益生菌 *Bifidobacterium lactis* V9 对多囊卵巢综合征患者肠道微生物组的调节作用

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摘要 多囊卵巢综合征是育龄期妇女常见的代谢紊乱性疾病。前期研究表明, 益生菌的干预可以有效改善肠道菌群并缓解以肥胖和2型糖尿病为代表的代谢类疾病, 而多囊卵巢综合征患者的临床指标及肠道菌群能否通过益生菌的干预得到改善则有待证实。本研究通过联合使用多组学技术揭示了多囊卵巢综合征患者食用4周益生菌 *Bifidobacterium lactis* V9后肠道微生物组发生的变化。结果表明, *Bifidobacterium lactis* V9在患者肠道中具有稳定的定殖能力, 4周干预期后, 患者肠道菌群结构和代谢均发生显著变化, *Clostridium*, *Collinsella*, *Coprococcus*和*Klebsiella*菌属的丰度显著降低, 而*Faecalibacterium*和*Lactobacillus*菌属的丰度显著增加。基于代谢组学的分析结果表明, 糖皮质激素代谢水平显著降低, 乙酸、丙酸和丁酸等短链脂肪酸和琥珀酸的代谢水平显著提高。通过PICRUSt功能预测显示, PPAR信号通路与脂肪细胞因子信号通路显著下降, 而基于差异性菌属与代谢功能单元的网络互作分析进一步显示了*Bifidobacterium lactis* V9对多囊卵巢综合征患者肠道微生物组的调节作用。因此, 以肠道微生物为靶点, 通过益生菌*Bifidobacterium lactis* V9干预可以有效调节多囊卵巢综合征患者的肠道微生物组, 而这些微生物指标的改善将有助于患者临床症状的缓解, 为益生菌治疗代谢类疾病提供更加客观详实的数据支撑。

关键词 益生菌, *Bifidobacterium lactis* V9, 多囊卵巢综合征, 肠道微生物组, 多组学, 短链脂肪酸

多囊卵巢综合征 (polycystic ovary syndrome, PCOS)是育龄期妇女常见的内分泌及代谢紊乱性疾病, 也是导致女性无排卵性不孕的最常见病因之一^[1,2]。临床共有4个表型, 诊断标准包括: 高雄激素和多毛症、多囊性卵巢和排卵障碍^[3], 患者同时伴随肥胖、痤疮、阻塞性睡眠呼吸暂停等症状^[4]。PCOS患者由于肥胖通常表现出较强的胰岛素抵抗, 随着胰岛素抵抗水平的增加, PCOS患者更易出现代谢综合征^[5]。

健康人肠道中居住数量庞大、种类繁多的微生物。

一个健康的成年人肠道微生物数量达 $10^{13} \sim 10^{14}$, 与体细胞的比值接近1:1, 基因数量是人类的100多倍^[6,7]。近年来研究发现, 机体肠道菌群的失衡及核心功能基因组的缺失会诱发或加剧肥胖^[8]、2型糖尿病^[9-10]、肝硬化^[11]、结肠癌^[12]、痛风^[13]和动脉粥样硬化^[14]等多种代谢类疾病。着眼于菌株和代谢发现通过高膳食纤维选择性富集肠道菌群缓解2型糖尿病具有显著功效^[15], 通过摄入乌龙茶中的活性成分可以显著降低厚壁菌门/拟杆菌门比值, 增加肠道菌群多样性, 从而减轻肥胖小鼠的体重^[16]。可见, 经外来饮

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食干预并通过肠道菌群为调节靶点预防、改善和治疗代谢类疾病切实可行。

益生菌的益生特性逐渐被揭示并被大众认可,其在维持肠道稳态方面表现出其他外因无法比拟的作用。口服益生菌可显著改善机体胰岛素抵抗水平和肝脂肪变性^[17],减轻酒精性脂肪肝大鼠的肥胖和炎症^[18]。从宏基因组角度发现益生菌介导的被高脂饮食破坏的肠道菌群向正常菌群结构方向移动,代谢组学研究表明,益生菌能够通过影响与代谢综合征相关的菌群从而缓减肥胖等代谢疾病并发症^[19]。因此通过益生菌调节肠道菌群达到改善和治疗代谢类疾病已具有一定基础。多组学技术包括宏基因组学、宏转录组学、蛋白组学、代谢组学。通过多组学技术的应用能更好地揭示患者肠道微生物组物种丰度、代谢水平强弱,从而进一步解析肠道微生物组与代谢类疾病之间的作用机制。*Bifidobacterium animalis* subsp. *lactis* V9(乳双歧杆菌V9, *Bifidobacterium lactis* V9, CP001892)分离自中国蒙古族健康儿童的粪便,已被证实具有较强的耐酸性,在人工胃液中也可以存活,且能够抑制致病菌的生长,研究还发现*Bifidobacterium lactis* V9可以通过调节肠道菌群有效的治疗便秘和腹泻^[20-22]。以该菌株为基础的产品已经面世,并被广泛证实为一株优良的益生菌。

本研究通过联合使用宏基因组学和代谢组学技术,从多组学的角度客观阐释益生菌*Bifidobacterium lactis* V9对多囊卵巢综合征患者肠道微生物组的调节作用,旨在探讨以肠道微生物组为靶点,通过益生菌的干预实现对多囊卵巢综合征的精准治疗,同时为益生菌在慢性代谢类疾病的预防治疗方面提供更加客观详实的数据支撑。

1 材料方法

(i) 菌粉来源。 *Bifidobacterium lactis* V9由内蒙古农业大学乳品生物技术与工程教育部重点实验室提供,成分组成*Bifidobacterium lactis* V9活菌体、脱脂乳粉、乳糖,该菌粉已完成安全性评价。

(ii) 粪便样品采集。 获得患者知情同意并登记注册伦理试验后,征集临床诊断为多囊卵巢综合征患者9例,年龄范围21~35岁,均为女性。采集患者干预治疗前(0周)的粪便样品。干预治疗期间每日服用150亿益生菌*Bifidobacterium lactis* V9,期间没有使用任何抗生素类药物,保持正常饮食,连续服用4周,

期间采集患者服用1, 2, 4周的粪便样品,粪便样品采集至无菌采样管中,4℃运送至实验室。用粪便DNA提取试剂盒,按操作手册要求提取细菌宏基因组总DNA,并进行纯度检测。

(iii) qPCR定量检测*Bifidobacterium lactis* V9与优势微生物菌属。应用已知数量的双歧杆菌属参考菌株绘制扩增标准曲线,用*Bifidobacterium lactis* V9特异性引物对患者粪便中的*Bifidobacterium lactis* V9进行PCR扩增,在95℃预变性30 s; 95℃变性5 s,退火30 s, 72℃延伸45 s,循环40次; 72℃终端延伸5 min的条件下进行PCR扩增。qPCR反应在20 μL体系中进行,包括Super MIX (SYBR Green) 10 μL,模板2 μL,上、下游引物各0.5 μL, Dye 0.4 μL,加无菌去离子水至20 μL,定量分析以扩增标准曲线为依据。制备外标准品,应用特异性引物扩增*Bacteroides* group, *Clostridium* group, *Faecalibacterium prausnitzii*, *Lactobacillus*的特异性片段,检测之后胶回收各自片段达到纯化的目的,将纯化的片段和pMD-19T载体连接,连接后转化到大肠杆菌感受态细胞中,挑选阳性克隆子进行酶切验证,将验证过的阳性克隆子提取质粒,测定浓度后对未知菌数的参考菌株计算拷贝数,得到*Bacteroides*, *Clostridium*, *Faecalibacterium*, *Lactobacillus*的标准曲线,然后以标准曲线为依据,对特异性引物扩增上述菌属后的特异性片段进行定量分析。

(iv) 代谢组学分析。使用GC-MS分析短链脂肪酸的组成^[23],通过使用Agilent1290的UPLC Q-TOF/MS分析除短链脂肪酸之外的代谢产物变化。样品经过C18柱分离,期间保持柱温40℃。LC-MS的流动相为一定梯度的0.2%的甲酸和乙腈,速度0.3 mL/min,梯度: 0~4 min, 5%~50%乙腈: 4~18 min, 50%~5%乙腈: 18~20 min, 5% 乙腈: 20~25 min, 5%~95%乙腈。待测样品进样量5 μL。质谱扫描范围是50~1000 m/z,采集速率为1.00谱/s。

(v) 16S rRNA高通量测序及PICRUSt功能预测。扩增16S rRNA的V3-V4区,引物为338F(5'-ACTCCTACGGGAGGCAGCA-3')和806R(5'-GGACTACHVGGGTWTCTAAT-3')^[24]。PCR产物经过定量和均一化后通过Illumina MiSeq平台进行测序。使用QIIME工具包进行分析^[25],根据97%的序列相似度将所有序列进行同源比对并聚类成OTUs(Operational Taxonomic Units,可操作分类单元)后与数据库

GreenGene进行比对以获得物种信息. 基于微生物OTU表进行PICRUST宏基因组功能预测^[26], 预测结果通过KEGG对基因进行分类得到二级和三级功能基因代谢通路的划分结果.

(vi) 统计学分析方法. 微生物菌属的含量以平均值±标准差表示, 采用Mann-Whitney检验和Kruskal-Wallis检验进行干预治疗前后差异性比较分析, 基于Weighted Unifrac距离的PCOA主坐标分析和Heatmap均采用QIMME分析, R程序做图. R程序计算Spearman相关性后, 通过Cytoscape构建网络图阐释患者肠道菌群和二级代谢通路之间的关系.

2 结果

2.1 益生菌*Bifidobacteria lactis* V9定量分析

应用qPCR定量研究益生菌干预期间患者粪便中*Bifidobacteria lactis* V9的数量, 定量结果见图1. 当服用*Bifidobacteria lactis* V9一周后, 粪便中该菌平均含量达到 $(6.98 \pm 0.45) \text{Log}_{10} \text{CFU/g}$ (即 $10^{6.98} \text{CFU/g}$); 服用2周时, 患者粪便中*Bifidobacteria lactis* V9平均含量为 $(7.26 \pm 0.42) \text{Log}_{10} \text{CFU/g}$ (即 $10^{7.26} \text{CFU/g}$). 服用第4周, 平均含量为 $(7.46 \pm 0.42) \text{Log}_{10} \text{CFU/g}$ (即 $10^{7.46} \text{CFU/g}$). 由此可见在每日服用150亿 *Bifidobacteria lactis* V9干预条件下, *Bifidobacteria lactis* V9在患者肠道中表现出稳定的定殖能力.

2.2 肠道微生物代谢组分析

通过UPLC Q-TOF/MS和GC-MS技术比较分析患者服用*Bifidobacteria lactis* V9四周前后代谢产物的变化, 经注释后, 结果表明具有显著变化($P < 0.05$)的代谢产物如表1所示. 共22个代谢产物含量显著增加, 10个代谢产物含量显著下降. Hydrocortisone cypionate 属于糖皮质激素, 人体长期大量存在会引起痤疮、多毛, 这与多囊卵巢综合征患者症状相似, 经*Bifidobacteria lactis* V9干预后, 患者体内该激素水平下降63万倍左右. Acetic acid, Propanoic acid, Succinic acid, Butanoic acid等对人体有益的短链脂肪酸增加显著, Succinic acid琥珀酸的含量也显著增加.

2.3 肠道优势菌属定量分析

qPCR定量检测患者粪便中优势菌属的结果如图2. 服用4周*Bifidobacteria lactis* V9后, 患者肠道中

*Bacteroides*和*Clostridium*的丰度显著减少, *Faecalibacterium*和*Lactobacillus*的丰度显著增加. 0周时, 各菌属含量: *Bacteroides* ($8.37 \pm 0.57 \text{Log}_{10} \text{CFU/100 ng}$) > *Clostridium* ($7.82 \pm 0.17 \text{Log}_{10} \text{CFU/100 ng}$) > *Lactobacillus* ($5.50 \pm 0.46 \text{Log}_{10} \text{CFU/100 ng}$) > *Faecalibacterium* ($5.32 \pm 0.85 \text{Log}_{10} \text{CFU/100 ng}$). 第4周, 各菌属含量: *Faecalibacterium* ($7.18 \pm 0.23 \text{Log}_{10} \text{CFU/100 ng}$) > *Bacteroides* ($6.87 \pm 0.24 \text{Log}_{10} \text{CFU/100 ng}$) > *Lactobacillus* ($6.63 \pm 0.38 \text{Log}_{10} \text{CFU/100 ng}$) > *Clostridium* ($6.22 \pm 1.22 \text{Log}_{10} \text{CFU/100 ng}$). 可见*Bifidobacteria lactis* V9能改变患者肠道优势微生物的结构, 且差异性显著.

2.4 肠道微生物群落结构与差异性菌属分析

基于Weighted Unifrac距离的 β 多样性分析, 并细化差异性菌属($P < 0.01$), 如图3(a)和(b). 结果表明, 服用*Bifidobacteria lactis* V9前后, 患者肠道菌群构成在主坐标上的位置明显分隔开(图3(a)), 内圈代表置信值为0.8, 外圈代表置信值为0.9, 4周较0周整体向左上方移动, 说明经*Bifidobacteria lactis* V9干预后定向改变了患者肠道菌群结构. 差异性菌属中*Bifidobacterium*, *Faecalibacterium*的丰度显著增加, 其他差异性菌属均显著减少, 包括*Clostridium*, *Collinsella*, *Ochrobactrum*, *Streptococcus*, *Prevotella*, *Coprococcus*, *Actinomyces*, 其中条件致病菌*Klebsiella*几乎全部消失.

图1 不同时期患者粪便中*Bifidobacteria lactis* V9含量

Figure 1 The content of *Bifidobacteria lactis* V9 in different periods stool

表 1 患者 0 与 4 周代谢组变化分析

Table1 Analysis of metabolic changes between week 0 and week 4

组分	P值	FC (abs)	4周 vs. 0周
Hebevinoside I	0.0038	3100089.30	Down
5,6,7,8-Tetrahydro-4-methylquinoline	0.0041	1522984.15	Down
10-hydroxy-hexadecan-1,16-dioic acid	0.0235	1140848.05	Down
Hydrocortisone cypionate	0.0153	631660.95	Down
Gabapentin	0.0268	612204.30	Down
Pleurostyline	0.0130	519480.35	Down
Pirbuterol	0.0240	513159.25	Down
Rifabutin	0.0038	519499.95	Down
Ursodeoxycholic acid	0.0071	324404.80	Down
Norselic acid	0.0120	231612.87	Down
Theogallinin	0.0384	110920.62	Down
cholesterol sulfate	0.0249	6063542.00	Down
3-Formyl-6-hydroxyindole	0.0083	1820451.50	Down
all-trans-hexaprenyl diphosphate	0.0384	1191700.63	Down
N-Acetylneuraminic Acid	0.0196	899362.05	Down
3-Chlorotyrosine	0.0384	624018.74	Down
Sodium Tetradecyl Sulfate	0.0013	540267.17	Down
Magnoshinin	0.0020	527900.05	Down
Proscillaridin	0.0083	566268.17	Down
Lauryl hydrogen sulfate	0.0015	443617.55	Down
N-Didesethylquinagolide sulfate	0.0015	642948.10	Down
7-Ketodeoxycholic acid	0.0132	323982.17	Down
Acetic acid	0.0184	3237058.79	Up
Propanoic acid	0.0039	2837493.07	Up
ethanesulfonic acid	0.0031	1711969.25	Up
Tauroursocholic acid	0.0238	1666336.25	Up
Succinic acid	0.0162	1295938.00	Up
Butanoic acid	0.0087	1165943.50	Up
Tetraacetylenediamine	0.0154	521588.99	Up
Torvoside	0.0287	504162.67	Up
Putaminoxin	0.0384	508534.16	Up
Butyl butyryllactate	0.0132	366183.17	Up

2.5 肠道微生物差异性功能单元分析

通过PICRUST预测功能基因组, 选取 $P < 0.01$ 的三级代谢通路如表2, 其中Cell cycle—Caulobacter, Apoptosis, Phosphatidylinositol signaling system, Glutathione metabolism和Transcription related proteins共5个代谢通路显著上调, Adipocytokine signaling pathway, PPAR signaling pathway, Bacterial invasion

of epithelial cells, Germination, Pantothenate and CoA biosynthesis和Transcription machinery共6个代谢通路显著下调. 与胰岛素抵抗和肥胖等脂代谢有关的PPAR(过氧化物酶体增殖剂激活受体)信号通路、Adipocytokine (脂肪细胞因子) 信号通路显著下降, 说明*Bifidobacteria lactis* V9可以通过改善代谢水平从而缓解患者相关临床症状.

图2 不同时期优势菌属定量结果

Figure 2 Quantitative results of dominant bacteria in different periods

图3 Beta多样性与差异性菌属分析

Figure 3 Analysis of Beta diversity and differential genus

2.6 优势菌属与二级代谢通路相关性分析

选取菌属平均含量大于1%，代谢通路贡献率大于3%，通过Spearman相关性计算 R 值， R 的绝对值大于0.4的菌属与二级代谢通路的关系被选择构建网络图如图4。灰色代表负相关，绿色代表正相关，用于

表示相关性强弱的线条颜色连续变化。二级代谢通路中主要与脂代谢、碳水化合物代谢、氨基酸代谢有关，其中辅酶因子和维生素代谢与*Bifidobacterium*和*Faecalibacterium*具有极强的正相关关系，碳水化合物代谢与*Bifidobacterium*、*Prevotella*、*Faecalibacterium*具有极强的负相关关系。*Blautia*只与碳水化合物代谢呈负相关，*Megamonas*只与能量代谢具有呈负相关。

3 讨论

益生菌经黏附与定植后才能在人体复杂的肠道环境下生长发育，良好的定植能力是益生菌发挥益生作用的关键。*Bifidobacterium lactis* V9能增加患者肠道中*Lactobacillus*的含量，酸化肠道环境，有利于*Bifidobacterium lactis* V9与患者肠道中其他菌群的竞争，增加其存活能力。*Bifidobacterium lactis* V9与碳水化合物代谢呈极强的负相关，干预后*Faecalibacterium*也会增加，而*Faecalibacterium*与碳水化合物代谢也呈极强的负相关，表明*Bifidobacterium lactis* V9可以通过调节肠道菌群，从而营造有利的肠道环境，逐渐实现在患者肠道的定植并发挥作用。同样发现产维生素B12的益生菌粪肠球菌LZ86和益生菌魏斯氏菌属能在一定胃酸和胆盐环境下存活并能黏附在与小肠上皮细胞结构和功能相似的Caco-2细胞上^[27,28]，该特性有利于益生菌在肠道定植。罗伊氏乳杆菌能产生有机酸、罗氏菌素等抗菌分子从而抑制病原微生物生长，改变宿主共生菌群结构而实现大量定植^[29]。由此可见优良的益生菌都具有大量定植的能力并能后续的益生作用奠定基础。

经*Bifidobacterium lactis* V9干预有效改善了患者肠道微生物组，厌氧菌*Bacteroides*和*Clostridium*的显著降低，显著增加的*Lactobacillus*可以生成乳酸从而促进产丁酸的*Faecalibacterium*的生长^[30]，研究发现通过摄入益生菌复合剂促进丁酸的产生可以抑制非酒精性脂肪肝大鼠的系统性肥胖^[18]，从代谢组可知其他短链脂肪酸，如乙酸、丙酸、丁二酸也显著增加，通过服用马里乳杆菌可以调节肠道菌群，同样表现为促进短链脂肪酸分泌^[31]，短链脂肪酸可以作用于 β 细胞促进胰岛素的分泌从而缓解肥胖^[32]。琥珀酸可以驱动于UCP1的褐色脂肪产热，从而抵抗饮食诱导的肥胖和血糖问题^[33]。除此之外，由于降低了碳水化合物合成水平，减少了能量的存储，进而缓解肥胖。因此通过益生菌*Bifidobacterium lactis* V9干预可

表 2 差异性功能基因组分析

Table2 Functional genes analysis of significant differences

代谢通路	相对贡献率(%)		中值, 范围(%)		P值
	0周	4周	0周	4周	
Adipocytokine signaling pathway	0.0712	0.0505	0.0719, 0.0520~0.0827	0.0085, 0.0028~0.0605	0.0007
PPAR signaling pathway	0.1434	0.1219	0.1439, 0.1336~0.1564	0.0748, 0.0655~0.1367	0.0009
Cell cycle—Caulobacter	0.4774	0.5109	0.4787, 0.4271~0.5334	0.5426, 0.5040~0.5853	0.0013
Apoptosis	0.0113	0.0245	0.0103, 0.0034~0.0222	0.0479, 0.0144~0.0621	0.0017
Phosphatidylinositol signaling system	0.1001	0.1182	0.0996, 0.0904~0.1134	0.1570, 0.1046~0.1634	0.0017
Bacterial invasion of epithelial cells	0.0038	0.0025	0.0036, 0.0016~0.0067	0.0005, 0.0000~0.0039	0.0047
Glutathione metabolism	0.2414	0.3206	0.2606, 0.1435~0.3542	0.4076, 0.2438~0.6022	0.0054
Transcription related proteins	0.0071	0.0113	0.0058, 0.0039~0.0129	0.0128, 0.0069~0.0391	0.0070
Germination	0.0935	0.0673	0.0728, 0.0348~0.1740	0.0370, 0.0055~0.0668	0.0071
Pantothenate and CoA biosynthesis	0.5253	0.5028	0.5069, 0.4801~0.6073	0.4874, 0.4243~0.5068	0.0071
Transcription machinery	0.8106	0.7556	0.8373, 0.6877~0.9462	0.6871, 0.6458~0.7594	0.0071

图 4 优势菌属与二级代谢通路相关性分析

Figure 4 Correlation analysis between dominant bacteria and secondary metabolic pathways

以多角度减轻PCOS患者的肥胖症。研究发现，PCOS患者与正常女性的体内维生素D含量存在差异^[34]，而PCOS患者体内低水平的维生素D与胰岛素抵抗、肥胖、多毛症、高雄激素等有关^[35]，经益生菌干预后，患者辅酶因子和维生素的代谢随之升高，呈极强的正相关，可见*Bifidobacterium lactis* V9可以调节患者机体维生素代谢。通过对肠道微生物的调节从而降

低糖皮质激素的代谢水平，并改善因体内长期大量存在糖皮质激素而引起的代谢紊乱，缓解痤疮、多毛的症状。脂肪信号细胞作用水平的改变会导致肥胖及相关代谢疾病^[36]，PPAR调节的基因下调可以促进代谢综合征的发展^[37]，*Bifidobacterium lactis* V9能够降低与糖、脂代谢有关的PPAR、脂肪细胞因子信号通路从而缓解肥胖等代谢症状。因此足以见得

Bifidobacterium lactis V9可以通过改善PCOS患者肠道微生物组从而改变机体代谢水平进而可能缓解临床症状。

本研究通过联合使用宏基因组学和代谢组学系统分析了益生菌*Bifidobacterium lactis* V9对多囊卵巢综合征患者肠道微生物组的调节作用。基于肠道微生物及其代谢通路的网络互作分析可以看出,*Bifidobacterium lactis* V9通过占领肠道生态位促进了有益菌的增长,同时抑制了条件致病菌的增殖。微生物群落的改变通过功能基因促使患者肠道代谢产物的改善,而这些微生物指标的改善将有助于患者临

床症状的缓解。然而本研究仍存在以下两点局限:首先,没有完成基于大规模人群的多囊卵巢综合征患者与健康志愿者临床指标和肠道微生物组的对比分析;其次,参与益生菌干预试验的人群数量偏少,仅可满足基本的统计学要求。而上述局限性也将是我们后续研究的发力点,在接下来的工作中将继续结合多组学技术在分析对比多囊卵巢综合征患者与健康人群临床指标和肠道微生物组差异性的基础上,寻找该疾病的生物标记物,并以此为靶点,应用益生菌开展更大规模的临床研究,为益生菌治疗代谢类疾病提供更加客观详实的数据支撑。

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Summary for “益生菌 *Bifidobacterium lactis* V9 对多囊卵巢综合征患者肠道微生物组的调节作用”

Probiotic *Bifidobacterium lactis* V9 regulates the intestinal microbiome in patients with polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is a common metabolic disorders among women of reproductive age worldwide. It is also one of the common causes of female infertility. The effect of probiotics has been gradually revealed and recognized by the public. Previous studies have shown that probiotic intervention can effectively improve intestinal microbiome and relieve metabolic diseases represented by obesity and type 2 diabetes. Although a few studies have investigated the intestinal microbiota profiles of women with PCOS, the regulatory effect of the probiotic *Bifidobacterium lactis* V9 has yet to be revealed. We hope that *Bifidobacterium lactis* V9 can improve the metabolism of patients by regulating the intestinal flora, thus slowing down the disease. To address this gap, in this study, metagenomics and metabolomics were used together to analyze the effects of *Bifidobacterium lactis* V9 on patients with PCOS. Nine patients with PCOS were selected to consume 15 billion probiotic *Bifidobacterium lactis* V9 every day during the intervention period. Fecal samples were collected from patients at week 0, week 1, week 2 and week 4. The results showed that *Bifidobacterium lactis* V9 had stable colonization ability in the intestinal tract of patients. The abundance of *Bifidobacterium* and *Faecalibacterium* significantly increased, while that of other bacteria significantly decreased, including *Clostridium*, *Collinsella*, *Ochrobactrum*, *Streptococcus*, *Prevotella*, *Coprococcus* and *Actinomyces* and the opportunistic pathogen *Klebsiella* is almost gone. The results of metabonomic analysis showed that the metabolic level of glucocorticoids was significantly reduced and the metabolic level of short chain fatty acids such as acetic acid, propionic acid and butyric acid was significantly increased. PPAR signal pathway and adipocytokine signal pathway associated with insulin resistance and lipid metabolism were decreased significantly based on PICRUSt. Furthermore, the regulation functions of *Bifidobacterium lactis* V9 on intestinal microbiome in patients with polycystic ovary syndrome was further revealed based on the analysis of the network interaction between different genus and metabolic functional units. As expected, *Bifidobacterium lactis* V9 can improve the body's metabolism by regulating the intestinal flora of patients with PCOS, which is conducive to slowing down the clinical symptoms of patients. To sum up, this study revealed the changes of intestinal microbiome and metabolites of patients with PCOS after 4 weeks of consumption of probiotic *Bifidobacterium lactis* V9 by using multi-group technique including metagenomics and metabolomics. Although we have observed that *Bifidobacterium lactis* V9 is beneficial to patients with PCOS, the regulatory mechanism needs to be revealed in larger clinical trials. At the same time, we need to find biomarkers and target them for the treatment of metabolic diseases through probiotics. The results of this study provide new insights into the treatment of PCOS and offer objective and detailed data supporting the use of probiotic treatments for metabolic diseases.

probiotics, *Bifidobacterium lactis* V9, polycystic ovary syndrome, intestinal microbiome, multi-omics, short-chain fatty acid

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