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专论与综述

浓香型白酒大曲微生物群落结构研究进展

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摘　要：大曲通常作为发酵剂用于酿造传统中国白酒，其提供各类微生物菌系和酶系启动白酒发酵，影响白酒风味和独特风格。近年来，对大曲微生物群落结构的研究成为研究热点，研究人员对大曲微生物群落结构、基因功能和功能微生物等进行了广泛而深入的研究，对大曲微生物组成、变迁规律和功能的认识逐渐清晰。本文综述了浓香型大曲微生物群落结构分析方法、主要微生物组成、重要功能微生物和微生物溯源，为研究大曲微生物群落结构、优化大曲生产工艺和改善白酒品质提供一定的理论依据。

关键词：大曲；微生物群落结构；微生物溯源；浓香型白酒

Research progress in microbial community structure in Nongxiangxing Daqu

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Abstract: Daqu, commonly used as a starter during the brewing of Chinese Baijiu, provides a variety of microbial strains and enzymes initiating the Baijiu fermentation, thus affecting the unique flavor and taste of Baijiu. In recent years, the microbial community structure of Daqu

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has become a research hotspot, and researchers have conducted extensive in-depth research on the microbial community structure, gene function, and functional microorganisms of Daqu. Therefore, the knowledge of the microbial composition, succession, and functions of Daqu has become increasingly richer. This review summarized the analytic methods of microbial community structure, the main microbial composition, functional microorganisms, and microbial traceability in the production of Daqu, aiming to provide a theoretical basis for investigating the microbial community structure of Daqu, optimizing the production process of Daqu, and improving the quality of Baijiu.

Keywords: Daqu; microbial community structure; microbial traceability; Nongxiangxing Baijiu

中国白酒具有历史悠长、工艺独特等特点，在世界六大蒸馏酒中独树一帜，其中浓香型白酒是中国最受欢迎的白酒之一，具有独特口感和典型风格等特点。大曲、粮食、水是酿造浓香型白酒的原料，其中大曲被称为酒之骨，在酿酒过程中提供微生物菌系和酶系启动白酒发酵。通常，白酒的风味和质量与整个生产过程中的微生物群落密切相关，特别是与大曲中的微生物有关，因为白酒发酵所需的原料中大曲大约占了20%[1]。近年来，随着分子生物学技术的完善与发展，研究人员对大曲中的微生物群落进行了广泛而深入的研究。本文从浓香型大曲微生物群落结构分析方法、主要微生物组成、重要功能微生物和微生物溯源等方面进行阐述，以期为浓香型大曲微生物组成的揭示和功能微生物资源利用提供参考。

1 大曲简介

大曲是一种微生物发酵剂，其中富含大量微生物、酶类(蛋白酶、淀粉酶、纤维素酶等)以及风味物质(醇、酸、醛、酯等)，具有糖化发酵、生香等功能，在白酒酿造过程中发挥着重要作用。多数大曲以小麦为主要原料，有的则以大麦或豌豆等作为主要原料，经浸泡、润料、粉碎、拌料、装箱上料等操作后入库储存[2](图 1)。大曲一般储存 3−4 个月后，可作为成熟大曲使用[3]。通常高温大曲的贮存期超过 3 个月，中温大曲的贮存期超过 5 个月，但最长不应超过 12 个月[4]。

大曲依据制曲品温不同，分为低温曲、中温曲和高温曲。温度变化会影响大曲微生物群落，导致大曲微生物菌系、酶系、风味物质产生差异[5-7]，一般品温低于 50 ℃的低温曲其酵母菌含量较高，对淀粉质原料的转化率高，主要应用于清香型白酒的生产[8]。通常品温在50−60 ℃范围内的中温曲，主要应用于浓香型白酒生产。品温高于 60 ℃的高温曲，则应用于酱香型白酒生产。高温曲中已长出的霉菌和酵母菌因为高温工艺导致增殖受阻，逐渐趋向衰老死亡，而耐高温的芽孢杆菌较多，这有利于蛋白质的热分解和糖的裂解并形成吡嗪之类的香气成分和褐变反应物质[9-10]。大曲依据生产原料的不同分为酱香型大曲、浓香型大曲和清香型大曲[11]。最后依据生产工艺差异可将大曲分为传统大曲、强化大曲和纯种大曲(表 1)。浓香型白酒作为中国最受欢迎的白酒之一，全球消费的浓香型白酒年产量达到 935 万 t，约占白酒整体市场份额的 51%[13]。大曲中的微生物启动白酒发酵，影响浓香型白酒的质量、特性和安全性，所以只有全面了解浓香型大曲中微生物群落结构和功能，深入解析群体微生物代谢机制，才能有助于进一步提升浓香型白酒质量和产量。

图 1　大曲制作流程

Figure 1　The production process of Daqu.

2　微生物群落结构分析方法及应用

大曲从发酵到储存的过程中，在环境中富集了大量微生物，这些微生物的群落演替和进化影响着白酒发酵过程的特性，因此大曲中的微生物备受研究人员的关注。微生物正常生长发育需要依据其本身的生长特性来配制相应的培养基，根据这一特性，可以将大曲中微生物大致类别分离纯化出来，为进一步深入了解微生物，需要利用显微镜对其形态进行观察，或者依据微生物在生长过程中呈现出来的生理生化特征和遗传特征来鉴定该微生物属于何属甚至鉴定到种[14-16]。然而，发酵环境中能直接培养的微生物微乎其微，只有 1%−10%左右[17]，所以传统培养法对研究微生物群落有一定限制，并不能真实反映微生态。随着分子生物学技术的完善与发展，免培养技术有助于研究大曲微生物群落组成和物种多样性。常见的免培养技术主要包括磷脂脂肪酸(phospholipid fatty acid,

表 1 大曲的分类[12]

Table 1 Classification of Daqu[12]

分类依据 Classification basis		种类 Types	酒种代表 Baijiu type representatives	酒体风格 Baijiu style
品温 Temperature	40−50 ℃	低温大曲 Low-temperature Daqu	汾酒，黄鹤楼 Fenjiu, Huanghelou	口感干净、醇甜柔和、余味悠长 Clean mouthfeel, mellow, sweet and soft, long aftertaste
	≤60 ℃	中温大曲 Medium-temperature Daqu	五粮液，泸州老窖 Wuliangye, Luzhou Laojiao	香甜果味、落口绵、尾净余长 Sweet and fruity, with a long, clean finish
	>60 ℃	高温大曲 High-temperature Daqu	茅台，郎酒 Maotai, Langjiu	空杯留香、幽雅而持久、口味细腻、回味悠长 Empty glass fragrance, elegant and lasting; Delicate taste, long aftertaste
原料 Raw materials	大麦、豌豆 Barley, peas	清香型大曲 Qingxiangxing Daqu	汾酒，黄鹤楼 Fenjiu, Huanghelou	口感干净，醇甜柔和、余味悠长 Clean mouthfeel, mellow, sweet and soft, long aftertaste
	主要为小麦 Mainly wheat	浓香型大曲 Nongxiangxing Daqu	五粮液，泸州老窖 Wuliangye, Luzhou Laojiao	香甜果味、落口绵、尾净余长 Sweet and fruity, with a long, clean finish
	主要为小麦 Mainly wheat	酱香型大曲 Jiangxiangxing Daqu	茅台，郎酒 Maotai, Langjiu	空杯留香、幽雅而持久、口味细腻、回味悠长 Empty glass fragrance, elegant and lasting; Delicate taste, long aftertaste
生产工艺 Production process	自然发酵 Spontaneous fermentation	传统大曲 Traditional Daqu		
	接入功能菌 Inoculation of functional microorganisms	强化大曲 Fortified Daqu		风味和性能有所提高 Improved flavor and performance
	单菌种发酵 Single strain fermentation	纯种大曲 Purebred Daqu		品质稳定、风味单一 Consistent quality and single flavor

PLFA)、荧光原位杂交(fluorescence *in situ* hybridization, FISH)、聚合酶链反应-单链构象多态性(polymerase chain reaction-single strand conformation polymorphism, PCR-SSCP)、聚合酶链反应-变性梯度凝胶电泳(polymerase chain reaction-denaturing gradient gel electrophoresis, PCR-DGGE)和 Illumina MiSeq/HiSeq 高通量测序(Illumina MiSeq/HiSeq high-throughput sequencing, Illumina MiSeq/HiSeq HTS)等技术(表 2)。这些研究方法从不同层次对微生物群落结构进行解析，各有优劣。磷脂脂肪酸(phospholipid fatty acid, PLFA)是生物细胞膜的骨架成分，只存在于活细胞中，当生物死亡后，PLFA 被迅速代谢，基于 PLFA 标记的微生物群落指纹图谱技

术是一种能定量或半定量表征微生物群落结构特征及动态变化的免培生物化学方法，尤其是研究土壤中主要微生物群落多样性的快捷方法，也能揭示胁迫对生物量的影响。Zhang 等[30]采用 PLFA 法对 3 种清香型大曲(清茬曲、红心曲、后火曲)进行了研究，获得了 3 种大曲的 PLFA 谱，结果表明，曲中的微生物群落由革兰氏阳性菌、革兰氏阴性菌以及放线菌组成；后火曲和清茬曲样品中的革兰氏阳性菌含量和种类相似，但显著高于红心曲，而 3 种大曲中的革兰氏阴性菌含量和种类显著不同。此外，PCR-DGGE 作为使用较为广泛的 DNA 指纹图谱技术之一，具有检测快速、检测限低、检测成本低和检测结果可靠的特点，通常用于研究环境中不可培养的微生物群落结构，并确定微生物群落动态变化。Yan 等[25]采用 PCR-DGGE 对大曲发酵过程中的酵母群落结构进行了研究，结果表明，在发酵过程中酵母群落表现出显著的多样

表 2　研究大曲微生物群落结构的主要免培养技术

Table 2　Main culture-independent technique for investigating the microbial community structure in Daqu

方法 Methods	特点 Characteristics	文献 References
PLFA	检测迅速、重现性高，但不能用于检测古细菌，只能鉴定到属水平；受环境和微生物生理状态影响大 Rapid detection and high reproducibility; And it cannot be used to detect archaea, and can only be identified at the genus level. This method is greatly influenced by the environment and physiological state of microorganisms	[18-19]
FISH	直接观察到 DNA 扩增、荧光强度反映扩增水平,可对微生物进行定性和定量研究；但是该方法步骤烦琐，不能达到 100%杂交 The DNA amplification was directly observed and the fluorescence intensity reflected the amplification level, allowing qualitative and quantitative studies of microorganisms; However, the method is complicated and cannot achieve 100% hybridization	[20-21]
PCR-SSCP	操作方便、快速、可自动化测序，但分析片段越长，检测准确率越低、操作条件越严苛 The operation is easy, fast, and can be automated sequencing; however, the longer the analyzed fragment, the lower the detection accuracy and the harsher the operating conditions	[22-24]
PCR-DGGE	检测快速、检测限低；只能检测样本中大于总量 1% 的微生物，分离片段必须在 200−700 bp 范围内 Rapid detection, low detection limit; only 1% of the total number of microorganisms in the sample can be detected, and the isolated fragments must be within the range of 200−700 bp	[25-27]
Illumina 高通量测序 Illumina high-throughput sequencing	能快速、大量地进行测序，具有省时、省力、花费少等优点；但读取序列长度较短、会检测出样品中无活性的微生物 It can be sequenced quickly and in large quantities, and has the advantages of saving time, effort and expense; However, the read sequence length is short, and inactive microorganisms in the sample are detected	[15,28-29]

性，且 *Pichia kudriavzevii* 和 *Saturnispora silvae* 为优势菌株。近年来，利用高通量测序的方法对群落结构进行分析成为研究的热点，基于 Illumina 的高通量测序广泛用于大曲、窖泥和糟醅的微生物群落分析，这种方法具有通量高、检测速度快等优点，可更加准确、真实地反映样本中微生物群落结构的组成。Wang 等[31]采用 Illumina MiSeq 高通量测序技术对 3 种不同高温大曲(白大曲、黑大曲和黄大曲)的细菌多样性进行了研究，结果表明，在 30 个高温大曲样品中鉴定出 7 个细菌门和 262 个细菌属，其中厚壁菌门是绝对优势细菌门，*Thermoactinomyces* 、*Staphylococcus* 和 *Lentibacillus* 是绝对优势细菌属。除了这些常见的技术外，宏组学也被应用于浓香型大曲样品的微生物功能和分类特征的研究中，尽管宏组学测序比靶向扩增子测序更昂贵，数据处理也更复杂，但分析大规模组学数据集对于理解大曲微生物群的相互关系具有很大的潜力[2,32]。但是这些方法最多提供种水平上的信息，而许多重要的信息在菌株水平上体现。培养组学则是一种在菌株水平上培养和识别微生物的高通量方法，它不仅补充了用于宏基因组分析的分类学和功能数据库的目录，还有助于合成可控的微生物群落[33-34]。因此，随着分析技术的进步，对大曲微生物群落组成和微生物功能的认识将逐渐深入和清晰。

3 浓香型大曲中微生物群落结构研究

大曲是白酒酿造过程中微生物的重要来源，并且大曲微生物组成一定程度上影响着白酒后续发酵过程的微生物繁殖代谢，因此，大曲中的微生物在中国传统白酒发酵中起着不可替代的作用。随着高通量测序技术的进步，利用基于高通量测序的免培养技术结合可培养方法，研究人员对大曲微生物群落结构、基因功能和功能微生物进行了广泛且深入的研究，对大曲微生物组成、变迁规律和功能的认识逐渐清晰，为优化大曲发酵工艺和提升大曲品质奠定了理论基础。大曲中的微生物受到环境的影响，群落结构会产生一定的差异，场地、制作方式和储存时间等因素都会对大曲质量和微生物群落结构造成影响。研究人员在不同酒厂中取样对浓香型大曲微生物群落结构进行探究，结果表明大曲中微生物相对丰度大于 1%的真菌属主要包括根霉属(*Rhizopus*)、热孢霉属(*Thermoascus*)、毕赤酵母属(*Pichia*)、曲霉属(*Aspergillus*)、威克汉姆酵母属(*Wickerhamomyces*)、嗜热真菌属(*Thermomyces*)和酵母属(*Saccharomyces*)等，细菌属主要包含魏斯氏菌属(*Weissella*)、乳酸杆菌属(*Lactobacillus*)、葡萄球菌属(*Staphylococcus*)、片球菌属(*Pediococcus*)、肠杆菌属(*Enterobacter*)、乳球菌属(*Lactococcus*)、芽孢杆菌属(*Bacillus*)和不动杆菌属(*Acinetobacter*)等，但含量有所差异[35-38](图 2)。

3.1 大曲细菌群落结构及功能研究

细菌在大曲生产和白酒酿造中都扮演着重要的角色，细菌主要通过产香影响大曲风味，进而影响白酒风味和香型。乳酸杆菌属(*Lactobacillus*)、魏斯氏菌属(*Weissella*)、芽孢杆菌属(*Bacillus*)、片球菌属(*Pediococcus*)和葡萄球菌属(*Staphylococcus*)是浓香型大曲中的优势菌[38-42]。

3.1.1 乳酸杆菌属(*Lactobacillus*)

在浓香型白酒大曲发酵过程中乳酸杆菌属(*Lactobacillus*)的平均相对丰度在 75%以上[35]。乳酸杆菌属在发酵开始时丰度通常较低，但在发酵前 5 天迅速增加成为主要的细菌属，之后随着

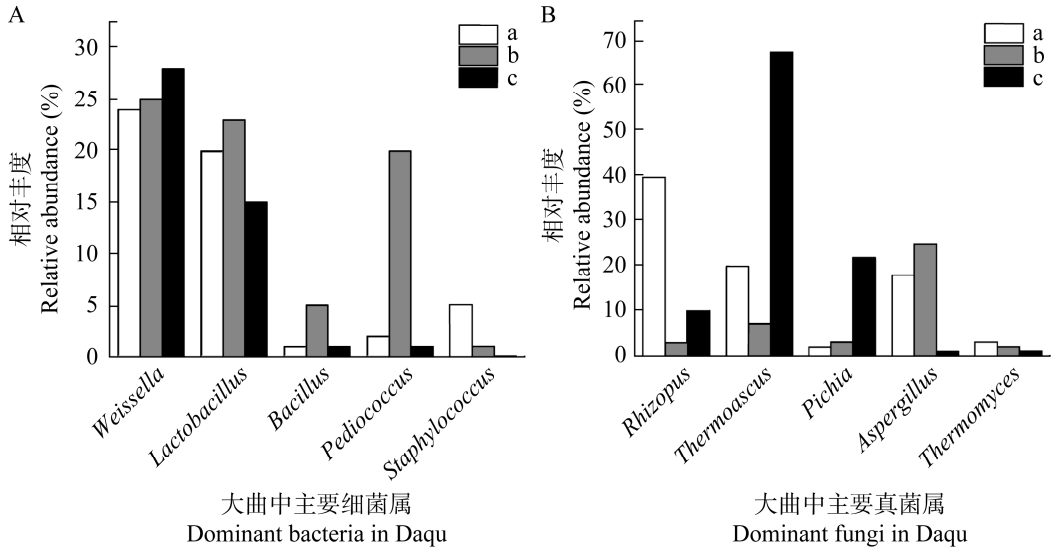

图 2 浓香型大曲主要细菌(A)和真菌(B)及相对丰度 a、b、c 代表不同来源的 3 种浓香型大曲

Figure 2 Relative abundance of dominant bacteria (A) and fungi (B) in Nongxiangxing Daqu. a, b and c represent three types of Nongxiangxing Daqu from different sources.

发酵的进行而逐渐减少[2,38,43-44]。但也有研究表明，乳酸杆菌属在发酵后期成为主要的细菌属，并且随着氧浓度的降低和乳酸的积累，乳酸杆菌属的丰度仍然很高[45]。乳酸杆菌可以利用糖类生产乙偶姻、乙醛和 2,3-丁二酮等挥发性风味物质，这些物质分别具有奶油香、果香、脂肪香的特征，赋予白酒丰富、多层次的香味，有助于提升白酒风味品质[46]。近年来，研究人员利用宏组学技术对大曲中微生物功能机制进行研究，有助于更好地了解它们的遗传背景和潜在功能。Du 等[47]利用宏转录组技术分析表明，乳酸杆菌在转录过程中涉及风味化合物及其前体(包括酯和酸)生物合成的基因很活跃，证明其与不同的风味化合物相关。Xia 等[48]利用宏基因组学技术研究大曲中微生物糖化功能时发现 α-葡萄糖苷酶基因主要在乳酸杆菌中编码。此外，较多学者对大曲中具有优良特性的乳酸杆菌株进行筛选，*Lactobacillus fuchuensis*、*L. lindneri*、*L. plantarum* E2、*L. plantarum* E11、*L. paralimentarius* LBM12001 和 *L. sanfranciscensis*[49-50]等菌株被分离鉴定出。同一微生物群落中分离出来的物种通常表现出菌株特异性，因此，在实验室中依据发酵特性使乳酸杆菌在菌株水平上分型，筛选出潜在应用中表现最好的菌株，为构建合成大曲微生物群落提供潜在的候选菌株，这可能有助于控制发酵过程和白酒质量。

3.1.2 芽孢杆菌属(*Bacillus*)

在整个大曲生产过程中，芽孢杆菌属(*Bacillus*)是主要的细菌属，然后随着发酵的进行，在发酵中期(10−25 d)达到最高丰度，随后其相对丰度保持相对稳定[15,44,51-52]。芽孢杆菌属具有分泌水解纤维素酶和半纤维素酶的潜力，并有助于在白酒发酵过程中形成挥发性化合物如双乙酰等[48,53]。此外，Yang 等[54]结合宏蛋白质组学等多组学方法，确定枯草芽孢杆菌(*Bacillus subtilis*)是促使群落在特定环境条件下形成相应稳定微生态的微生物，其通过调节大曲中五元杂环氨基酸代谢影响大曲微生态产生分化。大曲作

为浓香型白酒发酵微生物的主要来源,较多学者从大曲中分离得到了 *Bacillus subtilis*、*B. velezensis*、*B. licheniformi* 和 *B. amyloliquefaciens* 等[55-56]菌株,并将芽孢杆菌作为功能微生物添加到大曲中制作强化大曲来改善大曲风味品质,进而提高白酒风味品质,同时对芽孢杆菌强化大曲的作用机制进行探究,为定向增加白酒原位发酵系统的关键香气成分、控制白酒风味与品质奠定基础[53]。He 等[56]将 *B. velezensis* 和枯草芽孢杆菌(*B. subtilis*)加入大曲中生产强化大曲,结果表明,强化大曲中芽孢杆菌属、乳酸杆菌和念珠菌(*Candida*)相对丰度分别增加了 6.2%、5.3%和 16.8%;大曲液化力、糖化力和酯化力分别提高了 25.6%、9.0%和 15.2%;川芎嗪的含量增加了 1.9 倍;参与糖化、酒精发酵和香气生成的酶编码基因丰度明显增加。

3.1.3 魏斯氏菌属(*Weissella*)

魏斯氏菌属(*Weissella*)是酿酒过程中的有益细菌,能在酿酒过程中分泌多种水解酶并产生风味物质(乳酸),这些风味物质可以丰富中国白酒的醇厚口感[41,57-59]。Din 等[60]对浓香型大曲进行宏基因组学分析,鉴定出 *Weissella paramesenteroides*、*W. confuse* 和 *W. cibaria* 等菌株,并通过可培养和免培养法从浓香型大曲中分离鉴定出 *W. cibaria* 和 *W. confusa*,而在其他香型大曲中仅分离出 *W. paramesenteroides*[49]。其中,有研究[2,61]利用宏基因技术分析大曲中微生物的功能作用,结果表明 *W. paramesenteroides*、*W. confuse* 和 *W. cibaria* 是主要潜在的利用乳酸脱氢酶将丙酮酸转化为乳酸的菌种。同时,魏斯氏菌属是浓香型大曲中最丰富的细菌属之一,并且在发酵前期(0−10 d)成为优势细菌属,然后在后续发酵中丰度保持相对稳定[36-38,43]。然而 Li 等[52]利用 Illumina HiSeq 技术对浓香型大曲中微生物群落进行研究,结果表明,魏斯氏菌属在发酵开始时出现,然后随着发酵的进行而减少。这可能是由于环境中的微生物和大曲原料的不同导致了魏斯氏菌属在不同的浓香型大曲中呈现出不同的群落演替规律。

3.2 大曲真菌群落结构及功能研究

真菌是大曲中活跃的群落成员,能够分泌与淀粉代谢相关的关键酶且其群落结构和功能对大曲品质影响极大。根霉属(*Rhizopus*)、毕赤酵母属(*Pichia*)、曲霉属(*Aspergillus*)、威克汉姆酵母属(*Wickerhamomyces*)和酵母属(*Saccharomyces*)为浓香型大曲中的优势真菌[52,62-64]。

3.2.1 曲霉属(*Aspergillus*)

曲霉属(*Aspergillus*)是大曲中重要的功能真菌属,具有分泌耐酸、耐乙醇的胞外酶(α-淀粉酶、糖化酶、葡萄糖苷酶、内切葡聚糖酶和 β-葡萄糖苷酶等)的能力,可以有效地将发酵基质中的淀粉、蛋白质等大分子物质转化为小分子如葡萄糖和氨基酸,为微生物的生长提供必需的碳源和氮源[48,52,64]。目前,利用可培养方法从大曲中分离得到了多株曲霉,包括红曲(*Aspergillus ruber*)、烟曲霉(*A. fumigatus*)、谢瓦氏曲霉(*A. salwaensis*)、*A. chevalieri*、米曲霉(*A. oryzae*)和 *A. amstelodami* 等[45,65]。其中,Wang 等[66]第一次从浓香型大曲中分离出分泌特殊葡萄糖淀粉酶的米曲霉(*A. oryzae* LZ2),这种葡萄糖淀粉酶对分解葡萄糖产生的最终抑制产物不太敏感,这也表明大曲是葡萄糖淀粉酶的重要来源。同时,曲霉属也是浓香型大曲生产中的优势真菌属之一,在发酵过程中,曲霉属的相对丰度在发酵前期(0−10 d)达到峰值,然后随着发酵进行而减少,并且曲霉属在发酵过程中会产生许多的风味物质,有助于提升酒体的风味[43-44,64,67-68]。

3.2.2 根霉属(*Rhizopus*)

根霉属(*Rhizopus*)为浓香型大曲的核心真菌属之一,在发酵前期(4−6 d)出现并成为优势真菌

属，然后随着发酵进行而逐渐减少[15,35,37]。近年来，较多学者应用多组学技术多角度研究大曲中复杂微生物群落的糖化作用，发现根霉是重要的糖化菌。Wang 等[69]通过宏蛋白组学分析大曲中起糖化作用的糖化菌群，结果表明根霉属通过分泌丰富的糖化酶而成为大曲糖化过程中的主要驱动菌群，并证明大曲的容重是关键糖化菌群的主要驱动力。Wang 等[70]利用可培养方法从大曲中筛选出 *Rhizopus microsporus* 并证明 *R. microsporus* 是重要的糖化酶分泌菌株，这与宏蛋白组学分析结果相一致。随着研究人员对大曲中根霉的深入了解，将可能提升糖化剂与发酵食品的质量。同时，研究表明根霉属在生长过程中还能产除糖化酶以外的多种酶类和有机酸，如淀粉酶、蛋白酶、柠檬酸、琥珀酸和乳酸等，其中米根霉(*Rhizopus oryzae*)能分泌乳酸脱氢酶，这利于乳酸菌产出乳酸[[71-72]。

3.2.3 酵母属(*Saccharomyces*)

酵母属(*Saccharomyces*)是主要的优势真菌属之一，在开始发酵时其相对丰度极低，但在发酵第 6 天时增加到最大值，然后随着发酵进行而逐渐减少[52]。近年来，大曲中酵母菌的分离与鉴定及应用取得了较大进展，酿酒酵母(*Saccharomyces cerevisiae*)、*S. kudriavzevii*、*S. uvarum*、*S. carlsbergensis*、*S. servazzii* 和 *S. apiculatus* 等多株酵母菌从大曲中分离得到[73-75]。其中酿酒酵母(*Saccharomyces cerevisiae*)和 *Wickerhamomyces anomalus* 是重要的酵母菌株，Fan 等[72]将两者加入大曲中混合发酵提高大曲中乙酸乙酯含量。此外，Ban 等[35]利用扩增子测序和宏蛋白组学技术研究大曲微生物群落结构，结果表明在发酵过程中酵母属是优势菌群并且提供了主要的具有较高活性的蛋白，成为大曲中核心菌群。研究大曲中核心菌群(酵母属)与环境因素的相关性，根据试验数据建立数学预测模型，有助于达到通过控制发酵过程参数调控微生物的目的。

3.2.4 毕赤酵母属(*Pichia*)

毕赤酵母属(*Pichia*)是不同香型大曲中的优势微生物之一[38,49]。Li 等[52]对发酵过程大曲中的微生物群落结构进行探究时发现，毕赤酵母属在整个过程中均为优势真菌属，且在发酵第 6 天时其相对丰度达到峰值(占总真菌丰度 90%)，并在后续发酵中维持相对稳定。已从大曲中分离得到的菌株包括 *Pichia kluyveri* YE002、*P. kluyveri* YE004、*P. kudriavzevii* EP1、*P. burtonii*、*P. occidental* 和 *P. anomala* 等[13,76-77]。其中 *P. kudriavzevii* 是最常见的代表之一，并在浓香型大曲中大量存在[25]。Yang 等[36]对大曲中微生物群落的研究发现，*P. kudriavzevii* 占总真菌丰度 43%。而且毕赤酵母起着酯化的作用，与大曲中的酯和苯乙醇生成有关[44,76]。此外，毕赤酵母属是分泌糖苷酶和糖基转移酶的主要贡献者，其可以利用蔗糖和葡萄糖生成乙醇、乙酸乙酯和 4-羟基-2-丁酮等多种芳香化合物来提升白酒风味品质[78-79]。同时，Wang 等[69]依据宏蛋白质组学分析结果证明，毕赤酵母能分泌多种糖化酶和糖基转移酶，是酒曲糖化的关键菌群。

3.3 制作方式和储存对大曲群落的影响

目前，大曲生产存在机械化程度低、劳动强度大、大曲品质控制难等问题。虽然机制大曲(Mechanical Daqu)的工艺原理和传统大曲(Traditional Daqu)一样，但是在质量和微生物群落结构上仍然存在着差异[79-80]，研究机制大曲与传统大曲中微生物群落间的差异与联系，将有助于推进浓香型大曲的全自动化生产。Guan 等[37]利用高通量测序的方法对浓香型传统大曲和机制大曲之间微生物群落结构进行探究，结果表明，在机制大曲和传统大曲中鉴定到相对丰度大于 1%的优势真核菌属和原核菌属为根霉属

(*Rhizopus*)、热孢霉属(*Thermoascus*)、毕赤酵母属(*Pichia*)、芽孢杆菌属(*Bacillus*)、片球菌属(*Pediococcus*)、魏斯氏菌属(*Weissella*)和乳酸杆菌属(*Lactobacillus*)等；柠檬酸杆菌(*Citrobacter*)是机制大曲中特有的原核菌群，而 *Rasamsonia* 是传统大曲中特有的真菌群(图 3)。

大曲储存对大曲品质有重要影响。不同储存时间的大曲微生物群落结构会有所差异，导致最终产品的质量不一致[81]。He 等[44]利用高通量测序技术研究浓香型大曲在贮存阶段的理化性质、微生物群落和挥发性化合物的动态变化，结果表明，浓香型大曲中微生物组成在贮存 2 个月和 3 个月差异很小，群落结构较为稳定；将贮存 1 个月与贮存 2–3 个月的大曲中微生物群落进行比较，结果表明魏斯氏菌属(*Weissella*)在贮存阶段相对丰度变化不大，但 *Chloroplast* 随贮存时间延长而丰度降低，乳酸杆菌属(*Lactobacillus*)和 *Thermoactinomyces* 则在增加；贮存时间为 3 个月时，风味物质含量和种类最多。若将微生物群落结构不够稳定的大曲作为成曲使用可能造成同一批次大曲之间品质存在差异，导致白酒品质与风味不稳定[81]。因此，研究大曲在贮存期间的微生物群落变化可以为构建大曲储存工艺提供指导。

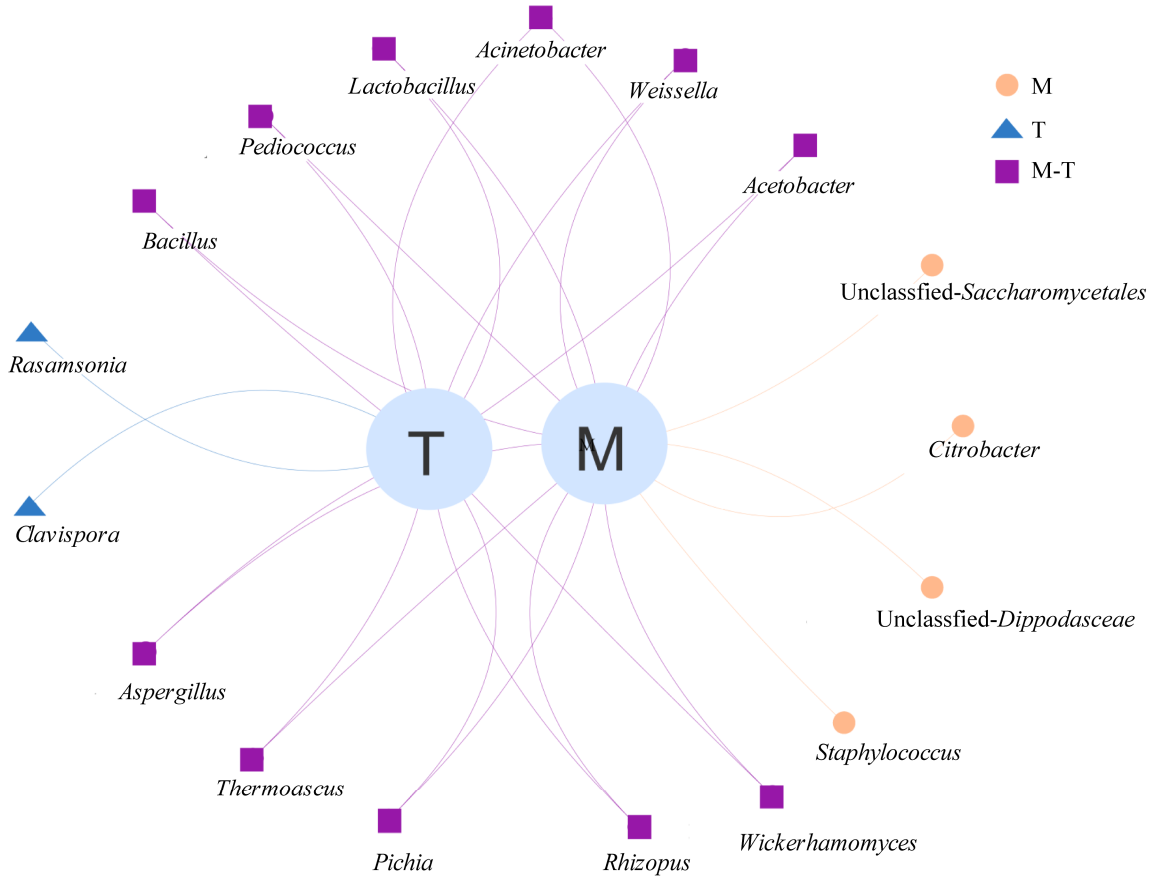

图 3　传统大曲与机制大曲之间微生物组成差异　T：传统大曲；M：机制大曲

Figure 3　Differences in microbial composition between traditional produced Daqu and mechanical produced Daqu. T: Traditional Daqu; M: Mechanical Daqu.

4 大曲中微生物溯源分析

已有较多学者对大曲中微生物进行研究，揭示了不同香型大曲在发酵和贮存过程中微生物群落演替规律以及微生物与风味物质间相关性，研究了环境因素对大曲微生物群落的影响，但缺少对大曲中微生物溯源的系统性研究[36-37,69-70,82-84]。由于传统大曲的原料未经灭菌且生产过程是在自然开放的环境中进行，其微生物富集和群落的多样性易受原料、环境(空气、地面和酿造工具)等多种因素的影响，这导致大曲中微生物群落结构的稳定性有一定的缺陷[62](表 3)。因此，采用微生物溯源分析手段分析大曲微生物的来源非常必要，目前溯源分析大多采用基于贝叶斯算法的微生物来源追踪技术(SourceTracker)。已有研究者[85-87]对大曲微生物和原料(小麦)之间的关系进行探索，结果表明，小麦是大曲中微生物的主要来源且大曲中的葡萄球菌属可能起源于小麦。Du等[88]结合高通量测序技术和 SourceTracker 对大曲中微生物来源进行了全面溯源分析，SourceTracker 结果显示，原料是大曲中细菌来源，并且大曲中的优势细菌来自原料的占比为80%左右；工具和室内地板则可能是大曲中真菌来源，并且大曲中的优势真菌来自工具的占比为 75%左右(图 4)。

表 3 大曲中微生物来源分布[85-91]

Table 3 Distribution of microbial sources in Daqu[85-91]

种类 Species	小麦 Wheat	空气 Air	曲房室外地板 Outdoor floor of Daqu room	曲房内地板 Indoor floor of Daqu room	酿造工具 Brewing tools	酿造用水 Brewing water
细菌 Bacteria	*Pantoea agglomerans*, *Enterobacter aerogenes*, *Pseudomonas koreensis*, *Sphingomonas desiccabilis*, *Staphylococcus*, *Saccharopolyspora*, *Kroppenstedtia*	*Pseudomonas*, *Staphylococcus*, *Bacillus*	*Alternaria*, *Cladosporium*	*Bacillus amyloliquefaciens*, *Pediococcus acidilactici*	*Bacillus amyloliquefaciens*, *Pediococcus acidilactici*	*Marivita*, *Prochlorococcus*
真菌 Fungi	*Candida athensensis*, *Botrytis cinerea*, *Sporobolomyces roseus*, *Thermoascus*	*Saccharomycopsis*, *Lichtheimia*		*Saccharomycopsis fibuligera*, *Rhizopus oryzae*, *Sterigmatomyces elviae*, *Aspergillus flavus/oryzae*, *Hyphopichia burtonii*, *Pichia kudriavzevii*, *Lichtheimia corymbifera*	*Saccharomycopsis fibuligera*, *Hyphopichia burtonii*, *Rhizopus flavus/oryzae*, *Sterigmatomyces elviae*, *Aspergillus*, *Pichia kudriavzevii*, *Lichtheimia corymbifera*	

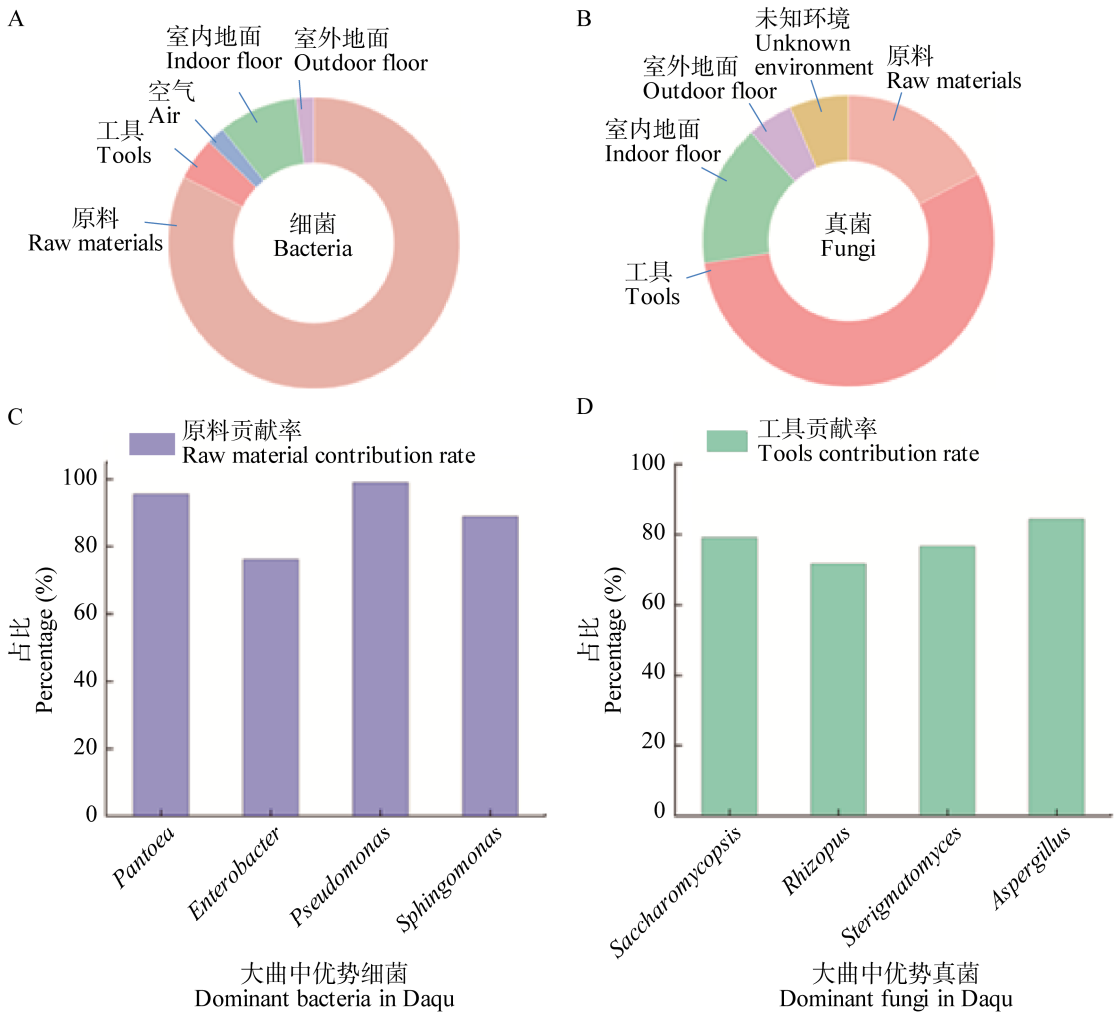

图 4　大曲微生物溯源分析　A：大曲中细菌溯源分析. B：大曲中真菌溯源分析. C：原料对大曲中优势细菌平均贡献率. D：工具对大曲中优势真菌平均贡献率

Figure 4　Microbial traceability analysis of Daqu. A: Bacterial traceability analysis in Daqu. B: Fungal traceability analysis in Daqu. C: Average contribution of raw materials to the dominant bacteria in Daqu. D: Average contribution of tools to the dominant fungi in Daqu.

5　总结与展望

大曲作为糖化发酵剂对白酒的风味和品质有重要影响，其中的微生物种类和数量决定着白酒的出酒率、酒质和风味成分。因此，对大曲微生物群落结构的研究有利于更深层次地认识大曲微生物群落结构与功能，揭示固态发酵机理。然而，由于技术的限制和认知的不完全，对大曲微生物群落的认识还不够全面，传统的微生物培养方法极大地限制了微生物菌群结构的研究。近年来，研究人员结合多组学技术与生物统计学分析，系统剖析了大曲群落结构和酶系形成机制，揭示了大曲中优势菌群以及功能作用，确定了驱动大曲群落演替的关键因素，从而进一步指导微生物群落功能的定向调控和合成微生物群落的构建。相信随着研究的不断深入，高通量测序技

术结合分子生物学技术在白酒行业中的应用将更加广泛，在微生物资源挖掘、品质控制及产品开发等方面将发挥巨大作用，为我国白酒行业的快速发展提供强有力的技术支撑。

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