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·多样性及代谢·

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组学技术在产油微生物中的应用

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摘 要: 微生物油脂是未来燃料和食品用油的重要潜在资源。近年来，随着系统生物学技术的快速发展，从全局角度理解产油微生物生理代谢及脂质积累的特征成为研究热点。组学技术作为系统生物学研究的重要工具被广泛用于揭示产油微生物脂质高效生产的机制研究中，这为产油微生物理性遗传改造和发酵过程控制提供了基础。文中对组学技术在产油微生物中的应用概况进行了综述，介绍了产油微生物组学分析常用的样品前处理及数据分析方法，综述了包括基因组、转录组、蛋白 (修饰) 组及代谢 (脂质) 组等在内的多种组学技术，以及组学数据基础上的数学模型在揭示产油微生物脂质高效生产机制中的研究，并对未来发展和应用进行了展望。

关键词: 基因组，转录组，蛋白组，代谢组，产油微生物

Application of omics technology in oleaginous microorganisms

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Abstract: Microbial oils are potential resources of fuels and food oils in the future. In recent years, with the rapid development of systems biology technology, understanding the physiological metabolism and lipid accumulation

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characteristics of oleaginous microorganisms from a global perspective has become a research focus. As an important tool for systems biology research, omics technology has been widely used to reveal the mechanism of high-efficiency production of oils by oleaginous microorganisms. This provides a basis for rational genetic modification and fermentation process control of oleaginous microorganisms. In this article, we summarize the application of omics technology in oleaginous microorganisms, introduced the commonly used sample pre-processing and data analysis methods for omics analysis of oleaginous microorganisms, reviewe the researches for revealing the mechanism of efficient lipid production by oleaginous microorganisms based on omics technologies including genomics, transcriptomics, proteomics (modification) and metabolomics (lipidomics), as well as mathematical models based on omics data. The future development and application of omics technology for microbial oil production are also proposed.

Keywords: genome, transcriptome, proteome, metabolome, oleaginous microorganisms

油脂在食品生产、营养补充剂、清洁剂、润滑剂和生物燃料等方面均有较高的需求。随着人口的增加和耕地面积的减少，微生物油脂(Microbial oils) 成为了近年来的研究热点，且被认为是未来食品和燃料用油重要的潜在资源[1-2]。微生物油脂，又称单细胞油脂 (Single cell oils，SCO)，是指由微生物发酵生产的脂质。微生物油脂具有生产周期短、不受季节气候影响、不占用耕地且易于大规模生产的特点，符合绿色可持续发展的理念[3]。通常情况下，微生物胞内积累的脂质含量达到菌体干重 20%以上即可被认为具有工业化生产油脂的潜力，称之为产油微生物[4-5]。产油微生物主要包括细菌、酵母、丝状真菌及藻类等，不同产油微生物的油脂产量相差巨大，其油脂积累量从细胞干重的 20%到 80%以上不等，且产油微生物有不同的产脂特点[1,6-7]。产油真菌(酵母和丝状真菌) 胞内脂质积累量在 30%–70%之间，产油酵母合成的脂肪酸以十六碳和十八碳的饱和、不饱和脂肪酸为主，而产油丝状真菌在胞内积累大量脂质以十八碳以上的多不饱和脂肪酸为主，如亚油酸、γ-亚麻酸、花生四烯酸及二十碳五烯酸等。产油藻类胞内脂质积累量通常在 20%–70%之间，藻类能够合成高附加值的长链多不饱和脂肪酸，尤其是二十二碳六烯酸。产油细菌胞内脂质积累量最高可达 80%以上，合成的脂质以类脂为主，主要成分为聚羟基烷酸。

1 产油微生物研究进展

关于微生物油脂的研究已有较长的历史。20 世纪 80 年代之前 (1980s)，揭示产油微生物油脂积累的生化基础是相关领域科研工作者关注的焦点[8]。在随后的 20–30 年里，随着 omega-3、omega-6 脂肪酸在包括人类在内的高等生物中的结构和功能作用被逐渐认识，利用微生物工业化生产高附加值油脂开始引起研究人员的兴趣。产油微生物脂质积累过程中涉及的脂质合成途径及关键酶的酶学性质在生化层面得到了更为全面的阐明和验证[9]。在此基础上提出了多种针对培养基组成、发酵过程控制和代谢工程改造的策略，微生物油脂逐渐进入工业化时代[10-14]。同时，在油脂高产菌株的诱变、育种工作方面[15]，相关的胞内油脂实时监测等高通量筛选方法被迅速开发[16-18]。随着近些年分子生物学、基因编辑以及高通量测序技术的快速发展，大量研究借助组学技术从系统生物学层面完成了产油模式菌株脂质合成全途径构建[19-21]。本文从代谢、调控、信号传导等多个层次系统地对产油微生物脂质积累的代谢及调控机制进行了解析和阐述，并以此为基础指导发酵过程控制、代谢工程改造和脂质合成细胞工厂的构建[22-23]。

2 组学技术在产油微生物中的应用

组学 (Omics) 主要包括基因组学、转录组

学、蛋白 (修饰) 组学、代谢 (脂质) 组学以及在其基础上构建的代谢网络模型，是以高通量检测与生物信息学技术为基础的系统生物学分析方法，能够从基因组规模反映生物体的全局转录、翻译及代谢情况。自组学概念提出以来，在技术方面一直处于较快的发展和持续的更新中，目前已在医药、农林、食品等多个领域的基础、临床、生产研究中广泛应用[24-27]。近些年，随着系统生物学与合成生物学的快速发展,基于组学分析的系统发酵优化以及遗传改造策略被提出,且已在工业微生物领域得到了广泛研究和成功应用[28-31]。

近几年，组学技术在产油微生物中得到广泛应用，相关研究内容主要集中在以下几方面。首先是组学分析方法的评估和优化,涉及样品收集、前处理、提取、检测及数据分析等流程[32-36]。第二方面是基于组学分析结果指导发酵工艺优化及过程控制。通过组学分析明确微生物在发酵过程中对环境及营养的响应，并针对性地对微生物发酵不同阶段的环境参数和包括基本营养物、中间代谢物在内的多种成分进行更为合理的阶段控制和补加[37]。第三方面主要是采用单一组学或多组学整合技术从不同维度解析影响产油微生物生长、代谢及脂质合成的代谢及调控机制[38-41]。通过组学分析确定菌株发酵过程中与菌株生长及目标产物合成相关的关键基因、蛋白和代谢途径，并提出合理的代谢工程策略[19,42]。

2.1 产油微生物组学技术分析方法学概况

组学分析主要可分为样品制备和检测分析两大部分[35,43-44]。样品制备主要包括样品收集、淬灭、提取等步骤[35,44-45]。由于不同样品 (细胞、组织、粪便、土壤等) 在来源和形态上存在较大差异，优化和评估样品前处理方法是保证后续检测结果稳定准确的前提条件。产油微生物样品制备方法主要参考微生物种类，微生物包括单细胞形式存在的大多数细菌、酵母及藻类等，也包括多细胞形式存在的丝状真菌，由于不同产油微生物菌体形态不同，在样品前处理上也有着明显差异。在样品收集方法上，细菌、酵母和藻类通常采用离心法分离细胞和发酵液[46-47]，而丝状真菌通常采用快速抽滤法 (表 1–4)[48]。在淬灭方法上，液氮快速冷冻在不同产油微生物中均最为常用[49]，也有部分研究在样品收集后进行超低温保藏 (表 1–4)[47,50]。对于目标物 (DNA、RNA、蛋白或代谢物) 提取，用于基因组和转录组分析所需的核酸提取方法在不同产油微生物间差异不大,目前主要采用 Trizol 试剂或商业化的提取试剂盒[47,51]。而对用于蛋白组、代谢组 (脂质组) 分析所需的胞内蛋白和代谢物的提取在提取试剂和步骤上差异较大 (表 1–4)。产油微生物胞内总蛋白提取目前常用方法包括两种，一种是在植物中广泛应用的 Tris-饱和酚提取法，另一种是比较通用的尿素裂解液提取法，部分实验组还考虑加入了一定量的十二烷基硫酸钠 (Sodium dodecyl sulfate，SDS) 或二硫苏糖醇 (Dithiothreitol，DTT)[52-53]。代谢物提取目前常用的提取溶剂包括甲醇-水、乙腈-水、乙醇-水 (热提取) 等[54]。甲醇-氯仿是目前产油微生物胞内脂质提取最为常用的提取剂[55]。近些年，考虑到提取试剂的安全性和提取操作方便性，甲基叔丁基醚也在细胞脂质提取中得到广泛应用[56-57]。通常情况下，产油微生物胞内物质提取过程中会采用液氮研磨或超声等手段对菌体进行破碎以提高目标物的浸出效率[58-59]。由于产油微生物胞内脂质积累量较多，在进行目标物提取时，选取丙酮、氯仿等在提取前或提取过程中对样品进行除脂处理可获得质量更高的目标物，以减少脂质对后期检测的干扰[60]。

对于目标物的检测，基因组和转录组测序目前均采用 Illumina 测序平台[61-66] (表 1–2)。代谢组检测主要采用的是液相色谱质谱联用 (LC-MS/MS) 和气相色谱质谱联用 (GC-MS)[67]，还有部分代谢组分析采用核磁 (NMR) 技术[68]，而脂质组分析均采用 LC-MS/MS 平台[69-70](表 4)。

组学分析另外一个重要的影响因素是参考数据库的选择。产油微生物基因组注释目前主要是参考已有的同源物种基因组信息，如借助 NCBI、UniPort、KEGG、GO 等数据库进行基因功能和代谢途径等注释。尽管目前转录组和蛋白组分析仍以无参分析最为常见，但随着测序技术快速的发展和费用降低，完成全基因组测序的产油菌株数量快速上升。因此，利用自身基因组构建本地库进行转录组和蛋白组结果注释 (有参分析) 也逐渐增多。对于代谢组代谢物注释，目前主要是借助一些商业化 (如 NIST、mzCloud 等) 和开源的数据库 (如 HMDB、MoNA 等) 来完成代谢物的鉴定工作，也有部分研究机构和商业化公司会自行构建代谢组数据库。

2.2 组学技术在产油微生物脂质积累机制研究中的应用

2.2.1 基因组分析

在基因组层面的应用主要通过比较基因组学分析，包括产油微生物彼此间比较 (如高低产菌株)、产油和非产油菌株间比较，以及基于基因组的代谢网络模型(Genome-scale metabolic model，GSMM)构建 (表 1)。随着测序技术的进步和费用的下降，完成全基因组测序的微生物菌株数量逐年上升，这为产油微生物比较基因组研究提供了基础[71]。笔者课题组前期完成了高山被孢霉 *Mortierella alpina* ATCC 32222 的全基因组测序，构建了高山被孢霉脂质合成全途径[72]，并在此基础上构建了高山被孢霉基因组规模的代谢网络模型，系统阐述了高山被孢霉脂肪酸合成所需还原力 NADPH 和前体乙酰辅酶 A 的主要来源途径[73]。Sheng 等[74]将产油酵母发酵丝孢酵母 *Trichosporon fermentans* CICC 136 基因组与其他产油微生物进行基因组比较发现，*T. fermentans* CICC 136 具有高度的基因重复性和独特的基因组组成，与圆红冬孢酵母 *Rhodosporidium toruloides* NP11 和解脂耶氏酵母 *Yarrowia lipolytica* CLIB122 相比，其脂肪酸延伸和降解相关的基因数量高出 3–4 倍，这些结果提示 *T. fermentans* CICC 136 具有较强的脂肪酸合成和代谢能力。Vorapreeda 等[75]将产油微生物和非产油微生物基因组进行对比发现，有 209 个直系同源蛋白序列存在于产油微生物中，分布于细胞的多个生物学过程，代谢分类发现这些基因主要参与脂肪酸和脂质前体，尤其是乙酰辅酶 A 的合成。该作者在产油微生物中还发现了一系列负责通过柠檬酸分解代谢、脂肪酸 β 氧化、亮氨酸代谢和赖氨酸降解来产生脂肪酸合成所需的关键双碳代谢物的同源蛋白，并指出在生物合成双碳代谢物乙酰辅酶 A 的过程中,碳水化合物、脂质和氨基酸代谢之间存在密切关系，有助于其脂质生成。Vongsangnak 等[76]通过对 3 株产油模式微生物基因组规模代谢模型比较分析发现，卷枝毛霉 *Mucorcircinelloides* WV1213 相比 *M. alpina* CY1106 和 *Y. lipolytica* YL619_PCP 具有更多参与碳水化合物、氨基酸和脂质代谢的基因，这有利于 *M. circinelloides* WV1213 营养利用的多样化，提高其脂质含量。

可以看出，目前产油微生物基因组比较主要是考察不同菌株间参与脂质合成途径基因数量、脂肪酸合成前体乙酰辅酶 A 合成能力以及对营养的利用差异。

2.2.2 转录组分析

在转录层面的应用主要包括转录组分析、转录因子挖掘、转录调控网络的构建。转录组是目前在产油微生物中应用最为广泛的组学技术 (表 2)。产油微生物自身具有较强的脂质积累能力，而特定营养或环境胁迫会诱导产油微生物胞内脂质积累量大幅提高。当外部营养或环境发生改变时，产油微生物最先做出的响应往往发生在调控层面，激发自身的抗逆信号响应途径，进而引发全局性代谢重编程。笔者课题组前期完成了对产油丝状真菌高山被孢霉 ATCC 32222 氮限制培养

过程的时间序列转录组分析，将高山被孢霉与其他产油微生物和非产油微生物全细胞转录本进行了比较，确定了磷酸戊糖途径在产油微生物脂质合成所需还原力 NADPH 供应上的重要作用[77]。Morin 等[78]对产油微生物 *Y. lipolytica* 从生物量合成转向脂质积累这一过程进行时间序列转录组分析，通过差异表达基因鉴定以及时间序列趋势聚类分析，作者详细描述了解脂耶氏酵母从生长转向脂质积累过程中不同阶段细胞基因表达特征，探究了解脂耶氏酵母作为产油微生物的潜力，鉴定出了参与脂质积累过程中的关键基因。Wang 等[79]以玉米浆作为氮源，通过比较培养基中不同玉米浆添加量对产油微生物破囊壶菌 *Aurantiochytrium* sp. YLH70 转录组的影响发现，高中低含量玉米浆培养的 *Aurantiochytrium* sp. YLH70 转录本间有着显著性差异，玉米浆含量 (氮含量) 对细胞产脂的影响是一个全局性的调控，特定信号传导及其相关转录因子在这一全局调控中发挥了重要作用。Ajjawi 等[80]在微拟球藻 *Nannochloropsis gaditana* 中通过敲除真菌同源的 $Zn(Ⅱ)_2Cys_6$ 编码基因使得菌株脂质产量相比野生型菌株提高了 2 倍。Hu 等[81]对 *Nannochloropsis* sp.进行了基因组规模转录因子及其结合位点的鉴定，通过比较 6 株微拟球藻基因组相关的 68 个转录因子结合位点 motifs，11 个转录因子被预测为与脂质代谢和光合作用相关，将微拟球藻 *Nannochloropsis oceanica* IMET1 的转录因子，转录因子结合位点 motifs 与植物参考数据库进行比对，共预测出 78 个转录因子-转录因子 motifs 互作对，这些互作对包括 34 个转录因子 (11 个参与甘油三酯合成)，30 个转录因子潜在位点 motifs 以及 2 368 个转录因子与目的基因间的调控连接点，这项研究为进一步在微拟球藻中构建用于微生物油脂生产的转录调控网络奠定了基础，同时也为基于全局转录调控的脂质高效合成策略提供了参考。

2.2.3 蛋白组分析

在蛋白组翻译层面的应用主要包括全细胞蛋白组分析、蛋白修饰组分析以及脂滴蛋白组分析 (表 3)。Guarnieri 等[83]通过比较蛋白组分析小球藻 *Chlorella vulgaris* 中可用于促进脂质合成的基因工程靶点，鉴定出了包括转录因子以及参与细胞信号和循环调控相关的新的靶点蛋白，这些结果为基因改良策略奠定了基础，同时也证明了蛋白组学分析在指导产油微生物遗传改造中发挥的重要作用。笔者课题组前期借助蛋白组以及比较蛋白组技术，分别考察限氮培养以及产油/非产油菌株蛋白谱差异，解析了产油真菌卷枝毛霉脂质积累的机制[82,93]。Pomraning 等[40]对 *Y. lipolytica* 氮限制培养过程中胞内蛋白进行了磷酸化蛋白组分析，共鉴定出 1 219 个新的蛋白磷酸化位点在氮限制发酵培养过程中有显著性变化，整合蛋白组、代谢组数据系统分析了氮限制诱导解脂耶氏酵母脂质积累的作用机制。除了对产油微生物进行全细胞组学分析外，亚细胞组学分析在近些年也受到了较多的关注，尤其是脂滴蛋白组分析。Zhu 等[84]考察了不同营养限制条件下 *R. toruloides* 胞内脂滴蛋白组组成，总计鉴定出 226 种脂滴蛋白，这些蛋白主要参与脂质代谢、脂滴形成和进化。通过比较营养限制对脂滴蛋白表达的影响，该团队进一步确定并证明了脂滴结构蛋白 Ldp1 作为脂滴中的标志蛋白在调控细胞脂滴动态变化中的重要作用。Yu 等[85]对产油微生物 *M. alpina* 脂滴蛋白组进行分析，检出蛋白超过 400 种，整合全细胞蛋白组和代谢组分析，从多个角度系统考察了细胞老化过程中高山被孢霉蛋白变化及其与细胞生长、脂质积累之间的关系。

2.2.4 代谢组分析

组学技术在代谢层面对产油微生物的考察主要包括代谢组学、脂质组学以及代谢流分析 (表 4)。代谢组分析主要是对产油微生物非胁迫或胁迫条件下胞内极性代谢物进行定性和定量分析，主要涉及碳代谢、氮代谢、氨基酸代谢、核酸代谢以及能量代谢等。脂质组分析主要是对产油微生物

表 1 基因组技术在产油微生物中的代表性应用案例

Table 1 Representative cases of genomics techniques application in oleaginous microorganisms

Strains	Omics	Sample collection/extraction	Platform	Research content	Reference
Mucor circinelloides	Genomics	Filtration/grinding with liquid nitrogen/Benzene Trizol extraction	Illumina	Comparative genome	[48]
Rhodosporidium toruloides	Genomics	Liquid nitrogen quenching after centrifugation/ phenol-chloroform-isopropanol extraction	Illumina GA Ⅱ or GA Ⅱx	/	[46]
Trichosporon fermentans	Genomics	Yeast DNA Extraction Kit	Illumina	Comparative genomics	[74]
/	Genomics	/	/	Comparative genomics	[75]
Nannochloropsis	Genomics	/	/	Transcription factor	[81]
Chlorella vulgaris	Genomics	/	Illumina HiSeq2 000	Genome sequencing	[71]
Mortierella alpina	Genomics	Filtration/grinding with liquid nitrogen/phenol-chloroform-isopropanol extraction	Sanger	/	[72]
Mucor circinelloides	GEM	/	/	Strains comparison	[76]

GEM: Genome-scale metabolic model.

表 2 转录组技术在产油微生物中的代表性应用案例

Table 2 Representative cases of transcriptomics techniques application in oleaginous microorganisms

Strains	Omics	Sample collection/extraction	Platform	Research content	Reference
Rhodosporidium toruloides	Transcriptomics	Centrifugation/grinding with liquid nitrogen/Trizol extraction	Illumina HiSeq2 000	N limitation/ medium	[46]
Nannochloropsis sp.	Transcriptomics	Plant RNA extraction kit extraction after centrifugation	IlluminaHiseq2 000	Different phase	[51]
Chlamydomonas Reinhardtii	Transcriptomics	Centrifugation/-80 °C storage/kit extraction	Illumina	Time-series	[47]
Chlamydomonas reinhardtii	Transcriptomics	Centrifugation/-80 °C storage/kit extraction	Illumina	N limitation	[50]
Phaeodactylum tricornutum	Transcriptomics	Centrifugation/liquid nitrogen quenching/kit extraction	Illumina	N limitation	[49]
Chlamydomonas reinhardtii	Transcriptomics	Centrifugation/-80 °C storage/kit extraction	Illumina HiSeq2000	Cold stress	[62]
Chlorella sp.	Transcriptomics	Centrifugation/grinding with liquid nitrogen/Trizol extraction	Illumina	UV stress	[63]
Neochloris Oleoabundans	Transcriptomics	Centrifugation/liquid nitrogen quenching/kit extraction	Illumina Hiseq2000	N limitation	[64]
Schizochytrium sp.	Transcriptomics	Centrifugation/-80 °C storage/ Trizol extraction	Illumina Hiseq2000	Oxygen	[65]
Aurantiochytrium sp.	Transcriptomics	Centrifugation/-80 °C storage/ Plant RNA extraction kit extraction	Illumina HiSeq	N limitatiom	[66]
Yarrowia lipolytica	Transcriptomics	Liquid nitrogen quenching/-80 °C storage/RNA extraction kit extraction	/	Different phase	[78]
Aurantiochytrium sp.	Transcriptomics	Centrifugation/ddH_2O Clearing/ -80 °C storage/Trizol extraction	Illumina	N source	[79]
Chlorella vulgaris	Transcriptomics	Centrifugation/grinding with liquid nitrogen/RNA extraction kit extraction	Illumina	N limitation	[58]
Mortierella alpina	Transcriptomics	Filtration/grinding with liquid nitrogen/Trizol extraction	Illumina GA Ⅱx sequencing platform	N limitation	[77]

表 3 蛋白组技术在产油微生物中的代表性应用案例

Table 3 Representative cases of proteomics techniques application in oleaginous microorganisms

Strains	Omics	Sample collection/extraction	Platform	Research content	Reference
Mucor circinelloides	Proteomics	Liquid nitrogen quenching after vacuum filtration/Tris saturated phenol extraction	2-DE/MALDI/TOF/TOF	N limitation	[82]
Rhodosporidium toruloides	Proteomics	Centrifugation/grinding with liquid nitrogen/urea extraction	2-DE/nanoLC/MS/MS analysis	N limitation	[46] [59]
Chlorella vulgaris	Proteomics	Centrifugation/liquid nitrogen quenching/ sodium chloride-DTT- glycerin extraction	GeLC/MS/label-free	N limitation	[83]
Chlamydomonas reinhardtii	Proteomics	-80 °C storage/freeze drying/acetone degreasing/urea extraction	2-DE/MALDI-TOF	Systematic evolution	[60]
Chlorella vulgaris	Proteomics	Centrifugation/liquid nitrogen quenching/ sodium chloride-DTT-glycerin extraction	GeLC/MS/MS	N limitation	[83]
Mortierella alpina	Proteomics	Washing with PBS, centrifuge/SDS extraction	Easy nLC/MS/MS	Aging	[54]
Yarrowia lipolytica	Proteomics	Liquid nitrogen quenching vacuum filtration/urea extraction, chloroform methanol degreasing	iTRAQ/NanoLC/LTQ Orbitrap/MS	N limitation	[40]
Chlorella vulgaris	Proteomics	Centrifugation/liquid nitrogen quenching/ sodium chloride-DTT-glycerin extraction	nanoLC/MS/MS	N limitation	[52]
Rhodosporidium toruloides	Proteomics	Extraction with chloroform and acetone after washing with ddH_2O	LC/LTQ/MS/MS	N/P limitation	[84]
Mortierella alpina	Proteomics	Cells are filtered with PBS, and gradient centrifugation/SDS-PAGE	LC/MS/MS	Aging	[85]
Mortierella alpina	Proteomics	Liquid nitrogen quenching after vacuum filtration/urea extraction	Easy nLC/MS/MS	Tine-resolved	[86]
Mortierella alpina	Proteomics	Centrifugation/SDS extraction	Easy nLC/MS/MS	Aging	[53]

表 4 代谢组技术在产油微生物中的代表性应用案例

Table 4 Representative cases of metabolomics techniques application in oleaginous microorganisms

Strains	Omics	Sample collection/extraction	Platform	Research content	Reference
Yarrowia lipolytica	Metabolomics	Liquid nitrogen quenching after vacuum filtration/chloroform-methanol water extraction	GC/MS	N limitation	[40]
Yarrowia lipolytica	Metabolomics	Centrifugation/liquid nitrogen quenching, vacuum drying/chloroform-methanol water extraction	GC/MS	Fermentation process	[55]
Mortierella alpina	Metabolomics	Liquid nitrogen grinding and ethanol thermal extraction	GC/TOF/MS	Aging	[54]
Yarrowia lipolytica	Metabolomics	ddH_2O after vacuum filtration/acetonitrile water extraction	GC/TOF MS	Medium	[87]
Yarrowia lipolytica	Metabolomics	Cold glycerol quenching/methanol water extraction	GCMS	N source	[88]
Mortierella alpina	Metabolomics	Liquid nitrogen quenching after vacuum filtration/ methanol water	GCMS	Method	[36]
Chlorella protothecoides	Metabolic flux	Centrifugal hydrochloric acid hydrolysis	GCMS/NRM	N limitation	[89]
Yarrowia lipolytica	Metabolic flux	Centrifugal hydrochloric acid hydrolysis	GCMS	N limitation	[90]
Yarrowia lipolytica	Lipidomics	Freeze drying after centrifugation/ chloroform-methanol extraction	UPLC/MS/MS	Fermentation process	[55]
Mortierella alpina	Lipidomics	Freeze-drying/MTBE	UPLC/MS/MS	N source	[91]
Ettlia oleoabundans	Lipidomics	Centrifugation/chloroform methanol water extraction	LC/QToF MS	Time series	[92]

胞内脂质种类 (甘油三酯、磷脂、固醇、甾体及脂肪酸)、含量和组成进行全面的考察。通过对产油微生物不同生长或脂质合成阶段胞内代谢物差异进行多元统计学、差异代谢物以及代谢途径富集分析，确定影响产油微生物脂质积累的关键代谢途径及其限制脂质高效合成的限速步骤，进而为培养基优化、发酵过程控制以及代谢工程改造提供依据和指导。Yun 等[87]将代谢组学用于揭示不同培养基和碳源对解脂耶氏酵母脂肪酸合成的影响。Zhao 等[88]借助代谢组结果将细胞代谢分为不同模块，通过计算代谢模块丰度来直接解释代谢组数据，通过对不同碳源条件下解脂耶氏酵母代谢模块丰度进行计算，确定了不同碳源培养条件下解脂耶氏酵母从生长到脂质积累过程中胞内代谢的具体变化情况，这一研究丰富了代谢组学技术在产油微生物中的应用，为利用代谢组学数据了解细胞代谢以提高目标产物的产量提供了新的视角。Matich 等[92]采用时间序列脂质组分析，对产油微藻富油新绿藻 *Ettlia oleoabundans* 在氮限制培养条件下胞内脂质组成及含量动态变化进行分析，揭示了氮限制条件下甘油三酯 (TAG) 积累以及其他脂类的降解与光合效率之间的关联性。笔者课题组前期对高山被孢霉胞内脂质组成进行了分析，借助脂质组学技术，系统考察了不同氮源影响产油真菌高山被孢霉 TAG 积累的作用机制，确定了 TAG 和脂肪酸合成途径中影响高山被孢霉脂质合成的关键基因[91]。基于 ^{13}C 标记实验，代谢流分析已成为一种整合实验和计算结果的有力工具，用于识别生化网络和定量细胞内代谢通量在整个中心碳代谢中的分布情况。近年来，代谢通量组学 (Fluxome) 已被用于探索产油微生物油脂积累的生化机制[94]。Xiong 等[89]对产油微藻原球藻 *Chlorella protothecoides* 0710 进行了 ^{13}C 标记实验，发现磷酸戊糖途径在限氮条件下相对活性增加，提供了脂质积累过程所需要的 NADPH。而在产油酵母丝孢酵母菌 *Trichosporon cutaneum* 中，代谢流分析表明胞质苹果酸酶是 NADPH 的主要来源，柠檬酸裂解酶(ACL) 是脂质积累过程中乙酰辅酶 A 的主要来源[95]。Zhang 等[90]研究以 ^{13}C 标记的葡萄糖为基础，对生长在富氮和限氮条件下的 *Y. lipolytica* 进行代谢流分析，证明氮限制条件下 NADPH 并不是解脂耶氏酵母脂质积累的限制因素，而氮限制显著提高了 ACL 对柠檬酸的裂解强度，该过程是脂肪酸合成所需前体乙酰辅酶 A 的主要来源。

2.2.5 多组学整合分析

微生物脂质合成是多层次网络相互调节作用的结果，单一组学的数据不足以全面、真实地反映细胞的代谢活动。近些年，多组学整合分析案例在产油微生物中的应用案例逐渐增多，如转录组-代谢组整合[96]、蛋白组-代谢组整合[97]、转录组-蛋白修饰组整合以及 3 种及以上组学的整合分析[98-99]。另外，在多组学数据基础上构建的基因组代谢网络模型也在产油微生物中得到了广泛应用。通过整合产油微生物发酵过程中宏观参数与多组学数据，能够更好地将细胞表型 (如理化指标、产脂特点) 与分子层面的全局代谢变化相关联，进而从信号调控、转录翻译、生化反应等多个维度提出精准靶向的工程改造策略，指导发酵工艺的优化及细胞工厂的构建。笔者课题组近期借助时间序列多组学技术，系统考察了氮限制培养过程中高山被孢霉胞内全局代谢动态变化，确定氮限制诱导的资源再分配在脂质积累过程中的关键作用，并确定了胞内资源再分配过程中对脂质合成发挥关键作用的基因、代谢途径及生物学过程[86]。Pomraning 等[40]整合蛋白组、磷酸化蛋白组学以及代谢组学对氮限制调控解脂耶氏酵母脂质代谢的作用机制进行了解析，用于鉴定操控脂质积累的靶向基因和调控因子。基因组代谢模型是研究生物体代谢的重要工具，该模型采用系统生物学方法通过整合基因组学、转录组学、蛋白组学和代谢组学等多组学数据构建生物体代谢

网络，目前在产油微生物中已广泛用于分析生物体代谢特征，预测生物生长表型，解释实验数据，并指导工程菌株构建。整合基因组信息以及蛋白表达数据，Vongsangnak 等[76,100]借助基因组代谢网络模型，深入剖析了脂质高产菌株 *M. circinelloides* WJ11 脂质高产的代谢特征以及其与其他菌株间的代谢差异，鉴定出一系列可作为代谢工程靶点的候选基因。

3 总结与展望

近年来，组学技术 (基因组学、转录组学、蛋白质组学和代谢组学) 和基于组学的数学模型在生命科学领域变得越来越流行，而基于组学分析的系统发酵优化和遗传改造策略在产油微生物中也得到广泛应用。一方面通过组学分析明确了产油微生物在发酵过程中对环境及营养的响应，另一方面采用单/多组学整合技术从不同维度解析了影响产油微生物生长代谢及脂质合成的调控机制。

大量研究表明，产油微生物胞内脂质积累对培养环境及营养条件敏感，特定的环境及营养胁迫能够诱导产油微生物胞内脂质大量积累，比如氮限制有利于胞内脂质和长链多不饱和脂肪的积累，但氮限制同样也会导致产油微生物生物量合成的减少。因此，基于压力胁迫的脂质调控策略一直未能够很好地应用到产油微生物脂质高效合成中。组学技术能够从系统水平考察环境及营养胁迫条件下产油微生物胞内细胞代谢及脂质代谢情况，解析胁迫环境诱导产油微生物脂质积累的机制。胁迫诱导脂质积累是一个涉及信号调控，蛋白转录、翻译、修饰以及物质代谢的全局调控过程，这就要求我们在后续的研究中更多地将多组学数据整合，从不同尺度确定影响产油微生物脂质积累的关键代谢途径和生物学过程，使我们能从全局角度提出更为精准地调控脂质合成的策略。

近些年来，随着测序技术的不断发展和进步，单细胞测序技术已成为新的研究热点，而单细胞测序技术在产油微生物还暂无报道，对产油微生物不同细胞产脂情况进行分析，并从单细胞组学层面解析不同细胞产脂差异的作用机制对于产油微生物菌种优化以及工程改造将提供更为精准和合理的参考。

此外，产油微生物胞内脂质积累是多层次网络调控作用的结果，在获得多种组学数据的基础上，借助数学模型构建产油微生物脂质合成的代谢及调控网络模型，如基因组代谢网络模型、基因组转录调控网络模型等，进而确认网络中的关键节点，发现其中的关键转录及调控因子。最终开展以 ^{13}C 和 ^{14}N 标记的碳氮代谢流分析，对产油微生物产脂过程中细胞代谢活动动态变化进行考察，使其中涉及的碳氮通量和胞内代谢物池能够更好地将细胞代谢活动为脂质合成提供动力。

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