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Research Paper 研究报告

光滑球拟酵母中AMP代谢对其生理功能的影响

靳丛丛[1,2]，陈修来[1,2]，陈瑞东[1,2]，刘立明[1,2*]

[1] 江南大学食品科学与技术国家重点实验室，江苏 无锡 214122
[2] 江南大学工业生物技术教育部重点实验室，江苏 无锡 214122

摘要：【目的】研究光滑球拟酵母(*Candida glabrata*)中AMP代谢影响其碳流代谢和耐酸胁迫的生理机制。【方法】同源重组法敲除基因*cgade12* (ORF CAGL0K05027g)和*cgade13*(ORF CAGL0B02794g)构建双缺菌株*cgade12Δade13Δ*，与出发菌株(*ATCC55*)比较ATP水平、碳流代谢酶活性和中间代谢物含量变化分析和AMP代谢对其碳流代谢的影响，对比菌株有机酸胁迫下生长情况及胞内环境变化，研究AMP代谢变化对光滑球拟酵母酸胁迫耐受性的影响。【结果】与出发菌株*ATCC55*相比，*cgade12Δade13Δ*的ATP水平下降了12.50%。与*ATCC55*相比，*cgade12Δade13Δ*中柠檬酸合成酶、苹果酸脱氢酶、异柠檬酸脱氢酶、α-酮戊二酸脱氢酶的活性分别上升了31.26%、19.45%、28.96%、18.36%，柠檬酸、α-酮戊二酸、苹果酸、琥珀酸含量分别提高了44.11%、73.60%、50.00%、65.68%。胞内丙酮酸浓度下降20.00%，丙酮酸产量下降73.11%。与*ATCC55*相比，*cgade12Δade13Δ*在0.4%丙酮酸、0.6%苹果酸和0.2%乙酸胁迫下的菌体浓度分别提高了8.71%、11.21%和12.71%。在0.2%乙酸胁迫下，菌株*cgade12Δade13Δ*的H^+-ATPase活性、细胞膜完整度、细胞膜电势分别比出发菌株*ATCC55*上升了7.04%、8.71%、25.14%，ROS水平下降了19.51%。【结论】基因*cgade12*、*cgade13*的缺失导致菌株的ATP水平下降，TCA循环活性和有机酸耐受性上升。

关键词：光滑球拟酵母，AMP代谢，碳流代谢，耐酸性

在微生物细胞内，辅因子作为重要的调控元件，为代谢网络提供稳定的自由能、氧化还原力和功能基团，全局性调控细胞的生理代谢[1]。ATP、NADH、NADPH和CoA等是微生物细胞内重要的辅因子，NAD(P)H/NAD(P)$^+$是代谢反应中最重要的氧化还原载体，为细胞代谢提供电子受体和还原力，辅酶A则作为代谢物的运输载体，在不同的反应之间传递不同的基团[2]。ATP作为细胞内的主要能量供体，是微生物细胞内的重要辅因子，影响菌株的生理功能[3]：(1) 作为代谢底物

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*通信作者。Tel/Fax：+86-510-85197875；E-mail：mingll@jiangnan.edu.cn

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或产物直接参与菌株的代谢；(2) 通过磷酸化或别构调节调节细胞内代谢关键节点的酶活性，影响菌株的生理代谢；(3) 影响菌株能量状态，调节耗能或产能的代谢过程及物质转运；(4) 通过影响菌株的信号通路，全局性调控菌株的生理功能。微生物细胞内AMP代谢可以调节胞内ATP水平[4]，参与细胞供能和生化反应，从而影响如主动运输、信号传导、蛋白质的迁移和磷酸化、细胞形态等许多复杂的生理过程。此外AMP还参与环AMP的合成，协调整个代谢网络尤其是碳代谢流的分配[5]。采用全基因组注释和基因组规模网络模型构建的方法，构建了光滑球拟酵母AMP代谢途径，如图1所示。AMP分解途径为通过*cgamd1*编码的腺苷甲硫氨酸脱羧酶催化分解成IMP；而合成途径则有：以PRPP为起始物的从头合成途径、次黄嘌呤为起始物的回收途径和腺嘌呤为起始物的回收途径。前2条合成途径在IMP处交叉，并通过*cgade12*基因将琥珀酸转化为腺苷酸基琥珀酸，这一中间产物在*cgade13*催化下释放琥珀酸，转化成AMP。酿酒酵母中的研究表明，AMP合成

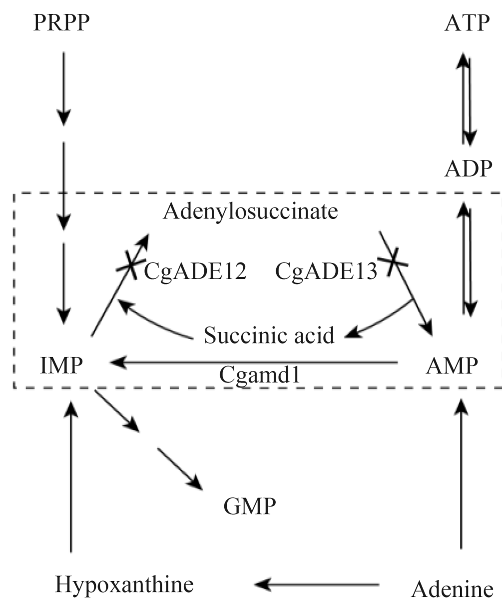

图 1. 光滑球拟酵母中AMP代谢途径[6]

Figure 1. Schematic of AMP metabolism in *C. glabrata*[6].

途径中基因*ade12*、*ade13*的缺失，导致胞内IMP浓度提高，AXP(ATP+ADP+AMP)水平降低。而AMD1的缺失则导致相反的结果，菌株的胞内ATP水平提高。当在培养基中添加腺苷时，AMD1缺失菌株的ATP水平进一步提高，GTP 水平下降，菌株的生长受到抑制。光滑球拟酵母是生产丙酮酸的重要菌株，其丙酮酸代谢受到ATP水平的影响，但AMP代谢对光滑球拟酵母生理功能的影响尚不清晰。

为此，本文以光滑球拟酵母*ATCC55*为出发菌株，构建*cgade12*和*cgade13*基因双缺失菌株*cgade12Δade13Δ*，并比较出发菌株*ATCC55*与*cgade12Δade13Δ*的ATP水平、丙酮酸生产性能、碳代谢中心关键酶活性和中间代谢物含量以研究AMP代谢改变对菌株碳代谢流分配的影响。及进一步比较2株菌的耐酸性及乙酸胁迫下的细胞质酸化、氧化及细胞膜功能参数，解析AMP代谢对菌株生理性能的影响。

1 材料和方法

1.1 材料

1.1.1 菌株和质粒：本研究中所使用的菌株见表1，所用质粒pMD19购自宝生物生物工程(大连)有限公司。

1.1.2 培养基：(1) LB培养基：蛋白胨10 g/L，酵母膏5 g/L，氯化钠10 g/L，121 °C灭菌15 min。(2) YNB培养基：葡萄糖 20 g/L，无氨基酵母氮源7.6 g/L (根据实验需要，添加一定量的不同种类的有机酸)，115 °C灭菌15 min。(3) 发酵种子培养基：葡萄糖20 g/L，大豆蛋白胨10 g/L，磷酸二氢钾1 g/L，七水硫酸镁0.5 g/L；121 °C灭菌15 min。(4) 发酵培养基：葡萄糖100 g/L，尿素7 g/L，磷酸二氢钾5 g/L，乙酸钠3 g/L，七水硫酸镁0.8 g/L，尿嘧啶0.04 g/L、组氨酸0.04 g/L、色氨酸0.04 g/L，115 °C灭菌15 min，使用前加入过滤除菌的维生

表1. 本研究所使用的菌株

Table 1. Strains used in this study

Strains	Relevant genotype and property	Source
ATCC2001	*C. glabrata* Wild type strain	Presented by Prof. Dr. Karl Kuchler[7]
ATCC55	*C. glabrata* ura4Δhis3Δtrp1Δ	Presented by Prof. Dr. Karl Kuchler[7]
cgade12Δade13Δ	*C. glabrataΔade12::his4; Δade13::trp1;Δura3*	This study
E. coli JM109	recA1, supE44, endA1, hsdR1, gyrA96	Stored in this lab

素液1 mL/L。(5) 维生素液：生物素4 mg，硫胺素2 mg，吡哆醇40 mg，烟酸0.8 g，使用2 mol/L盐酸溶解定容至1 L，过滤除菌。

1.1.3 培养方法：(1) 摇瓶培养：500 mL锥形瓶中培养基为50 mL，于30 °C、200 r/min培养。(2) 5 L罐发酵：发酵培养基体积3 L，接种量10%，搅拌转速为400 r/min，通气量2.0 vvm，用8 mol/L NaOH控制pH在5.5左右。接种后将400 r/min，通气量2.0 vvm条件下的溶氧水平标定为100%，溶氧水平降低至40%及20%后偶联转速将溶氧稳定在相应的数值上。

1.1.4 主要试剂和工具酶：主要工具酶有*rTaq*、*ExTaq*、Primstar、限制酶等购自宝生物生物工程(大连)有限公司；各类试剂盒购自北京天根生物技术公司；荧光探针CFPA-SE [5(6)-Carboxy fluorescein diacetate N-succinimidyl ester]、离子载体CCCP (Carbonyl cyanide 3-chlorophenyhydrazone)、探针DCFH-DA (2’,7’-Dichlorofluorescin diacetate)购自Sigma公司。蛋白胨、酵母提取物购自OXOID公司，乙腈为国产色谱纯。化学试剂均为分析纯，分别购自上海国药集团或上海生工生物技术公司。引物合成和测序工作由上海桑尼生物技术公司完成。

1.1.5 引物：本研究所用引物见表2。

1.2 缺失菌株的构建与验证

敲除框的构建流程按照文献的方法进行[7]：基因*cgade12*和*cgade13*的敲除分别以*cghis3*和*cgtrp1*为标记基因，以*C. glabrata ATCC2001*基因组作为模板PCR获取敲除框片段，构建敲除框。将苯酚氯仿抽提获得的高浓度敲除框电转化导入酵母感受态。在含有尿嘧啶、色氨酸的YNB平板上筛选*cgade12*缺失菌株，在含有尿嘧啶的YNB平板上筛选*cgade12*和*cgade13*的双缺菌株。提取阳性转化子的基因组并以相应的验证引物对转化子进行验证。取适量阳性转化子的对数期细胞，PBS洗涤后迅速置于液氮冷冻2 min，4 °C解冻，PBS稀释至OD_{600}=1利用超声破碎，破碎条件：温度2 °C，工作时间10 min，工作1 s停2 s。裂解液用于比色法检测腺苷琥珀酸合成酶和腺苷琥珀酸裂解酶的活性，具体操作参考文献中进行[8–9]。

1.3 胞内ATP水平测定[6]

取适量对数期的菌体，液氮研磨破碎细胞，加入适量预冷的PBS溶液(0.2 mol/L、pH 7.4，下同)，4 °C、12000 r/min离心2 min，利用HPLC迅速测定上清。色谱条件：Agilent ZORBAX SB-Aq反相柱，检测波长UV 254 nm，柱温35 °C，进样量10 μL，流速1 mL/min，流动相为磷酸盐缓冲液(10.93 g 磷酸二氢钠，3.04 g 磷酸氢二钠，3.22 g四正丁基溴化铵，超纯水定容至1 L)：乙腈=86∶14。利用试剂盒测定上清可溶蛋白量。ATP水平=ATP浓度/蛋白浓度。

1.4 丙酮酸及葡萄糖浓度、碳代谢关键酶活性检测

丙酮酸及葡萄糖浓度利用HPLC法进行测定，碳代谢中关键酶的活性利用偶联比色法进行测定，具体方法参考Zhou等[10]。

表2. 本研究所使用的引物

Table 2. PCR primers used in this study

Primer name	Primer sequence(5′→3′)	Function
P1	ATCAAAACTGGCAATACCTAACT	*cgade12Δ* Verification
P2	TTCTTAGGTAGAGCATTTGCG	*cgade12Δ* Verification
P3	TCAGGTTCTAAAGTAACAATGCC	*cgade12Δade13Δ* Verification
P4	CTATCTTTATTTCAAACAGCGTG	*cgade12Δade13Δ* Verification
P5	CGGGATCCTGACAAATGTGTTTTCTTCTG	*cgade12* deletion
P6	TGTTTGCTAGTTATTAATATAGAATGGCGTTTGTTAAGAGGG	*cgade12* deletion
P7	ACTAAGGGTGTCCTAGCATAGTTGAAAAGCAAATTTAAGTCT	*cgade12* deletion
P8	CGGAATTCCCTGAAATTATCGACTGTATG	*cgade12* deletion
P9	GACTTAAATTTGCTTTTCAACTATGCTAGGACACCCTTAGTG	*cgade12* deletion
P10	TCTTAACAAACGCCATTCTATATTAATAACTAGCAAACAATC	*cgade12* deletion
P11	CGGGATCCCTTTTGCCTTAATTTCTACTTCT	*cgade13* deletion
P12	AAAGGAATTTACTATTGAAAATGTCATTTGATTCGTTACTCG	*cgade13* deletion
P13	CAAAATGCAAAGAAACAATGATGATCAAATATTCCTCGGACT	*cgade13* deletion
P14	CGGAATTCGACTAATTCTGTGAAATGCAAG	*cgade13* deletion
P15	AGTCCGAGGAATATTTGATCATCATTGTTTCTTTGCATTTTG	*cgade13* deletion
P16	ACGAATCAAATGACATTTTCAATAGTAAATTCCTTTTATTAT	*cgade13* deletion

"_" indicates homologous sequence of *Bam*H I.

1.5 生长测定方法

生长曲线测定及细胞存活率分析根据文献[7]描述的方法进行。

1.6 胞内ROS水平及胞内pH测定

利用DCFH-DA荧光标定法测定胞内ROS水平，具体方法参考Machida等[11]。利用cFDA-SE荧光标定法测定胞内pH，具体方法参考Bracey等[12]。

1.7 H^+-ATPase 活性、过氧化物酶活性测定

适量对数期细胞，PBS洗涤后迅速置于液氮冷冻2 min，4 °C解冻，PBS稀释至OD_{600}=1。利用超声破碎，破碎条件：温度2 °C，工作时间10 min，工作1 s停2 s。细胞膜的制备及H^+-ATPase活性的检测参考Nakamura[13]等。愈创木酚法检测过氧化物酶活性，具体方法参考Lopes等[14]。

1.8 细胞膜完整性分析

对数期细胞接种至YNB及YNB-AC培养基，使初始OD_{600}=1，30 °C培养2 h。PBS洗涤后重悬，加入PI至终浓度为5 μg/mL，30 °C温育2 h。利用荧光显微镜观察并进行计数。

1.9 细胞膜电势分析

对数期细胞接种至YNB及YNB-AC培养基，使初始OD_{600}=1，30 °C培养2 h。PBS洗涤后重悬，加入Rhodamine123至终浓度为0.5 mol/L，避光温育10 min。PBS清洗3次，多功能酶标仪检测荧光强度(激发光488 nm，发射光530 nm)。

2 结果和分析

2.1 突变菌株*cgade12Δade13Δ*的构建

以*cghis3*为标记基因构建*cgade12*缺失菌株。以P3、P4为引物验证转化子敲除情况，其在2000 bp处有特异性条带，而*ATCC55*的条带在3000 bp处，证实*cgade12*缺失菌株构建完成，将其命名为

cgade12Δ (图2-A)。在菌株*cgade12Δ*基础上，以*cgtrp1*为标记基因敲除*cgade13*。以P5、P6为引物验证其敲除情况，其在 2000 bp处有特异性条带，而*cgade12Δ*的条带在3000 bp处，表明*cgade12*、*cgade13*双缺菌株*cgade12Δade13Δ*构建完成(图2-B)。比色法检测*cgade12Δade13Δ*腺苷琥珀酸合成酶和腺苷琥珀酸裂解酶的活性，发现其活性分别比*ATCC55*下降了98.27%和97.73%。

2.2 敲除基因*cgade12*和*cgade13*对胞内ATP水平的影响

5 L发酵罐中不同溶氧水平下基因*cgade12*和*cgade13*的缺失对能量代谢的影响如图3所示。发现：(1) 在不同溶氧水平下，与出发菌株*ATCC55*相比，菌株*cgade12Δade13Δ*胞内ATP水平均下降，在发酵前、中、后期分别下降了12.50%、19.35%、18.23% (DO=100%，图3-A)；8.44%、6.92%、17.85%(DO=40%，图3-B)；10.47%、10.23%、27.32% (DO=20%，图3-C)。(2) 突变菌株*cgade12Δade13Δ*中ATP水平对溶氧变化更为敏感。与DO=100%时比较，DO=20%时在发酵前、中、后期，菌株*ATCC55*中ATP水平分别下降了53.91%、39.92%、27.74%；而菌株*cgade12Δade13Δ*中ATP水平则分别下降了58.41%、54.64%、37.58%。同时，与DO=20%比较，DO=40%时在发酵前、中、后期，菌株*ATCC55*的ATP水平分别上升了144.55%、67.73%、16.67%；而*cgade12Δade13Δ*中ATP水平则上升了177.32%、147.24%、23.46%。

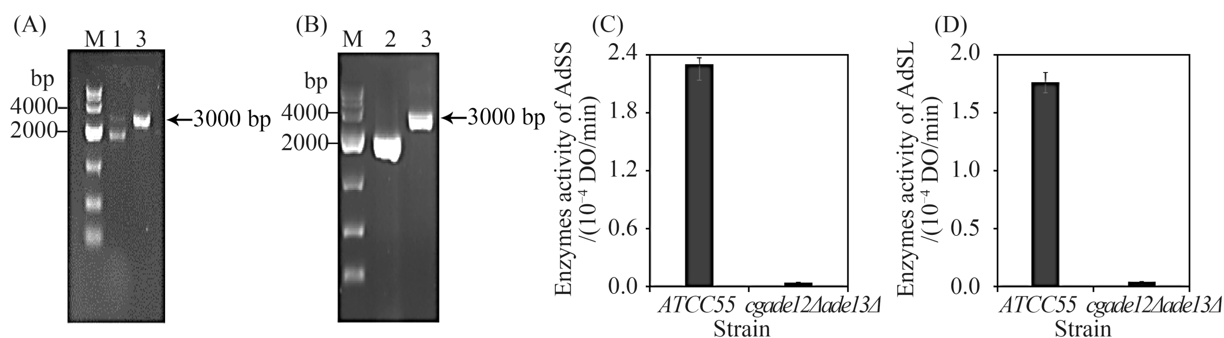

图 2. 菌株*cgade12Δade13Δ*的PCR和酶活性验证

Figure 2. Verification of *cgade12Δade13Δ* by gel electrophoresis of PCR products and enzymes activity. A: Verification of *cgade12Δ*. B: PCR verification of *cgade12Δade13Δ*. C: enzyme activity of adenylosuccinate synthetase (AdSS) in *ATCC55* and *cgade12Δade13Δ*. D: enzyme activity of adenylosuccinate lyase (AdSL) in *ATCC55* and *cgade12Δade13Δ*. M: 10000 bp DNA marker; lane 1: PCR product of *cgade12Δ*; lane 2: PCR product of *cgade12Δade13Δ*; lane 3: PCR product of the control group.

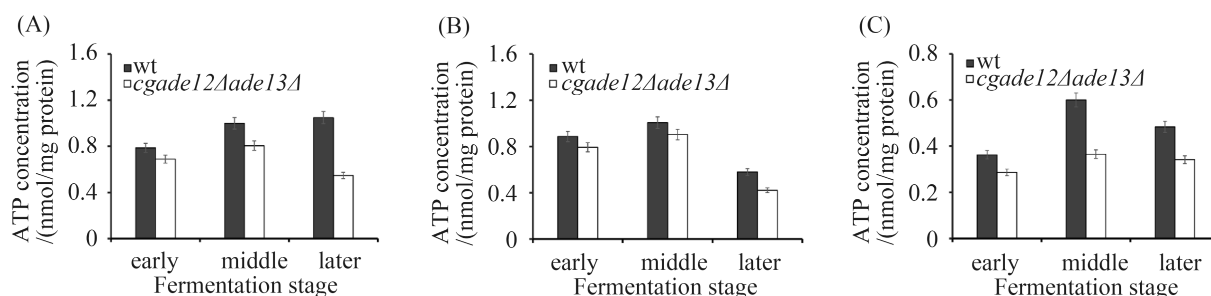

图 3. *cgade12Δade13Δ*在不同溶氧水平下的胞内ATP水平变化

Figure 3. Changes of the intracellular ATP level in strain *cgade12Δade13Δ* at different dissolved oxygen concentration. The intracellular ATP levels of *cgade12Δade13Δ* were measured controlling dissolved oxygen concentration at 100% (A), 40% (B), 20% (C).

2.3 敲除基因*cgade12*、*cgade13*对丙酮酸生产的影响

在5 L发酵罐研究了突变株*cgade12Δade13Δ*丙酮酸的生产情况，列于表3。与出发菌株*ATCC55*相比，菌株*cgade12Δade13Δ*的细胞干重、丙酮酸产量、糖耗、丙酮酸生产强度、葡萄糖消耗速度、丙酮酸得率和单位细胞生产丙酮酸能力分别下降了4.93%、73.11%、24.10%、77.50%、64.0%、35.71%和71.51%，因此导致发酵周期增加了20%。

进一步分析菌株丙酮酸生产发生变化的生理原因，发现：(1) 糖酵解途径的已糖激酶(HXK)、磷酸果糖激酶(PFK)、3-磷酸甘油醛脱氢酶(TDH)、丙酮酸激酶(PYK)的酶活水平分别上升了9.36%、5.54%、5.96%和6.27%；(2) TCA循环中柠檬酸合成酶(CIT)、苹果酸脱氢酶(MDH)、异柠檬酸脱氢酶(IDH)、a-酮戊二酸脱氢酶(KGD)酶活分别上升了31.26%、19.45%、28.96%、18.36% (图4-A)。(3) 与出发菌株*ATCC55*相比，菌株*cgade12Δade13Δ*胞内柠檬酸、a-酮戊二酸、苹果酸和琥珀酸的含量分别提高了44.11%、73.60%、50.00%和65.68%，表明菌株中TCA循环的效率提高(图4-B)。(4) 但胞内丙酮酸含量下降了20.03%，说明菌株内的丙酮酸分解加强导致细胞内丙酮酸含量下降，从而导致了菌株丙酮酸产量下降(图4-B)。

2.4 敲除基因*cgade12*、*cgade13*对酸胁迫耐受性的影响

不同有机酸及其浓度胁迫下出发菌株*ATCC55*与菌株*cgade12Δade13Δ*的生长情况，如图5所示。发现：(1) 在YNB培养基中添加0.4%丙酮酸、0.6%苹果酸和0.2%乙酸，菌株*cgade12Δade13Δ*的菌体浓度比出发菌株*ATCC55*分别提高了8.71%、11.21%和12.71%。(2) 在YNB培养基中添加1%丙酮酸、2%苹果酸和0.5%乙酸，*cgade12Δade13Δ*与*ATCC55*均不能生长。温育12 h后，菌株*cgade12Δade13Δ*的菌体浓度比出发菌株*ATCC55*分别提高了72.12%、20.18%和121.25%。这一结果表明，菌株*cgade12Δade13Δ*对丙酮酸、苹果酸和乙酸的耐受性要高于出发菌株*ATCC55*，其中在乙酸胁迫下的优势最为显著。

表3. 丙酮酸发酵过程参数比较

Table 3. Comparison of parameters during pyruvic acid fermentation process

Parameter	Strains		Change/%
	ATCC55 (A)	*cgade12Δade13Δ* (B)	
Culture time/h	60.00	72.00	20.00
Maximum dry cell weight/(g/L)	13.81	13.12	–4.92
The titer of pyruvic acid/(g/L)	23.80	6.40	–73.11
Glucose consumption/(g/L)	97.00	73.62	–24.10
Growth rate/[g/(L·h)]	0.23	0.18	–21.74
Pyruvic acid production/[g/(L·h)]	0.40	0.09	–77.50
Glucose consumption rate/[g/(L·h)]	1.62	1.02	–37.04
Yield of pyruvic acid on glucose/(g/g)	0.25	0.09	–64.00
Yield of pyruvic acid on DCW/(g/g)	1.72	0.49	–71.51
Yield of DCW on glucose/(g/g)	0.14	0.19	35.71

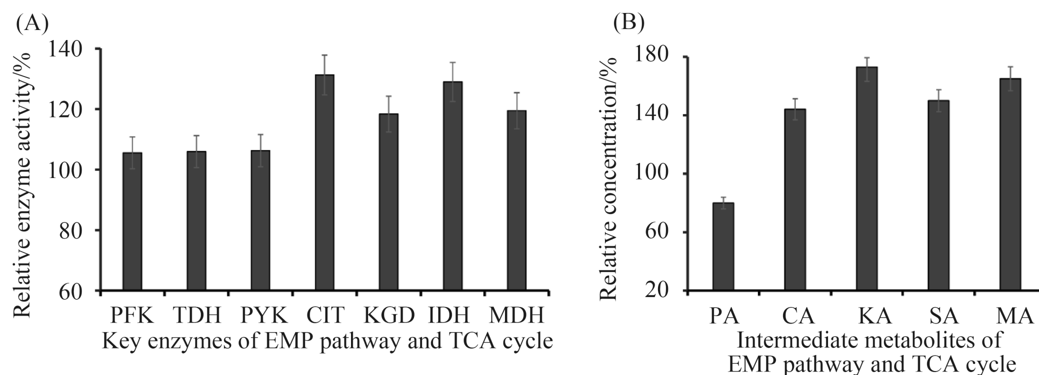

图 4. 菌株*cgade12Δade13Δ*胞内碳流代谢变化

Figure 4. Changes of carbon flow metabolism in strain *cgade12Δade13Δ*. HXK, PFK, TDH, PYK means hexokinase, phosphofructokinase, phosphoglyceraldehyde dehydrogenase, pyruvate kinase and CIT, KGD, IDH, MDH means citrate synthetase, α-ketoglutarate dehydrogenase, isocitrate dehydrogenase, malate dehydrogenase, respectively. PA, CA, KA, SA, MA means pyruvic acid, citric acid, α-ketoglutaric acid, succinic acid, malic acid respectively. Strain *ATCC55* was used as control strain when relative enzyme activities and inter-metabolite concentrations were analyzed.

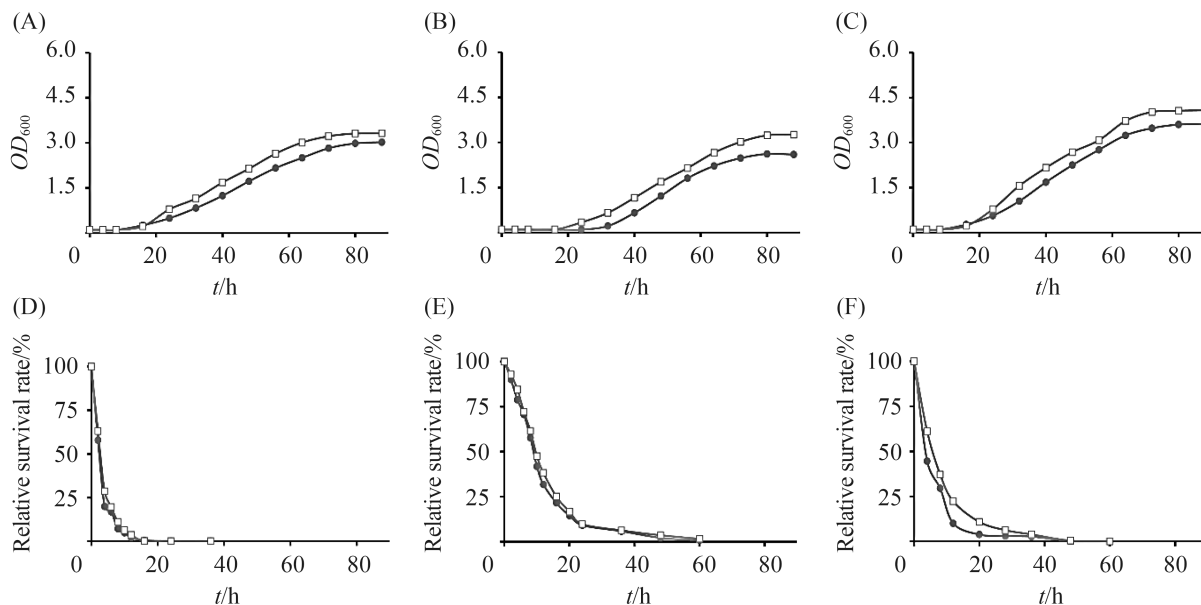

图 5. 出发菌株*ATCC55*与菌株*cgade12Δade13Δ*的耐酸性比较

Figure 5. Comparison of strain *ATCC55* and mutant *cgade12Δade13Δ* for acid tolerance. Cell concentrations of *ATCC55* and mutant *cgade12Δade13Δ* were measured in YNB medium with 0.4% (A) and 1.0% (D) pyruvic acid, 0.6% (B) and 2.0% (D) malic acid, 0.2% (C) and 0.5% (F) acetic acid respectively. –●–: *ATCC55*; –○–: *cgade12Δade13Δ*.

2.5 敲除基因*cgade12*、*cgade13*对乙酸胁迫下胞内微环境的影响

出发菌株*ATCC55*和菌株*cgade12Δade13Δ*在0.2%乙酸胁迫下的胞内微环境的变化如图6所示。发现：(1) 在YNB培养基上，与出发菌株*ATCC55*相比，菌株*cgade12Δade13Δ*的H^+-ATPase活性、胞内pH、过氧化物酶活性、ROS水平、细胞膜完整性和细胞膜电势分别下降了0%、0%、4.17%、26.05%、0%、0%；(2) 但在0.2%乙酸胁迫下，菌株*cgade12Δade13Δ*的H^+-ATPase活性、胞内pH、

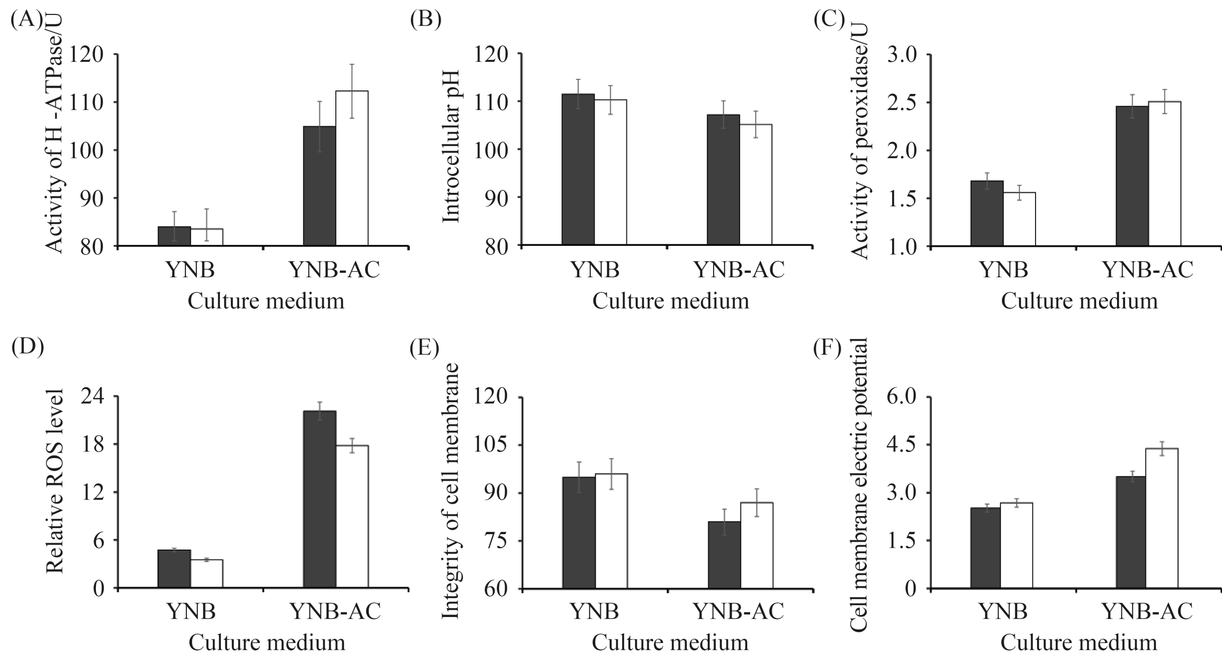

图 6. 胁迫条件下菌株*ATCC55*与*cgade12Δade13Δ*的胞内环境的差异

Figure 6. Differences between strain *ATCC55* and *cgade12Δade13Δ* in intracellular environment under stress condition. □: *ATCC55*; ■: *cgade12Δade13Δ*.

过氧化物酶活性、ROS水平、细胞膜完整性和细胞膜电势分别提高了0.04%、–3.50%、10.36%、–19.51%、8.71%和25.14%。

3 讨论

光滑球拟酵母是生产丙酮酸的重要菌株，调节胞内ATP水平可以提高丙酮酸的生产强度和产量[10]。敲除AMP合成基因*cgade12*和*cgade13*使得胞内ATP水平显著下降。有机酸的发酵过程中，产物的不断积累降低了环境pH，抑制了菌株的生长，降低了有机酸产量。提高菌株的有机酸耐受性是提高有机酸产量的重要途径之一[10]。*cgade12*和*cgade13*的缺失提高了菌株的有机酸耐受性。在丙酮酸、苹果酸和乙酸胁迫下，*cgade12Δade13Δ*的菌体浓度均高于出发菌株*ATCC55*。有机酸胁迫下的胞内微环境稳定是维持细胞活性的基础，H^+-ATPase和过氧化物酶通过清除胞内H^+和ROS维持细胞内环境稳态[15]。*cgade12Δade13Δ*的过氧化物酶活性提高，胞内酸化和氧化程度降低，细胞膜功能增强，胞内环境相对稳定。*cgade12*和*cgade13*的缺失降低了菌株的胞内ATP水平，提高了TCA循环代谢效率，增强了有机酸胁迫下的胞内环境稳定性和有机酸耐受性。这一研究结果有助于深入了解光滑球拟酵母生产丙酮酸及有机酸耐受的生理机制，为采取代谢工程等策略提高菌株的有机酸耐受性奠定了基础。

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Effects of AMP metabolism on physiological function of *Candida glabrata*

Congcong Jin[1,2], Xiulai Chen[1,2], Ruidong Chen[1,2], Liming Liu[1,2*]

[1] State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi 214122, Jiangsu Province, China

[2] Key Laboratory of Industrial Biotechnology, Ministry of Education, Jiangnan University, Wuxi 214122, Jiangsu Province, China

Abstract: [**Objective**] The aim of this study is to investigate the effects of AMP metabolism on the physiological function of *Torulopsis glabrata*. [**Methods**] Strain *cgade12Δade13Δ* was constructed by deleting *cgade12* and *cgade13* with homologous recombination, and was used to study the effects of AMP metabolism on carbon metabolism by comparing the ATP levels, enzymes activity and inter-metabolite concentrations of carbon metabolism to that of *ATCC55*. And the effects of AMP on metabolisms on organic acid tolerance were studied by compared the cell growth and intracellular environment of *cgade12Δade13Δ* to that of *ATCC55* under organic acid stress. [**Results**] The ATP levels of mutant *cgade12Δade13Δ* was decreased by 12.50% when compared with that of strain *ATCC55*. The enzymes activity of citrate synthetase, malate dehydrogenase, isocitrate dehydrogenase and α-ketoglutarate dehydrogenase was increased by 31.26%, 19.45%, 28.96%, 18.36% and the intracellular citric acid, α-ketoglutarate, malic acid, succinic acid contents were increased by 44.11%, 73.60%, 50.00%, 65.68%, respectively, compared with the corresponding value of strain *ATCC55*. However, the intracellular concentration of pyruvic acid in mutant *cgade12Δade13Δ* was decreased by 20.00% which led to a 73.11% reduction of pyruvic production in fermentation broth. Compared with strain *ATCC55*, the cell concentrations of *cgade12Δade13Δ* were increased by 8.71%, 11.21% and 12.71% grown in YNB with 0.4% pyruvic, 0.6% malic acid and 0.2% acetic acid, respectively. Grown in YNB with 0.2% acetic acid the H^+-ATPase activity, cell membrane integrity, cell membrane electric potential of mutant *cgade12Δade13Δ* was increased by 7.04%, 8.71%, 25.14% than that of strain *ATCC55*, respectively, while the ROS concentration was decreased by 19.51%. [**Conclusion**] The deletion of genes *cgade12* and *cgade13* resulted in a reduction in ATP level but led to an increase in activity of TCA cycle and organic acid tolerance.

Keywords: *Candida glabrata*, AMP metabolism, carbon metabolism, organic acid tolerance

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[*]Corresponding author. Tel/Fax: +86-510-85197875; E-mail: mingll@jiangnan.edu.cn

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