生 物 工 程 学 报
Chinese Journal of Biotechnology
http://journals.im.ac.cn/cjbcn
DOI: 10.13345/j.cjb.230026



Nov. 25, 2023, 39(11): 4463-4481
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·综 述·

# 肠道微生物资源库的构建：进展、方法和展望

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冯赛赛，柳利平，张亮亮，徐建国. 肠道微生物资源库的构建：进展、方法和展望[J]. 生物工程学报，2023，39(11): 4463-4481.
FENG Saisai, LIU Liping, ZHANG Liangliang, XU Jianguo. Construction of gut microbial culture banks: advances, methods and perspectives[J]. Chinese Journal of Biotechnology, 2023, 39(11): 4463-4481.

**摘 要：**基于肠道微生物组的活体药物(live biotherapeutics, LBPs)开发、菌株与宿主互作的分子机制研究及新型抗菌肽、酶、代谢途径的挖掘使得肠道微生物资源库的构建成为必然。本文对近年来国内外不同研究团队肠道微生物资源库构建进行系统比较，分析了不同构建方法间的差异，以期为国内外不同研究者在构建和丰富现有肠道微生物资源库方面提供帮助。目前，肠道微生物资源库共有 1 000 多种肠道细菌，分属于 12 个门、22 个纲、39 个目、96 个科和 358 个属，厚壁菌门(Firmicutes)、变形菌门(Proteobacteria)、拟杆菌门(Bacteroidota)、放线菌门(Actinomycetota)菌株最多。测序结果显示人肠道细菌物种丰富度在 2 000 左右，因此目前分离到的菌株远未达到饱和。在构建方法上，一般对粪便样本进行或不进行乙醇处理，使用非选择性培养基(以 Gifu 厌氧培养基为代表)进行涂布分离培养，最后进行纯化培养。使用较为简单的培养方法即可培养得到多数常见的重要肠道微生物类目，如双歧-乳杆菌属(*Lactobacillus-bifidobacteria*)菌株、阿克曼氏菌(*Akkermansia muciniphila*)、普拉梭菌(*Faecalibacterium prausnitzii*)、普雷沃氏菌属(*Prevotella*)及 S24-7 科菌株等。为满足功能研究和产品开发的需要，肠道微生物菌种资源库的样本来源应该进一步覆盖更多地域和生活习惯、疾病及健康状态具有显著差异的人群，从而进一步丰富肠道关键物种的菌株多样性。

**关键词：**肠道菌；资源库；构建

资助项目：来晋优博科研经费(02010011/0113)
This work was supported by the Research Foundation for Outstanding Doctor in Shanxi (02010011/0113).
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Received: 2023-01-12; Accepted: 2023-04-24; Published online: 2023-05-09

# Construction of gut microbial culture banks: advances, methods and perspectives

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**Abstract:** Recently, the gut microbiota-based live biotherapeutics (LBPs) development, the interaction between gut microbial species and the host, and the mining of new antimicrobial peptides, enzymes and metabolic pathway have received increasing attention. Culturing gut microbial species is therefore of great importance. This review systemically compared the construction advances of gut microbial culture banks and also analyzed the differences of methods used by research groups to give insight into the construction and enrichment of gut microbial resources. Presently, the gut microbial culture banks have included more than 1 000 bacterial species, belonging to 12 phyla, 22 classes, 39 orders, 96 families, and 358 genera. Among these, Firmicutes, Proteobacteria, Bacteroidota, and Actinomycetota exhibited the greatest diversities at the species level. The sequencing data showed that there are more than 2 000 species inhibited in the human gut. Therefore, the cultured gut microbial species are far from saturation. In terms of the construction method, the stool samples were pre-treated with ethanol or directly spread and cultured in the non-selective nutritional rich medium (represented by Gifu anaerobic medium) to obtain single colony. Then single colony was further purified. Generally, a simplified isolation and culture method is sufficient to obtain the most common and important intestinal bacterial species, such as *Bifidobacteria-Lactobacillus*, *Akkermansia muciniphila*, *Faecalibacterium prausnitzii*, *Prevotella* and S24-7 family strains. Finally, microbial resources with great diversities at the strain level are required for further functional research and product development. Samples covering hosts with distinct physiological status, diets or regions are necessary.
**Keywords:** gut microbes; culture bank; construction

近年来，随着测序技术的成熟及人类微生物计划(Human Microbial Project, HMP)启动，肠道微生物的组成和功能受到了广泛的关注，已被证明可以影响宿主代谢、免疫及情绪行为[1-3]。16S rRNA扩增子测序及宏基因组测序技术表明肠道微生物组成和功能的紊乱与疾病的发生和发展密切相关，如肥胖及 II 型糖尿病患者肠道中拟杆菌门(Bacteroidota)与厚壁菌门(Firmicutes)的比例显著降低、癌症免疫疗法响应的患者肠道中阿克曼氏菌的丰度显著升高等[4-5]。动物实验模型表明补充特定菌株可以改善或逆转宿主的疾病表型，如补充霍氏真杆菌(*Eubacterium hallii*)可以显著改善糖尿病小鼠(ob/ob)的胰岛素抵抗，补充复合卵形拟杆菌(*Bacteroides ovatus*)可以显著增加小鼠肠道中免疫球蛋白 A (immunoglobulin A, IgA)的水平[6-7]。临床研究结果提示肠道微生物制剂可能在疾病干预中发挥重要作用，如菌群药物SER-109在基于182名复发性艰难梭菌(*Clostridium difficile*)感染患者的 3 期双盲随机对照试验中显著降低了抗生素治疗后患者复发率，其中SER-109组复发率12%，安慰剂组复发率40%[8]。

基于肠道共生菌在改善宿主健康及疾病干

预中的巨大潜力，2016 年美国食品药品监督管理局(Food and Drug Administration, FDA)发布了活菌药物(live biotherapeutic products, LBPs)指南[9]。指南中将活菌药物定义为：能够起到预防、治疗或治愈某种疾病作用的活的微生物、死菌或代谢产物(非疫苗)。O'Toole 等[10]称其为下一代益生菌，主要与具有较长食用历史、安全性得到肯定的传统益生菌区分开来。最具潜力的下一代益生菌包括阿克曼氏菌、多形拟杆菌(*Bacteroides thetaiotaomicron*)、普拉梭菌、丁酸梭菌(*Clostridium butyricum*)等。

值得一提的是，益生菌尤其是下一代益生菌或活菌药物走向大规模的食品或临床应用还有很长的路要走。近期多个大型临床队列研究显示，特定肠道微生物在干预疾病发生和发展方面无效甚至恶化疾病表型。例如，基于 1 315 个 23−30 周婴儿的随机双盲对照人群实验表明，短双歧杆菌(*Bifidobacterium breve*) BBG-001 对预防早产儿坏死性小肠结肠炎无效；基于 21 个健康个体的前瞻性纵向队列研究表明，益生菌干预不利于抗生素使用后的菌群复原[11-12]。要合理解释不同报道中的相悖的结果，真正意义上做到通过调控肠道菌群或基于菌群设计干预疾病需要更加深入的机制研究，明确微生物与宿主互作的分子机制。此外，肠道微生物资源库也是挖掘新型抗菌肽、活性酶等的储备库，随着计算机算法领域与微生物基因组信息的交叉融合、多组学和各个数据库的联用，使得从复杂多样的肠道微生物中挖掘和开发可用的微生物资源成为可能[13-14]。综上所述，依赖于可培养肠道微生物的机制研究、功能验证、微生物资源挖掘和产品开发要求在体外分离纯化和培养不同肠道微生物物种，构建人或其他动物尤其是模式动物的肠道微生物资源库。

## 1 国内外肠道微生物资源库构建的现状

2014 年，Rajilić-Stojanović 等[15]综述了 1 057 个可培养肠道微生物物种，其中包括 957 种细菌、8 种古菌、92 种真核微生物。2019 年，基于不同国家、地区 3 810 份粪便样本的宏基因组研究结果表明，当以平均核苷酸一致性指数(average nucleotide identity, ANI) 95%为阈值界定种时，人体肠道微生物包括 2 058 个不同种水平上的分类单元[16]。自 2007 年以来，国内外各个团队开始筛选和培养肠道微生物物种。表 1 列出了目前国内外各个研究团队构建的肠道微生物资源库的大致情况。已构建的肠道微生物资源库，宿主来源以人、小鼠为主，此外还包括猴子、猪等，宿主生理状态以健康人和小鼠为主，也包括肥胖、肠炎及肠应激综合征的个体。此外，小鼠及猪粪便样本还使用了不同基因型、不同饲养单位的动物。人粪便样本来源还涉及中国、美国、英国等多个不同国家和地区。

对表 1 中不同研究团队构建的肠道微生物资源库中代表菌株类目进行统计汇总，剔除少数没有明确种属分类关系的菌株及 2 株古细菌，共得到 1 066 种肠道细菌(详细分类及菌株信息等见附表 1–2，文中所有附表和附图均已上传在国家微生物科学数据中心，编号 NMDCX0000194)。这些细菌分属于 12 个门、22 个纲、39 个目、96 个科和 358 个属，其中厚壁菌门(669 种)、变形菌门(132 种)、放线菌门(121 种)和拟杆菌门(100 种)菌株数目远远超过其他 6 个门中的菌株(表 2)，其他分类水平的统计信息见附表 3–6)。厚壁菌门、变形菌门、放线菌门、拟杆菌门及其他菌门的菌株在更低分类层级上的组成情况见附图 1−5。

虽然目前分离得到的肠道菌株远没有覆盖肠道微生物种属水平(尤其是种水平)上的多样性，但是与肠道微生态或宿主生理紧密联系的基石菌类目已经被成功分离培养。表 3 列出了目前肠道

**表 1** 国内外已构建的肠道微生物资源库概览*

Table 1 Summary of constructed gut microbial culture banks*

| Source of fecal samples | Isolation conditions | Number of representative (total) isolated strains; Number of species (taxonomy) |
|---|---|---|
| Culturable Genome Reference, CGR[17] (BGI-Shenzhen, China) | | |
| Healthy Chinese individuals (115) | 11 (pretreatment 11) | 1 502 (6 487); 338 |
| Mouse Gut Microbial Biobank, mGMB[18] (Institute of Microbiology, Chinese Academy of Sciences, China; Liu C., et al.; 2020) | | |
| ob/ob mice (12) | 4 (pretreatment 2, culture medium 2) | 244 (1 437); 126 (77 newly identified species) |
| human Gut Microbial Biobank, hGMB[19] (Institute of Microbiology, Chinese Academy of Sciences, China) | | |
| Healthy Chinese individuals (239) | 67 (pretreatment 11, culture medium 18) | 1 170 (10 558); 400 (102 newly identified species, 28 newly isolated genera, 3 newly families) |
| *Macaca fascicularis* Gut Microbial Biobank, MfGMB[20] (Institute of Microbiology, Chinese Academy of Sciences, China) | | |
| *Macaca fascicularis* | 73 (culture conditions 73) | 250 (4 100); 97 (63 genera, 25 families, 4 phyla) |
| The Broad Institute-OpenBiome Microbiome Library, BIO-ML[21] (Massachusetts Institute of Technology, USA) | | |
| Healthy American individuals (11) | 19 (pretreatment 3, culture medium 12) | 7 758; 984 taxonomic units (133 genera, 40 families, 16 orders, 11 classes, 6 phyla) |
| Human Gut Bacteria Culture Collection[22] (Washington University School of Medicine, USA) | | |
| Healthy American individuals (2) (Four time points each person, totally 8 samples were collected) | 1 (GMM) | Approximately 30 000 colonies; 316 taxonomic units |
| The Human Gastrointestinal Bacteria Culture Collection, HBC[23-24] (Wellcome Sanger Institute, UK) | | |
| British (8), North Americans (12) | 2 (pretreatment 2, YCFA) | 737 (more than 10 000); 273 (105 newly isolated species, 31 families) |
| The Mouse Intestinal Bacterial Collection, miBC[25] (Technical University of Munich, Germany) | | |
| 8 genotype mice, 6 animal feeding facilities | 18 (culture mediums 18) | 100 (1 500); 76 (26 families) |
| Human Gut Bacteria Culture Collection[26] (McMaster University, Canada) | | |
| Healthy individuals and IBS patients (5) | 66 (culture medium 33, aerobic or anaerobic culture 2) | 79 to 27 taxonomic units |
| Human Gut Bacteria Culture Collection[27] (Aix Marseille Université, France) | | |
| African (2 thin individuals); European (1 obese individual) | 212 | 340 (32 500); 340 (174 newly isolated species, 117 genera, 7 phyla) |
| Human Gut Bacteria Culture Collection[28] (Aix Marseille Université, France) | | |
| Feces or samples collected from other body sites from both healthy individuals and patients(973) | Comparing numbers (70, 18) of culture conditions suitable for covering the majority of species | 1 057 (901 364); 1 057 (human gut species 531, human non-gut species 146, newly isolated species 197) |
| Pig Intestinal Bacterial Collection, PiBAC[29] (RWTH University Hospital, Germany) | | |
| Pig feces(APC1311/+, P53R167H/+, wild type; Germany animal feeding facility 1, American animal feeding facilities 2, Canadian farm 1; pigs with different ages, both males and females) | Culture medium 24 | 117 (more than 1 000): 100 (40 families, 9 phyla) |
| Human Microbial Project, HMP[30-31] (2007-To date) | | |
| Human | | Approximately 300 species |

*: Lines with a grey background describe the name and constructers of gut bacterial culture collections. Lines with a white background show the sample source, number of isolation conditions used in their studies as well as obtained strains through these culture conditions. Culture conditions include a combination of pretreatment methods, culture media, and also culture temperatures, and aerobic or anaerobic culture.

**表 2　已分离菌株门水平分布情况**

Table 2　Distribution of isolated gut microbial species at the phylum level

| Phylum | Species No. |
|---|---|
| Bacillota | 669 |
| Pseudomonadota | 132 |
| Actinomycetota | 121 |
| Bacteroidota | 100 |
| Campylobacterota | 16 |
| Fusobacteriota | 11 |
| Thermodesulfobacteriota | 6 |
| Synergistota | 4 |
| Fibrobacterota | 3 |
| Lentisphaerota | 2 |
| Spirochaetota | 1 |
| Verrucomicrobiota | 1 |
| Total (12 phyla) | 1 066 |

微生物资源库中基石菌的主要类目及功能，这些菌株从门水平到属水平上的分布情况见图 1。这些工作使得以前进化关系较为混乱的分类单元如梭菌纲(Clostridia)变得清晰明确。之前的研究表明梭菌纲菌株具有非单源、分类关系复杂等特点。事实上，随着梭菌纲菌株被不断分离培养和测序，现在认为常见的梭菌簇 XIVa 和 IV 菌株并非在进化关系上密切相关的分类单元[32]。近年来，根据这些类群菌株间的进化关系，梭菌纲菌株被划分到不同种属的分类单元中，如梭菌科(Clostridiaceae)、真细菌科(Eubacteriaceae)、毛螺菌科(Lachnospiraceae)、颤螺菌科(Oscillospiracease)和消化链球菌科(Peptostreptococcaceae)等，这些菌株在宿主生理中发挥着重要作用，如产生短链脂肪酸(short chain fatty acids, SCFAs)、调节宿主肠道免疫稳态等[33]。通过非特异性肠道微生物培养，共得到真细菌目菌株 400 种，不同研究中新分离和命名的菌株多属于真细菌目菌株。S24-7(后命名为 Muribaculaceae，拟杆菌门)为此前在 16S 扩增子测序中常注释到的科水平的分类单元，和阿克曼氏菌一起被认为具有在稳态条件下诱导免疫球蛋白 G (immunoglobulin G, IgG)免疫应答的能力，揭示其可能具有独特的免疫刺激活性，目前构建的肠道细菌资源库中已经可以分离得到该科 6 个属 8 种肠道菌[34]。阿德勒克罗伊茨菌属(*Adlercreutzia*)代表菌株 *A. equolifaciens* 可以代谢大豆异黄酮，产生具有雌激素活性的雌马酚，被报道和肝脏疾病密切相关，目前共分离得到 5 个种的该属菌株，代表菌株 *A. equolifaciens* 已经被不同的研究团队分离得到[35]。此外，通过非特异性的分离培养还可以成功分离得到之前报道的较难分离的阿克曼氏菌、普拉梭菌等。

**表 3　肠道基石菌类目及其潜在功能**

Table 3　Gut keystone species list and their potential functions

| Genus; Species | Potential functions |
|---|---|
| *Adlercreutzia*; *A. caecimuris*, *A. equolifaciens*, *A. faecis*, *A. mucosicola*, *A. muris* | Metabolizing isoflavonoids; producing equol; a reducing abundance in colitis individuals[36-38] |
| *Akkermansia*; *A. muciniphila* | Anti-obesity; anti-diabetes (both type I & II); a biomarker for responders who accepting immunotherapy against tumors; Amuc_1100 (cell surface pill-like protein) with TLR2 activation capability; phospholipid with non-canonical TLR2-TLR1 heterodimer activation capability; P9 (secreting protein) promoting GLP-1 production by L cells in an IL-6 dependent manner[39-41] |
| *Alistipes*; *A. communis*, *A. finegoldii*, *A. hominis*, *A. indistinctus*, *A. massiliensis*, *A. muris*, *A. onderdonkii*, *A. putredinis*, *A. senegalensis*, *A. shahii*, *A. timonensis* | Enriched in population with the high-fat diet; tolerance to bile acids; associated with anxiety and depression populations; linked to anxiety and depression[42-44] |

(待续)

(续表 3)

| Genus; Species | Potential functions |
|---|---|
| *Bacteroides*; *B. acidifaciens*, *B. caccae*, *B. caecimuris*, *B. capillosus*, *B. cellulosilyticus*, *B. clarus*, *B. coagulans*, *B. coprocola*, *B. coprophilus*, *B. difficilis*, *B. dorei*, *B. eggerthii*, *B. facilis*, *B. faecichinchillae*, *B. faecis*, *B. finegoldii*, *B. fluxus*, *B. fragilis*, *B. hominis*, *B. intestilis*, *B. koreensis*, *B. kribbi*, *B. massiliensis*, *B. multiformis*, *B. nordii*, *B. oleiciplenus*, *B. ovatus*, *B. parvus*, *B. pectinophilus*, *B. rodentium*, *B. salyersiae*, *B. sartorii*, *B. stercoris*, *B. thetaiotaomicron*, *B. uniformis*, *B. vulgatus*, *B. xylanisolvens* | With diversified and abundant carbohydrate utilizing capabilities; its colonization relating to the intestinal development and mature; zwitterionic polysaccharides of *B. fragiles* inducing the production of IL-10 with an anti-inflammatory effects in EAS mice model; harboring rich bile salt hydrolases; enhancing the efficiencies of immunotherapy against tumors[45-47] |
| *Bifidobacterium*; *B. adolescentis*, *B. angulatum*, *B. animalis*, *B. bifidum*, *B. boum*, *B. breve*, *B. catenulatum*, *B. coryneforme*, *B. dentium*, *B. faecale*, *B. gallicum*, *B. longum*, *B. pseudocatenulatum*, *B. pseudolongum*, *B. rumintium*, *B. stercoris*, *B. thermophilum*, *B. tsurumiense* | With breast milk oligosaccharides or mucin utilizing capabilities; linked to host health, especially infants; Traditional probiotics, benefiting the host health; harboring rich bile salt hydrolases[48-50] |
| *Bilophila*; *B. wadsworthia* | Enriched in population with high-fat or high-protein diet; tolerance to bile acids; pro-inflammation; aggravating the high-fat diet induced metabolic syndrome[51] |
| *Blautia*; *B. beijingensis*, *B. caecimuris*, *B. celeris*, *B. coccoides*, *B. difficilis*, *B. faecis*, *B. glucerasea*, *B. hansenii*, *B. hydrogenotrophica*, *B. intestilis*, *B. lenta*, *B. luti*, *B. massiliensis*, *B. obeum*, *B. ovalis*, *B. producta*, *B. schinkii*, *B. segnis*, *B. simiae*, *B. stercoris*, *B. tarda*, *B. wexlerae* | With a low abundance in obese and colitis individuals; harboring bile acid hydrolases; producing bacteriocin and S-adenosyl methionine[52-55] |
| *Butyricimonas*; *B. faecihominis*, *B. hominis*, *B. paravirosa*, *B. virosa* | Butyrate-producing bacteria[56] |
| *Clostridium*; *C. baratii*, *C. beijerinckii*, *C. boliviensis*, *C. bornimense*, *C. botulinum*, *C. butyricum*, *C. cadaveris*, *C. celatum*, *C. celerecrescens*, *C. cochlearium*, *C. cocleatum*, *C. colinum*, *C. dakarense*, *C. difficile*, *C. diolis*, *C. disporicum*, *C. facile*, *C. faecis*, *C. glycolicum*, *C. glycyrrhizinilyticum*, *C. hathewayi*, *C. herbivorans*, *C. hominis*, *C. hylemoe*, *C. innocuum*, *C. lactatifermentans*, *C. lentum*, *C. mangenotii*, *C. methoxybenzovorans*, *C. methylpentosum*, *C. mobile*, *C. nexile*, *C. orbiscindens*, *C. oroticum*, *C. paraperfringens*, *C. paraputrificum*, *C. perfringens*, *C. polysaccharolyticum*, *C. porci*, *C. propionicum*, *C. puyanii*, *C. ramosum*, *C. rectum*, *C. saccharogumia*, *C. saccharolyticum*, *C. sardiniense*, *C. saudiense*, *C. scindens*, *C. segne*, *C. senegalense*, *C. septicum*, *C. simiarum*, *C. sphenoides*, *C. sporogenes*, *C. symbiosum*, *C. tertium*, *C. thiosulfatireducens*, *C. xylanolyticum*, *C. xylanovorans* | Core genus in the gut; spores-producing genus; certain species with T-regulatory-cells-inducing capability and then anti-inflammation; producing short fatty acids and contributing to gut homeostasis; involved in the metabolism of bile acids and influencing the composition of bile acid pools[57-58] |
| *Collinsella*; *C. aerofaciens*, *C. gabonensis*, *C. intestilis*, *C. stercoris*, *C. takaei* | Pro-inflammation; with a low abundance in obese or low-fiber intake individuals as well as patients with nonalcoholic fatty liver diseases[59-60] |
| *Coprococcus*; *C. catus*, *C. comes*, *C. eutactus*, *C. hominis* | Butyrate-producing bacteria; with a reducing abundance in depressed individuals and Parkinson's patients[61] |
| *Desulfovibrio*; *D. desulfuricans*, *D. legallii*, *D. piger*, *D. porci* | Pro-inflammation; using sulfate as an electron acceptor and producing H2S[62-63] |
| *Dorea*; *Candidatus D. massiliensis*, *D. formicigenerans*, *D. hominis*, *D. longicate* | Main gas-producing bacteria in human gut; with a higher abundance in patients with irritable bowel syndrome (IBS)[64] |
| *Enterococcus*; *E. asini*, *E. avium*, *E. canintestini*, *E. casseliflavus*, *E. cecorum*, *E. dispar*, *E. durans*, *E. faecalis*, *E. faecium*, *E. flavescens*, *E. gallinarum*, *E. gilvus*, *E. hirae*, *E. mundtii*, *E. pallens*, *E. phoeniculicola*, *E. raffinosus*, *E. saccharolyticus*, *E. thailandicus*, *Enterococcus xiangfangensis* | Opportunistic pathogens; certain strains with probiotic capability; antagonizing pathogens[65-66] |
| *Eubacterium*; *E. biforme*, *E. callanderi*, *E. contortum*, *E. cylindroides*, *E. desmolans*, *E. difficile*, *E. dolichum*, *E. eligens*, *E. hominis*, *E. infirmum*, *E. limosum*, *E. maltosivorans*, *E. muris*, *E. oxidoreducens*, *E. ramulus*, *E. rectale*, *E. rumintium*, *E. segne*, *E. siraeum*, *E. sulci*, *E. tenue*, *E. tortuosum*, *E. ventriosum*, *E. xylanophilum* | Main butyrate-producing bacteria; metabolizing bile acids and cholesterol; with a low abundance in obese and diabetic individuals as well as colitis patients[67-68] |
| *Faecalibacterium*; *F. cf*, *F. hominis*, *F. prausnitzii* | *F. prausnitzii*, main butyrate-producing species, with significant anti-inflammatory effects[6,69-70] |

(待续)

(续表 3)

| Genus; Species | Potential functions |
|---|---|
| *Lachnospira*; *L. hominis*, *L. multipara*, *L. pectinoschiza* | Previously been classified as members of *Clostridium* XIVa cluster; main butyrate-producing bacteria; its abundance changed significantly in the pathological states[71-74] |
| *Lactobacillus*; *L. acidophilus*, *L. amylolyticus*, *L. amylovorus*, *L. crispatus*, *L. delbrueckii*, *L. equicursoris*, *L. gasseri*, *L. helveticus*, *L. intestilis*, *L. johnsonii*, *L. kalixensis*, *L. porci*, *L. rodentium*, *L. rogosae*, *L. taiwanensis*, *L. ultunensis* | Traditional probiotics, with many beneficial functions; with a relatively high abundance and significantly associated with gut health[75-79] |
| *Lactococcus*; *L. formosensis*, *L. garvieae*, *L. lactis* | Producing short fatty acids and bacteriocin[80] |
| *Megamonas*; *M. funiformis*, *M. hypermegale*, *M. rupellensis* | Increasing abundance in obese and colitis individuals and patients with autism[81-83] |
| *Odoribacter*; *O. laneus*, *O. muris*, *O. splanchnicus* | Metabolizing certain bile acids with anti-inflammatory and antibacterial effects[84] |
| *Oscillospira*; − | Previously classified as *Clostridium* IV, only limited culturable strains; its abundance changed significantly in the pathological states[85-89] |
| *Parabacteroides*; *P. acidifaciens*, *P. distasonis*, *P. faecis*, *P. goldsteinii*, *P. gordonii*, *P. hominis*, *P. intestilis*, *P. johnsonii*, *P. merdae*, *P. muris*, *P. segnis* | Metabolizing bile acids; producing succinic acid; protecting host against abnormal glucose and lipid metabolism[90-91] |
| *Paraprevotella*; *P. clara*, *P. xylaniphila* | With proteolytic activity; protecting IgA from being degraded by trypsin[92] |
| *Peptostreptococcus*; *P. aerobius*, *P. porci*, *P. stomatis* | Utilizing glutamic acid and tryptophan; producing indole derivatives; protecting gut health; opportunistic pathogens (certain species) and promoting colon cancer[93-94] |
| *Phascolarctobacterium*; *P. succitutens*, *P. faecium*, *P. succitutens* | Succinate supports its growth; utilizing mucin; inhibiting the infection of *Clostridium difficile*[95] |
| *Prevotella*; *P. bivia*, *P. copri*, *P. denticola*, *P. disiens*, *P. intermedia*, *P. jejuni*, *P. melaninogenica*, *P. mizrahii*, *P. oralis*, *P. ruminicola*, *P. salivae*, *P. stercorea*, *P. veroralis* | With plant-derived complex carbohydrates utilization capabilities and associated with high plant-based diet; with proteolytic activity; involved in gut microbiota circadian rhythm fluctuations; associated with high blood pressure, success or failure of weight loss[96-100] |
| *Roseburia*; *R. zhanii*, *R. cecicola*, *R. difficilis*, *R. faecis*, *R. hominis*, *R. intestilis*, *R. inulinivorans*, *R. lenta*, *R. mobilis*, *R. muris*, *R. porci*, *R. rectibacter*, *R. yibonii* | Producing short fatty acids; with high abundance in population with a Mediterranean diet; associated with obesity, inflammation, neurological or immune-related diseases; cell surface protein, molecularly similar to β2GPI (core antigen epitope in patients with antiphospholipid syndrome), can induce disease in genetic sensitive mice[101-105] |
| *Ruminococcus*; *R. albus*, *R. bicirculans*, *R. bromii*, *R. callidus*, *R. difficilis*, *R. faecis*, *R. gauvreauii*, *R. gvus*, *R. hominis*, *R. intestilis*, *R. lactaris*, *R. lentus*, *R. muris*, *R. obeum*, *R. torques* | Utilizing resistant starch; producing short fatty acids; species-dependent beneficial or pathogenic effects; polysaccharide of *R. gnavus* aggravating colitis; Cell surface protein can serve as super-antigens and relate to immune imbalance associated diseases, such as allergies and asthma[106-109] |
| *Sutterella*; *S. parvirubra*, *S. stercoricanis*, *S. wadsworthensis* | Associated with colitis; with IgA degrading capabilities, which may damage the protective mucosal immune response[110] |
| *Veillonella*; *V. atypica*, *V. dispar*, *V. hominis*, *V. parvula*, *V. rogosae*, *V. tobetsuensis* | Organic acid (lactic acid, pyruvate, malic acid, oxaloacetate and etc.) fermentation bacteria, without carbohydrate or amino acid fermentation capabilities, the end fermentation product is acetic acids or propionic acids[111] |

−: Without named species.

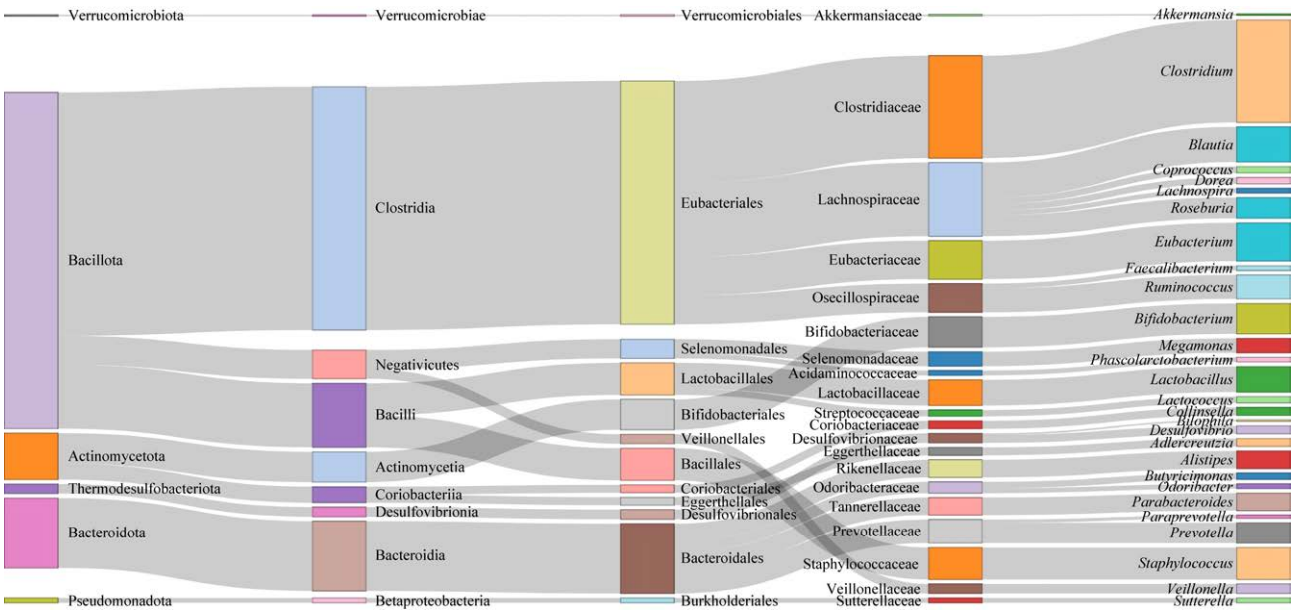


**图 1　肠道基石菌从门到属水平上的物种桑基图**

Figure 1　Sankey plot displays the distribution of core gut microbes from phylum to genus level.

## 2　国内外肠道微生物资源库的构建方法

肠道微生物的体外分离培养是肠道微生物资源库构建的核心。虽然在培养肠道共生菌之初，可培养的物种仅占到全部肠道微生物的10%–25%[15]。但是，近年来随着培养技术的不断改进和完善，多数与宿主疾病和健康紧密相关的基石物种已经被工程分离培养。基于分离菌株基因组测序结果构建的肠道微生物参考基因组库，使得宏基因组测序数据的比对率得到显著的提高。Zou 等[17]从 155 份中国健康人样本中分离培养得到 6 487 株肠道菌，并测序了其中 1 502 株菌，使得中国人肠道宏基因组的比对率从 52.00%提升至 76.88%。

肠道微生物的分离培养一般包括，前处理、粪便样本的梯度稀释、固体平板的分离培养、液体增菌培养和固体平板多次纯化及鉴定。

### 2.1　前处理

前处理主要包括 3 类，分别是乙醇处理、热处理和预培养(不同预处理条件见附表 7)。乙醇处理使用的乙醇浓度在 35%–70%之间，处理时间多在 1–4 h[19,21]。通过乙醇处理可以抑制乙醇敏感的菌株或芽孢菌的营养体，为其他肠道菌提供生长优势。乙醇处理结合含 1 g/L 牛磺胆酸钠(刺激孢子萌发)固体培养基进行分离培养即可有效地分离产孢的厚壁菌门菌株。热处理的温度一般选择 65 ℃或 80 ℃，处理时间多为 10–30 min，与乙醇处理的目的类似[20]。预培养不同于乙醇和热处理，主要是为了活化肠道中活性较差、生长受到抑制的肠道物种，从而分离得到尽可能多的未培养菌株。预培养主要使用血清培养瓶，硫基乙酸、瘤胃液、粪便基质、绵羊血或后续分离培养基厌氧培养 2–30 d[20,28]。虽然 Lagier 等[27-28]使用不同预培养条件可以得到更多不同类目的肠道菌，但是不同预培养条件对分离结果的影响并未得到系统性的比较和评价。

### 2.2 梯度稀释

要获得粪便样本的代表菌株，需要对粪便样本进行梯度稀释，并选择合适的稀释度进行后续的分离纯化。一般每克粪便样本溶解于 10–25 mL 缓冲溶液中，此时粪便样本的稀释倍数在 10–25 倍之间，粪便样本用量在 0.1–1 g 之间。Goodman 等[22]报道样本稀释度在 10 000 倍时，显微镜下不可见或不生长的样本占到总测序数据的 2%，而稀释度 100 000–1 000 000 倍时，可以分离得到多数可培养的菌体细胞。此外，Goodman 等[22]建议将 $10^{-4}$ 稀释度下的粪便样本涂布 150 mm 直径的非选择性平板，可以得到密集且区分明显的菌落，每个平板菌落数约 5 000 个，涂布 6–7 个平板可以覆盖样本的微生物组成特征，分离到的菌落达到饱和。

### 2.3 固体平板分离培养

肠道微生物分离培养基包括非选择和选择性培养基(附表 8)。常用的非选择性培养基有 YCFA 或改良 YCFA 培养基、GAM 或 MGAM 培养基(modified Gifu anaerobic medium)、GMM 或改良 GMM 培养基(gut microbiota medium)、PYG 或 MPYG 培养基(modified peptone yeast glucose medium)、苛性厌氧琼脂(fastidious anaerobic agar, FAA)、胰蛋白大豆琼脂(tryptic soy agar, TSA)或改良 TSA 琼脂、脑心浸出液琼脂(brain infusion broth, BHI)或哥伦比亚血琼脂。利用非选择性培养基分离培养肠道共生菌时，常添加的其他成分包括维生素 K、氯化血红素、维生素溶液、短链脂肪酸、脱纤维羊血、澄清瘤胃液及复合碳源。研究发现澄清瘤胃液可以显著提高肠道菌分离培养的活率[28]。培养基中额外添加其他碳源，包括纤维二糖、麦芽糖、半乳糖、甘露糖、山梨糖、阿拉伯糖、菊粉、几丁质、低聚果糖和粘蛋白等，可以增加碳源复杂度、分离更为多样的肠道细菌。此外，常使用稀释的培养基降低营养物质的浓度以支持生长缓慢的共生菌，如改良的 PYG 培养是将 PYG 培养基稀释 10 倍使用。Rettedal 等[112]的研究表明，固体培养基固化剂的选择也会影响到特定菌株分离到的概率，结冷胶可以富集到更多乳杆菌科的菌株，而琼脂则富集到了更多的肠球菌科的菌株。但在不同营养成分或培养条件下，固化剂富集到的菌株可能不尽相同，因此琼脂仍是最常用的固化剂。常用的选择性培养基包括乳酸菌筛选用 MRS 培养基、拟杆菌分离用拟杆菌胆汁七叶苷琼脂培养基(bacteroides bile esculin agar, BBE)、梭菌分离用强化梭菌培养基(reinforced clostridium media, RCM)、双歧富集培养基(bifidobacterium enrichment medium, BEM)及韦氏球菌培养基(veilonella Media, Veil)等。比较不同培养基上可分离到的肠道细菌类目，发现基础培养基上可获得的类目极少，GAM 中添加多种碳源、澄清牛瘤胃液等营养成分丰富的培养基上可以得到数量最多的肠道细菌类目，而稀释的 GAM 这类低营养成分的培养基虽然得到的肠道细菌类目不如前者，但由于较低营养成分抑制了很多快速生长的细菌，故而可以分离到更多特殊的种属[112]。

肠道微生物的培养时间多为 2–7 d。由于部分肠道菌生长极其缓慢且菌落微小，几乎肉眼不可见，故在进行单菌落挑取的过程中，可以借助放大镜，或进一步延长培养时间[28]。阿克曼氏菌在添加黏蛋白的 BHI 培养基上，培养 2–4 周时形成的菌落较大，便于后续操作[113]。

### 2.4 液体增菌培养

液体富集过程并不是肠道微生物分离过程中的必需步骤，在多数研究中并不包含这一步。部分肠道微生物体外培养困难，尤其在传代过程中可能存在失活、固体平板转接固体平板失败等现象。因此，将新分离得到的单菌落转接液体管

进行复苏和增菌培养后，再用固体平板进一步分离纯化，可以显著提升菌株的存活率。Liu 等[18]在构建小鼠肠道微生物资源库时，即挑取单菌落接种 96 孔板液体培养基进行培养，为降低工作量，仅保留了测序后仅包含单菌落的孔。

### 2.5 纯化及鉴定

分离菌株经过纯化后可以进行生理生化及分子鉴定。目前常用的鉴定方法有两种：经典的 16S 核糖体 RNA 基因测序鉴定和基质辅助激光解析电离-飞行时间质谱(matrix-assisted aser desorption/ionization time of flight mass spectrometry, MALDI-TOF-MS)鉴定。其中，基于 16S rRNA 基因测序的分子鉴定方法最为普遍，适用于几乎全部肠道细菌的分类鉴定。MALDI-TOF-MS 最初应用于致病菌的快速检测，近来随着培养组学的发展，开始被广泛应用于肠道微生物的快速分离鉴定。MALDI-TOF-MS 在细菌分类鉴定上的优势在于高通量、高效率，可以并行检测 96、384、1 536 个样本，从样本预处理到得到比对结果仅需 3 h，但是更为广泛的应用有赖于进一步克服技术壁垒，如构建更加完整的微生物谱库、提高实验的可重复性等[114]。

值得一提的是，根据肠道微生物资源库构建目的的不同，在预处理、分离用培养基及鉴定方法的选择上可能存在显著不同。旨在分离肠道微生物的主要类目时，在预处理和分离用培养基的选择上可以较为单一，甚至可以忽略预处理步骤，直接使用常规非选择性培养基进行；旨在分离更多未培养肠道菌时，需要设计尽可能多的预处理及培养基组合，配合各种不同培养条件，因此往往需要更高效率和通量的鉴定方法，如 MALDI-TOF-MS。因此，上述肠道微生物资源库的构建过程中，从预处理到分离用培养基及培养条件的组合可以从单一到多达 200 多种。此外，除了较为传统的肠道微生物分离方法，也涌现出更多新兴的技术，如基于分离芯片装置(isolation chip, iChip)的原位分离培养技术、基于互生共养体系的分离培养技术等[115]，这些技术已被尝试应用于土壤、海洋等环境样本中微生物的分离培养，进一步优化和应用于肠道微生物将扩大可培养菌类目、丰富对肠道微生物资源的认知。

## 3 肠道微生物资源库构建的展望

一直以来，使用各种培养基及多样化的培养条件，分离和培养肠道中不可培养的物种是肠道微生物研究领域的一个重要方向。近年来，随着肠道基石菌种与宿主互作关系及功能被不断阐明和揭示，针对特定关键肠道物种的多样性研究尤为重要。肠道微生物组成和功能多样性受到饮食、地域、文化及宿主基因型等因素的影响。最新研究表明宿主微生物之间的差异性也体现在菌株水平上[116]。Zhao 等[117]发现从同一个体中分离得到的拟杆菌菌株单核苷酸多态性(single nucleotide polymorphisms, SNP)位点数约为 100 个，而不同个体之间 SNP 个数在 10 000 个以上。Brito 等[116]对斐济岛上不同地域人群肠道和口腔微生物进行深度测序表明不同地域的肠道和口腔微生物在菌株基因型和可移动微生物组成上呈现出显著的地域特征。此外，西方人群和非西方人群肠道内的普雷沃氏菌(*Prevotella copri*)具有显著差异，非西式饮食人群的普雷沃氏菌代谢复杂纤维的能力更强，西式人群的普雷沃氏菌基因组中含有更多参与药物代谢相关的基因[100]。同种菌株虽然在基因组及生理生化上具有很多共性，但是也存在差异性，如不同拟杆菌及副拟杆菌菌株对植物多糖的结合和代谢能力存在显著差异[118]。最典型的例子为拟杆菌属菌株的 *SusC*/*SusD* 基因对，两者编码的膜蛋白具

有结合碳水化合物的活性，在拟杆菌属基因组中存在多个拷贝，这些拷贝之间的变异程度决定了其对多种碳水化合物结合能力的差异，也使得拟杆菌菌株具有多样的碳水化合物代谢能力[119]。因此，基于不同人群(生理状态、饮食、地域、生活习惯等)的样本采集及菌种资源库构建可以进一步丰富肠道微生物资源库的多样性和代表性。

此外，随着测序和组学技术的发展，肠道微生物资源库不仅包含有分离株，还包括分离株的基因组信息、生理生化特性等。基于菌种资源和数字信息的肠道微生物数据库共享将进一步促进交流和协作，如基于国家基因库生命大数据平台(China National GeneBank DataBase, CNGBdb)的数据开放共享等[120]。

## 4 结语

综上所述，肠道微生物资源库的构建越来越受到研究人员及相关行业的重视。目前，已构建的肠道微生物资源库包含 1 000 多个种，主要分布在厚壁菌门、拟杆菌门、变形菌门及放线菌门。构建方法逐渐成熟，可以使用有限的处理条件及培养基组合，实现肠道中多种基石微生物的分离。为满足菌种资源开发和功能研究的需要，肠道微生物菌种资源库的样本来源可以覆盖更多的不同疾病与健康状态、生活习惯和地域差异较大的人群，从而进一步丰富肠道关键物种的菌株多样性。

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