells [J]. *Biotechnol Bioeng*, 1989, **34** (6): 882-884

4 Nielsen MK, Arneborg N. The effect of citric acid and pH on growth and metabolism of anaerobic *Saccharomyces cerevisiae* and *Zygosaccharomyces bailii* cultures [J]. *Food Microbiol*, 2007, **24** (1): 101-105

5 Yalcin SK, Yesim OZ. Effects of pH and temperature on growth and glycerol production kinetics of two indigenous wine strains of *Saccharomyces cerevisiae* from Turkey [J]. *Braz J Microbiol*, 2008, **39** (2): 325-332

6 刘兴艳. 低pH对酿酒酵母酒精发酵的影响及酵母应答酸胁迫机制初探[D]. 北京: 中国农业大学, 2015 [Liu XY. Effect of low pH on alcoholic fermentation and primary study of acid stress response in *Saccharomyces cerevisiae* [D]. Beijing: China Agricultural University, 2015]

7 Koichi T, Yukari I, Jun O, Jun S. Enhancement of acetic acid tolerance in *Saccharomyces cerevisiae* by overexpression of the *HAA1* gene, encoding a transcriptional activator [J]. *Appl Environ Microbiol*, 2012, **78** (22): 8161-8163

8 Fernandes AR, Mira NP, Vargas RC, Canelhas I, Sa-Correia I. *Saccharomyces cerevisiae* adaptation to weak acids involves the transcription factor Haa1p and Haa1p-regulated genes [J]. *Biochem Biophy Res Commum*, 2005, **337**: 95-103

9 Arroyo-Lopez FN, Orli'c S, Querol A. Effects of temperature, pH and sugar concentration on the growth parameters of *Saccharomyces cerevisiae*, *S. kudriavzevii* and their interspecific hybrid [J]. *Int J Food Microbiol*, 2009, **131** (2-3): 120-127

10 Wu J, Chen X, Cai L, Tang L, Liu L. Transcription factors Asg1p and Hal1p regulated pH homeostasis in *Candida glabrata* [J]. *Front Microbiol*, 2015, **6**: 843

11 郑昌欣, 诸葛斌, 陆信曜, 宗红, 李静. 渗透压对产甘油假丝酵母磷酸果糖激酶和6-磷酸葡萄糖脱氢酶的调控[J]. 应用与环境生物学报, 2017, **22** (1): 123-128 [Zheng CX, Zhuge B, Lu XY, Zong H, Li J. Effects of osmostress on phosphofructokinase and 6-phosphate glucose dehydrogenase of *Candida glycerinogenes* [J]. *Chin J Appl Environ Biol*, 2017, **22** (1): 123-128]

12 Looke M, Kristjuhan K, Kristjuhan A. Extraction of genomic DNA from yeasts for PCR-based applications [J]. *Biotechniques*, 2011, **50** (5): 325-328

13 王晨莹. 产甘油假丝酵母高渗甘油应答途径关键基因*CgHOG1*的研究[D]. 无锡: 江南大学, 2013 [Wang CY. Study on the key gene of high osmolarity glycerol pathway-*CgHOG1* in *Candida glycerinogenes* [D]. Wuxi: Jiangnan University, 2013]

14 Guo XK, Fang HY, Zhuge B. *Bud*C knockout in *Klebsiella pneumonia* for bioconversion from glycerol to 1,3-propanediol [J]. *Biotechnol Appl Biochem*, 2013, **60** (6): 557-563

15 Takao K, Kenro T, Hediheko S, Katsuhiro K, Naoto I, Makoto H, Haruo T, Takao I. Construction of a *β*-glucosidase expression system using the multistress-tolerant yeast *Issatchenkia orientalis* [J]. *Appl Microbiol Biotechnol*, 2010. **87** (5): 1841-1853

16 赵心清, 张明明, 徐桂红, 许建韧, 白凤武. 酿酒酵母乙酸耐性分子机制的功能基因组进展[J]. 生物工程学报, 2014, **30** (3): 368-380. [Zhao XQ, Zhang MM, Xu GH, Xu JR, Bai FW. Advances in functional genomics studies underlying acetic acid tolerance of *Saccharomyces cerevisiae* [J]. *Chin J Biotechnol*, 2014, **30** (3): 368-380]

17 Destruelle M, Holzer H, Klionsky DJ. Identification and characterization of a novel yeast gene: the YGP1 gene product is a highly glycosylated secreted protein that is synthesized in response to nutrient limitation [J]. *Mol Cell Biol*, 1994, **14** (4): 2740-2754

18 Orij R, Brul S, Smits GJ. Intracelluar pH is a tightly controlled signal in yeast. *Biochim Biophys*, 2011, **1810** (10): 933-944

19 Liu Z, Evan R. Simpson. Molecular mechanism for cooperation between Sp1 and steroidogenic factor-1 (SF-1) to regulate bovine CYP11A gene expression [J]. *Mol Cell Endocrinol*, 1999, **153** (1-2): 183-196

20 Yoshito S, Gengo K, Anandhakumar C, Sefan A, Guo CX, Shinsuke S, Kaori H, Toshikazu B, Hiroshi S. Sequence-specific DNA binding by long hairpin pyrrole-imidazole polyamides containing an 8-amino-3,6-dioxaoctanoic acid unit [J]. *Bioorgan Med Chem*, 2016, **24** (16): 3603-3611

21 Klimova N, Yeung R, Kachurina N, Turcotte B. Phenotypic analysis of a family of transcriptional regulators, the zinc cluster proteins, in the human fungal pathogen *Candida glabrata* [J]. *Gene Genomes Genet (Bethesda)*, 2014, **4** (5): 931-940