

研究报告

EM 复合菌对新疆色素辣椒生长及根际细菌群落的影响

魏红青¹, 宋旭², 徐海娟², 夏光富², 张登录³, 傲耐³, 李吉鲁⁴, 万翠翠⁵, 刘聪¹, 王军^{*1}

1 中国海洋大学海洋生命学院, 山东 青岛 266003

2 巴州绿领职业技能培训学校, 新疆 巴音郭楞蒙古自治州 841400

3 博湖县农业科技推广中心, 新疆 巴音郭楞蒙古自治州 841400

4 新疆农业广播电视学校博湖分校, 新疆 巴音郭楞蒙古自治州 841400

5 博湖县农残检测中心, 新疆 巴音郭楞蒙古自治州 841400

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摘要:【背景】Effective microorganisms (EM)复合菌在我国农业种植上的应用越来越广泛, 但对色素辣椒的促生作用与根际细菌群落结构的影响未见报道。【目的】评估 EM 复合菌对新疆色素辣椒的促生长作用, 并分析其对色素辣椒根际细菌群落组成的影响。【方法】通过随水灌溉方式将 EM 复合菌接种到色素辣椒根部, 在收获期测定辣椒生长指标、土壤养分和酶活活性, 明确 EM 复合菌对辣椒生长和土壤质量的影响; 利用 16S rRNA 基因高通量测序技术测定 EM 复合菌对辣椒根系细菌群落组成和结构的影响。【结果】与对照组相比, EM 复合菌的施用使辣椒株高、鲜重、单个果重和单株结果数分别提高 23.89%、85.41%、42.31%和 46.04%; 土壤中碱解氮和速效磷含量分别提高 5.83%和 13.39%, 土壤中脲酶、蔗糖酶和过氧化物酶的活性分别提高 11.47%、9.42%和 21.43%; 施用 EM 复合菌显著改变辣椒根际微生物群落的 α 多样性和 β 多样性, 提高有益菌群变形菌门(*Proteobacteria*)、酸杆菌门(*Acidobacteria*)、芽单胞菌门(*Gemmatimonadetes*)、放线菌门(*Actinobacteria*)和厚壁菌门(*Firmicutes*)的相对丰度, 其中变形菌门黄单胞菌科(*Xanthomonadaceae*)的相对丰度增加 119.32%; 在属的水平上, 施用 EM 复合菌显著增加了藤黄色杆菌属(*Luteitalea*)、藤黄单胞菌属(*Luteimonas*)、鞘脂单胞菌属(*Sphingobacterium*)和盐单胞菌属(*Halomonas*)的相对丰

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*Corresponding author. E-mail: wangjun@ouc.edu.cn

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度,尤其是藤黄单胞菌属的丰度提高 244.17%,同时显著降低黄杆菌属(*Flavobacterium*)的相对丰度。此外,与土壤理化指标呈正相关的微生物菌群相对丰度也显著升高。【结论】EM 复合菌能够通过提高土壤营养成分与酶活活性,调控根系微生物群落结构,富集大量在盐碱地生存能力较强的有益菌群,进而起到促进色素辣椒生长的功效。

关键词: EM 复合菌; 色素辣椒; 促生作用; 根际细菌群落结构; 土壤酶活活性

EM biofertilizer affects growth and rhizosphere bacterial community of pigment pepper in Xinjiang of China

WEI Hongqing¹, SONG Xu², XU Haijuan², XIA Guangfu², ZHANG Denglu³, AO Nai³, LI Jilu⁴, WAN Cuicui⁵, LIU Cong¹, WANG Jun^{*1}

1 College of Marine Life Sciences, Ocean University of China, Qingdao 266003, Shandong, China

2 Bazhou Green-collar Vocational Skills Training School, Bayingol Mongolian Autonomous Prefecture 841400, Xinjiang, China

3 Agricultural Technology Promotion Center of Bohu County, Bayingol Mongolian Autonomous Prefecture 841400, Xinjiang, China

4 Bohu Branch of Xinjiang Agricultural Radio and Television School, Bayingol Mongolian Autonomous Prefecture 841400, Xinjiang, China

5 Pesticide Residue Testing Center of Bohu County, Bayingol Mongolian Autonomous Prefecture 841400, Xinjiang, China

Abstract: [Background] Effective microorganisms (EM) have been widely used in the farming in China, while the available information is limited regarding the impacts of EM biofertilizer on the growth and rhizosphere bacterial community structure of pigment pepper. **[Objective]** To clarify the growth-promoting effect of EM biofertilizer on pigment pepper in Xinjiang of China and uncover the influence of the biofertilizer on rhizosphere bacterial community structure. **[Methods]** The EM biofertilizer was inoculated into the roots of pigment pepper plants through irrigation. We determined the growth indexes, soil nutrient levels, and enzyme activities at the harvest stage to assess the impacts of EM biofertilizer on the pepper growth and soil quality. High-throughput sequencing of 16S rRNA was employed to investigate the impacts of EM biofertilizer on the composition and structure of rhizosphere bacterial community. **[Results]** Compared with the control group, the application of EM biofertilizer increased the plant height, fresh weight, single fruit weight, and number of fruits per plant by 23.89%, 85.41%, 42.31%, and 46.04%, respectively. Moreover, it increased the available nitrogen, the available phosphorus, and the activities of urease, sucrase, and peroxidase in the soil by 5.83%, 13.39%, 11.47%, 9.42%, and 21.43%, respectively. The application of EM biofertilizer significantly altered the alpha and beta diversity of bacteria in the rhizosphere soil and increased the relative abundance of beneficial bacteria including *Proteobacteria*, *Acidobacteria*, *Gemmatimonadetes*, *Actinobacteria*, and *Firmicutes*. Especially, the relative abundance of *Xanthomonadaceae* belonging to *Proteobacteria* was increased by 119.32%. At the genus level, the application of EM biofertilizer increased the relative abundance of *Luteitalea*, *Luteimonas*, *Sphingobacterium*,

and *Halomonas*. Among them, the relative abundance of *Luteimonas* was increased by 244.17%, whereas that of *Flavobacterium* was lower than that in the control group. The microbial taxa showing positive correlations with soil physicochemical indices presented significantly increased relative abundance after the application of EM biofertilizer. **[Conclusion]** The application of EM biofertilizer regulates the rhizosphere microbial community structure by changing soil nutrient levels and enzyme activities, which further enriched beneficial bacteria with strong survival ability in saline-alkali land and promoted the growth of pigment pepper.

Keywords: EM biofertilizer; pigment pepper; growth-promoting effect; rhizosphere bacterial community structure; soil enzyme activity

Effective microorganisms (EM)复合菌是由乳酸菌、酵母菌、放线菌和光合细菌等组成的有效微生物菌群^[1], 在土壤污染修复、克服连作障碍、拮抗病原菌、提高作物的抗逆能力、产量和品质等方面发挥积极的作用^[2-5]。研究发现长期施用 EM 菌肥可以显著提高小麦秸秆生物量、籽粒产量、秸秆和籽粒营养^[6]。此外, EM 菌还能够在减少无机化肥施用量的同时, 显著提高棉花的产量, 促进棉花植株中氮、磷和钾元素的含量^[7]。EM 复合菌中的乳酸菌(lactic acid bacteria, LAB)具有直接促进植物生长和种子发芽的能力, 并能缓解盐碱化等非生物胁迫^[8]。与植物促生微生物(plant growth promoting microbes, PGPM)一样, EM 菌通过氮固定、产铁载体、矿化溶解营养元素和改善土壤理化性质等机制提高作物产量^[9]。随着人们对作物产量和品质的重视, EM 复合菌在农业生产上的应用越来越广泛。

除了对作物生长与土壤质量发挥积极作用之外, EM 复合菌还能改善作物根系微生物的群落结构。微生物群落分泌的植物激素可以被根吸收利用, 植物的根际分泌物同样也可以被微生物加工分解^[10]。因此, 根系微生物结构的改变会直接影响植物的生长和健康状况。例如, 德尔塔变形菌门(*Deltaproteobacteria*), 浮霉菌门(*Planctomycetes*)和硝化螺旋菌门(*Nitrospirae*)的

丰度增加与水稻根际土壤中氮素的循环紧密相关^[11]。褐紫曲霉菌(*Aspergillus brunneoviolaceus*)则能够提高小白菜(*Brassica chinensis* L.)根际中具有促生特性的微生物, 包括黄单胞菌科(*Xanthomonadaceae*)、噬几丁质科(*Chitinophagaceae*)和红杆菌科(*Rhodanobacteraceae*)的丰度^[12]。研究表明, 最初的微生物群落结构会影响植物最终的健康状况, 并间接影响番茄的产量和品质^[13]。然而, 目前对于 EM 菌影响微生物群落结构的研究多集中于堆肥发酵, 对于作物根际微生物的影响研究较少^[14]。为全面了解 EM 复合菌剂的作用, 还应考虑施用 EM 复合菌对根际细菌群落结构的影响。

辣椒(*Capsicum annuum* L.)属于茄科, 是全球最重要的经济作物之一, 年人均消费量约为 3.50 kg^[15-16]。中国是世界上最大的辣椒生产国, 在 28 个省份均有种植^[17]。新疆自然条件优越, 日照时间长, 昼夜温差大, 为色素辣椒的种植提供了良好的环境。然而, 由于长期连作和化肥的大量不合理使用, 近年来新疆色素辣椒的产量与品质出现了明显下降^[18]。本研究以新疆色素辣椒为研究对象, 分析 EM 复合菌施用对色素辣椒生长、土壤理化性质和根际细菌群落结构的影响, 评估其对辣椒生长与土壤生态环境的改良作用, 以期 EM 复合菌在新疆辣椒种植中的大面积应用提供科学依据。

1 材料与方法

1.1 材料

色素辣椒选用“疆红 868”品种,由巴州绿领职业技能培训学校提供。供试 EM 复合菌液由山东启宏生物科技有限公司生产,主要成分为乳酸菌、酵母菌、光合细菌和放线菌。

土壤脲酶测定试剂盒、土壤过氧化氢测定试剂盒、土壤蔗糖酶测定试剂盒和土壤过氧化物测定试剂盒,北京索莱宝科技有限公司; E.Z.N.A.[®] Soil DNA Kit, Omega Bio-Tek 公司。QuantiFluor[™]-ST 蓝色荧光定量系统, Promega 公司; pH 计, 上海仪电科学仪器股份有限公司; 紫外分光光度计, 上海元析仪器有限公司。

1.2 大田试验及样品采集

2022 年 4 月 15 日, 将六叶一心的辣椒幼苗移栽到新疆巴音郭楞蒙古自治州博湖县的大田中(86°19'00"—87°26'00"E, 41°33'00"—42°14'00"N)。试验设置对照组(control)和处理组(treatment), 每个试验组都包括 5 个地块, 每个地块面积约为 1 333.34 m²。对照组施用常规肥料(尿素 0.090 kg/m²+磷酸二氢钾 0.067 kg/m²), 处理组除了施用常规肥料外, 还在 4 月 17 日、5 月 6 日和 5 月 21 日以滴灌的方式在每个地块施用 0.007 kg/m² EM 复合菌液。在 8 月 20 日, 采用抖根法取辣椒根际土壤, 每个试验组取 4 个重复。将土样置于干冰中保存, 并带回实验室, 各取 50 g 保存于-80 °C 冰箱中, 用于 16S rRNA 基因测序, 其余土壤风干后用于理化性质测定。

1.3 测定方法

利用游标卡尺和卷尺测定辣椒的茎粗、株高、根长和叶长、叶宽。利用直尺测量果长和果宽, 并测定植株鲜重、果实重量和单株结果数。土壤过 10 目筛后, 用于碱解氮和速效磷的测定, 过 100 目筛后用于有机质的测定。将土

壤和蒸馏水以 1:2.5 (质量体积比)混合后用 pH 计测定土壤 pH^[19]。土壤碱解氮采用碱扩散法测定^[20]。将土壤用 0.50 mol/L NaHCO₃ 浸提后用钼锑抗比色法测定速效磷含量^[21]。有机质采用重铬酸钾(K₂Cr₂O₇)容量法-稀释热法测定^[22]。4 种酶活(脲酶、过氧化氢酶、蔗糖酶和过氧化物酶)使用紫外分光光度法测定, 具体步骤按照试剂盒说明书进行。

1.4 根际土壤细菌高通量测序

使用 E.Z.N.A.[®] Soil DNA Kit 提取土壤样品的 DNA。通过琼脂糖凝胶电泳检测 DNA 提取质量。使用通用引物 27F (5'-AGRGTTYGATY MTGGCTCAG-3')和 1492R (5'-RGYTACCTTG TTACGACTT-3')扩增细菌 16S rRNA 基因^[23]。PCR 反应条件: 95 °C 5 min; 95 °C 30 s, 58 °C 30 s, 72 °C 45 s, 28 个循环; 72 °C 10 min。PCR 反应体系参考文献[24]。PCR 产物用 QuantiFluor[™]-ST 蓝色荧光定量系统进行检测定量后按照测序量要求进行相应比例的混合, 每个样本重复 3 次。在完成修复补平、连接接头形成类似哑铃型的结构和外切酶去除未连接接头的片段的基础上构建 PacBio 文库。随后, 由 PacBio 三代测序平台进行双端测序, 使用 SMRTLINK (v9)获得一致性 circular consensus sequencing (CCS)序列, CCS 序列的准确性达到 QV20 (99%准确率)水平。高通量测序由上海凌恩生物科技有限公司完成。

1.5 数据分析

通过 Mothur (v.1.35.1)计算 α 多样性指数, 并结合 R 语言等相关软件(R 3.5.1)进行 β 多样性分析。使用 SPSS Statistics 22 (IBM)进行数据处理和统计学分析。通过单因素方差分析(one-way ANOVA)将统计显著性水平设定在 0.05 水平。通过线性判别分析(linear discriminant analysis effect size, LEfSe)获得 EM 复合菌处理

后色素辣椒根系土壤中的微生物标志物, 其中筛选阈值设置为 >3.50 。进行扩增子分析时, 基于 98.65%相似度的 OTU 聚类^[25]。

2 结果与分析

2.1 EM 复合菌对辣椒生长发育和产量的影响

与对照组相比, EM 复合菌处理组的辣椒株高和鲜重分别提高 23.89%和 85.41% ($P<0.001$, 表 1), 能够明显观察到处理组色素辣椒的生长状态优于对照组(图 1)。EM 复合菌施用导致辣椒株高显著升高(是对照组的 1.24 倍), 这会相应增加植株鲜重和叶片数量, 表明 EM 复合菌能够有效促进辣椒植株的生长。同时, 施用 EM 复合菌使得单个果重增加 42.31%, 单株结果数增加 13 个, 最后的成品椒个数增加 4 个/株。另外, EM 复合菌还在一定程度上增加辣椒的果重(图 2)。可见, EM 复合菌的施用能够提高色素辣椒产量。

2.2 EM 复合菌对土壤理化指标的影响

EM 复合菌的施用提高了土壤有机质、碱解氮和速效磷的含量, 尤其是碱解氮和速效磷的含量分别提高 5.83%和 13.39% (图 3)。EM

复合菌的施用还显著提高脲酶、蔗糖酶和过氧化物酶的活性, 分别比对照组提高 11.47%、9.42%和 21.43%。新疆种植色素辣椒的土壤本身为碱性, 而 EM 复合菌的施用并未对土壤 pH 产生显著影响。

2.3 EM 复合菌对辣椒根际细菌 α 和 β 多样性的影响

根际土壤样本经测序后一共得到 176 987 条有效序列, 在 98.65%相似性对非重复序列(不含单序列)进行聚类后得到 19 671 个特征 OTU。所有曲线均逐渐达到饱和(图 4A), 表明本研究所有样本的序列结果能够真实地反映辣椒根系细菌群落组成结构。

α 多样性指数包括 Chao1 指数、Shannon 指数、Simpson 指数和 ACE 指数, 能够表征物种的多样性、均匀度及物种丰度。EM 复合菌的施用并未提高细菌群落 α 多样性, 反而有所降低(表 2), 其中 Chao1 指数、Shannon 指数和 ACE 指数均显著性降低($P<0.01$)。 β 多样性结果显示对照组和 EM 复合菌液处理组的群落结构能够明显区分开, PCoA1 和 PCoA2 的解释度分别为 37%和 25% (图 4B), 表明 EM 复合菌显著改变了辣椒根际细菌群落结构。

表 1 施用 EM 复合菌后色素辣椒生长指标和产量变化

Table 1 Changes of pigment pepper growth indexes and yield after inoculation with EM biofertilizer

Items	Control	Treatment
株高 Plant height (cm)	62.50 \pm 0.87	77.43 \pm 0.90***
茎粗 Stem diameter (mm)	0.94 \pm 0.08	1.10 \pm 0.11
根系长 Root length (cm)	22.50 \pm 1.11	22.80 \pm 1.41
植株鲜重 Fresh weight (g)	621.33 \pm 5.51	1 152.00 \pm 103.12***
叶长 Leaf length (cm)	8.80 \pm 0.49	9.63 \pm 0.49
叶宽 Leaf width (cm)	4.58 \pm 0.21	4.95 \pm 0.17
单株结果数 Number of fruit per plant	30.00 \pm 5.03	43.00 \pm 4.73*
单株成品椒个数 Number of finished fruit per plant	27.00 \pm 2.89	31.00 \pm 1.53

*: $P<0.05$; ***: $P<0.001$. The same below.

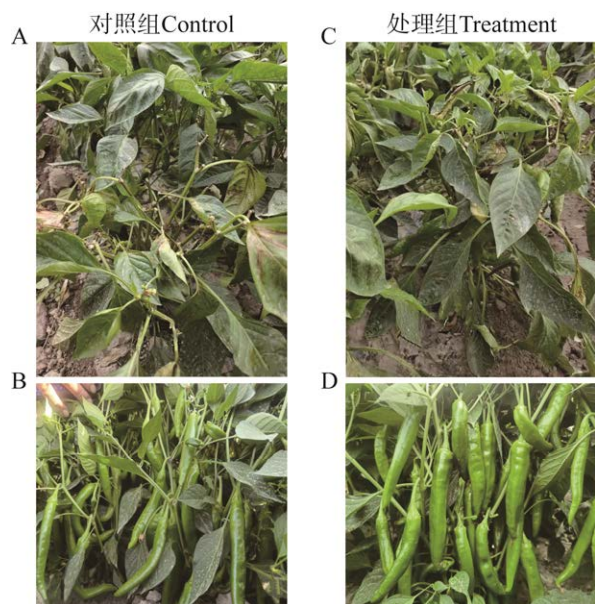


图 1 施用 EM 复合菌对色素椒植株和果实的影响 A、B: 分别为对照组的植株和果实. C、D: 分别为处理组的植株和果实

Figure 1 Effects of EM biofertilizer on pigment pepper plants and fruits. A, B: Plants and fruits in control group. C, D: Plants and fruits in treatment group.

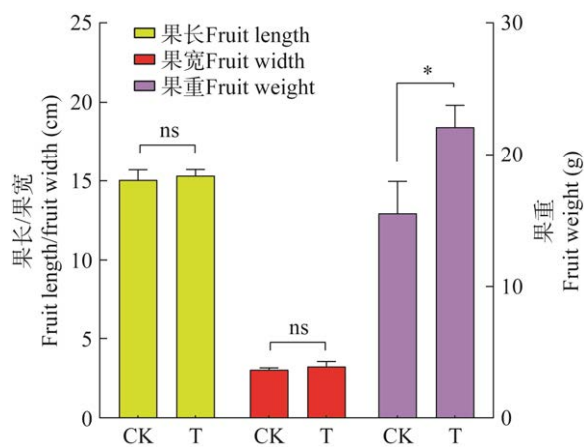


图 2 施用 EM 复合菌对色素椒果长、果宽和果重的影响 CK: 对照组(control); T: 处理组(treatment). ns: 无显著差异. 下同

Figure 2 Effects of EM biofertilizer on fruit length, fruit width and fruit weight of pigment pepper. CK: Control group (control); T: Treatment group (treatment). ns: No significance. The same below.

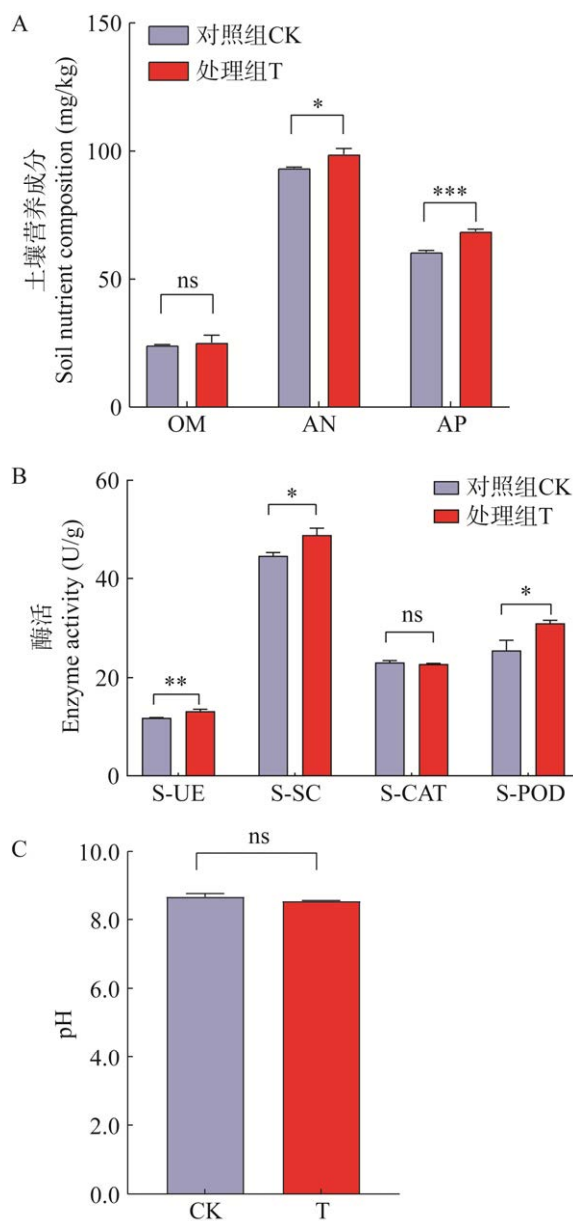


图 3 接种 EM 复合菌液后的土壤 pH、营养成分和酶活变化 A: 土壤中的有机质(OM)、碱解氮(AN)和速效磷(AP)含量. B: 土壤中脲酶(S-UE)、蔗糖酶(S-SC)、过氧化氢酶(S-CAT)和过氧化物酶(S-POD)的活性. C: 土壤 pH 值

Figure 3 Effects of EM biofertilizer on soil pH, nutrients and enzyme activity. A: Content of organic matter (OM), alkali-hydrolyzed nitrogen (AN) and available phosphorus (AP) in soil. B: Activities of urease (S-UE), sucrase (S-SC), catalase (S-CAT) and peroxidase (S-POD) in soil. C: pH of soil.

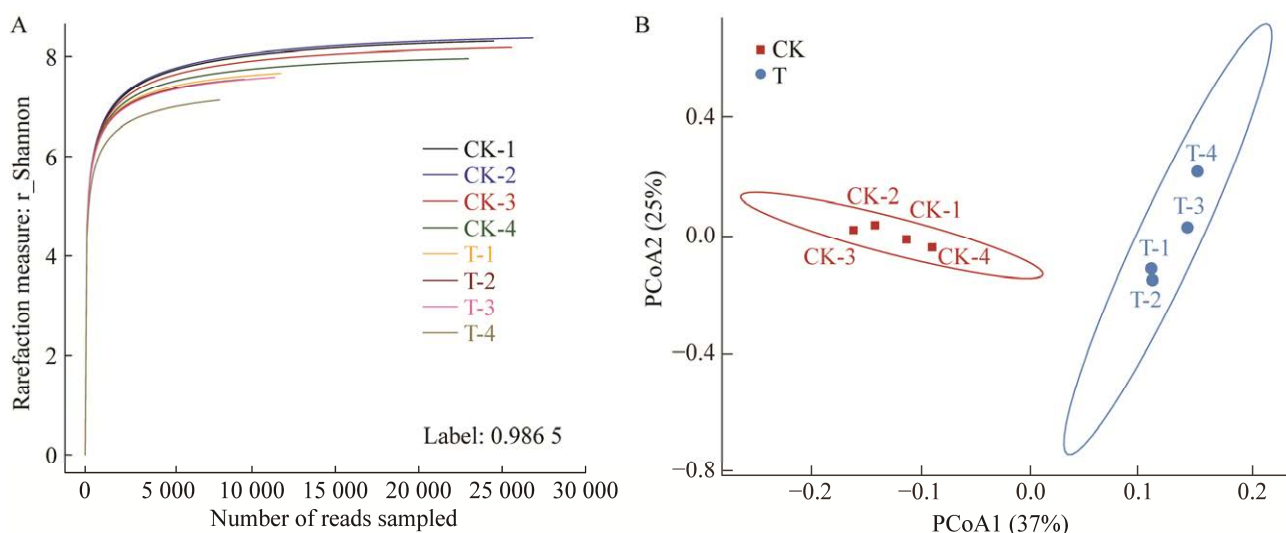


图 4 EM 复合菌施用后的根际细菌群落稀释曲线图(A)和 PCoA 分析(B)

Figure 4 The rarefaction curves of rhizosphere bacterial community after inoculation of EM biofertilizer (A) and the PCoA analysis (B).

表 2 EM 复合菌施用后的根际细菌 α 多样性指数
Table 2 The alpha diversity indexes of rhizosphere bacterial community after inoculation of EM biofertilizer

Items	对照组 Control	处理组 Treatment
Chao1	7 618.81±549.10	7 268.01±719.68**
Shannon	11.83±0.27	11.60±0.44**
Simpson	0.000 875±0.000 126	0.000 925±0.000 171
ACE	8 262.81±497.49	7 884.04±741.26**

** $P < 0.01$. The same below.

2.4 EM 复合菌对辣椒根际细菌群落结构的影响

对 OTU 代表序列进行分类学分析, 归属到 41 个门, 其中变形菌门(*Proteobacteria*)的相对丰度最高, 在对照组与 EM 复合菌处理组的占比分别为 28.94%和 35.10%。并且该门类黄单胞菌科(*Xanthomonadaceae*)的相对丰度相对于对照组增加了 119.32%。施用 EM 复合菌后, 浮霉菌门(*Planctomycetes*)和疣微菌门(*Verrucomicrobia*)的相对丰度分别下降到 9.76%和 1.04%(图 5A–5C)。此外, 放线菌门(*Actinobacteria*)和厚壁菌门(*Firmicutes*)的相对丰度则分别增加 4.81%和 1.57%。这可能是因为 EM 复合菌液中的厚壁菌门乳酸菌

与放线菌门类细菌在土壤中存活并繁殖导致的, 也表明有益菌能够定殖于色素辣椒根系, 并招募其他有益菌种, 重塑根系微生物群落结构。

在属水平上, 所有样本获得 851 个属。除了 unclassified, 对照组和 EM 复合菌处理组中细菌相对丰度最高的均是藤黄色杆菌属(*Luteitalea*), 分别为 5.23%和 7.81%。*Gemmatirosa* 是 EM 复合菌处理组中的第二大属, 其次依次为 *Longimicrobium*、*Litorilinea*、鞘氨醇杆菌属(*Sphingobacterium*)、*Pyrinomonas*、藤黄单胞菌属(*Luteimonas*)、寡养单胞菌属(*Stenotrophobacter*)、小梨形菌属(*Pirellula*)、*Gimesia* 和盐单胞菌属(*Halomonas*)。将对照组与 EM 复合菌处理组的优势属相对丰度进行差异分析, 发现 EM 复合菌的施用显著增加藤黄色杆菌属和藤黄单胞菌属的相对丰度, 尤其是藤黄单胞菌属, 提高 244.17%, 同时显著降低黄杆菌属(*Flavobacterium*)的相对丰度(图 5D 和 5E)。推测 EM 复合菌能够招募藤黄色杆菌和藤黄单胞菌属, 形成稳定菌群, 共同促进色素辣椒生长, 其产生的抗菌物质可能抑制了黄杆菌属等的生长。

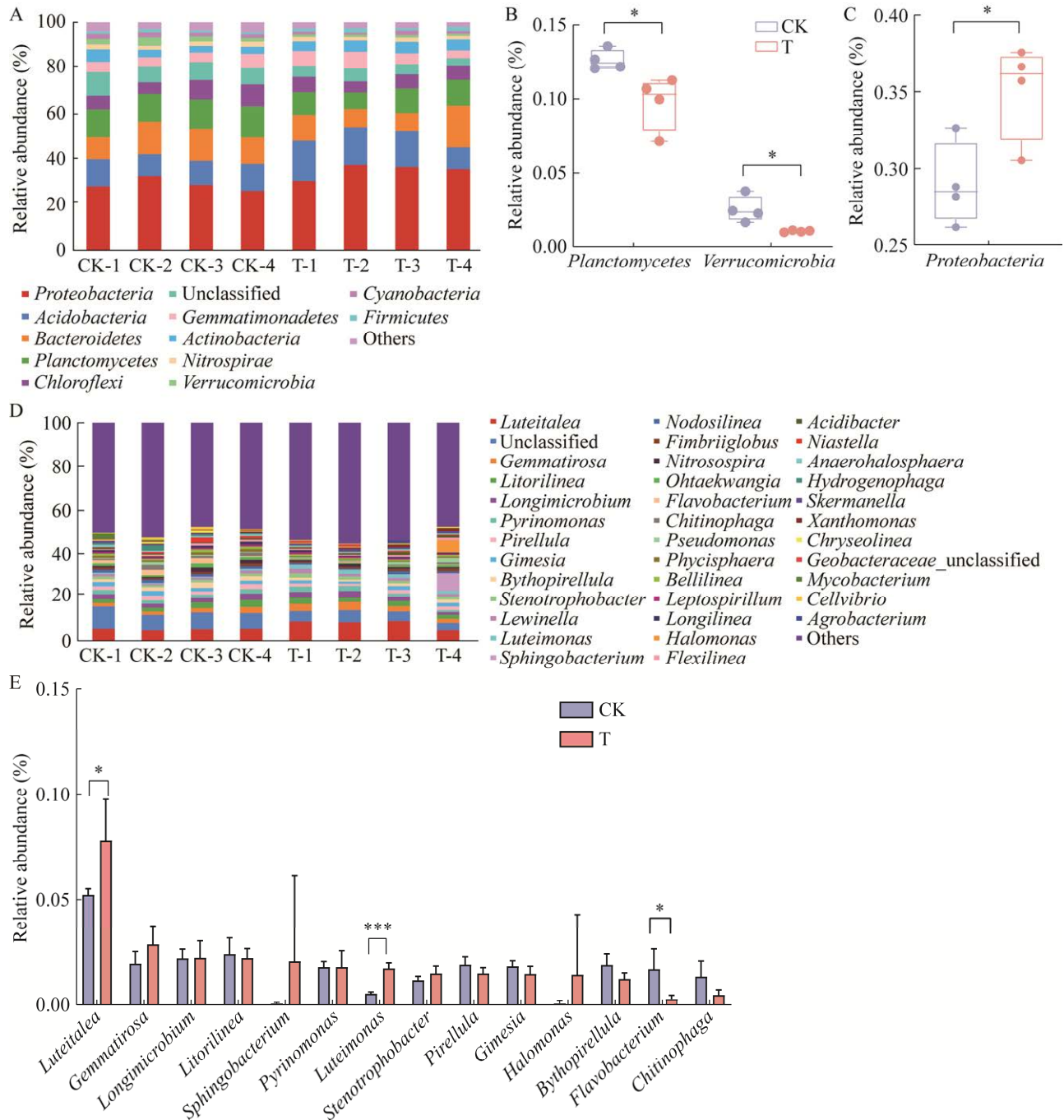


图 5 EM 复合菌施用后的根际细菌群落结构分析 A: 门水平下细菌种类相对丰度柱形图. B、C: 空白组和处理组中在门水平上具有显著性差异的微生物. D: 属水平下细菌种类相对丰度柱形图. E: 空白组 (CK) 和处理组中在属水平上具有显著性差异的微生物

Figure 5 Analysis of bacteria community structure after inoculation of EM biofertilizer. A: Column diagram of relative abundance of bacteria at the phylum level. B, C: Microorganisms with significant differences at the phylum level in control group and treatment group. D: Column diagram of relative abundance of bacteria at the genus level. E: Microorganisms with significant differences at the genus level in control group and treatment group.

为进一步研究不同分组所存在的特殊标志性微生物, 本实验基于线性判别分析 LDA 得到 LefSe 分析图。结果显示处理组辣椒根际土壤显著富集了变形菌门(*Proteobacteria*)中的阿尔法变形菌门(*Alphaproteobacteria*)、黄单胞菌科(*Xanthomonadaceae*)和藤黄单胞菌属。对照组中占优势类群在门水平上则为疣微菌门(*Verrucomicrobia*), 在目水平上有小梨形菌目(*Pirellulales*), 从属于浮霉菌纲(*Planctomycetia*)

(图 6)。

2.5 微生物与土壤理化性质相关性分析

以处理组与对照组的微生物在属水平上的相对丰度为分析对象, 通过 *t* 检验筛选出两组之间具有显著性差异($P \leq 0.01$)的菌属, 一共得到 36 种菌属(图 7A), 包括酸微菌属(*Acidimicrobium*)、藤黄单胞菌属(*Luteimonas*)和硫菌属(*Sulfuriferula*)等。将这些菌属与土壤理化指标进行相关性分析, 发现微生物与土壤理化性质具有明显的相关

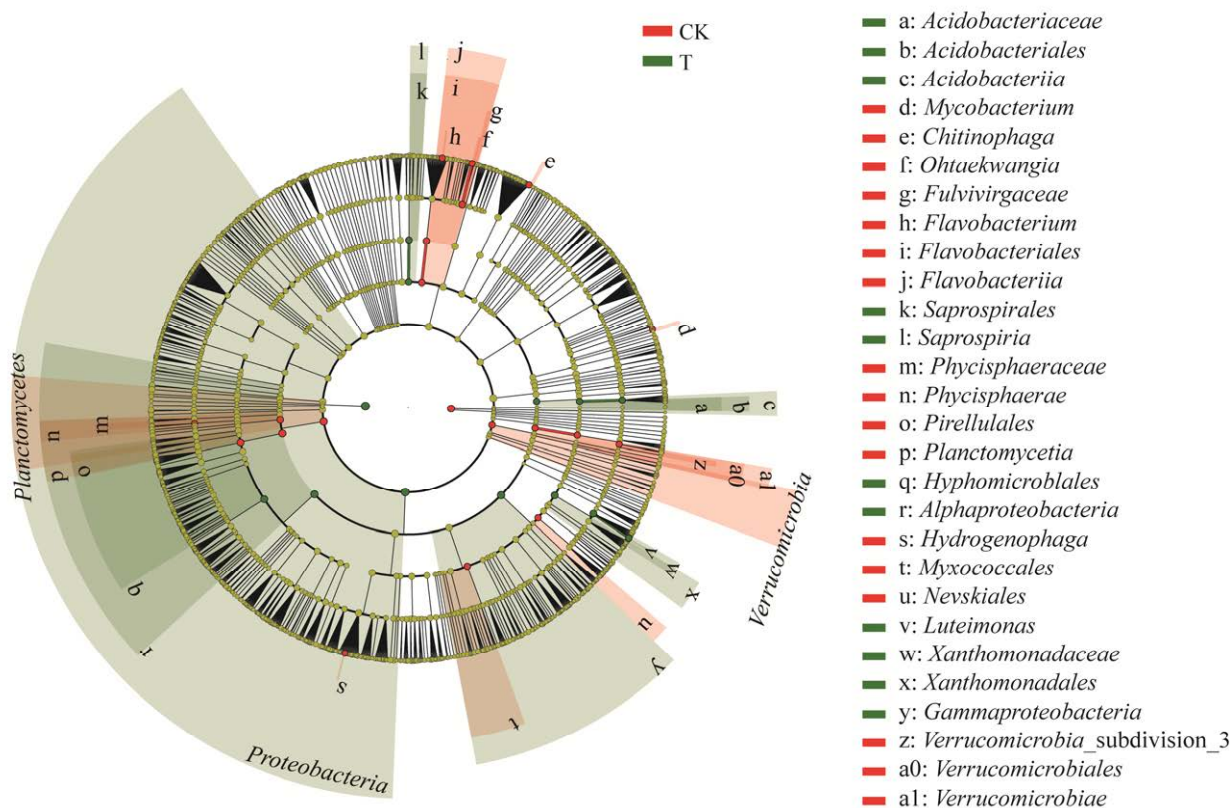


图 6 EM 复合菌施用后根际细菌群落的 LefSe 分析 对照组和处理组的根际土从门到属分类水平的微生物群落结构响应。红色表示对照组显著富集的分类群, 绿色表示处理组显著富集的分类群。仅显示符合线性判别分析显著性阈值 >3.5 的分类单元, 并用颜色进行标记

Figure 6 LefSe analysis of bacteria microbial community after inoculation of EM biofertilizer. Response of rhizosphere soil microbial community from phylum to genus classification level in control group and treatment group. Red represents significantly enriched taxa in the control group and green represents significantly enriched taxa in the treatment group. The threshold value of difference feature LDA is 3.5, and color-coded.

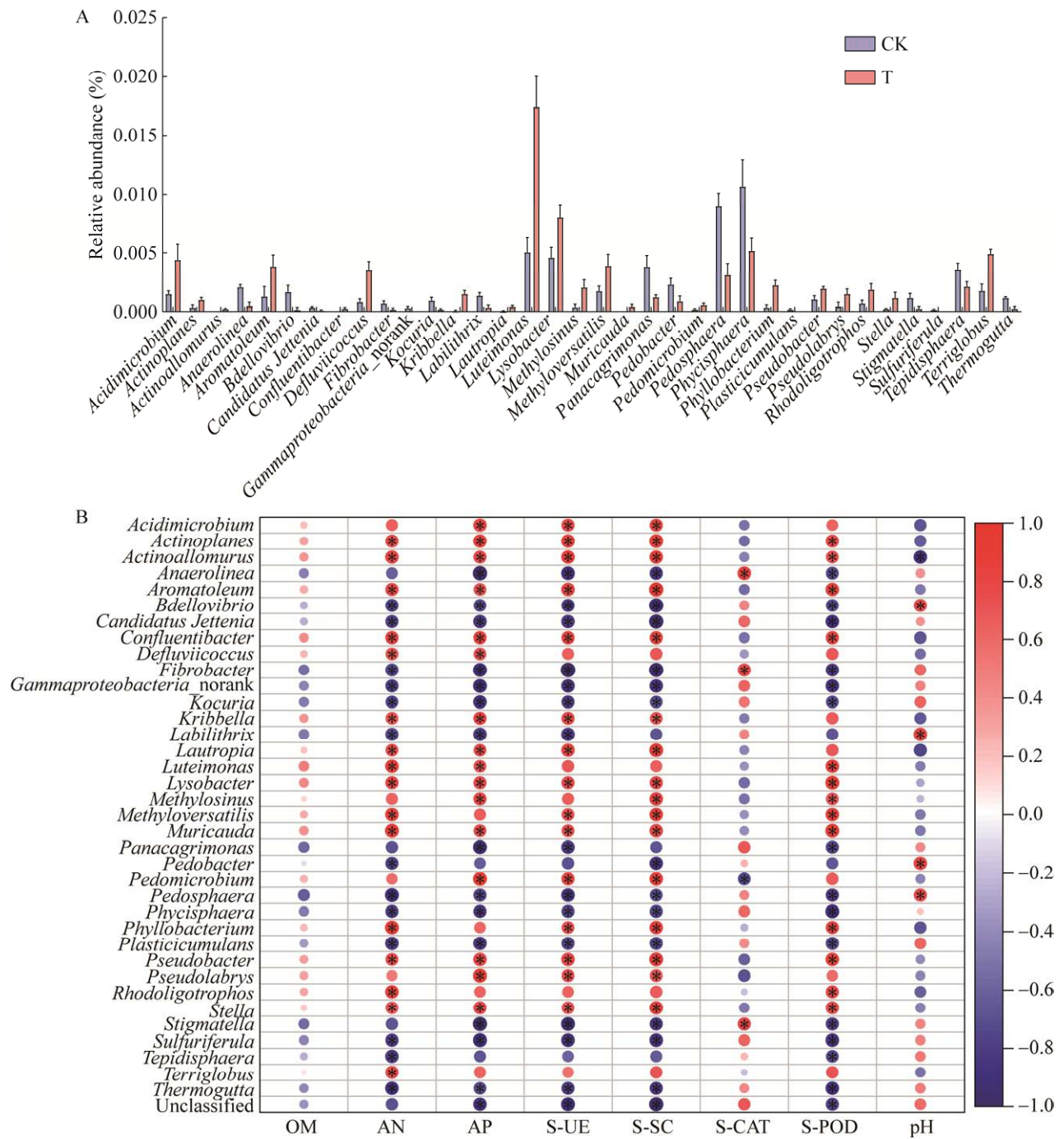


图7 微生物相对丰度图(A)和相关性分析图(B) A: 微生物在属的水平上的相对丰度. B: 微生物与土壤理化指标的相关性分析. 所选属为处理组和对照组通过 t 检验筛选出的显著性 $P \leq 0.01$ 的属. 相关性分析的显著性设为 $P \leq 0.05$

Figure 7 Abundance map of microorganisms (A) and correlation analysis map (B). A: Abundance of microorganisms at the genus level. B: Correlation analysis between microorganisms and soil physicochemical indexes. The significance of the selected genera in treatment group and control group was screened by t test ($P \leq 0.01$). The significance of correlation analysis was set as $P \leq 0.05$.

性,尤其是碱解氮、速效磷、脲酶、蔗糖酶和过氧化物酶。其中,酸微菌属(*Acidimicrobium*)、放线异壁酸菌属(*Actinoallomurus*)、游动放线菌属(*Actinoplanes*)和藤黄单胞菌属(*Luteimonas*)等与土壤理化性质呈现显著正相关,蛭弧菌(*Bdellovibrio*)、*Pedospaera* 和 *Thermogutta* 等菌属与土壤理化性质呈现显著负相关(图 7B)。与对照组相比,EM 复合菌处理组中与土壤理化指标呈现正相关的菌属相对丰度均有所升高,表明 EM 复合菌液的施用增加了对土壤肥力有益的微生物菌属。

3 讨论与结论

本研究发现施加 EM 复合菌可以显著提高新疆色素辣椒的株高和鲜重、增加单株果重和结果数,证实 EM 复合菌能够提高色素辣椒的产量。EM 菌的促生能力在松柳、油菜种子和菠菜上也得到了验证,并且针对不同作物,EM 菌存在一个最适浓度范围^[26]。此外,施用 EM 复合菌还能改善土壤盐碱化,显著提高土壤肥力,增加土壤碱解氮和速效磷含量,进而促使辣椒吸收更多的营养成分。例如,EM 菌能够保护四季豆(*Phaseolus vulgaris* L.)免受盐度的不利影响,并提高四季豆产量^[27]。除了土壤盐碱化,经济作物还面临着连作障碍问题,作物根系微生态被破坏,导致参与营养元素循环的酶活性降低。土壤酶活是评估土壤肥力的重要指标,并且与碳和氮的转变密切相关^[28]。研究发现施用 EM 菌能够提高连作大蒜(*Allium sativum* L.)根际土壤酶活性,减少有害物质的累积^[29]。本试验发现,接种 EM 复合菌液后土壤中脲酶、蔗糖酶和过氧化物酶活性显著提高。脲酶和蔗糖酶活性分别与有效氮浓度和总氮浓度呈现显著正相关性^[30],表明施用 EM 复合菌可能会增加土壤的固氮能力。EM 复合菌的施用降低

了 α 多样性指数值,可能是因为 EM 复合菌中的乳酸菌能够合成抗生素,抑制了有害细菌的生长^[31]。在成品辣椒保存期,乳酸菌分泌的 1-戊醛(1-pentanal)能够抑制黄曲霉(*Aspergillus flavus*)污染^[32],从而延长成品辣椒的保存期。在育种和幼苗期,EM 菌液同样能提高种子发芽率和质量^[33],并且维持茎根比在 1.28–2.50 之间,以获得优质辣椒幼苗^[34]。处理组富集的黄单胞菌科(*Xanthomonadaceae*)能够分泌次生代谢产物,也能抑制病原菌^[35]。李凤等^[36]在番茄栽培中接种枯草芽孢杆菌(*Bacillus subtilis*) Z54 之后,番茄根系土壤中细菌群落的丰富度和多样性指数显著性降低,这与本研究结果一致。针对于新疆土壤现状,多样性的降低也许和病原菌丰度降低有关,这一点需要更进一步地研究。PCoA 分析证实了 EM 复合菌液的使用改变了根际细菌群落结构,对细菌群落的 β 多样性产生明显影响。

高通量测序结果显示,EM 复合菌的施用改善了辣椒根际土壤细菌的多样性,显著提高了变形菌门中的阿尔法变形菌门(*Alphaproteobacteria*)和伽马变形菌门(*Gammaproteobacteria*)。酸杆菌门(*Acidobacteria*)在酸性条件下容易被富集,与有机酸释放导致的 pH 下降有关^[37],本试验中 EM 复合菌的施用提高了辣椒根际酸杆菌门(*Acidobacteria*)的丰度,对偏碱性的土壤有所改善,但是无显著性差异。EM 复合菌处理组的厚壁菌门(*Firmicutes*)的相对丰度有所提高,但是属于该门的乳杆菌属(*Lactobacillus*)丰度无明显变化,这可能与植物促生细菌在盐碱地中的生存适应性较差有关^[38],说明菌肥中的有效菌种不是通过提高某种有益菌的丰度促进辣椒生长,而是通过改善辣椒根际微生物群落结构起到促生作用。另外,数据分析发现藤黄单胞菌属(*Luteimonas*)是黄单胞菌科(*Xanthomonadaceae*)富

集的主要原因。在处理组中,鞘脂单胞菌属(*Sphingobacterium*)的相对丰度显著升高,成为优势菌属。研究发现鞘脂单胞菌属(*Sphingobacterium*)、固氮菌属(*Azotobacter*)和伯克氏菌属(*Burkholderia*)结合使用并配比 75%氮磷钾肥,洋葱产量能够提高 3.70%^[39]。嗜盐单胞菌属(*Halomonas*)也属于变形菌门,根据 pH 值可知新疆土壤呈现碱性,而 EM 复合菌液的施用恰好富集了该属,表明该属更能适应碱性土壤环境,并在促生菌的协同下得到富集,具有促进盐生植物生长的能力^[40]。Martínez 等^[41]将一株嗜盐单胞菌(*Halomonas maura*, 一种中等嗜盐细菌)与一株固氮菌(*Ensifer meliloti*)结合使用能够提高盐碱地中苜蓿产量。*Gemmatirosa* 通常在有机质较高和水分充足的条件下富集^[42],因此该属的相对丰度显著增加说明 EM 复合菌的施用提高了土壤质量。研究发现,藤黄色杆菌属(*Luteitalea*)是一种化学有机营养细菌,能够在很宽的 pH 和温度范围内生长^[43],说明在碱性土壤条件下,生命力较强的有益菌群得到富集,并且该属相对丰度的提高与硅元素的吸收密切相关^[44]。大量研究表明,黄杆菌属(*Flavobacterium*)具有促生能力,可作为植物促生菌使用^[45-48],但是本研究中该属的相对丰度降低,可能与微生物之间的生存竞争有关系。LEfSe 分析表明在处理组辣椒根际相对丰度降低的门类反而在对照组中被富集,比如拟杆菌门(*Bacteroidetes*)、浮霉菌门(*Planctomycetes*)和疣微菌门(*Verrucomicrobia*)。可见,EM 复合菌可以重塑辣椒根际微生物群落结构,构成稳定生物网络。此前,Zhao 等^[49]通过接种放线菌发现辣椒根际微生物群落结构和功能的改变与辣椒品质有密切关联。因此,针对 EM 复合菌液对辣椒品质的研究需要进一步试验。

土壤肥力是作物生长的关键,同时也会影

响土壤中微生物群落结构^[50]。研究表明,利用根瘤菌作为生物接种剂可以提高土壤中氮、磷和钾等营养元素的有效性^[51]。值得注意的是,EM 复合菌需要结合化肥施用。EM 复合菌与氮肥混合使用能够减轻盐胁迫对辣椒生长和产量的损害,显著提高辣椒素、蛋白质和可溶性糖的含量^[52]。也有研究发现结合生物炭使用,EM 复合菌能够显著提高辣椒植株生长、产量、宏微量养分浓度、脱水耐受性和灌溉利用效率^[53]。因此,EM 菌在作物生长过程中以协调辅助作用为主,而不是提供营养物质。本研究中 EM 复合菌的施用提高了土壤中蔗糖酶、脲酶和过氧化物酶的活性,进而刺激有效形式的养分缓慢释放到土壤中,这与 Igalavithana 等^[54]的研究一致。Xu 等^[55]发现有机质、速效磷和脲酶活性与细菌群落具有密切关联性,其中,藤黄单胞菌属(*Luteimonas*)刺激了磷酸酶活性,进而提高速效磷含量。酸微菌属(*Acidimicrobium*)的丰度升高也许和碱解氮含量相关^[56],由此可见,C、N 和 P 含量也是根际微生物群落组成发生变化的主要贡献者^[57]。

综上所述,EM 复合菌能够应用于色素辣椒种植,表现出改善土壤生态环境质量与促进辣椒生长发育的有益作用。研究结果可为色素辣椒专用微生物肥开发,以及微生物-作物互作研究提供参考。然而,考虑到新疆偏碱性的土壤,微生物菌中有效活菌种的定殖能力需要进一步的研究,同时还要关注对色素辣椒的品质提升作用。

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