

王麒,李奎,彭成林,等.球形赖氨酸芽孢杆菌WH07对潜育化水稻土的改良效果和土壤微生态的影响[J].华中农业大学学报,2024,43(4):169-181.DOI:10.13300/j.cnki.hnlkxb.2024.04.019

球形赖氨酸芽孢杆菌WH07对潜育化水稻土的改良效果和土壤微生态的影响

王麒^{1,2},李奎¹,彭成林³,罗治华^{1,2},陈涛^{1,4},易菊平¹,龚艳¹,胡洪涛¹

1.湖北省生物农药工程研究中心,武汉430064;2.长江大学生命科学学院,荆州434025;
3.湖北省农业科学院植保土肥研究所,武汉430064;4.武昌首义学院城建学院,武汉430064

摘要 为建立潜育化稻田微生物改良技术,采用MWMM(modified wolfe's mineral medium)培养基富集微好氧FeOB,结合16S rRNA测序等技术鉴定菌株种类,分别采用100 mL 10⁶ (T1)、10⁷ (T2)、10⁸ (T3) CFU/mL菌株发酵液处理潜育化水稻土,评价菌株对潜育化水稻土壤的还原性物质、土壤养分、氮循环功能基因丰度和水稻秧苗生长的影响,并利用16S rRNA高通量测序技术评价该菌株对土壤微生态的影响。结果显示:筛选到的对Fe²⁺具有较强氧化作用的FeOB为球形赖氨酸芽孢杆菌WH07(*Lysinibacillus sphaericus* WH07);相比于CK,土壤氧化还原电位(Eh)显著提高($P<0.05$),并由负电位转为正电位;T1、T2、T3处理土壤还原性物质总量分别减少26.47%、41.53%、53.19%,亚铁含量分别减少0.37%、21.50%、50.09%,亚锰含量分别减少7.84%、21.57%、37.25%。土壤碱解氮含量分别显著增加15.50%、27.38%、48.90%($P<0.05$),速效磷分别显著增加12.52%、17.34%、27.38%($P<0.05$),速效钾分别显著增加11.56%、17.20%、19.34%($P<0.05$),有机质分别显著增加8.66%、22.22%、45.05%($P<0.05$),pH显著分别增加3.40%、8.94%、16.99%($P<0.05$)。土壤AOA-amoA基因丰度分别增加11.94%、14.68%、33.83%,nosZ基因丰度分别增加42.97%、75.78%、118.75%,nifH基因丰度分别增加38.29%、51.05%、216.13%,UreC基因丰度分别增加16.74%、54.51%、60.94%。水稻株高分别增加5.44%、10.98%、36.00%,叶龄分别增加10.21%、23.42%、36.94%,鲜质量增加分别12.61%、22.52%、28.38%,白根数分别增加10.14%、32.92%、46.81%。土壤微生物多样性指数Chao1和Shannon指数相比于CK均显著降低($P<0.05$)。门水平上相对丰度前10的土壤细菌中,有8个显著下调($P<0.05$),如Proteobacteria等、2个(Bacteroidetes和Firmicutes)显著上调。在相对丰度前50的属中,3个处理分别有20、19、22个属显著上调($P<0.05$),包括Macellibacteroides等6个FeOB;25个属在3个处理中均显著下调($P<0.05$),包括MBNT15等4个铁还原菌。调控网络分析显示菌株WH07潜在地与FeOB协同改善土壤理化性质和生物活性,最终促进了秧苗生长。结果表明,应用菌株WH07显著改善了潜育化水稻土壤理化性质,改变了土壤微生物群落结构和功能。

关键词 潜育化稻田;土壤改良;球形赖氨酸芽孢杆菌;还原性物质;氧化还原电位;氮循环功能基因;土壤微生态

中图分类号 S156.99;S154.3 文献标识码 A 文章编号 1000-2421(2024)04-0169-13

潜育化稻田是我国南方主要低产田类型,面积约有346万hm²^[1],且近来有扩大趋势,潜育化稻田中的水稻生长发育不良、产量仅为正常的一半,这严重制约着南方地区水稻稳产和增产。潜育化是由于土壤长期在渍水条件下严重缺氧,土壤中有毒还原性物质如Fe²⁺等累积、氧化还原电位(Eh)下降、土壤变成蓝灰色或青灰色的土壤退化现象^[2]。潜育化土

壤中有毒还原性物质累积、生物活性不高、矿化度低、有效养分偏少等导致水稻生长慢、分蘖少、黑根多、僵苗不发、迟熟低产^[1]。然而,潜育化稻田主要分布于肥水条件较好的低洼地区,土壤富含有机质和全量养分,具有较大的增产潜力^[2],对这类稻田进行有效改良和利用将有利于我国粮食生产安全。目前,潜育化稻田主要采用以下5种方法进行改良。(1)农

收稿日期:2023-12-11

基金项目:湖北省重点研发计划项目(2021BBA075);湖北省农业科技创新团队项目(2021-620-000-001-027)

王麒,E-mail:1289326605@qq.com

通信作者:胡洪涛,E-mail:hongtaohu@hbaas.com

业工程法。修建排水措施,如开挖排水沟、埋设暗沟、暗管等^[3],该方法可显著降低地下水位和改变土壤长期渍水情况,可提高水稻产量10.1%~13.3%^[2]。农业工程建设对治理潜育化土壤行之有效,但限于自然条件和成本等原因,在生产中难以大面积推广。(2)优化种植制度。改冬沤为冬晒,减少土壤淹水时间。改冬闲为冬种,在部分光照条件好、地下水位不高、潜育化程度较弱的农田种植绿肥、油菜等,实行水旱轮作^[4]。(3)增肥改土。增施磷钾肥、暖性肥料和生石灰^[5],提高养分供给、水温和土壤pH,减轻对禾苗根系伤害,但长期大量使用生石灰易造成土壤板结。(4)实行垄作。对传统的平作田做好垄沟,水不浸过垄面,使垄面泥土长期保持浸润,垄上种稻,长期自然免耕^[6],但该措施操作难度较大,不易大面积推广。(5)化学改良法。采用释氧剂,如过氧化物、过氧化尿素等作肥料^[7],释氧剂施入稻田后,氧气快速释放出来,有毒有害还原物质得以削减,水稻根系活力和产量得以提高^[8]。然而,释氧剂在土壤中反应迅速、持效期短,难以在整个水稻生育期内发挥作用^[7]。目前采用微生物改良潜育化稻田的研究未见报道,但土壤中 Fe^{2+} 受到FeRB(Fe^{3+} reducing bacteria)与FeOB(Fe^{2+} oxidizing bacteria)的调控和转化。其中FeRB是一类能将 Fe^{3+} 还原为 Fe^{2+} 的微生物,能降低土壤Eh、加重土壤潜育化;而微好氧FeOB是一类对氧气需求量少的土壤微生物,能够适应稻田低氧环境,对铁氧化作用净反应速率的平均贡献可达44.6%,能显著降低 Fe^{2+} 半衰期,加快二价铁氧化速率^[9]。为此,应用此类微生物有可能改良潜育化土壤,并且微生物施用简便、环境兼容性好,能在土壤中长期存活,可在相对长的时间段发挥作用。然而,目前关于FeOB的研究尚不多见,且对潜育化水稻土壤改良效果也未见报道。本研究从潜育化土壤中筛选获得FeOB,通过盆栽试验验证该菌株对潜育化土壤的改良效果,并通过实时荧光定量PCR技术(qPCR)和高通量测序技术探讨该菌株对氮循环功能基因丰度和土壤微生物态的影响,以期为潜育化土壤高效微生物改良剂研发奠定基础。

1 材料与方 法

1.1 FeOB的富集和分离

采集荆州市沙市区观音垱镇丫角村(112.25°E, 30.31°N)潜育化水稻土,立即装入厌氧袋于4℃条件下带回。采用 $\text{Fe}(\text{II})-\text{O}_2$ 逆浓度梯度管法^[10]富集FeOB。在灭菌的15 mL具塞玻璃瓶底部中加入

2.0 g FeS粉末,并沿管壁加入10 mL MWMM半固体培养基^[11]与100 μL 的土壤1 000倍浸提稀释液后,置于30℃培养箱中避光培养。培养14 d后,采用梯度稀释法对FeS氧化带中的细菌进行单菌落分离和纯化,纯化后的菌株采用LB液体培养基于28℃、150 r/min发酵48 h,将100 μL 浓度为 10^6 、 10^7 、 10^8 CFU/mL发酵菌液再次回接到加有FeS粉末的MWMM培养基中,以LB培养基做对照,置于30℃培养箱中避光培养,10 d后观测FeS氧化情况,将有明显氧化现象的菌株使用甘油管于-80℃保存。

1.2 菌株鉴定

1)形态学鉴定。筛选获得的菌株经结晶紫染色后,在BX43光学显微镜[奥林巴斯(中国)有限公司]下进行形态学观测。

2)生理生化鉴定。参照文献^[12-13]进行生理生化鉴定,包括革兰氏染色、 H_2O_2 酶试验、甘露糖利用、果糖利用、硝酸盐还原试验、葡萄糖利用等。

3)16S rRNA鉴定。采用细菌基因组DNA提取试剂盒(天根生化科技有限公司)进行基因组DNA提取,采用16S rRNA通用引物27F(5'-AGAGTTT-GATCCTGGCTCAG-3')/1492R(5'-CTACGGC-TACCTTGTTACGA-3')进行PCR扩增^[14],测序序列在NCBI网站上进行BLAST比对,并利用MEGA 11.0软件采用邻接法构建系统发育树^[15]。

1.3 对潜育化水稻土的改良效果评价

1)菌液制备。将-80℃甘油管保藏的FeOB于LB培养基上活化24 h,挑取单菌落接种于LB液体培养基中,于28℃、150 r/min条件下培养48 h,调整菌液浓度为 10^6 、 10^7 、 10^8 CFU/mL,4℃保存备用。

2)试验处理。将荆州市沙市区观音垱镇丫角村采集的潜育化水稻土分装至直径20 cm、高25 cm的塑料钵中,表面覆盖2~3 cm水层,保持5~7 d后用于盆栽试验。分别将100 mL浓度为 10^6 (T1)、 10^7 (T2)、 10^8 (T3) CFU/mL菌株WH07发酵液采用直径2 cm的塑料管分5个点注入到距地表10~15 cm的土层中,对照(CK)使用等量LB培养基,每个处理3个重复,每个重复5钵。菌液处理后,将催芽的水稻种子按每钵10粒播入盆栽钵内,置于温室中培养:温度30℃,12 h光照,12 h黑暗。

3)土壤氧化还原电位测定。处理前和处理后每隔10 d,使用TR-901型土壤ORP仪(上海仪电科学仪器股份有限公司)按照厂家说明书测量土壤的氧

化还原电位。

4)土壤样本采集和保存。处理后30 d,采用五点法采集每钵水稻表层5~10 cm土壤样品,每个重复为1组(5钵/组),每组土壤样品分为2部分:一部分液氮速冻后储存于-80 °C冰箱,用于土壤基因组DNA提取,另一部分在4 °C下保存,用于土壤理化性质测定。

5)土壤理化性质测定。参照文献[16]中的测定方法,土壤pH的测定采用电位法($m_{\text{水}}:m_{\text{土}}=2.5:1$),土壤有机质(soil organic matter, SOM)含量的测定采用重铬酸钾氧化-容量法,碱解氮(alkaline hydrolyzable nitrogen, AHN)含量的测定采用碱解扩散法,速效磷(available P, AP)含量的测定采用碳酸氢钠浸提法,速效钾(available K, AK)含量的测定采用乙酸铵提取法,土壤还原性物质总量的测定采用重铬酸钾氧化法,亚铁含量的测定采用邻菲罗啉比色法,亚锰含量的测定采用高碘酸钾比色法。

6)土壤细菌16S rRNA基因高通量测序及数据处理。采用FastDNA SPIN Kit试剂盒(安诺伦生物科技有限公司)对采集的土样进行基因组DNA提取,使用NanoDrop 2000和1%琼脂糖凝胶电泳测定DNA浓度和质量。首先采用通用引物338F(5'-ACTCCTACGAGAGGCAGCAG-3')/806R(5'-GGACTACHVGGGTWTCTAAT-3')[17]对细菌16S rRNA基因的V3-V4区域进行PCR扩增,PCR扩增程序如下:98 °C变性5 min;98 °C变性30 s,52 °C退火30 s,72 °C延伸1 min,25个循环;72 °C延伸5 min。PCR扩增产物采用Agencourt AMPure

Beads试剂盒(贝克曼库尔特商贸(中国)有限公司)纯化后,使用PicoGreen dsDNA检测试剂盒(美国英杰生命技术有限公司)进行定量,然后在上海派森诺生物科技有限公司Illumina MiSeq平台上进行pair-end测序(2×300 bp)。测序数据上传至NCBI数据库,登录号为PRJNA1020755。

测序所得原始数据使用QIIME v1.8.0软件[18]DADA2方法对测序原始数据进行质控、去噪、双端拼接、嵌合体去除,获得扩增序列变体(amplicon sequence variants, ASVs),代表序列与SILVA v132数据库中序列进行同源序列比对并进行物种分类注释。使用Mothur软件[19]计算微生物 α 多样性(Chao1和Shannon指数),并基于Bray-curtis算法通过相似性分析(analysis of similarities, ANOSIM)对不同污染条件下的土壤细菌群落差异进行检验。

7)土壤氮循环功能基因qPCR分析。采用qPCR技术测定4个土壤氮循环功能基因(nitrogen cycling functional gene, NCFG)的丰度,包括固氮酶基因(*nifH*)、氨单加氧酶基因(*AOA-amoA*)、氧化亚氮还原酶基因(*nosZ*)和脲酶基因(*UreC*)。qPCR反应总体积为20 μ L,体系为:10 μ L SYBR Green Master Mix(Bio-Rad Laboratories Inc., USA)、上下游引物各1 μ L、ddH₂O 3 μ L、稀释50倍的DNA模板5 μ L,每个样品3次重复,反应条件设置为:95 °C保温3 min,PCR反应40个循环(95 °C变性20 s,退火20 s,72 °C延伸40 s),72 °C保温2 min。以10倍梯度稀释质粒DNA绘制标准曲线。所用引物见表1。

表1 qPCR分析所用引物

Table 1 Primers for qPCR analysis

基因 Genes	引物名称 Primer names	引物序列(5'→3') Primer sequences	退火温度/°C Annealing temperature	参考文献 References
<i>nifH</i>	nifHF	AAAGGYGGWATCGGYAARTCCACCAC	60	[20]
	nifHRb	TGSGCYTTGTCYTCRCGGATBGGCAT		
<i>AOA-amoA</i>	CrenamoA23f	ATGGTCTGGCTWAGACG	55	[21]
	CrenamoA616r	GCCATCCATCTGTATGTCCA		
<i>nosZ</i>	F-1181	CGCTGTTCTCGACAGYCAC	55	[22]
	R-1880	ATGTGCAKIGCRTGGCAGAA		
<i>UreC</i>	UreC-F	TGGGCCTTAAAATHCAYGARGAYTGGG	55	[23]
	UreC-R	GGTGGTGGCACACCATNANCATRTC		

8)水稻生理指标测定。施用菌剂30 d后,在每个重复中随机选取10株水稻苗测定水稻株高、叶龄、白根数以及植株鲜质量。

1.4 数据处理

利用IMB SPSS Statistics 27软件,采用Duncan's

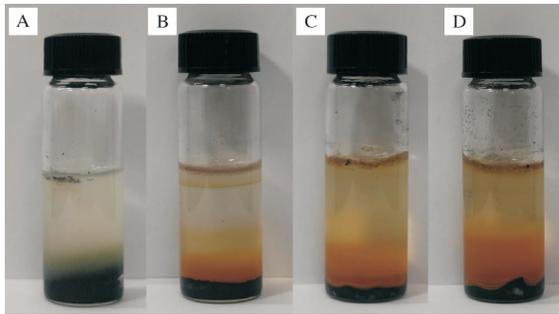
法对土壤氧化还原电位、土壤理化性质、水稻秧苗素质等进行单因素方差分析(ANOVA),采用Pearson法对土壤赖氨酸芽孢杆菌丰度、6个FeOB(*Pseudomonas*、*Rheinheimera*、*Aquaspirillum*、*Comamonas*、*Zoogloea*和*Bacillus*)丰度、4个FeRB(*MBNT15*、*An-*

aeromyxobacter、*Geothrix* 和 *Ignavibacterium*) 丰度、土壤还原性物质含量、土壤 NCFG 丰度、土壤理化性质、水稻秧苗生长指标进行相关性分析。采用 Microsoft 进行图表绘制。

2 结果与分析

2.1 FeOB 对 Fe²⁺氧化能力评价

在接种 WH07 菌株 10 d 后,在 Fe(II)-O₂ 梯度管内培养基与硫化亚铁交界处形成明显的红褐色铁氧化物细菌混合带(图 1),且随着浓度的增加,含有菌株 WH07 的 Fe(II)-O₂ 梯度管中的红褐色铁氧化物细菌混合带越明显,表明该菌株对硫化亚铁有着明显的氧化作用。



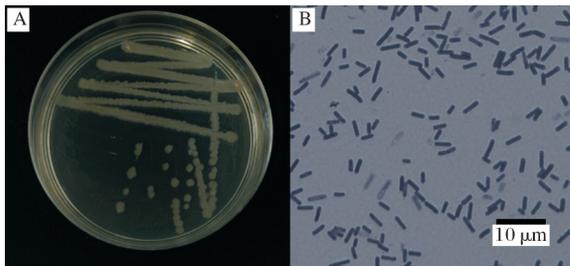
A: CK; B: 10⁶ CFU/mL; C: 10⁷ CFU/mL; D: 10⁸ CFU/mL.

图 1 不同浓度的 WH07 对硫化亚铁氧化结果

Fig. 1 Results of the strain WH07 with different concentration on FeS oxidation

2.2 菌株鉴定

1) 形态学观测。菌株 WH07 菌落呈暗白色,表面光滑,边缘不规则(图 2A),营养体形态为杆状(图 2B),长 3.1~5.0 μm,芽孢形态为近圆球状,大小为 0.7~1.2 μm。



A: LB 培养基上的菌落; B: 菌株 WH07 形态。A: The colonies on LB medium. B: The morphology of the strain WH07.

图 2 菌株 WH07 菌落和菌体图

Fig. 2 The colonies and image of cells of the strain WH07

2) 生理生化鉴定。由表 2 可见,菌株 WH07 革兰氏染色、蛋白水解、淀粉酶活性、果糖利用、半乳糖利

用、H₂O₂ 酶试验、甘露糖利用、葡萄糖利用呈阳性反应,V-P 试验、硝酸盐还原试验、甲基红测试、明胶水解呈阴性反应。

表 2 菌株 WH07 的生理生化分析结果

Table 2 Physiological and biochemical tests of the strain WH07

检测项目 Test items	结果 Results	检测项目 Test items	结果 Results
革兰氏染色 Gram stain	+	硝酸盐还原 Nitrate reduction	-
蛋白水解 Proteolysis	+	过氧化氢酶 Catalase	+
淀粉酶 Amylase	+	甲基红测试 Methyl red test	-
V-P 测试 V-P test	-	甘露糖 Mannose	+
果糖 Fructose	+	葡萄糖 Glucose	+
半乳糖 Galactose	+	明胶水解 Gelatin hydrolysis	-

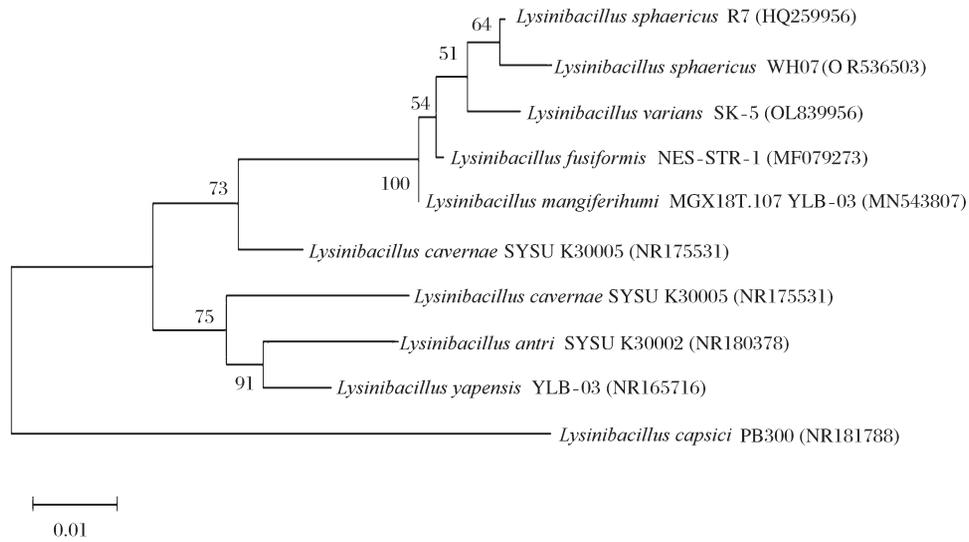
注: +: 阳性反应; -: 阴性反应。Note: +: Positive reaction; -: Negative reaction.

3) 16S rRNA 基因测序。菌株 WH07 的 16S rRNA 长度为 1 000 bp (GenBank 登录号: OR536503), 该序列与 *Lysinibacillus sphaericus* R7 (Genbank 登录号: HQ259956) 相似性最高(图 3), 为 98.90%。综合形态学、生理生化和 16S rRNA 鉴定结果, 将该菌株鉴定为球形赖氨酸芽孢杆菌 (*Lysinibacillus sphaericus*) WH07。

2.3 菌株 WH07 对潜育化水稻土改良效果

1) 菌株 WH07 对土壤 Eh 的影响。由表 3 可见, 菌株 WH07 处理 10 d 后, T1、T2、T3 处理土壤 Eh 值分别为 -5.00、19.00 和 42.67 mV, 均显著高于 CK (-31.67 mV) ($P < 0.05$)。处理 20 d 后, 土壤 Eh 分别为 7.33、40.67、106.67 mV, 均显著高于 CK (-26.33 mV) ($P < 0.05$)。处理 30 d 后, Eh 分别为 10.67、48.33、115.00 mV, 均显著高于 CK (-16.33 mV) ($P < 0.05$)。

2) 菌株 WH07 对土壤还原性物质的影响。在处理 30 d 后, T1、T2、T3 处理土壤还原性物质总量分别为 8.64、6.87、5.50 cmol/kg (图 4), 分别较 CK (11.75 cmol/kg) 减少 26.47%、41.53%、53.19%, 其中 T2 和 T3 处理显著减少 ($P < 0.05$)。土壤还原性亚铁含量分别为 5.33、4.20、2.67 cmol/kg, 分别较 CK (5.35 cmol/kg) 减少 0.37%、21.50%、50.09%, 其中 T2 和 T3 显著减少 ($P < 0.05$)。土壤还原性亚锰含量分别



括号中序号代表菌株的登录号;分支点上的数字代表bootstrap值;标尺代表进化距离。Numbers in parentheses represent the accession number of the strain in GenBank; Numbers on the branch point represent the bootstrap; the scales represent the evolutionary distance.

图3 菌株WH07基于16S rRNA基因序列的系统发育树

Fig. 3 The phylogenetic tree of the strain WH07 constructed on base of 16S rRNA gene sequences

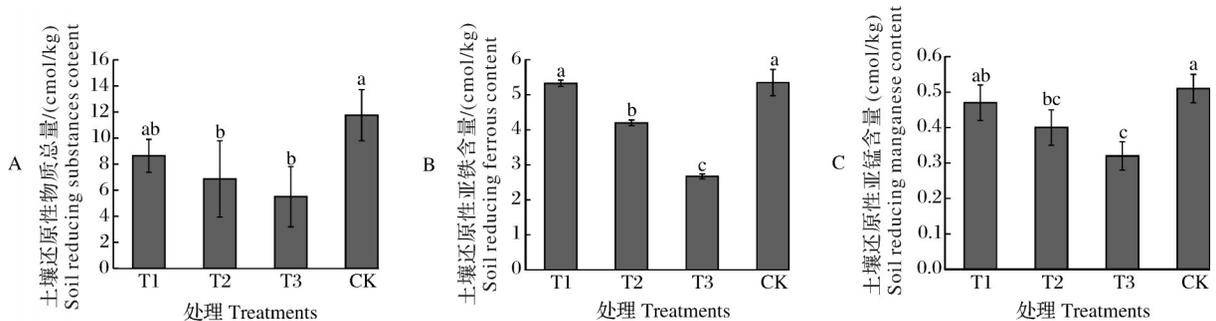
表3 菌株WH07处理对土壤Eh的影响

Table 3 Effects of the strain WH07 treatments on soil Eh

mV

处理 Treatments	处理前Eh Eh before treatments		处理后Eh Eh after treatments	
	0 d	10 d	20 d	30 d
T1	-61.00±6.08a	-5.00±7.21c	7.33±4.04c	10.67±3.51c
T2	-52.00±4.36a	19.00±4.36b	40.67±3.79b	48.33±1.53b
T3	-63.33±9.02a	42.67±8.02a	106.67±6.81a	115.00±5.20a
CK	-63.00±5.29a	-31.67±4.51d	-26.33±5.51d	-16.33±5.51d

注:表中同列数据后不同小写字母表示0.05水平差异显著,下同。Note: Different lowercase letters behind the data in the same columns in the table indicate significant differences at the level of 0.05, the same as below.



不同小写字母表示处理间差异显著($P < 0.05$),下同。Different lowercase letters indicate significant different ($P < 0.05$), the same as below.

图4 菌株WH07处理对土壤还原性物质的影响

Fig. 4 Effects of the strain WH07 treatments on reducing substances in soil

0.47、0.40、0.32 cmol/kg,除T1处理外,分别较CK (0.51 cmol/kg)减少7.84%、21.57%、37.25%,其中T2和T3显著减少($P < 0.05$)。

3)菌株WH07对土壤理化性质的影响。菌株WH07处理后土壤理化性质测定结果见表4。T1、T2、T3处理AHN分别为95.60、105.43、124.07 mg/kg,均显著高于CK(82.77 mg/kg) ($P < 0.05$),分别较CK增加15.50%、27.38%、49.90%。AP含量分别为8.63、9.00、9.77 mg/kg,显著高于CK(7.67 mg/kg) ($P < 0.05$),较CK增加12.52%、17.34%、

27.38%。AK含量分别为39.57、41.57、42.33 mg/kg ($P < 0.05$), 较CK增加8.66%、22.22%、45.05%。土壤pH分别为5.78、6.09、6.54, 显著高于CK (5.59) ($P < 0.05$), 较CK增加3.40%、8.94%、16.99%。

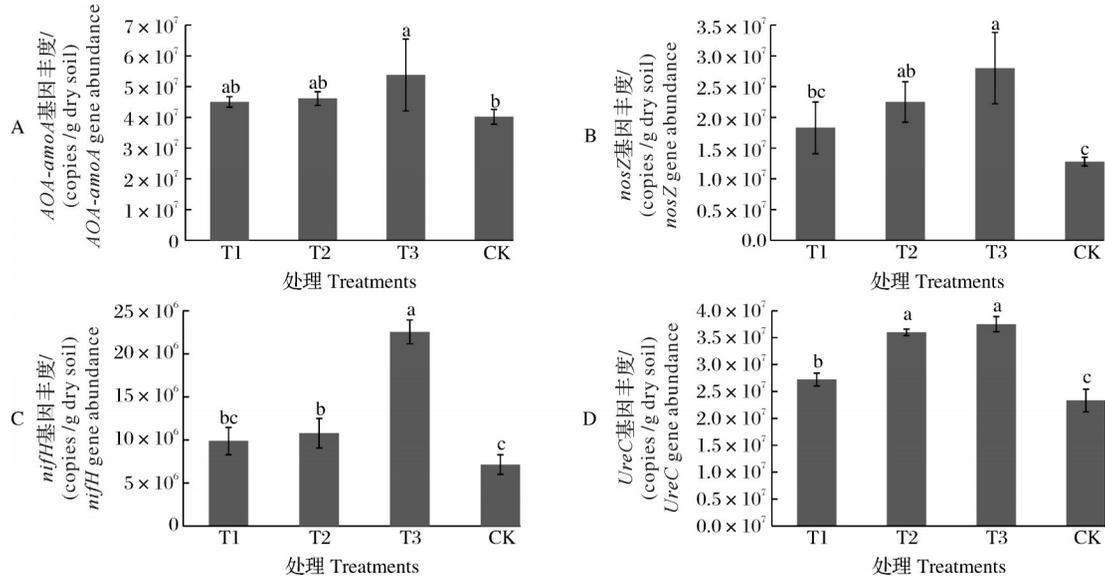
表4 菌株WH07处理对土壤理化性质的影响

Table 4 Effects of the strain WH07 treatments on the soil physiochemical properties

处理 Treatments	碱解氮/(mg/kg) AHN	速效磷/(mg/kg) AP	速效钾/(mg/kg) AK	有机质/(g/kg) SOM	pH
T1	95.60±2.49c	8.63±0.25b	39.57±0.21c	23.47±0.25c	5.78±0.09c
T2	105.43±2.90b	9.00±0.36b	41.57±0.32b	26.40±0.10b	6.09±0.06b
T3	124.07±1.84a	9.77±0.45a	42.33±0.21a	31.33±0.15a	6.54±0.09a
CK	82.77±1.83d	7.67±0.21c	35.47±0.32d	21.60±0.30d	5.59±0.06d

4) 菌株WH07对土壤NCFG丰度的影响。由图5可见, 菌株WH07处理后T1、T2、T3处理AOA-*amoA*丰度分别为 4.50×10^7 、 4.61×10^7 、 5.38×10^7 copies/g, 比CK (4.02×10^7 copies/g) 丰度增加了11.94%、14.68%和33.83%, 仅T3处理增加显著($P < 0.05$) (图5A)。*nosZ*丰度分别为 1.83×10^7 、 2.25×10^7 、 2.80×10^7 copies/g, 较CK (1.28×10^7 copies/g) 丰度增加了42.97%、75.78%、118.75%, 除T1处理

外均显著增加($P < 0.05$) (图5B)。*nifH*丰度分别为 9.86×10^6 、 10.77×10^6 、 22.54×10^6 copies/g, 较CK (7.13×10^6 copies/g) 丰度增加了38.29%、51.05%、216.13%, 除T1处理外, 均与CK差异显著($P < 0.05$) (图5C)。*UreC*丰度分别为 2.72×10^7 、 3.60×10^7 、 3.75×10^7 copies/g, 与CK (2.33×10^7 copies/g) 比较丰度显著增加了16.74%、54.51%、60.94% ($P < 0.05$) (图5D)。

图5 菌株WH07处理后土壤AOA-*amoA*(A)、*nosZ*(B)、*nifH*(C)和*UreC*(D)基因丰度Fig. 5 AOA-*amoA*(A), *nosZ*(B), *nifH*(C) and *UreC*(D) gene abundance after strain WH07 treatments

5) 菌株WH07对水稻秧苗生长的影响。由表5可见, 施用菌剂30 d后, T1、T2、T3处理水稻株高分别为10.66、11.22、13.75 cm, 分别较CK (10.11 cm) 增加了5.44%、10.98%、36.00%, 但仅T3处理显著增加($P < 0.05$)。叶龄分别为3.67、4.11、4.56, 较CK (3.33) 分别增加了10.21%、23.42%、36.94%, 仅T3

处理显著增加($P < 0.05$)。水稻鲜质量分别为2.50、2.72、2.85 g/10 plants, 较CK (2.22 g/10 plants) 分别增加12.61%、22.52%和28.38%, T2和T3处理显著增加($P < 0.05$)。白根数分别为9.67、11.67、12.89, 较CK (8.78) 分别增加了10.14%、32.92%、46.81%, T2和T3处理显著增加($P < 0.05$)。

表5 菌株 WH07 处理对水稻秧苗生长的影响

Table 5 Effects of the strain WH07 treatments on the growth of rice seedlings

处理 Treatments	株高/cm Plant height	叶龄 Leaf age	鲜质量/(g/10 plants) Fresh weight	白根数 White roots number per plant
T1	10.66±0.74b	3.67±0.58ab	2.50±0.12ab	9.67±2.03bc
T2	11.22±0.71b	4.11±0.51ab	2.72±0.20a	11.67±0.33ab
T3	13.75±0.66a	4.56±0.19a	2.85±0.23a	12.89±0.19a
CK	10.11±0.53b	3.33±0.67b	2.22±0.19b	8.78±1.07c

2.4 菌株 WH07 对土壤微生物的影响

1) 菌株 WH07 高通量测序数据分析。T1、T2、T3 与 CK 处理分别获得 7 375、6 241、8 043、10 437 条扩增子测序变体(amplicon sequence variants, ASVs) (图6), 合并为 24 511 个 ASVs, 其中 4 个处理共有的 ASVs 数为 679 (2.77%), T1、T2、T3 与 CK 处理特有的 ASVs 数分别为 4 765 (19.44%)、3 124 (12.75%)、4 039 (16.48%)、7 653 (31.22%)。

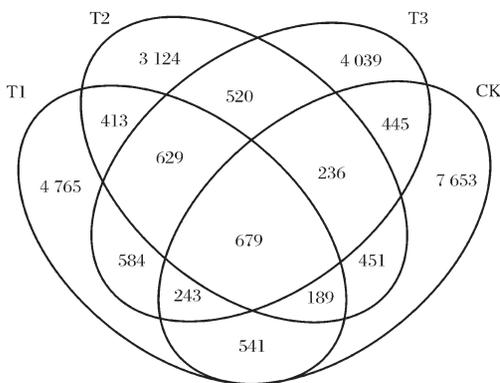


图6 土壤细菌 ASVs 韦恩图

Fig. 6 Venn diagram of soil bacterial ASVs

2) 菌株 WH07 对土壤细菌 α 多样性影响。T1、T2、T3 处理 Chao1 指数分别为 4 031.11、3 340.89、3 635.57 (图7A), 均显著低于 CK (4 747.70) ($P < 0.05$)。Shannon 指数分别为 9.25、8.47、9.23 (图7B), 均显著低于 CK (11.03) ($P < 0.05$)。

3) 菌株 WH07 对土壤细菌 β 多样性的影响。PCoA 分析(图8)结果显示, 第一主成分和第二主成分的分差贡献率分别为 49.2% 和 12.9%, 累计方差贡献率为 62.1%, 主成分 1(PCo1) 是最主要的变量, 占总变异的 49.2%。ANOSIM 分析结果显示, CK 与 T1 ($R=1, P=0.091$)、T2 ($R=1, P=0.106$)、T3 ($R=1, P=0.097$) 处理土壤细菌群落结构间没有显著差异。

4) 菌株 WH07 对土壤细菌群落组成的影响。门水平相对丰度前 10 的土壤细菌组成见图 9A, 相比于

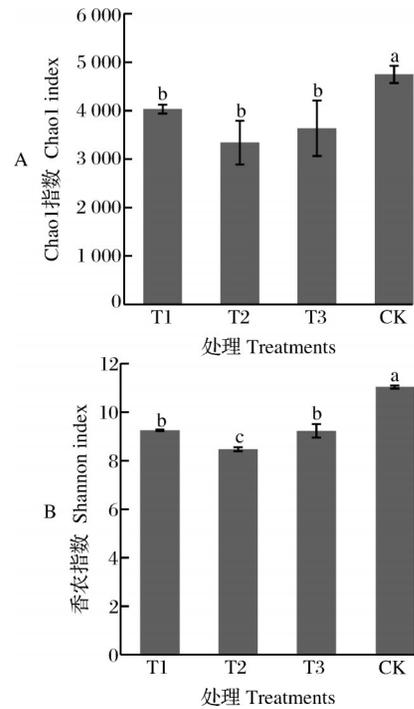


图7 菌株 WH07 处理的土壤细菌 α 多样性指数
Fig. 7 Soil bacterial α diversity indexes under treatments of the strain WH07

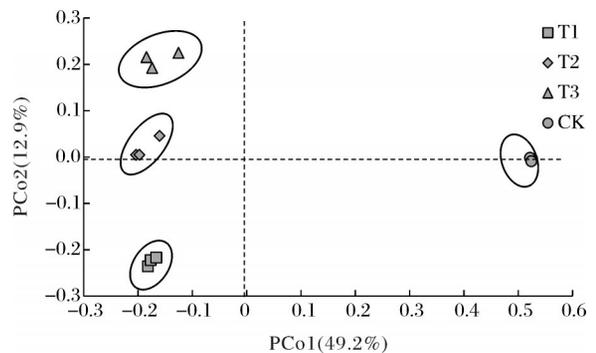


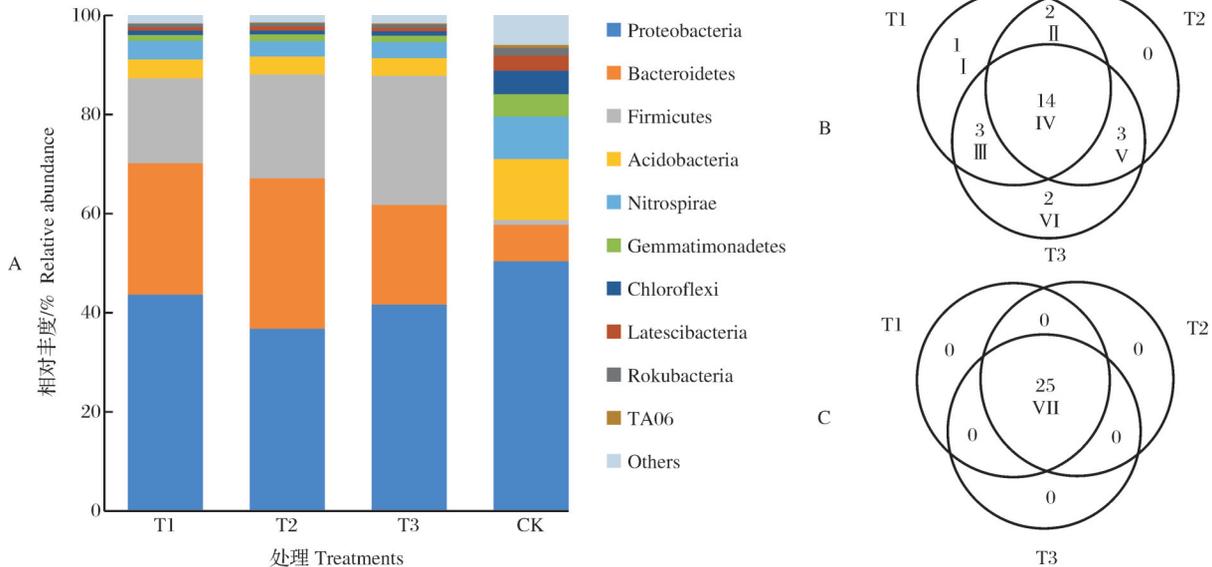
图8 菌株 WH07 处理细菌群落结构 PCoA 分析
Fig. 8 PCoA analysis of bacterial community structure under the strain WH07 treatments

CK, 其中 8 个门在 3 个处理中均显著下调 ($P < 0.05$), 包括 Proteobacteria、Acidobacteria 等, 倍数变化(ln of fold changes) 范围为 -2.00 ~ -0.14; 2 个门 (Bacteroidetes 和 Firmicutes) 在 3 个处理中均显著上调 ($P <$

0.05), 倍数变化分别为1.29、2.90。

在属水平相对丰度前50的土壤细菌中, T1、T2、T3处理分别有20、19、22个属丰度显著上调(图9B), 其中3个处理共有14个属丰度上调, 包括6个FeOB (*Pseudomonas*、*Rheinheimera*、*Aquaspirillum*、

Comamonas、*Zoogloea*和*Bacillus*), 倍数变化范围为0.59~9.57。25个属在3个处理中均显著下调(图9C), 包括4个FeRB (*MBNT15*、*Anaeromyxobacter*、*Geothrix*和*Ignavibacterium*), 倍数变化范围为-1.56~-1.04。



A: 土壤细菌群落在门水平的组成; B: 土壤细菌丰度上调属韦恩图; C: 土壤细菌丰度下调属韦恩图。A: The community of soil bacteria at phylum level. B: Venn diagram of up-regulated soil bacteria at genus level in abundance. C: Venn diagram of down-regulated soil bacteria at genus level in abundance. I: *Aquaspirillum*; II: *Aeromonas*, *Acidaminobacter*; III: *Azoarcus*, *Zoogloea*, *Bacillus*; IV: *Macellibacteroides*, *Pseudomonas*, *Sedimentibacter*, *Rheinheimera*, *Hydrogenoanaerobacterium*, *Acetobacteroides*, *Comamonas*, *Vogesella*, *Clostridium_sensu_stricto_13*, *Clostridium_sensu_stricto_12*, *Thauera*, *Chryseobacterium*, *Paraclostridium*, *Clostridium_sensu_stricto_16*; V: *Lysinibacillus*, *Clostridium_sensu_stricto_1*, *Peptoclostridium*; VI: *Hydrogenophaga*, *Sporacetigenium*; VII: *MBNT15*, *Sva0485*, *Thiobacillus*, Subgroup_6, *Latescibacteria*, *Bacteroidetes_vadin* HA17, *Aminicenanales*, *BSV26*, *Anaeromyxobacter*, Subgroup_18, 4-29-1, *Geobacter*, *Thioalkalispira*, Subgroup_22, Sh765B-TzT-35, *MND1*, *Desulfatiglans*, *Rokubacteriales*, *NB1-j*, *B1-7BS*, *Haliangium*, *Sva0081_sediment_group*, *Geothrix*, *Ignavibacterium*, *S0134_terrestrial_group*.

图9 菌株WH07处理土壤微生物群落组成结构

Fig. 9 Composition and structure of soil microbial community under the strain WH07 treatments

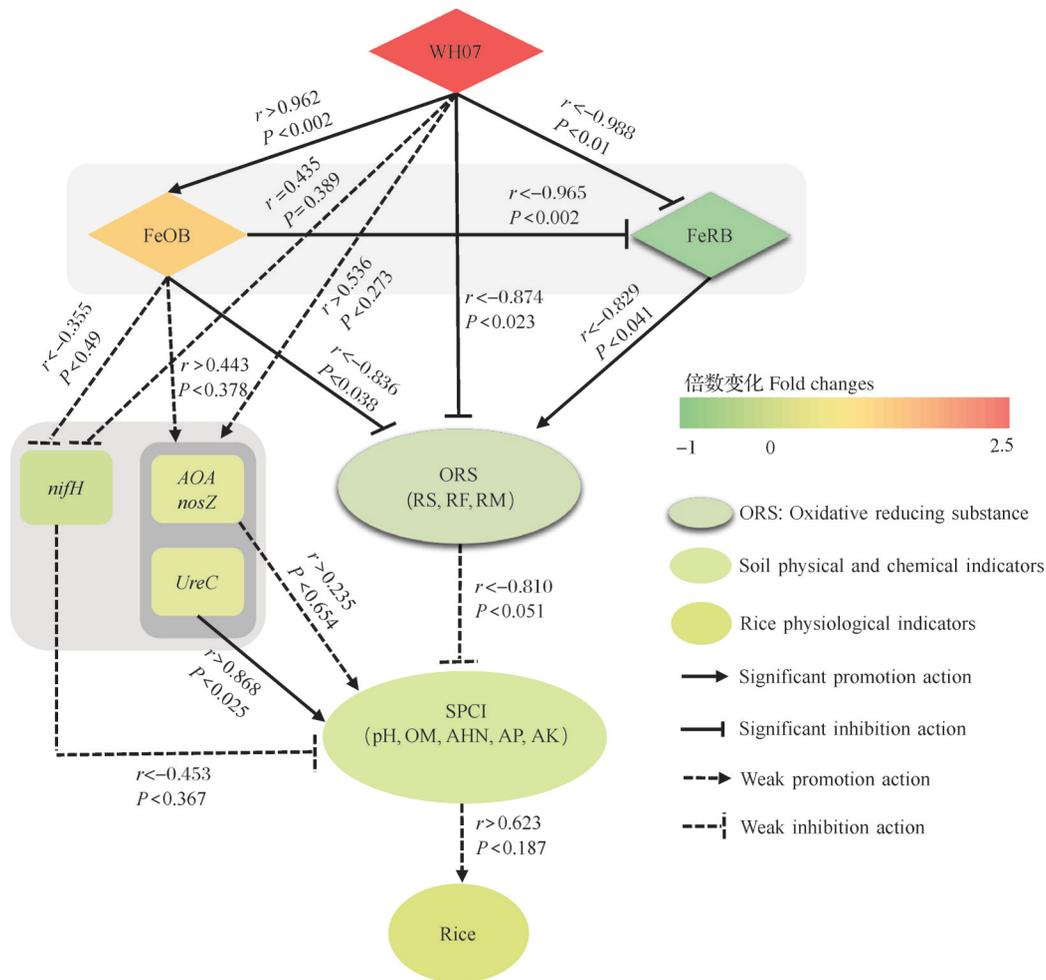
2.5 WH07改良潜育化水稻土壤的调控网络分析

菌株WH07改良潜育化水稻土壤的调控网络见图10。菌株WH07处理显著增加了FeOB丰度($r > 0.963$, $P < 0.002$), 但显著降低了FeRB丰度($r < -0.988$, $P < 0.001$)。在与FeOB和FeRB的协同作用下, 显著降低了土壤还原性物质含量($P < 0.05$), 同时增加了土壤*nifH*、*nosZ*和*UreC*基因丰度($P < 0.05$)与AOA-*amoA*基因丰度($P > 0.05$), 最终改善了土壤理化性质、促进了水稻生长。

3 讨论

土壤微生物是土壤物质循环和转化的关键调控者, 在氮、碳等物质循环和转化以及 Fe^{2+} 和 Mn^{2+} 的氧化还原中扮演重要角色^[24]。土壤长期处在淹水嫌

气条件下, 土壤有毒还原性物质累积, 土温与水温低, 土壤微生物活性降低, 物质分解转化缓慢, 土壤有效养分供应不足, 导致水稻生长迟缓和产量减少^[1]。因此, 有效减少土壤有毒还原性物质含量和提高土壤生物活性是潜育化土壤改良的关键。本研究通过 $Fe(II)-O_2$ 梯度管试验证明赖氨酸芽孢杆菌WH07对亚铁离子具有较好的氧化能力, 可形成明显的氧化带。盆栽试验结果显示, 菌株WH07处理潜育化土壤10 d后, 土壤Eh显著提高且该菌株有效作用时间长达20 d(表3); 同时, 菌液浓度越大, 土壤Eh上升幅度越大。在土壤Eh提升的同时, 土壤还原性物质总量、亚铁与亚锰离子含量显著降低(图4), 表明土壤Eh值与土壤中还原物质密切相关, 且受到土壤FeOB、FeRB等微生物的调控^[25]。菌株WH07的



铁氧化细菌 FeOB: *Rheinheimera*, *Zoogloea*, *Bacillus*, *Comamonas*, *Aquaspirillum*, *Pseudomonas*; 铁还原细菌 FeRB: MBNT15, *Geothrix*, *Ignavibacterium*, *Anaeromyxobacter*.

图 10 菌株 WH07 改良潜育化水稻土壤的调控网络

Fig. 10 Regulatory network of improving gleyed paddy soil mediated by the strain WH07

加入导致土壤 pH 显著增加,当土壤 pH 升至 6.0 以上时,土壤还原性物质显著降低, Fe^{2+} 被大量氧化成 Fe^{3+} 。据报道赖氨酸芽孢杆菌可通过结合铵态氮和硝酸盐来调节 pH^[26],而不同 pH 值对不同类型土壤的 Fe^{2+} 氧化还原过程有着不同的影响, pH 的升高有利于土壤还原性物质的氧化^[27]。WH07 处理增加了土壤中 *AOA-amoA*、*nosZ*、*UreC*、*nifH* 基因丰度,其中 *nosZ* 是反硝化作用过程相关功能基因,而反硝化过程与 Fe^{2+} 氧化之间存在偶联关系^[28],另外 *nifH* 的固氮作用与 *UreC* 脲酶对有机氮的降解作用可能是导致 AHN 增加从而促进水稻生长的主要原因。

除菌株 WH07 自身对土壤还原性物质具有较好氧化能力之外,菌株 WH07 处理还可通过减少土壤还原性物质含量,导致土壤细菌微生物群落组成与结构发生改变、土壤微生物多样性降低,增加土壤 FeOB 丰度、降低 FeRB 丰度、协同改善潜育化土壤环

境。Proteobacteria 是土壤细菌在门分类水平上丰度最高的优势菌,大多数 FeOB 属于该分类单元^[24],包括 α 门、 β 门、 γ 门、 δ 门和 ζ 门。在属水平上, *Pseudomonas* 是优势菌属之一,该菌具有较高的 Mn^{2+} 耐受能力与 Mn^{2+} 氧化能力^[29],本研究结果表明菌株 WH07 与土壤还原性物质呈显著负相关,另外 *Pseudomonas* 还是一种反硝化细菌(*nosZ*),能将硝态氮还原为 N_2O ^[30],在促进土壤氮循环的同时,也潜在地促进了 Fe^{2+} 的氧化。 *Rheinheimera*、*Zoogloea*、*Bacillus*、*Comamonas*、*Lysinibacillus*、*Aquaspirillum* 丰度也显著增加,其中 *Rheinheimera*、*Comamonas*、*Aquaspirillum* 被报道是一种 FeOB^[31-33],可在厌氧条件下利用 Fe^{2+} 生成 Fe^{3+} 化合物, *Lysinibacillus*、*Zoogloea*、*Bacillus* 被报道具有锰氧化能力^[34-36],可将亚锰离子转化为锰离子,且 *Lysinibacillus* 与土壤还原性物质含量呈显著负相关,这可推测 *Lysinibacillus*

潜在地参与还原性物质的氧化,菌株 WH07 处理富集了大量 FeOB,从而降低了土壤还原性物质含量。另外 *Thiobacillus* 也是一种氧化菌,具有将 Fe^{2+} 氧化生成 Fe^{3+} 的能力^[37],同时与 *Thioalkalispira* 属于硫氧化细菌^[38-39],本研究发现,WH07 处理后,*Thiobacillus* 与 *Thioalkalispira* 丰度显著减少,可能是 WH07 与 *Thiobacillus*、*Thioalkalispira* 存在着代谢竞争,抑制了 *Thiobacillus* 与 *Thioalkalispira* 的增殖。MBNT15、*Geothrix*、*Ignavibacterium*、*Anaeromyxobacter* 被报道具有铁还原能力^[40-43],能将 Fe^{3+} 还原为 Fe^{2+} ,本研究发现它们的丰度与土壤还原性物质含量呈正相关,且在处理组中其丰度显著降低,说明 WH07 处理能显著抑制 FeRB 的增殖,减少 Fe^{2+} 的累积。因此,菌株 WH07 的应用改变了土壤微生物结构和功能,增加了 FeOB 丰度但减少了 FeRB 的丰度,导致土壤中还原性物质减少,并改善了土壤理化性质,从而促进水稻的生长。

参考文献 References

- [1] 魏文学,谢小立,秦红灵,等.促进南方红壤丘陵区农业可持续发展的复合农业生态系统长期观测研究[J].中国科学院院刊,2019,34(2):231-243.WEI W X, XIE X L, QIN H L, et al. Long-term observational studies of complex agro-ecosystem promotes agricultural sustainable development in hilly red soil region of south China[J]. Bulletin of Chinese Academy of Sciences, 2019, 34(2): 231-243 (in Chinese with English abstract).
- [2] 李大明,余喜初,柳开楼,等.工程排水和农业措施改良鄱阳湖区潜育化稻田的效果[J].植物营养与肥料学报,2015,21(3):684-693.LI D M, YU X C, LIU K L, et al. Remediation effect of the combination of ditching drainage and agronomic managements on gleyed paddy field in Poyang Lake Region, China [J]. Journal of plant nutrition and fertilizers, 2015, 21(3):684-693 (in Chinese with English abstract).
- [3] 王飞,李清华,余广兰,等.开沟排渍与优化施肥对冷浸田生产力的耦合效应[J].土壤通报,2015,46(3):703-708.WANG F, LI Q H, YU G L, et al. Coupling effects of drainage by opening ditch and formulated fertilization on productivity of cold-waterlogged paddy field [J]. Chinese journal of soil science, 2015, 46(3): 703-708 (in Chinese with English abstract).
- [4] 李清华,王飞,林诚,等.水旱轮作对冷浸田土壤碳、氮、磷养分活化的影响[J].水土保持学报,2015,29(6):113-117.LI Q H, WANG F, HE C M, et al. Effects of paddy-upland rotation on the nutrient activation of soil carbon, nitrogen and phosphorus in cold waterlogged paddy field [J]. Journal of soil and water conservation, 2015, 29(6): 113-117 (in Chinese with English abstract).
- [5] 周利军,叶会财,李大明,等.配施有机肥对潜育化水稻土的培肥效果[J].中国土壤与肥料,2016(1):89-93.ZHOU L J, YE H C, LI D M, et al. Effect combined application of organic fertilizer on fertility of gleying paddy soil [J]. Soil and fertilizer sciences in China, 2016(1): 89-93 (in Chinese with English abstract).
- [6] 王思潮,曹凑贵,李成芳,等.耕作模式对冷浸田水稻产量和土壤特性的影响[J].中国生态农业学报,2014,22(10):1165-1173.WANG S C, CAO C G, LI C F, et al. Effects of cultivation patterns on rice yield and soil properties in cold waterlogged paddy fields [J]. Chinese journal of eco-agriculture, 2014, 22(10): 1165-1173 (in Chinese with English abstract).
- [7] 胡柯鑫,董春华,罗尊长,等.不同释放速率过氧化钙对模拟潜育环境下稻田土壤理化特性的影响[J].土壤,2020,52(4):853-861.HU K X, DONG C H, LUO Z C, et al. Effects of different release rates of calcium peroxide on physicochemical properties of paddy soil under simulated gley environment [J]. Soils, 2020, 52(4): 853-861 (in Chinese with English abstract).
- [8] 赵锋,王丹英,徐春梅,等.根际增氧模式的水稻形态、生理及产量响应特征[J].作物学报,2010,36(2):303-312.ZHAO F, WANG D Y, XU C M, et al. Response of morphological, physiological and yield characteristics of rice (*Oryza sativa* L.) to different oxygen-increasing patterns in rhizosphere [J]. Acta agronomica sinica, 2010, 36(2): 303-312 (in Chinese with English abstract).
- [9] 林超峰,龚骏.嗜中性微好氧铁氧化菌研究进展[J].生态学报,2012,32(18):5889-5899.LIN CF, GONG J. Recent progress in research on neutrophilic, microaerophilic iron(II)-oxidizing bacteria [J]. Acta ecologica sinica, 2012, 32(18): 5889-5899 (in Chinese with English abstract).
- [10] 陈娅婷,李芳柏,李晓敏.水稻土嗜中性微好氧亚铁氧化菌多样性及微生物成矿研究[J].生态环境学报,2016,25(4):547-554.CHEN Y T, LI F B, LI X M. Diversity and biomineralization of microaerophilic iron-oxidizing bacteria in paddy soil [J]. Ecology and environmental sciences, 2016, 25(4): 547-554 (in Chinese with English abstract).
- [11] 李晓敏,牟山,陈娅婷,等.稻田土壤微生物驱动的微好氧亚铁氧化耦合碳同化过程[J].中国科学:地球科学,2019,49(12):1948-1959.LI X M, MOU S, CHEN Y T, et al. Microaerobic Fe(II) oxidation coupled to carbon assimilation processes driven by microbes from paddy soil [J]. Scientia sinica (Terrae), 2019, 49(12): 1719-1729 (in Chinese with English abstract).
- [12] BRENNER D J, KRIEG N R, STALEY J T, et al. Bergey's manual® of systematic bacteriology [M]. Boston, MA: Springer US, 2005.
- [13] 东秀珠,蔡妙英.常见细菌系统鉴定手册[M].北京:科学出版社,2001.DONG X Z, CAI M Y. Handbook of identification of common bacterial systems [M]. Beijing: Science Press, 2001

- (in Chinese).
- [14] 杨东亚, 祁瑞雪, 李昭轩, 等. 黄瓜茄病镰刀菌拮抗芽孢杆菌的筛选、鉴定及促生效果[J]. 生物技术通报, 2023, 39(2): 211-220. YANG D Y, QI R X, LI Z X, et al. Screening, identification and growth-promoting effect of antagonistic *Bacillus spp.* against cucumber *Fusarium solani* [J]. Biotechnology bulletin, 2023, 39(2): 211-220 (in Chinese with English abstract).
- [15] HALL B G. Building phylogenetic trees from molecular data with MEGA [J]. Molecular biology and evolution, 2013, 30(5): 1229-1235.
- [16] 鲁如坤. 土壤农业化学分析方法[M]. 北京: 中国农业科技出版社, 1998. LU R K. Soil agrochemical analysis methods [M]. Beijing: China Agricultural Science and Technology Press, 1998 (in Chinese).
- [17] CAPORASO J G, LAUBER CL, WALTERS W A, et al. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample [J]. PNAS, 2011, 108(Suppl 1): 4516 - 4522.
- [18] ESTAKI M, JIANG L, BOKULICH NA, et al. QIIME 2 enables comprehensive end-to-end analysis of diverse microbiome data and comparative studies with publicly available data [J/OL]. Current protocols in bioinformatics, 2020, 70(1): e100 [2023-12-11]. <https://doi.org/10.1002/cpbi.100>.
- [19] NEVES A L A, LI F, GHOSHAL B, et al. Enhancing the resolution of rumen microbial classification from metatranscriptomic data using Kraken and Mothur [J/OL]. Frontiers in microbiology, 2017, 8: 2445 [2023-12-11]. <https://doi.org/10.3389/fmicb.2017.02445>.
- [20] CAVALIERI A, BAK F, GARCIA-LEMOS A M, et al. Effects of intra- and interspecific plant density on rhizosphere bacterial communities [J/OL]. Frontiers in microbiology, 2020, 11: 1045 [2023-12-11]. <https://doi.org/10.3389/fmicb.2020.01045>.
- [21] MERBT SN, BERNAL S, PROIA L, et al. Photoinhibition on natural ammonia oxidizers biofilm populations and implications for nitrogen uptake in stream biofilms [J]. Limnology and oceanography, 2017, 62(1): 364-375.
- [22] ANDRUS J M, PORTER M D, RODRÍGUEZ L F, et al. Spatial variation in the bacterial and denitrifying bacterial community in a biofilter treating subsurface agricultural drainage [J]. Microbial ecology, 2014, 67(2): 265-272.
- [23] HAMMES F, BOON N, DE VILLIERS J, et al. Strain-specific ureolytic microbial calcium carbonate precipitation [J]. Applied and environmental microbiology, 2003, 69(8): 4901-4909.
- [24] MAKITA H. Iron-oxidizing bacteria in marine environments: recent progresses and future directions [J/OL]. World journal of microbiology and biotechnology, 2018, 34(8): 110 [2023-12-11]. <https://doi.org/10.1007/s11274-018-2491-y>.
- [25] WU Y X, XU L G, WANG Z L, et al. Microbially mediated Fe-N coupled cycling at different hydrological regimes in riparian wetland [J/OL]. The science of the total environment, 2022, 851 (Pt 1): 158237 [2023-12-11]. <https://doi.org/10.1016/j.scitotenv.2022.158237>.
- [26] 管立平, 谢晶晶, 李伟豪, 等. 一种赖氨酸芽孢杆菌对凡纳滨对虾生长和水质影响 [J]. 渔业现代化, 2022, 49(6): 77-83. GUAN L P, XIE J J, LI W H, et al. Effects of a *Lysinibacillus* strain on the growth and water quality of *Litopenaeus vannamei* [J]. Fishery modernization, 2022, 49(6): 77-83 (in Chinese with English abstract).
- [27] 李金珠, 王旭刚, 袁雪红, 等. pH与石灰性水稻土铁氧化还原过程的关系 [J]. 土壤学报, 2014, 51(1): 143-149. LI J Z, WANG X G, YUAN X H, et al. Relationship between pH and iron redox cycle in calcareous paddy soil [J]. Acta pedologica sinica, 2014, 51(1): 143-149 (in Chinese with English abstract).
- [28] LIU T, CHEN D, LI X, et al. Microbially mediated coupling of nitrate reduction and Fe(II) oxidation under anoxic conditions [J/OL]. FEMS microbiology ecology. 2019, 95(4): fiz030 [2023-12-11]. <https://doi.org/10.1093/femsec/fiz030>.
- [29] 廖凤凤, 单美丽, 牟琼丽, 等. Mn(II)氧化细菌 *Pseudomonas aeruginosa* L3 的分离、鉴定及氧化特性 [J]. 微生物学通报, 2023, 50(5): 1787-1800. LIAO F F, SHAN M L, MOU Q L. Isolation, identification, and characterization of an Mn(II) oxidizing bacterium *Pseudomonas aeruginosa* L3 [J]. Microbiology China, 2023, 50(5): 1787-1800 (in Chinese with English abstract).
- [30] ZHAO W L, VERMACE R R, MATTES T E, et al. Impacts of ammonia loading and biofilm age on the prevalence of nitrogen-cycling microorganisms in a full-scale submerged attached-growth reactor [J]. Water environment research, 2021, 93(5): 787-796.
- [31] HE Y, ZENG X, XU F, et al. Diversity of mixotrophic neutrophilic thiosulfate- and iron-oxidizing bacteria from deep-sea hydrothermal vents [J/OL]. Microorganisms, 2022, 11(1): 100 [2023-12-11]. <https://doi.org/10.3390/microorganisms11010100>.
- [32] BLÖTHE M, RODEN E E. Microbial iron redox cycling in a circumneutral-pH groundwater seep [J]. Applied and environmental microbiology, 2009, 75(2): 468-473.
- [33] YAMAZAKI T, OYANAGI H, FUJIWARA T, et al. Nitrite reductase from the magnetotactic bacterium, a novel cytochrome cd1 with Fe(II): nitrite oxidoreductase activity [J]. European journal of biochemistry, 1995, 233(2): 665-671.
- [34] YANG Y, ALI A, SU J, et al. Phenol and 17 β -estradiol removal by *Zoogloea* sp. MFQ7 and in-situ generated biogenic manganese oxides: performance, kinetics and mechanism [J/OL]. Journal of hazardous materials, 2022, 429: 128281 [2023-12-11]. <https://doi.org/10.1016/j.jhazmat.2022.128281>.
- [35] ZAKHAROVA Y R, PARFENOVA V V, GRANINA L Z, et al. Distribution of iron- and manganese-oxidizing bacteria in

- the bottom sediments of Lake Baikal[J].Inland water biology, 2010,3(4):313-321.
- [36] TANG W, LIU Y, GONG J, et al. Analysis of manganese oxidase and its encoding gene in *Lysinibacillus* strain MK-1[J]. Process safety and environmental protection, 2019, 127: 299-305.
- [37] 鲁敏, 关晓辉, 赵浩然, 等. 嗜酸氧化亚铁硫杆菌对低品位硫化镍矿的生物浸出[J]. 材料导报, 2014, 28(12): 51-54. LU M, GUAN X H, ZHAO H R, et al. Research on bio-leaching of nickel sulfide ore by *Thiobacillus ferrooxidans*[J]. Materials reports, 2014, 28(12): 51-54 (in Chinese with English abstract).
- [38] VISSER J M, STEFESS G C, ROBERTSON L A, et al. *Thiobacillus* sp. W5, the dominant autotroph oxidizing sulfide to sulfur in a reactor for aerobic treatment of sulfidic wastes[J]. Antonie van Leeuwenhoek, 1997, 72(2): 127-134.
- [39] 刘阳, 姜丽晶, 邵宗泽. 硫氧化细菌的种类及硫氧化途径的研究进展[J]. 微生物学报, 2018, 58(2): 191-201. LIU Y, JIANG L J, SHAO Z Z. Advances in sulfur-oxidizing bacterial taxa and their sulfur oxidation pathways[J]. Acta microbiologica sinica, 2018, 58(2): 191-201 (in Chinese with English abstract).
- [40] BEGMATOV S, BELETSKY AV, DEDYSH SN, et al. Genome analysis of the candidate phylum MBNT15 bacterium from a boreal peatland predicted its respiratory versatility and dissimilatory iron metabolism[J]. Frontiers in microbiology, 2022, 13: 951761 [2023-12-11. <https://doi.org/10.3389/fmicb.2022.951761>].
- [41] SMITH J A, LOVLEY D R, TREMBLAY P L. Outer cell surface components essential for Fe(III) oxide reduction by *Geobacter metallireducens*[J]. Applied and environmental microbiology, 2013, 79(3): 901-907.
- [42] NEVIN KP, LOVLEY DR. Mechanisms for accessing insoluble Fe(III) oxide during dissimilatory Fe(III) reduction by *Geothrix fermentans*[J]. Applied and environmental microbiology, 2002, 68(5): 2294-2299.
- [43] STERN N, MEJIA J, HE S, et al. Dual role of humic substances as electron donor and shuttle for dissimilatory iron reduction[J]. Environmental science & technology, 2018, 52(10): 5691-5699.

Effects of *Lysinibacillus sphaericus* WH07 on improving soil and soil microecology in gleyed paddy field

WANG Qi^{1,2}, LI Kui¹, PENG Chenglin³, LUO Zhihua^{1,2},
CHEN Tao^{1,4}, YI Juping¹, GONG Yan¹, HU Hongtao¹

1. Hubei Biopesticide Engineering Research Center, Wuhan 430064, China;

2. College of Life and Sciences, Yangtze University, Jingzhou 434025, China;

3. Institute of Plant Protection and Soil Fertilizer, Hubei Academy of Agricultural Sciences, Wuhan 430064, China;

4. Institute of Urban Construction, Wuchang Shouyi University, Wuhan 430064, China

Abstract FeOB were enriched with modified wolf's mineral medium (MWMM) to screen microaerobic Fe²⁺ oxidizing bacteria (FeOB) with better oxidative abilities to Fe²⁺, evaluate its effects on improving soil and the microbial communities and functions of soil in gleyed paddy field, and lay a foundation for establishing technologies of improving microorganism in gleyed paddy fields. The FeOB strain was taxonomically identified with combined technologies including 16S rRNA sequencing. The 100 mL fermentation liquid of the strain with different concentration of 10⁶ (T1), 10⁷ (T2), 10⁸ (T3) CFU/mL was used to treat the soil in gleyed paddy field and evaluate the effects of the strain on the reducing substances, nutrients, abundances of functional genes for nitrogen cycling (NCFG) and rice seedlings of soil in gleyed paddy field. 16S rRNA high-throughput sequencing technology was used to evaluate the effect of this strain on the soil microecology. The results showed that the FeOB strain screened with strong oxidative effect on Fe²⁺ was identified as *Lysinibacillus sphaericus* WH07. Compared to CK, the soil redox potential (Eh) was sig-

nificantly increased ($P < 0.05$) and shifted from negative potential to positive potential. The total amount of reducing substance in soil treated with T1, T2, and T3 decreased by 26.47%, 41.53%, and 53.19%, respectively. The content of ferrous decreased by 0.37%, 21.50%, and 50.09%, while the content of manganese decreased by 7.84%, 21.57%, and 37.25%. The content of alkaline hydrolyzed nitrogen in soil significantly increased by 15.50%, 27.38%, and 48.90% ($P < 0.05$), while the available phosphorus significantly increased by 12.52%, 17.34%, and 27.38% ($P < 0.05$). The available potassium significantly increased by 11.56%, 17.20%, and 19.34% ($P < 0.05$), and the organic matter significantly increased by 8.66%, 22.22%, and 45.05% ($P < 0.05$). The pH significantly increased by 3.40%, 8.94%, and 16.99% ($P < 0.05$). The abundance of *AOA-amoA* gene in soil increased by 11.94%, 14.68%, and 33.83%, respectively. The abundance of *nosZ* gene increased by 42.97%, 75.78%, and 118.75%, while the abundance of *nifH* gene increased by 38.29%, 51.05%, and 216.13%. The abundance of *UreC* gene increased by 16.74%, 54.51%, and 60.94%. The plant height of rice increased by 5.44%, 10.98%, 36.00%, the leaf age of rice increased by 10.21%, 23.42%, 36.94%, the fresh weight of rice increased by 12.61%, 22.52%, 28.38%, and the white root number of rice increased by 10.14%, 32.92%, and 46.81%. The Chao1 and Shannon index of microbial diversity in soil was significantly decreased compared to CK ($P < 0.05$). Among the top 10 bacteria in soil with relative abundance at the Phylum level, 8 phyla including Proteobacteria were significantly downregulated ($P < 0.05$), while 2 phyla including Bacteroidetes and Firmicutes were significantly upregulated. Among the top 10 bacteria in soil with relative abundance at the Phylum level, 8 phyla including Proteobacteria were significantly downregulated ($P < 0.05$), while 2 phyla including Bacteroidetes and Firmicutes were significantly upregulated. Among the top 50 bacteria in soil with relative abundance at the genus level, 20, 19, and 22 genera including 6 FeOBs such as *Macellibacteroides* et al were significantly upregulated ($P < 0.05$) in the three treatments, while 25 genera including 4 Fe reducing bacteria such as MBNT15 et al were significantly down-regulated in all three treatments ($P < 0.05$). The results of analyzing regulatory network showed that the strain WH07 had the potential to synergistically improve the physicochemical properties and biological activity of soil with FeOB, ultimately promoting the growth of rice seedlings. It is indicated that the application of strain WH07 significantly improves the physicochemical properties of soil in the gleyed paddy field and change the structure and function of microbial community in soil.

Keywords gleyed paddy field; soil improvement; *Lysinibacillus sphaericus*; reducing substances; redox potential; functional genes for nitrogen cycling; soil microecology

(责任编辑:张志钰)