◆特邀专稿◆

酵母耐高温分子机制的研究进展*

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摘要:耐高温是酵母在工业生产中的重要特性之一。近年来,人们对耐高温酵母的应用研究日益重视并取得较大的进展,但其耐高温分子机制尚未明确。目前的研究结果表明,酵母耐高温机制涉及代谢网络调控、信号转导途径及多基因/蛋白共同作用等方面。本文从代谢途径、基因表达、酶活特性和蛋白质相互作用等4个方面综述当前酵母耐高温分子机制的研究进展,为酵母耐热性能的基因工程定向改造提供理论依据。

关键词:酵母　耐高温　代谢途径　基因表达　热激蛋白

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酵母作为真菌研究中的模式生物,在工业生产中应用十分广泛,如用于生产啤酒、葡萄酒、面包、异源蛋白质、疫苗和高价值代谢物等[1-4]。不同于实验室的培养条件,工业发酵的酵母需要应对的一个重大挑战是发酵环境的变化,如温度、低pH值、高渗和高浓度乙醇等,并在此过程中产生高价值代谢物,因此酵母菌株耐受胁迫的能力对工业生产十分重要[5-7]。在酵母多重胁迫耐受机制的研究中,耐高温是目前国内外发酵行业研究中十分热门且重要的课题。酵母的最优生长及发酵温度通常为28-35℃,适宜在37℃以上生长的酵母为高温酵母[8]。目前工业上常用的发酵菌株为酿酒酵母,其最适生长温度一般不超过37℃。有报道称,某些酿酒酵母菌株可在高达43℃的温度下产生乙醇,但这种耐高温的酿酒酵母菌株资源极其有限,因此人们常从自然界中分离出多株耐高温的非传统酵母菌株,包括耶氏酵母(*Yarrowia*)、克鲁维酵母(*Kluyveromyces*)、念珠菌(*Candida*)和毕赤酵母(*Pichia*)等,这些酵母菌株在高温下生长良好且能产生有价值的次级代谢产物[9-13]。一个典型的例子是酵母利用木质纤维素原料发酵生产乙醇,该过程需经过高温预处理和糖化步骤,即将木质素分解为微生物发酵可利用的五碳糖或六碳糖,随后酵母在中低温条件下将糖转化为生物乙醇[14,15]。同步糖化发酵法(Simultaneous Saccharification and Fermenta-

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tion,SSF)是乙醇生产的首选方法,其优点是水解和发酵可在同一个发酵罐中进行,操作简单、成本低、完成时间短,缺点是糖化和发酵对温度的要求不同[16]。因此,获得具有耐高温特性的酵母是生物乙醇产业中提高工业生产能力、降低生产成本的关键策略。

酵母的耐高温特性是一个复杂的性状,由多基因协同表达、多蛋白共同作用来决定。目前的研究发现,酵母已经进化出复杂而微妙的机制,可通过调节特定的代谢途径以保护机体免受高温损伤,或通过激活和调节特定的耐热相关基因来合成特定的化合物[17,18]。为鉴定新基因并阐明酵母耐高温的复杂机制,研究者们对酵母耐高温特性进行了深入的研究。本文主要从代谢途径、基因表达、酶活特性和蛋白质相互作用等 4 个方面对酵母耐高温分子机制进行综述(图 1),为耐高温酵母的筛选、改造及应用提供理论基础,有助于酵母耐热性的合理设计,提高工业生产的效率并降低生产成本。

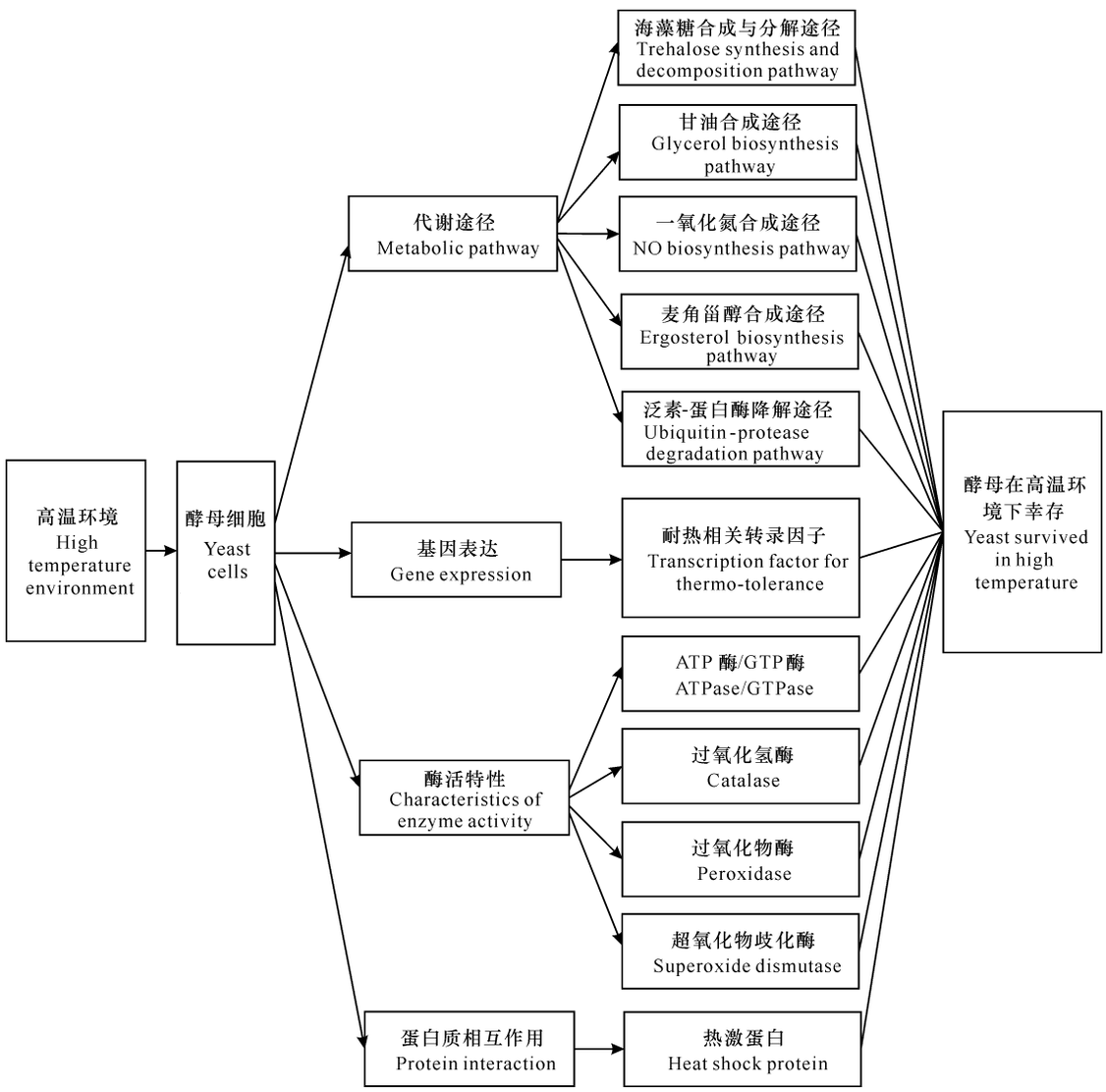

图 1　酵母耐高温分子机制

Fig.1　Molecular mechanism of thermo-tolerance in yeast

1　代谢途径

新陈代谢为细胞发挥功能提供能量和基础,新陈代谢的调节需要对不同的外部信号(如环境变化)和内部信号(如细胞周期状态)作出反应。当酵母处于高温环境下,其代谢途径可根据温度的变化及时调整以适应环境的变化,其中海藻糖合成与分解途径、甘油合成途径、麦角甾醇合成途径、一氧化氮合成途径、泛素-蛋白酶降解途径在应对高温胁迫方面发挥重要的作用[19-22]。

海藻糖是由两分子葡萄糖组成的非还原性双糖,存在于多种生物体中,如细菌、真菌、昆虫和植物等。对称的葡萄糖分子结构具有较好的稳定性,可作为细胞蛋白质和生物膜的应激保护剂以抵抗环境胁迫[23,24]。在酿酒酵母中,海藻糖由一种酶复合物合成,该酶复合物由 *tsl1*、*tps1*、*tps2* 和 *tps3* 编码的蛋白组成,其中基因 *tps1* 和 *tps2* 分别编码海藻糖-6-磷酸合成酶和海藻糖-6-磷酸酶,而 *tps3* 和 *tsl1* 编码复合物的2个调节亚基[25]。海藻糖的分解代谢主要由中性海藻糖酶和酸性海藻糖酶负责[26,27]。*nth1* 和 *nth2* 基因编码的胞浆中性海藻糖酶受 cAMP 依赖的磷酸化过程、营养物质和温度的调节,负责海藻糖的细胞内稳态或循环;由 *ath1* 基因编码的空泡酸性海藻糖酶受营养物质的调控,负责利用胞外海藻糖[28,29]。当酵母细胞受到高温胁迫时,海藻糖合成与分解途径中参与合成与分解的基因表达水平升高,促进海藻糖的合成与分解,使得细胞既可利用其作为高温保护剂,也可分解为单糖后进入中心糖代谢为细胞应对高温环境供能;当细胞远离胁迫环境时,细胞内海藻糖-6-磷酸含量的增加可抑制 Tps1 的活性,不利于海藻糖的合成[19,30]。

甘油是酵母在高温条件下的主要保护剂之一,其主要作用原理是甘油的积累使酵母重新调整细胞膜的渗透压以保证在高温胁迫下细胞功能的正常发挥,从而维持酵母正常的生命活动。在酵母发酵的过程中,甘油是仅次于乙醇和二氧化碳的第三大发酵产物,其合成途径受2个关键基因 *gpd1* 和 *gpd2* 的调控[31,32]。有研究证实,*gpd1* 和 *gpd2* 基因是细胞在高温胁迫下生长所必需的,且受高渗甘油(High Osmolarity Glycerol,HOG)途径的调节[33,34]。当酵母受到热刺激时,通过 Sho1-Ste20-Ste50-Ste11 蛋白级联将信息传递下去可激活 HOG 途径中关键的蛋白激酶 Hog1 或通过 Nts1 将细胞壁完整性(Cell Wall Integrity,CWI)途径与 HOG 途径连接起来。Hog1 由 Pbs2 磷酸化后进入细胞核激活 *gpd1* 和 *gpd2* 基因的表达,同时甘油分泌途径中 Fsp1 通道关闭,导致甘油分泌减少而间接增加细胞内的甘油含量,保护细胞免受高温的侵害[20,35]。

一氧化氮作为一种信号分子,参与多种细胞功能的调节,其主要来源是精氨酸代谢。当酵母受到高温冲击时,精氨酸可转化为瓜氨酸和一氧化氮,低水平的一氧化氮对酵母具有一定的保护作用[36]。在酵母合成一氧化氮的过程中,除一氧化氮合成酶发挥作用外,酵母的黄素蛋白 Tah18 也参与其中。基因敲除结果表明,Tah18 依赖的一氧化氮合成赋予酵母细胞耐高温胁迫的能力[22]。

麦角甾醇是真菌细胞膜的重要组成部分,决定膜相关蛋白的流动性、通透性和活性[37]。酵母在高温应激下生长可使细胞膜的流动性增加,影响蛋白质折叠和稳定性,破坏细胞骨架结构以及细胞代谢平衡[38]。有研究表明,甾醇脱氢酶 Erg3 和 Erg5 的缺失与酵母的耐热性相关,“扁平”甾醇如麦角-8(9),22-二烯醇或麦角-5,7,22,24(28)-三烯醇替代麦角甾醇不会增加酿酒酵母的耐热性,但“弯曲”甾醇如粪甾醇可增加酵母的耐热性。因此,*erg3* 基因突变后会导致甾醇甲基转移酶 Erg6 活性增强,Erg6 催化酵母甾醇甲基化形成粪甾醇,从而引起“弯曲”甾醇的积累,赋予酵母更高的耐热性[39];*erg5* 基因的缺失,导致细胞积累更多具有饱和侧链的甾醇促使细胞膜具有更好的排序效果,更好地维持细胞膜的流动性,因此可使 *erg5* 突变体比未突变 *erg5* 的酵母菌株具有更好的耐热性,并且该基因的功能在真菌中具有一定的保守性[21]。此外,有研究表明 ABC 转运蛋白基因 *Pdr18* 的缺失会导致细胞内麦角甾醇合成前体的积累,这种积累可能触发 Erg1 表达的反馈抑制,使得酵母细胞表现出更高的渗透性以及对多种环境的挑战,如高温环境的敏感性[40]。

高温环境会引起酵母细胞中蛋白的错误折叠从而影响蛋白活性,因此细胞可通过泛素-蛋白酶降解途径降解错误折叠的蛋白以保护细胞免遭功能失调的危机,例如酵母中 *rsp5* 基因的过度表达可使细胞具有更高的耐热性[41,42]。*rsp5* 基因编码 E3 泛素连接酶,参与蛋白质的泛素化,调节细胞结构中蛋白质的运输和最终降解[43,44]。此外,Rsp5 可与 Bul1、Bul2、泛素结合蛋白 Ubc1、泛素蛋白酶 Ubp4 和 Ubp16 等蛋白相互作用以提高应激反应基因 *spi1* 的表达水平,进而提高细胞对热休克和氧化应激的耐受能力[45]。

此外,通过转录组、蛋白质组等技术,已有对高温酵母整体代谢变化趋势的相关研究。例如,通过对定量蛋白质组学和转录组学的数据进行综合分析,Li 等[46]发现,酵母高温发酵后,马克斯克鲁维酵母参与转录、翻译、氧化磷酸化和脂肪酸代谢的蛋白下调,一些分子伴侣和蛋白酶体蛋白上调,ATP 酶活性显著降低,总脂肪酸逐渐积累。Fu 等[13]通过转录组分析发现,高温可刺激马克斯克鲁维酵母的线粒体呼吸,

抑制三羧酸 TCA 循环,导致活性氧(Reactive Oxygen Species,ROS)含量增加,并产生更多麦角甾醇来应对乙醇胁迫。Chamnipa 等[47]发现,库德氏毕赤酵母 RZ8-1 在高温 42℃乙醇发酵过程中,热休克蛋白基因(*ssq1*、*hsp90*)、乙醇脱氢酶基因(*adh1*、*adh2*、*adh3*、*adh4*)以及甘油醛-3-磷酸脱氢酶基因(*tdh2*)的表达水平升高,表明有些基因的表达不仅限于乙醇发酵途径,也与酵母的耐高温相关。总之,酵母在高温与适温下的代谢调节存在显著差异,细胞可通过控制耐高温相关代谢基因的表达来调节代谢产物的含量,从而有利于酵母在高温下生存。酵母细胞与耐热相关的代谢途径详见表 1。

表 1 酵母细胞与耐热相关的代谢途径

Table 1 Metabolic pathways related to thermo-tolerance in yeast cells

与耐热相关的代谢途径 Metabolic pathways related to thermo-tolerance	功能 Function	参考文献 Reference
海藻糖合成与分解途径 Trehalose sythesis and decomposition pathway	海藻糖可作为细胞的热保护剂,亦可分解为葡萄糖进入中心糖代谢途径 Trehalose can be used as a thermal protective agent and decomposed into glucose into the central glucose metabolic pathway	[19,29]
甘油合成途径 Glycerol biosynthesis pathway	甘油作为细胞的热保护剂 Glycerol as a thermal protective agent for yeast cells	[20,35]
一氧化氮合成途径 NO biosynthesis pathway	一氧化氮作为信号分子传递信息 NO as a signal molecule transmitting information	[22,36]
麦角甾醇合成途径 Ergosterol biosynthesis pathway	麦角甾醇可调节细胞膜的流动性、稳定性及活性 Ergosterol can regulate the fluidity, stability and activity of cell membrane	[37]
泛素-蛋白酶降解途径 Ubiquitin-protease degradation pathway	该途径可降解高温产生的错误蛋白,避免由错误蛋白所引起的细胞功能紊乱 This pathway can degrade misfolded proteins and avoid cell dysfunction caused by high temperature	[41]

2 基因表达

生物体耐高温是多基因协同表达以赋予细胞在异常温度下存活和生长的特性。通过分子生物学、分子遗传学、生物信息学、组学分析等手段,目前已有许多耐高温相关基因被鉴定,使得对生物体耐高温的遗传基础有了深入的了解。酵母的耐高温特性是酵母在工业发酵中十分重要的特点之一,当遭遇高温胁迫时,酵母通过调控自身的基因表达水平以适应高温环境。

有研究表明,酵母的转录因子 Hsf1、Msn2、Msn4、Yap1、Hac1、Rlm1、Cad1 在高温环境下可调控多个基因的表达水平,以提高细胞耐高温的能力[48-54]。热休克转录因子 Hsf1 非常保守,其核心结构包括翼螺旋-转螺旋 DNA 结合域以及对三聚体形成至关重要的疏水螺旋区域,此外还有两个分别位于 N 端和 C 端的反式激活域。正常情况下 Hsf1 是不活跃的单体或二聚体形式,当酵母受到高温胁迫时可形成三聚体并高度磷酸化,然后与热应激相关基因的启动子 nGAAn 反向重复序列结合,发挥其转录活性,激活相关基因的转录[55-57]。Msn2 和 Msn4 已被证实是酿酒酵母中两个对温度敏感的转录激活子,其氨基酸序列具有一定的同源性且识别 DNA 序列的氨基酸残基高度保守[58]。正常条件下 Msn2 和 Msn4 蛋白位于细胞质,当细胞处于高温环境则被运输到细胞核,随后蛋白中的锌指结构可直接结合到 DNA 序列上或通过其他蛋白间接调控约 200 个基因的表达[59-61]。氧化休克因子 Yap1 通过共价作用将传感器蛋白 Gpx3 激活,使其向细胞核移动,上调抗氧化基因的表达,如 Yap1 可诱导 *gsh1* 和 *gsh2* 的表达,以便酵母细胞在处于热休克期间合成还原型谷胱甘肽,从而提高细胞的耐受性[62-64]。转录因子 Hac1 作为一种碱性亮氨酸拉链蛋白,可与未折叠蛋白反应(Unfolded Protein-Response,UPR)元件结合从而调节参与 UPR 的基因表达[52]。Rlm1 作为蛋白激酶 C(Protein Kinase C,PKC)途径的 1 个转录因子,其转录活性被 MAP 激酶 Mpk1 通过 Ser427 和 Thr439 的磷酸化来调节,进而调控至少 25 个与细胞壁合成相关基因的表达,用于维持细胞壁的完整性[53]。Cad1 作为转录激活剂,参与蛋白质稳定性相关基因的表达调控[54]。除上述转录因子外,Skn7 可与 Hsf1 相互作用共同激活 *hsp12*、*hsp26*、*hsp70*、*hsp82* 和 *hsp104* 基因以响应温度变化带来的氧化应激[65]。

3 酶活特性

细胞需要不同的氧化环境以促进蛋白质折叠和活性,保证生命活动的正常进行。当细胞遇高温、高渗透压等不利的环境条件时,会导致细胞氧化还原功能障碍,影响细胞生长代谢过程,如信号转导,RNA、DNA 和蛋白质的合成以及细胞周期调节等,因此细胞可通过 ATP 酶催化 ATP 水解产生能量,维持细胞稳态[66,67]。酵母的质膜 H^+-ATP 酶(Plasma Membrane H^+-ATPase,Pma1)由基因 *pma1* 编码,属于分布广泛的一类重要的 P2 型 ATP 酶[68]。Pma1 是产生电化学质子梯度的重要酶,可穿过质膜,为次级溶质运输系统提供能量,维持离子稳态和细胞内 pH 值,并间接调节细胞代谢[69]。

生物体赖以生存的环境中营养可用性、渗透压平衡、温度和有害物质在不断变化。为抵御这些外部压力,所有需氧生物都配备了广泛的分子伴侣,如氧化还原分子伴侣[70,71]。酵母胞浆过氧化物酶 PrxS 可根据环境条件从低分子量(Low Molecular Weight,LMW)形式转变为高分子量(High Molecular Weight,HMW)形式。当其处于 LMW 形式时,PrxS 主要发挥过氧化物酶功能;而处于 HMW 形式时,PrxS 可作为分子伴侣发挥功能,这种过氧化物酶-分子伴侣功能的转换赋予酵母细胞抗应激能力[72,73]。有研究表明,酵母细胞遭遇热应激后,可诱导 Ytp1(一种 GTP 酶)可逆聚成 HMW 形式,这种结构变化使 Ytp1 失去原有的 GTP 酶活性,但赋予该蛋白一种新的分子伴侣活性;当细胞从热应激中恢复,Ytp1 蛋白会回到 LMW 形式,发挥其 GTP 酶功能。Ytp1 蛋白在 HMW 和 LMW 形式之间的变换,赋予酵母细胞适应不同温度环境的能力[74,75]。此外,Rho1 也是一个具有 GTP 酶活性且与酵母氧化应激相关的蛋白,已有报道证实 Rho1 的突变体对温度敏感且会使胞质中 ROS 积累水平升高,因此 Rho1 可通过调节包括 Ycf1 在内的多种下游靶点来保护酵母细胞免受氧化应激带来的损害[76,77]。

在正常的细胞代谢过程中,活性氧 ROS 主要通过呼吸和光合电子传递链不断产生[78]。这些高反应性分子可与多种细胞成分发生反应,破坏 DNA、蛋白质和脂质[79]。因此,细胞必须有一定的防护机制严格控制 ROS 的浓度。当酵母细胞遭受热应激后,会导致细胞的氧化损伤,因此酵母细胞也含有超氧化物歧化酶 SOD、过氧化氢酶和过氧化物酶 Prx 等抗氧化系统,以去除细胞中的活性氧[19]。酵母细胞中的超氧化物歧化酶,包括线粒体基质中的锰超氧化物歧化酶 SOD2 和胞浆/膜间隙中的铜锌超氧化物歧化酶 SOD1,可将过氧化氢歧化为水和二氧化碳,保障电子传递链的正常运行,维持细胞稳态[80]。过氧化物酶体过氧化氢酶 A(Cat1)和细胞溶质过氧化氢酶 T(Ctt1)在细胞受到不同的应激条件(如热应激)时可被诱导表达,因此这两种酶的活性对于保护酵母细胞在高温条件下免遭氧化损伤的侵害至关重要[81]。过氧化物酶可分为 4 种类型:2-Cys-Prx,1-Cys-Prx,Prx-Q 和Ⅱ型 Prx(PrxⅡ),这 4 种类型的过氧化物酶均具有同样的反应机理,即催化半胱氨酸接收来自过氧化氢的氧原子,生成磺酸后,再由不同的分子(如硫氧还蛋白 TRX)将其再生为还原形式,从而达到去除过氧化氢的目的。酿酒酵母的过氧化物酶主要是 2-Cys-Prx(Tsa1、Tsa2、Ahp1 和 Dot5)和 1-Cys-Prx(Prx1)两种类型,其中 Tsa1 和 Tsa2 已被证实与酵母耐热性相关,而 Prx1 能够以硫氧还蛋白特有的方式去除过氧化氢,维持线粒体在高温条件下的稳定性[79]。酵母细胞中与耐热相关的酶见表 2。

表 2 酵母细胞中与耐热相关的酶

Table 2 Enzymes related to thermotolerance in yeast cells

与耐热相关的蛋白酶 Enzymes related to thermo-tolerance	功能 Function	参考文献 Reference
ATP 酶 ATPase	为细胞提供能量 Provide energy for cells	[66]
GTP 酶 GTPase	为细胞提供能量或作为分子伴侣 Provide energy or act as molecular chaperones for cells	[72]
过氧化氢酶 Catalase	清除细胞中 ROS,保证细胞免受氧化损伤 Remove ROS in cell to protect the cells from oxidative damage	[19]
过氧化物酶 Peroxidase	清除细胞中 ROS,保证细胞免受氧化损伤 Remove ROS in cell to protect the cells from oxidative damage	[81]
超氧化物歧化酶 Superoxide dismutase	清除细胞中 ROS,保证细胞免受氧化损伤 Remove ROS in cell to protect the cells from oxidative damage	[80]

4 蛋白质相互作用

细胞体内含有多种多样的蛋白质,蛋白质发挥自身活性作用或与其他蛋白质相互作用构成细胞的生化网络,维持细胞的生命活动。热休克蛋白可作为分子伴侣在酵母热应激期间协助蛋白质折叠以及复性错误蛋白或激活沉默蛋白,以维持细胞的正常功能。有研究表明,酵母热休克转录因子 Hsf1 的活性抑制依赖于热休克蛋白 Hsp70-Ssa1 的直接结合,即 Hsp70-Ssa1 可利用两个暴露于蛋白表面的半胱氨酸残基与 Hsf1 结合来抑制 Hsf1 的活性,当细胞缺乏 Hsp70-Ssa1 时,转录因子 Hsf1 不能有效失活[82-84]。除 Hsp70 外,转录因子 Hsf1 也可与 Hsp90 相互作用,增加细胞短期的热适应[85]。Hsp90 作为酵母细胞中重要的热休克蛋白,有 Hsp82 和 Hsc82 两种亚型,除能与转录因子 Hsf1 相互作用外,其活性和靶蛋白特异性受到其他分子伴侣,如 Sti1、Cns1、Cdc37、Sba1、Aha1、Cpr6、Cpr7 和 Ppt1 的调控。例如 Sti1 和 Cns37 可促进 Hsp70/Hsp90 的相互作用,Cdc37 可调节 Hsp90 作为折叠蛋白酶的活性,防止某些蛋白的聚集,而 Sba1、Aha1、Cpr6、Cpr7 和 Ppt1 可控制 Hsp90 作为 ATP 酶活性的分子伴侣来调控细胞的能量供应[86-89]。热休克蛋白 Hsp104 在进化上是 Hsp100 家族中非常保守的一员,作为一种应激诱导型分子伴侣,可形成六聚体与 Hsp70、Hsp40 协同作用激活一些损伤蛋白,从而保护酵母细胞免受高温环境胁迫引起的损伤[90]。除上述的热休克蛋白 Hsp70 和 Hsp90 外,小热休克蛋白 sHsp26 通常以二十四聚体的形式存在,当细胞遭受高温时可及时解聚成二聚体与 Hsp70、Hsp104 相互作用对相关底物进行复性[91,92]。上述研究表明,酵母可利用不同的热休克蛋白 Hsp、小热休克蛋白 sHsp、热休克转录因子等蛋白之间的相互作用,提高细胞对温度变化的适应能力,从而促进细胞在高温环境下的生存能力。

5 结束语

酵母作为工业上应用广泛的微生物之一,其耐高温的性能对工业发酵具有十分重要的作用。尽管人们对酵母耐高温的分子机制研究已取得较大的进展,但细胞耐受高温胁迫是一个复杂的过程,仍存在许多未解之谜亟待研究,如酵母细胞对高温环境感知与应答的分子机制,是否有其他未报道的转录因子参与耐高温的调控,非传统酵母与传统酵母的耐高温分子机制存在哪些差异,非传统酵母在耐高温胁迫方面优于传统酵母的原因等。因此,对酵母耐高温分子机制进行深入研究,不仅有利于我国发酵工业对耐高温菌株的选育,而且有利于能耗的降低和水资源的利用,简化和优化发酵过程,促进我国发酵工业的环境友好型转变。

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Research Progress on Chemical Substance Basis and the Development and Utilization of Comprehensive Health Products of *Siraitia grosvenorii* in Guangxi

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Abstract: Mogroside is the main active components of *Siraitia grosvenorii*, and they are also a functional natural sweetener with great application prospect. In this article, the structural characteristics, formation rules, relationship between structure and sweetness, efficacy, characteristics of metabolism and product distribution in vivo, and the development and application status of comprehensive health products were reviewed. It is hope to lay the foundation for further development and utilization of mogrosides.

Key words: *Siraitia grosvenorii*; chemical composition; biological activity; metabolite; comprehensive health products

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Research Progress on Molecular Mechanism of Thermo-tolerance in Yeast

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Abstract: Thermo-tolerance is one of the important characteristics of yeast in industrial production. In recent years, more and more attentions have been paid to the application of thermo-tolerant yeast and great progress has been made, but the molecular mechanism of thermo-tolerance has not been clearly explained. The current research results indicated that the mechanism of yeast thermo-tolerance involved metabolic network regulation, signal transduction pathway and the interaction of multiple genes/proteins. In this article, the current research progress on molecular mechanisms of thermo-tolerance in yeast is reviewed from four aspects, including metabolic pathways, gene expression, characteristics of enzyme activity and protein interactions, which will provide a theoretical basis for rational design and improvement of yeast heat resistance and reduction of industrial production cost.

Key words: yeast; thermo-tolerance; metabolic pathway; gene expression; heat shock protein

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