果 树 学 报 2018,35(3):358-366
Journal of Fruit Science

DOI:10.13925/j.cnki.gsxb.20170385

# 利用拮抗酵母防治水果采后病害的研究进展

喻最新,贺明阳*,王日葵,韩 冷,周 炼

(西南大学柑桔研究所•西南大学食品科学学院•中国农业科学院柑桔研究所•国家柑桔工程技术研究中心,重庆北碚 400712)

**摘 要**:随着人们安全意识的提高,化学杀菌剂由于安全性差、且具致癌性等问题逐渐被淘汰,新型绿色、环保的生物防治逐渐成为当下研究的热点。拮抗酵母以其来源广泛、对营养条件要求低、生长迅速、安全性高等特点,在水果采后病害防治方面应用广泛。笔者在对拮抗酵母防治水果采后病害的来源途径、可能的拮抗机制以及增效途径进行综述的基础上,针对限制拮抗酵母商业化应用的原因进行了分析,即目前对拮抗酵母拮抗机制的研究还有待深入,同时工业上大规模生产的拮抗酵母制剂很难实现在实验室规模下发酵所具有的性质。通过组学等新的技术进一步揭示拮抗酵母防治采后病害的作用机制,进一步探讨拮抗酵母的分离干燥方式、佐剂以及剂型的选择,逐步完善适合拮抗酵母特性的商业化生产、销售方法和技术。

**关键词**:水果;拮抗酵母;生物防治;组学技术;商业化应用

**中图分类号**:S66 **文献标志码**:A **文章编号**:1009-9980(2018)03-0358-09

## Advances in the application of antagonistic yeasts to manage postharvest diseases in fruit

YU Zuixin, HE Mingyang*, WANG Rikui, HAN Leng, ZHOU Lian

*(Citrus Research Institute, Southwest University · College of Food Science, Southwest University · Citrus Research Institute, Chinese Academy of Agricultural Sciences · National Citrus Engineering Technology Research Center, Chongqing 400712, China)*

**Abstract**: Postharvest decay of fruits causes significant economic losses. Biological control of postharvest decay of fruits was developed as one of several promising alternatives to chemical fungicides. This paper provides a brief overview of the application of yeasts as postharvest biocontrol agents, which includes information on the sources which yeast antagonists have been isolated from, proposed biological mechanisms, and approaches to improve their efficiency and commercial applications. Yeast species have several attributes that make them suitable for use as biocontrol agents in fruit. Yeasts are tolerant to extreme environmental conditions (e.g., low and high temperatures, desiccation, wide range of relative humidity, low oxygen levels, pH fluctuations and UV radiation) that prevail before and after harvest. Also, yeasts have unique adaptation strategies to the fruit micro-environment (such as high sugar concentration, high osmotic pressure and low pH). Yeasts can grow rapidly on inexpensive substrates in fermenters. Large quantities of yeasts can be produced. Yeasts have simple nutritional requirements, so they can colonize dry surfaces for long periods of time. This paper also discusses the problems of yeast used in postharvest biocontrol, and suggests new ideas for future research, such as newly developed technologies, genomics, transcriptomics, metabolomics and bioinformatics, which could be used for research on antagonistic yeast in a biocontrol system. Yeast species have been isolated from a variety of sources, including fruit surfaces, the phyllo sphere, soil and sea water, et al. The proposed mechanisms of yeasts that are responsible for their antagonistic activity include competition for nutrients and space, parasitism of the pathogen, secretion of antifungal compounds, induction of host resistance, and biofilm

收稿日期:2017-10-25 接受日期:2017-12-18

基金项目:重庆市科技专项(cstc2016shms-ztzx80005);国家科技支撑计划(2015BAD16B07);中央高校基本科研业务费(XDJK2017C016);重庆市重点实验室基金(CKLC201302)

作者简介:喻最新,女,在读硕士研究生,研究方向为农产品加工与贮藏。Tel:13101375855,E-mail:1506757720@qq.com

*通信作者 Author for correspondence. Tel:15086681983,E-mail: hemingyang@cric.cn

(C)1994-2019 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net

formation. Competition for nutrients and space are considered to be the primary mechanisms. Yeast is able to use limited resources more efficiently than the pathogen. Yeasts have the ability to use specific features facilitating their adherence, colonization and multiplication to successfully colonize on fruit surfaces. This feature is associated with the formation of a biofilm. Yeasts can produce antifungal compounds, such as killer toxins, peptides and antibiotic metabolites. Yeasts have the capability to interact with the host tissue, particularly the wounds, increasing the cicatrization processes. These antagonistics were much more effective when applied before pathogen inoculation. Yeast cells could induce resistance processes in fruit skin. The performance of a biocontrol agent can be seen as the result of complex mutual interactions between all the biotic (organisms) and abiotic (environmental) components of the system. Although these interactions have been the subject of postharvest biocontrol research for many years, our understanding is still very incomplete. When studying mechanisms of action, a systems approach should be employed to investigate the network of interactions. Such an approach, that takes into account all the components of the system, may provide the greatest understanding of biocontrol systems. The exploration into the overall diversity and composition of microbial communities on fruit and how these communities vary across produce types, their function, the factors that influence the composition of the microbiota after harvest and during storage, and the distribution of individual taxa is needed. Information on the dynamics and diversity of microbiota may be useful to developing a new paradigm in postharvest biocontrol that is based on constructing synthetic microbial communities that provide superior control of pathogens. The availability of more cost-efficient, high throughput DNA/RNA and proteomic technologies, along with bioinformatics, have provided new opportunities and tools to obtain deeper insights into the mechanisms and interactions that have already been established. Developments in deep sequencing, transcriptomics, MS-MS proteomics, metagenomics, and comparative and functional genomics can be utilized to determine changes in the physiological status of biocontrol agents, and the effect of environmental stress on its intracellular machinery. Changes in the level of expression of related genes during mass production, formulation and storage, or in response to exposure and contact with host plant tissue after application can now be more readily investigated. Omic techniques (genomic, transcriptomic or proteomic) have been utilized, studies of postharvest biocontrol agents have been sparse and it is expected that greater details about interactions in the entire biocontrol system will be forthcoming. Acceptable and consistent performance under commercial conditions is critical to the success of any biocontrol agent. Economical production of large quantities of yeast in a formulation needs to ensure reasonable shelf life and maintain efficacy during large-scale testing. Industrial fermentation is accomplished under conditions quite different from those in shake culture. The process must be cost-effective, using industrial by-products as nutrients and fermentation must be completed within 24-30 h. Downstream processing involves various steps, such as drying, addition of volume materials, adhesives, emulsifiers and adjuvants. All these actions may affect the properties of the selected biocontrol agent. It is essential that a formulated product retains the properties of the initial lab-grown cultures. The formulation must retain its species purity (not be contaminated) and the microbial cells must retain their genetic stability, cell viability, and their attributes as colonizers on fruit surfaces, as well as other aspects of their mechanisms of action.

**Key words**: Fruit; Antagonistic yeast; Biological control; Omics; Commercial application

水果在采摘、加工、贮藏以及运输过程中容易造成机械损伤,病原菌极易通过伤口侵染果实造成腐烂。我国每年约有8 000万t水果因采后病害而无法销售,损失达近800亿元,水果采后病害严重制约了

(C)1994-2019 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net

我国水果产业的发展[1]。目前防治水果采后病害应用最广泛的仍然是化学杀菌剂。但是化学杀菌剂污染环境，危害人类健康，破坏生态平衡；连续使用同一类型杀菌剂容易导致病原菌形成抗性，防治效果变差[2]。因此，人们一直试图寻找一些防治效果好、特异性强、对环境无害、对人畜安全的新型防治方法。近年来食品添加剂因其安全高效的特点而被广泛用于防治水果采后病害，如盖智星等[3]研究证实，肉桂酸钾对柑橘采后主要病原真菌具有较强的抑制作用，甚至是杀伤作用，适于作为柑橘保鲜剂。利用微生物对水果采后病害进行生物防治展现出巨大的应用前景[4]。酵母具有遗传稳定、抑菌谱广、对营养条件要求低、生长迅速、干燥条件下能够在水果表面定殖很长一段时间等优点[5]。拮抗酵母对水果采前以及采后常见的极端环境（低温和高温、大范围波动的湿度、低氧、pH值波动、紫外线辐射）具有极强的耐受性，能够适应水果伤口处独特的微环境（高糖、高渗透压、低pH值）[4]。拮抗酵母一般不产生对人体有害的代谢产物，对大多数化学杀菌剂不敏感，能与物理、化学方法结合使用[6]。与此同时，拮抗酵母的使用一般不会对水果的主要品质指标如硬度、可溶性固形物含量、可滴定酸含量、维生素C含量等产生不良影响[1]。因而拮抗酵母作为生物防治的研究对象被广泛关注。在过去的研究中，张红印等[7]、耿鹏等[8]、林晓敏等[9]对拮抗酵母防治水果采后病害的应用现状、作用机制以及应用前景进行了系统的阐述。近几年来，拮抗酵母在来源途径、防治水果采后病害的拮抗机制以及增效途径等方面均有了许多新的研究进展，同时组学等新的技术的迅速发展也为拮抗酵母的商业化提供了有力的技术支持。笔者旨在通过综述近几年拮抗酵母防治水果采后病害的研究进展，探讨拮抗酵母应用于实际生产需要解决的问题，从而为加快推进拮抗酵母的商业化应用提供参考。

## 1 拮抗酵母的来源途径

研究表明，拮抗酵母的获得途径主要有以下3种：（1）从果蔬表面自然生长的微生物中分离拮抗酵母[10-15]。果实表面尤其是伤口处含有丰富的营养物质，为拮抗酵母提供良好地生长环境，同时从果实表面分离到的拮抗酵母能够很好地适应水果表面的环境，容易在水果表面定殖；（2）从植物的叶围、根际，土壤，冰川以及海水中分离拮抗酵母[16-21]。Lu等[18]从中国东海近海海域中分离到海洋红冬孢酵母（*Rhodosporidium paludigenum*），并研究了该酵母对柑橘转录组的影响。海洋红冬孢对多种果蔬的多种采后病害具有良好抑制效果。从极端环境中如冰川以及海洋中分离得到的拮抗酵母通常具有一般酵母所不具备的耐盐、耐低温、耐高渗透压等优点，在逆境中的生存能力更强，在防治果实采后病害中尤其是现代冷链物流中具有独特的优势；（3）从已有的微生物菌株中筛选拮抗酵母[4, 22-23]。例如，Yang等[4]从中国普通微生物菌种保藏管理中心筛选到的解脂耶氏酵母（*Yarrowia lipolytica*）能够显著抑制葡萄表面*Talaromyces rugulosus*孢子的萌发以及芽管伸长。解脂耶氏酵母能够利用的底物非常广泛，尤其能利用有机酸如柠檬酸、异柠檬酸，蛋白类如蛋白酶、磷酸酶以及烷烃类等廉价物质作为底物分泌大量的代谢产物。解脂耶氏酵母不产生对人体有害的代谢产物，安全性较高，目前广泛应用于食品和药物生产上，但在生物防治方面的应用较少。

## 2 拮抗酵母的拮抗机制

### 2.1 营养与空间的竞争

水果采后病害多是由病原微生物入侵引起的。病原微生物进入果实的途径一般有2种，一是通过果实上的自然通道（皮孔、气孔等）侵入，二是由机械伤形成的伤口侵入，后者为主要途径。当果实有伤口时，果皮表面的拮抗酵母和病原微生物同时开始抢占营养丰富的伤口，拮抗酵母能够在相当短的时间内利用伤口营养大量繁殖，迅速消耗掉伤口营养并占领全部空间，使得病原微生物得不到合适的营养与空间条件，不能生栖繁衍，从而抑制病害的发生[24]。研究表明，季也蒙毕赤酵母（*Pichia guilliermondii*）防治苹果灰霉病的主要拮抗机制是对糖类以及硝酸盐的竞争[25]。Kwasiborski等[26]研究了安诺拉毕赤酵母（*P. anomala*）接种在苹果伤口上的生长情况，在灰霉病菌（*Botrytis cinerea*）存在的情况下，安诺拉毕赤酵母在对数生长期磷酸戊糖途径增强，为酵母的生长繁殖提供充足的核酸和能量，促进酵母充分利用苹果中的营养物质，竞争性抑制灰霉病菌的生长。另外酵母还具有迅速长大、形成胞外多糖荚膜的特性，多糖荚膜可以促进酵母吸附在水果表面覆盖整个伤口[27]。

(C)1994-2019 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net

### 2.2　形成生物膜

生物膜是指微生物细胞相互聚集，黏附于活体或非活体组织表面，并被自身分泌的胞外多聚基质包裹形成的微生物细胞群体。同细菌生物膜一样，酵母在一定的条件下也具有类似的聚集现象。近年来，形成生物膜也被认为是拮抗酵母防治采后病害的作用机制之一。拮抗酵母在水果表面定殖需要有一定的黏附特性，形成生物膜有助于增强拮抗酵母黏附特性从而促进酵母在水果表面的生长繁殖。研究发现当*Pichia kudriavzevii*和*Candida diversa*从琼脂含量（$\omega$，后同）为2%的酵母蛋白胨葡萄糖培养基（YPD）转移到琼脂含量为0.3%的YPD培养基中时，其细胞形态会发生变化即形成生物膜，同时，*P. kudriavzevii*和*C. diversa*对热激、氧化胁迫的耐受性以及生物防治效力都显著增强[14, 28]。深刻理解生物膜形成的机制以及外界环境对拮抗酵母形态转变的影响有助于找到黏附能力强、生长速度快、防治效果好的拮抗酵母。

### 2.3　产生抑菌物质

某些酵母可以产生抑菌物质直接抑制病原菌的生长从而达到防治水果采后病害的效果。拮抗酵母产生的抑菌物质包括挥发性和非挥发性2种。某些酵母具有嗜杀活性，即能分泌抗菌化合物如蛋白质类毒素，杀死其他菌株，但自身具有免疫力[29]。目前研究较多的嗜杀酵母是酿酒酵母，酿酒酵母有3种不同类型的嗜杀菌株K1、K2以及K28[30]。膜醭毕赤酵母（*P. membranefaciens*）可以产生2种嗜杀毒素PMKT以及PMKT2，这2种毒素可以杀死酵母以及其他真菌[27]。短梗霉素A（aureobasidin A）是由出芽短梗霉（*Aureobasidium pullulans*）分泌的环状九肽，是对许多真菌如灰霉属、链核盘菌属、青霉属起作用的广谱抗生素[27]。真菌是大多数水果的主要致病因素，破坏真菌的细胞壁需要各种各样的酶参与。许多酵母可以产生细胞壁降解酶，例如*Metschnikowia fructicola*能够分泌几丁质酶，出芽短梗霉分泌葡聚糖酶、几丁质酶以及蛋白酶。Friel等[31]将安诺拉毕赤酵母中编码外切$\beta$-1,3-葡聚糖酶的基因敲除后，安诺拉毕赤酵母防治苹果灰霉病的效果显著变差。Banani等[32-33]将*Metschnikowia fructicola*中的几丁质酶基因和出芽短梗霉中碱性丝氨酸蛋白酶导入到*P. pastoris*细胞中表达，获得了可大规模生产的重组几丁质酶和重组蛋白酶，重组几丁质酶可以抑制桃中*Monilinia fructicola*和*M. laxa*孢子萌发以及芽管生长，达到防治桃褐腐病的效果，重组蛋白酶可以抑制苹果中多种病原微生物的生长。

挥发性抑菌物质主要是一些醇类以及酯类物质。Liu等[34]从柠檬克勒克酵母（*Kloeckera apiculate*）中分离到的2-苯基乙醇能够防治柑橘青霉病。在苹果伤口处接种安诺拉毕赤酵母后氧气浓度逐渐下降，安诺拉毕赤酵母利用酒精发酵产生挥发性抗菌物质，如乙醇和乙酸乙酯，抑制灰霉病菌[26]。Huang等[35]从培养*S. pararoseus*的培养基中提取得到能够显著抑制灰霉病菌分生孢子萌发以及菌丝体生长的挥发性有机化合物。

分泌抑菌物质的拮抗酵母为防治水果采后病害打开了新思路，可以从拮抗酵母中分离纯化得到抑菌物质直接用于水果采后病害的防治，从而克服了在拮抗酵母商业化应用的过程中难以保持其细胞活力和防治效果这一难题，同时产生挥发性抗菌物质的拮抗酵母为通过生物熏蒸防治水果采后病害提供了可能性。但是寄生在水果和人体的病原菌可能会逐渐对酵母分泌的抑菌物质产生抗性，因此需要对安全性做进一步的评估。

### 2.4　诱导抗性

许多酵母均能诱导多种果实的抗性及抗性相关反应，提高果实自身抗性，减少采后腐烂。拮抗酵母在生理生化以及分子水平诱导果实产生抗性已经得到证实。Lu等[36]研究证实，海洋红冬孢酵母可以诱导柑橘组织内的一些防御相关酶如$\beta$-1,3-葡聚糖酶、苯基丙氨酸脱氨酶、过氧化物酶、多酚氧化酶的等并使其活性显著提高。在葡萄柚伤口表面用橄榄假丝酵母（*C.oleophila*）处理能够增加葡萄柚乙烯生物合成，促进苯丙氨酸氨裂解酶活性提高和植保素积累，增加几丁质酶和$\beta$-1,3-葡聚糖酶含量，抑制青霉孢子萌发和胚芽生长[37]。

近年来，拮抗酵母诱导水果产生抗性有了许多分子层面的报道。Chan等[38]研究证实膜璞毕赤酵母处理桃会增强桃过氧化氢酶基因转录和翻译水平，诱导桃对青霉病的抗性。薛耀碧等[39]报道拮抗酵母可以激发果实病程相关蛋白如PR-1、几丁质酶、PR-5等基因的表达，诱导果实的抗病性。Lu等[18]研究证实，苯丙氨酸代谢通路及其中间代谢产物在基因表达层面和物质合成层面参与了拮抗酵母海洋红冬孢诱导柑橘抗病的过程。Guo等[13]研究证实在

(C)1994-2019 China Academic Journal Electronic Publishing House. All rights reserved.　http://www.cnki.net

罗伦隐球酵母(*Cryptococcus laurentii*)的诱导下，*LePR5*基因mRNA表达量在24 h后出现显著上调并呈持续上升趋势，*LePR5*基因作为抗性相关基因参与果实抗性反应。Zhang等[40]用转录组学分析证实解脂耶氏酵母诱导苹果产生的抗性受到水杨酸(SA)、茉莉酸(JA)和乙烯(ET)介导的基本防卫信号通路交叉对话的调控。拮抗酵母防治水果采后病害的作用机制如表1所示。

**表1 拮抗酵母防治水果采后病害的作用机制**

**Table 1 Representative modes of action involved in antagonizing yeast to control postharvest disease in fruit**

| 作用机制 Mode of action | 拮抗酵母 Antagonizing yeast | 采后病害 Postharvest diseases | 水果 Fruit | 参考文献 Reference |
|---|---|---|---|---|
| 形成生物膜 Biofilm formation | *Pichia kudriavzevii* | 胶孢炭疽病 *Colletotrichum gloeosporioides* | 梨 Pear | [28] |
| | *Pichia kudriavzevii* | 灰霉病 *Botrytis cinerea* | 梨 Pear | [28] |
| | *Candida diversa* | 灰霉病 *Botrytis cinerea* | 苹果 Apple | [29] |
| | *Candida diversa* | 灰霉病 *Botrytis cinerea* | 猕猴桃 Kiwifruits | [29] |
| 产生抑菌物质 Production of antimicrobial compounds | *Pichia anomala* | 灰霉病 *Botrytis cinerea* | 苹果 Apple | [32] |
| | *Metschnikowia fructicola* | 灰霉病 *Botrytis cinerea* | 桃 Peach | [33] |
| | *Aureobasidium pullulans* | 褐腐病 *Monilinia fructicola* | 苹果 Apple | [34] |
| | *Aureobasidium pullulans* | 青霉病 *Penicillium expansum* | 苹果 Apple | [34] |
| | *Aureobasidium pullulans* | 灰霉病 *Botrytis cinerea* | 苹果 Apple | [34] |
| | *Aureobasidium pullulans* | 褐腐病 *Monilinia fructicola* | 苹果 Apple | [34] |
| | *Pichia anomala* | 黑腐病 *Alternaria alternata* | 苹果 Apple | [26] |
| | *Kloeckera apiculate* | 青霉病 *Penicillium expansum* | 柑橘 *Citrus* | [35] |
| | *Sporidiobolus pararoseus* | 灰霉病 *Botrytis cinerea* | 草莓 Strawberry | [36] |
| 诱导抗性 Induction of resistance | *Rhodosporidium paludigenum* | 青霉病 *Penicillium expansum* | 柑橘 *Citrus* | [37] |
| | *Candida oleophila* | 青霉病 *Penicillium expansum* | 葡萄柚 Grapefruit | [38] |
| | *Pichia membranefaciens* | 青霉病 *Penicillium expansum* | 桃 Peach | [39] |
| | *Rhodosporidium paludigenum* | | 柑橘 *Citrus* | [18] |
| | *Cryptococcus laurentii* | 黑腐病 *Alternaria alternata* | 樱桃番茄 Cherry tomato | [13] |
| | *Yarrowia lipolytica* | 青霉病 *Penicillium expansum* | 苹果 Apple | [40] |

注：由于营养和空间的竞争是基本的作用机制，因此不在本表中列出。

Note: As the competition for nutrients and space is the basic mechanism, it is not listed in this table.

但是，拮抗酵母的防治效果是水果、拮抗酵母、病原微生物以及水果表面的其他微生物等多重因素相互作用的结果[41]。近年来，研究证实水果表面的其他微生物可以通过与水果、病原微生物和拮抗酵母的相互作用来影响拮抗酵母的防治效果[5]，深入了解这些相互作用有助于开发新型的生物防治方法。例如水果表面某些“辅助”菌株虽不能直接拮抗病原微生物，但可以通过增强拮抗酵母的生存能力从而间接起到生物防治的作用，而这些“辅助”菌株商业化的难度可能远远低于拮抗酵母[6]。然而水果表面微生物的种类、功能以及影响采后水果表面微生物多样性的因素还有待进一步深入研究。近年来高通量测序、蛋白质组学以及生物信息学等新技术的迅速发展为进一步深刻揭示拮抗酵母的作用机制以及生物防治系统中的相互作用提供了强有力的工具[26]。Massart等[42]使用分子技术揭示了真菌生物防治剂的作用机制，Massart等[43]对利用组学技术研究生物防治的作用机制进行了综述。组学技术可用于鉴定拮抗酵母的基因组、转录组或蛋白质组特征，比较具有不同生物防治功效的拮抗酵母菌株及其突变株的性质，鉴定和表征涉及拮抗酵母作用机制的基因、mRNA和蛋白质，以及研究水果、病原微生物、拮抗酵母和水果表面的其他微生物之间的多重相互作用。组学等新的技术为进一步揭示拮抗酵母防治采后病害的作用机制提供了有力的技术支持。

## 3 提高拮抗酵母生物防治效力的方法

### 3.1 通过逆境培养，提高拮抗酵母生存能力和拮抗效力

酵母细胞具有很强的应激反应能力，当它受到紫外线照射、热激或化学损伤等胁迫时，迅速合成许多具有自我修复功能的保护性物质，以抵御这些不良的条件。

研究发现，盐胁迫预处理海洋红冬孢能够提高其在冷冻处理时的细胞存活率[44]。以轻度的热激预处理*M. fructicola*能够诱导其细胞内海藻糖水平的

(C)1994-2019 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net

提高，促进活性氧的清除，提高酵母的生物防治活性[45]。亚致死氧化处理橄榄假丝酵母能够提高其对随后致死水平的高氧胁迫、高温胁迫以及低pH胁迫的耐受性[46]。用温和的热激预处理胶红酵母（*Rhodotorula mucilaginosa*）后，胶红酵母对高温胁迫、高氧胁迫、高渗透压胁迫以及低pH值胁迫的耐受性均增强[47]。

当酵母处于亚致死胁迫时，它们将适应这种胁迫从而对致死胁迫产生抗性。在大规模生产拮抗酵母制剂时会遇到许多极端的环境条件，降低了酵母细胞的存活力以及生物防治效力。通过逆境培养提高拮抗酵母的拮抗效力是加快拮抗酵母制剂走向市场的有效途径之一。

### 3.2　与物理方法结合

拮抗酵母与物理方法结合处理也是提高其生物防治效力的有效途径。研究发现38 ℃的热空气结合季也蒙毕赤酵母处理樱桃24 h能够显著减少其采后病害[48]。罗伦隐球酵母结合38 ℃的热空气处理樱桃番茄12 h能够显著降低樱桃番茄灰霉病的发生率，结合处理的效果比二者单独处理的效果更好[23]。短波紫外线结合热带假丝酵母（*Candida tropicalis*）处理的菠萝与单独用酵母处理的果实相比硬度保持得更好，果实对病原菌的抗性增强[49]。

### 3.3　与化学方法结合

拮抗酵母结合特定的化学物质可以大幅度提高生物防治效力。添加葡萄糖作为保护剂后，季也蒙毕赤酵母在高温胁迫下的存活率提高[50]。Liu等[51]证实经甜菜碱处理的*Cystofilobasidium infirmominiatum*细胞内活性氧的聚集能力减弱，应答氧化胁迫的蛋白质羰基化水平降低。几丁质可显著提高海洋红冬孢的生长效率，加快活性氧代谢，提高苹果中多酚氧化酶和超氧化物歧化酶的活性，从而增强酵母对苹果青霉病的防治效果[52]。卵磷脂能够促进葡萄汁有孢汉逊酵母（*Hanseniaspora uvarum*）在果实伤口处的生长，提高酵母与病原菌竞争营养与空间的能力，抑制指状青霉在果实伤口处的孢子萌发以及菌丝生长[53]。Fu等[54]研究表明，经β-葡聚糖诱导后罗伦隐球酵母生长速度明显变快，对氧化胁迫的耐受性增强，防治柑橘青霉病的效果显著提高。另外，拮抗酵母与水杨酸[55-56]、茉莉酸甲酯[57]、抗坏血酸[58]、植酸[59]等结合处理也能提高拮抗酵母的防治效果。

## 4　拮抗酵母的商业化应用以及未来的研究方向

目前市场上基于拮抗酵母的生物防治产品很少：Shemer™（基于拮抗酵母*M. fructicola*，以色列）、Candifruit™（基于拮抗酵母*C. sake*，西班牙），以及Boni-Protect™（基于拮抗酵母*A. pullulans*，德国）[60]。拮抗酵母走向商业化最大的障碍是工业上大规模生产的拮抗酵母制剂很难实现在实验室规模下发酵所具有的性质。在大规模生产中，保持拮抗酵母制剂的纯度以及拮抗酵母的细胞活力、遗传稳定性以及在植物表面定殖的能力都具有挑战性。工业发酵是在与实验室摇瓶培养发酵完全不同的条件下完成的，该过程必须具有经济效益，一般选用工业副产品作为营养物质培养菌种，发酵过程必须在24~30 h内完成[61]。发酵的下游加工过程还包括许多步骤，例如从培养基中分离拮抗酵母，干燥，加入大量惰性材料、黏合剂、乳化剂和佐剂[61]。这些操作都可能直接或间接地影响拮抗酵母的生防效果。

拮抗酵母制剂的制备是通过一定手段使酵母细胞的生长繁殖处于休眠状态。拮抗酵母菌制剂主要有液体型和固体型2种剂型。液体型菌剂是将拮抗酵母菌培养后的发酵液离心，收集菌体，直接加入保护剂配成菌悬液，然后在不同条件下保存的剂型。液体型菌剂制作过程简单，但是携带不便，其保存原理也需深入研究。固体型菌剂是除去酵母细胞中的水分后制成粉状或颗粒状菌体的剂型。固体型菌剂在制备过程中需要加一些保护剂、表面活性剂等以提高拮抗酵母菌的存活率。大多数拮抗酵母菌剂都为固体[6]。固体型菌剂常用的干燥方法有冷冻干燥、喷雾干燥以及流化床干燥[6]。冷冻干燥有利于保持酵母细胞的活力但是成本较高。喷雾干燥效率高，成本较低，但是喷雾干燥过程中温度比较高，一般高于拮抗酵母的最适生长温度，对拮抗酵母有直接破坏作用。流化床干燥是一种比较经济的干燥方式，干燥温度较低，可应用于对热敏感的酵母。进一步探讨拮抗酵母分离干燥方式、佐剂以及剂型的选择，逐步完善适合拮抗酵母特性的商业化生产、销售方法和技术，完善拮抗酵母及其配方的安全性试验是拮抗酵母商业化应用的研究重点。

(C)1994-2019 China Academic Journal Electronic Publishing House. All rights reserved.　http://www.cnki.net

## 5 结论与展望

综上所述，利用拮抗酵母防治水果采后病害已经得到了广泛的关注，国内外对拮抗酵母的来源途径、可能的拮抗机制以及提高拮抗酵母生物防治效果的方法进行了大量研究。然而，目前已经商业化应用的拮抗酵母却是屈指可数。一方面，目前对拮抗酵母拮抗机制的研究有待深入；另一方面，工业发酵是在与实验室摇瓶培养发酵完全不同的条件下完成的，工业上大规模生产的拮抗酵母制剂难以实现在实验室规模下发酵所具有的性质。因此笔者认为，今后应通过组学等新的技术进一步揭示拮抗酵母防治采后病害的作用机制，探讨拮抗酵母分离干燥方式、佐剂以及剂型的选择，逐步完善适合拮抗酵母特性的商业化生产、销售方法和技术。期待未来拮抗酵母完全取代化学杀菌剂，在防治水果采后病害中发挥出巨大的作用。

### 参考文献 References：


[1] 胡浩 . 西藏罗伦隐球酵母对水果采后病害的防治效果及相关机制研究[D]. 杭州：浙江大学，2016.
HU Hao. Biocontrol of fruit postharvest decay by Tibetan *Cryptococcus laurentii* and its action mechanism[D]. Hangzhou: Zhe jiang University，2016.

[2] ZHANG H，APALIYA M T，MAHUNU G K，CHEN L，LI W. Control of ochratoxin A-producing fungi in grape berry by microbial antagonists: A review[J]. Trends in Food Science & Technology，2016，51: 88-97.

[3] 盖智星，王日葵，贺明阳，周炼，韩冷，黄小兰 . 肉桂酸钾对柑橘采后主要病原真菌的抑制作用[J]. 食品与发酵工业，2016，42（3）: 109-113.
GE Zhixing，WANG Rikui，HE Mingyang，ZHOU Lian，HAN Leng，HUANG Xiaolan. Antifungal acitivity of potassium cinnamate against the main postharvest fungal pathogens of citrus fruits[J]. Food and Fermentation Industries，2016，42（3）: 109-113.

[4] YANG Q，WANG H，ZHANG H，ZHANG X，APALIYA M T，ZHENG X，MAHUNU G K. Effect of *Yarrowia lipolytica* on postharvest decay of grapes caused by *Talaromyces rugulosus* and the protein expression profile of *T. rugulosus*[J]. Postharvest Biology and Technology，2017，126: 15-22.

[5] DE PAIVA E，SERRADILLA M J，RUIZ- MOYANO S，CÓRDOBA M G，VILLALOBOS M C，CASQUETE R，HERNÁNDEZ A. Combined effect of antagonistic yeast and modified atmosphere to control *Penicillium expansum* infection in sweet cherries cv. Ambrunés[J]. International Journal of Food Microbiology，2017，241: 276-282.

[6] DROBY S，WISNIEWSKI M，TEIXIDÓ N，SPADARO D，JIJAKLI M H. The science, development, and commercialization of postharvest biocontrol products[J]. Postharvest Biology and Technology，2016，122: 22-29.

[7] 张红印，蒋益虹，郑晓冬，席玮芳，孙萍 . 酵母菌对果蔬采后病害防治的研究进展[J]. 农业工程学报，2003，19（4）: 23-27.
ZHANG Hongyin，JIANG Yihong，ZHENG Xiaodong，XI Yufang，SUN Ping. Research advances on the biological control of postharvest diseases of fruits and vegetables with yeast[J]. Transactions of the Chinese Society of Agricultural Engineering，2003，19（4）: 23-27.

[8] 耿鹏，杨柳，郝卫宁，李辉，胡美英 . 拮抗酵母菌控制果蔬采后病害的研究进展[J]. 北方园艺，2010（20）: 220-225.
GENG Peng，YANG Liu，HAO Weining，LI Hui，HU Meiying. Research advances on the biological control of postharvest diseases of fruits and vegetables with antagonistic yeasts[J]. Northern Horticulture，2010（20）: 220-225.

[9] 林晓敏，谭晓荣 . 拮抗酵母菌防治果蔬采后病害的研究[J]. 北方园艺，2015（1）: 182-186.
LIN Xiaomin，TAN Xiaorong. Study on antagonistic yeasts control of biological postharvest diseases of fruit and vegetables[J]. Northern Horticulture，2015（1）: 182-186.

[10] BASTIAANSE H，DE LAPEYRE DE BELLAIRE L，LASSOIS L，MISSON C，JIJAKLI M H. Integrated control of crown rot of banana with *Candida oleophila* strain O, calcium chloride and modified atmosphere packaging[J]. Biological Control，2010，53（1）: 100-107.

[11] ZENG L，YU C，FU D，LU H，ZHU R，LU L，ZHENG X，YU T. Improvement in the effectiveness of *Cryptococcus laurentii* to control postharvest blue mold of pear by its culture in $\beta$-glucan amended nutrient broth[J]. Postharvest Biology and Technology，2015，104: 26-32.

[12] CAMPOS- MARTÍNEZ A，VELÁZQUEZ- DEL VALLE M G，FLORES- MOCTEZUMA H E，SUÁREZ- RODRÍGUEZ R，RAMÍREZ-TRUJILLO J A，HERNÁNDEZ-LAUZARDO A N. Antagonistic yeasts with potential to control *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. and *Colletotrichum acutatum* J. H. Simmonds on avocado fruits[J]. Crop Protection，2016，89: 101-104.

[13] GUO D，YANG B，REN X，ZHU L. Expression of the *LePR5* gene from cherry tomato fruit induced by *Cryptococcus laurentii* and the analysis of *LePR5* protein antifungal activity[J]. Postharvest Biology and Technology，2016，111: 337-344.

[14] LI G，CHI M，CHEN H，SUI Y，LI Y，LIU Y，ZHANG X，SUN Z，LIU G，WANG Q，LIU J. Stress tolerance and biocontrol performance of the yeast antagonist, *Candida diversa*, change with morphology transition[J]. Environmental Science and Pollution Research，2016，23（3）: 2962-2967.

[15] ZHIMO V Y，DILIP D，STEN J，RAVAT V K，BHUTIA D D.


(C)1994-2019 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net

Antagonistic yeasts for biocontrol of the banana postharvest anthracnose pathogen colletotrichum musae[J]. Journal of Phytopathology,2017,165(1): 35-43.

[16] LONG C,WU Z,DENG B. Biological control of *Penicillium italicum* of *Citrus* and *Botrytis cinerea* of grape by strain 34-9 of *Kloeckera apiculata*[J]. European Food Research and Technology,2005,221(1/2): 197-201.

[17] VERO S,GARMENDIA G,GONZALEZ M B,BENTANCUR O,WISNIEWSKI M. Evaluation of yeasts obtained from antarctic soil samples as biocontrol agents for the management of postharvest diseases of apple (*Malus×domestica*)[J]. FEMS Yeast Research,2013,13(2): 189-199.

[18] LU L,WANG J,ZHU R,LU H,ZHENG X,YU T. Transcript profiling analysis of *Rhodosporidium paludigenum*-mediated signalling pathways and defense responses in mandarin orange[J]. Food Chemistry,2015,172(172): 603-612.

[19] MAHUNU G K,ZHANG H,YANG Q,ZHANG X,LI D,ZHOU Y. Improving the biocontrol efficacy of *Pichia caribbica* with phytic acid against postharvest blue mold and natural decay in apples[J]. Biological Control,2016,92(1): 172-180.

[20] ARRARTE E,GARMENDIA G,ROSSINI C,WISNIEWSKI M,VERO S. Volatile organic compounds produced by antarctic strains of *Candida sake* play a role in the control of postharvest pathogens of apples[J]. Biological Control,2017,109(1): 14-20.

[21] KALOGIANNIS S,TJAMOS S E,STERGIOU A,ANTONIOU P P,ZIOGAS B N. Selection and evaluation of phyllosphere yeasts as biocontrol agents against grey mould of tomato[J]. European Journal of Plant Pathology,2006,116(1): 69-76.

[22] HU H,XU Y,LU H,XIAO R,ZHENG X,YU T. Evaluation of yeasts from Tibetan fermented products as agents for biocontrol of blue mold of Nashi pear fruits[J]. Journal of Zhejiang Universityence B,2015,16(4): 275-285.

[23] WEI Y,XU M,WU H,TU S,PAN L,TU K. Defense response of cherry tomato at different maturity stages to combined treatment of hot air and *Cryptococcus laurentii*[J]. Postharvest Biology and Technology,2016,117: 177-186.

[24] 刘海波,田世平. 水果采后生物防治拮抗机制的研究进展[J]. 植物学报,2001,18(6): 657-664.
LIU Haibo,TIAN Shiping. Recent advances on the mechanism of postharvest biocontrol[J].Chinese Bulletin of Botany,2001,18(6): 657-664.

[25] ZHANG D,SPADARO D,GARIBALDI A,GULLINO M L. Potential biocontrol activity of a strain of *Pichia guilliermondii* against grey mold of apples and its possible modes of action[J]. Biological Control,2011,57(3): 193-201.

[26] KWASIBORSKI A,BAJJI M,RENAUT J,DELAPLACE P,JIJAKLI M H. Identification of metabolic pathways expressed by *Pichia anomala* Kh6 in the presence of the pathogen *Botrytis cinerea* on apple: new possible targets for biocontrol improvement[J]. PLoS One,2014,9(3): e91434.

[27] SPADARO D,DROBY S. Development of biocontrol products for postharvest diseases of fruit: The importance of elucidating the mechanisms of action of yeast antagonists[J]. Trends in Food Science & Technology,2016,47(49): 39-49.

[28] CHI M,LI G,LIU Y,LIU G,LI M,ZHANG X,SUN Z,SUI Y,LIU J. Increase in antioxidant enzyme activity, stress tolerance and biocontrol efficacy of *Pichia kudriavzevii* with the transition from a yeast-like to biofilm morphology[J]. Biological Control,2015,90(2): 113-119.

[29] 杨东升,罗先群,王新广. 嗜杀酿酒酵母对海南山兰甜酒发酵品质的影响[J]. 中国酿造,2013,32(11): 59-63.
YANG Dongsheng,LUO Xianqun,WANG Xinguang. Effect of killer brewery yeast on the fermentation quality of Hainan shanlan rice wine[J]. China Brewing,2013,32(11): 59-63.

[30] BREINIG F,TIPPER D J,SCHMITT M J. Kre1p, the plasma membrane receptor for the yeast K1 viral toxin[J]. Cell,2002,108(3): 395-405.

[31] FRIEL D,GOMEZ PESSOA N M,VANDENBOL M,JIJAKLI M H. Separate and combined disruptions of two exo-$\beta$-1,3-glucanase genes decrease the efficiency of *Pichia anomala* (strain K) biocontrol against *Botrytis cinerea* on apple[J]. Molecular Plant-microbe Interactions,2007,20(4): 371-379.

[32] BANANI H,SPADARO D,ZHANG D,MATIC S,GARIBALDI A,GULLINO M L. Biocontrol activity of an alkaline serine protease from *Aureobasidium pullulans* expressed in *Pichia pastoris* against four postharvest pathogens on apple[J]. International Journal of Food Microbiology,2014,182-183(6194): 1-8.

[33] BANANI H,SPADARO D,ZHANG D,MATIC S,GARIBALDI A,GULLINO M L. Postharvest application of a novel chitinase cloned from *Metschnikowia fructicola* and overexpressed in *Pichia pastoris* to control brown rot of peaches[J]. International Journal of Food Microbiology,2015,199: 54-61.

[34] LIU P,CHENG Y,YANG M,LIU Y,CHEN K. Mechanisms of action for 2-phenylethanol isolated from *Kloeckera apiculata*, in control of *Penicillium*, molds of citrus fruits[J]. BMC Microbiology,2014,14(1): 242.

[35] HUANG R,CHE H J,ZHANG J,YANG L,JIANG D H,LI G Q. Evaluation of *Sporidiobolus pararoseus* strain YCXT3 as biocontrol agent of *Botrytis cinerea* on post-harvest strawberry fruits [J]. Biological Control,2012,62(1): 53-63.

[36] LU L,YE C,GUO S,SHENG K,SHAO L,ZHOU T,YU T,ZHENG X. Preharvest application of antagonistic yeast *Rhodosporidium paludigenum* induced resistance against postharvest diseases in mandarin orange[J]. Biological Control,2013,67(2): 130-136.

[37] DROBY S,VINOKUR V,WEISS B,COHEN L,DAUS A,GOLDSCHMIDT E E,PORAT R. Induction of resistance to *Penicillium digitatum* in grapefruit by the yeast biocontrol agent *Candida oleophila*[J]. Phytopathology,2002,92(4): 393-399.

[38] CHAN Z,QIN G,XU X,LI B,TIAN S. Proteome approach to

(C)1994-2019 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net

characterize proteins induced by antagonist yeast and salicylic acid in peach fruit[J]. Journal of Proteome Research，2007，6（5）: 1677-1688.

[39] 薛耀碧，周雅涵，邓丽莉，曾凯芳. 采后果实病程相关蛋白研究进展[J]. 食品工业科技，2015，36（6）: 391-394.
XUE Yaobi，ZHOU Yahan，DENG Lili，ZENG Kaifang. Advances on pathogenesis-related proteins of postharvest fruits[J]. Science and Technology of Food Industry，2015，36（6）: 391-394.

[40] ZHANG H，CHEN L，SUN Y，ZHAO L，ZHENG X，YANG Q，ZHANG X. Investigating proteome and transcriptome defense response of apples induced by *Yarrowia lipolytica*[J]. Molecular Plant-Microbe Interactions，2017，30（4）: 301-311.

[41] MASSART S，MARTINEZ-MEDINA M，JIJAKLI M H. Biological control in the microbiome era: Challenges and opportunities [J]. Biological Control，2015，89: 98-108.

[42] MASSART S，JIJAKLI H M. Use of molecular techniques to elucidate the mechanisms of action of fungal biocontrol agents: A review[J]. Journal of Microbiological Methods，2007，69（2）: 229-241.

[43] MASSART S，PERAZZOLLI M，HOFTE M，PERTOT I，JIJAKLI M H. Impact of the omic technologies for understanding the modes of action of biological control agents against plant pathogens[J]. Biocontrol，2015，60（6）: 725-746.

[44] WANG Y，WANG P，XIA J，YU T，LOU B. Effect of water activity on stress tolerance and biocontrol activity in antagonistic yeast *Rhodosporidium paludigenum*[J]. International Journal of Food Microbiology，2010，143（3）: 103-108.

[45] LIU J，WISNIEWSKI M，DROBY S，TIAN S，HERSHKOVITZ V，TWORKOSKI T. Effect of heat shock treatment on stress tolerance and biocontrol efficacy of *Metschnikowia fructicola*[J]. Fems Microbiology Ecology，2011，76（1）: 145-155.

[46] LIU J，WISNIEWSKI M，DROBY S，NORELLI J，HERSHKOVITZ V，TIAN S，FARRELL R. Increase in antioxidant gene transcripts, stress tolerance and biocontrol efficacy of *Candida oleophila* following sublethal oxidative stress exposure[J]. Fems Microbiology Ecology，2012，80（3）: 578-590.

[47] CHENG Z，CHI M，LI G，CHEN H，SUI Y，SUN H，WISNIEWSKI M，LIU Y，LIU J. Heat shock improves stress tolerance and biocontrol performance of *Rhodotorula mucilaginosa* [J]. Biological Control，2016，95: 49-56.

[48] ZHAO Y，TU K，SU J，TU S，HOU Y，LIU F，ZOU X. Heat treatment in combination with antagonistic yeast reduces diseases and elicits the active defense responses in harvested cherry tomato fruit[J]. Journal of Agricultural and Food Chemistry，2009，57（16）: 7565-7570.

[49] OU C，LIU Y，WANG W，DONG D. Integration of UV-C with antagonistic yeast treatment for controlling post-harvest disease and maintaining fruit quality of *Ananas comosus*[J]. Biocontrol，2016，61（5）: 591-603.

[50] SUI Y，LIU J. Effect of glucose on thermotolerance and biocontrol efficacy of the antagonistic yeast *Pichia guilliermondii*[J]. Biological Control，2014，74（3）: 59-64.

[51] LIU J，WISNIEWSKI M，DROBY S，VERO S，TIAN S，HERSHKOVITZ V. Glycine betaine improves oxidative stress tolerance and biocontrol efficacy of the antagonistic yeast *Cystofilobasidium infirmominiatum*[J]. International Journal of Food Microbiology，2011，146（1）: 76-83.

[52] LU H，LU L，ZENG L，FU D，XIANG H，YU T，ZHENG X. Effect of chitin on the antagonistic activity of *Rhodosporidium paludigenum* against *Penicillium expansum* in apple fruit[J]. Postharvest Biology and Technology，2014，92: 9-15.

[53] LI W，ZHANG H，LI P，APALIYA M T，YANG Q，PENG Y，ZHANG X. Biocontrol of postharvest green mold of oranges by *Hanseniaspora uvarum* Y3 in combination with phosphatidylcholine[J]. Biological Control，2016，103: 30-38.

[54] FU D，ZENG L，ZHENG X，YU T. Effect of $\beta$-glucan on stress tolerances and biocontrol efficacy of *Cryptococcus laurentii* against *Penicillium expansum* in pear fruit[J]. BioControl，2015，60（5）: 669-679.

[55] CHAN Z，TIAN S. Induction of $H_2O_2$-metabolizing enzymes and total protein synthesis by antagonistic yeast and salicylic acid in harvested sweet cherry fruit[J]. Postharvest Biology and Technology，2006，39（3）: 314-320.

[56] YU T，CHEN J，CHEN R，HUANG B，LIU D. Biocontrol of blue and gray mold diseases of pear fruit by integration of antagonistic yeast with salicylic acid[J]. International Journal of Food Microbiology，2007，116（3）: 339-345.

[57] GUO J，FANG W，LU H，ZHU R，LU L，ZHENG X，YU T. Inhibition of green mold disease in mandarins by preventive applications of methyl jasmonate and antagonistic yeast *Cryptococcus laurentii*[J]. Postharvest Biology and Technology，2014，88: 72-78.

[58] YANG Q，LI Y，LI C，ZHANG H，JIANG Z，ZHANG X，SERWAH B N A，MAHUNU G K，ZHU S. Antioxidative enzymes and substances involve in the activity of improving the oxidative tolerance of *Pichia caribbica* by ascorbic acid[J]. Biological Control，2017，108: 83-88.

[59] MAHUNU G K，ZHANG H，YANG Q，ZHANG X，LI D，ZHOU Y. Improving the biocontrol efficacy of *Pichia caribbica* with phytic acid against postharvest blue mold and natural decay in apples[J]. Biological Control，2016，92(1): 172-180.

[60] LIU J，SUI Y，WISNIEWSKI M，DROBY S，LIU Y. Review: Utilization of antagonistic yeasts to manage postharvest fungal diseases of fruit[J]. International Journal of Food Microbiology，2013，167（2）: 153-160.

[61] DROBY S，WISNIEWSKI M，MACARISIN D，WILSON C. Twenty years of postharvest biocontrol research: Is it time for a new paradigm?[J]. Postharvest Biology and Technology，2009，52（2）: 137-145.

(C)1994-2019 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net