土壤食细菌线虫的原位富集培养方法

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摘要:采用两种孔径的尼龙网袋(1mm 和 5μm),将盆钵供试土壤分成内外两层,内层土壤混合猪粪或稻草,并以不添加猪粪和稻草的供试土壤作为空白,外层直接接入供试土壤,进行培养,以获取土著食细菌线虫大量富集的试验土壤。结果表明:添加基质(猪粪和稻草)显著地促进了土壤线虫的繁殖,大量繁殖的线虫通过 1mm 网袋迁移至外层未加基质的土壤,而采用 5μm 网袋则限制了线虫向外层土壤的迁移。添加猪粪的 1mm 网袋处理经过 28d 培养后,外层土壤线虫数是空白处理的 9.1 倍;添加稻草的 1mm 网袋处理经过 35d 培养后,外层土壤线虫数是空白处理的 5.9 倍。添加两种基质的 5μm 网袋处理,外层土壤线虫数和空白处理差异不大。添加基质主要是促进了食细菌线虫的繁殖,在培养结束时,添加猪粪的 1mm 网袋处理的食细菌线虫比例达到 98.2%,添加稻草的 1mm 网袋处理的食细菌线虫比例达到 90.5%,两个处理食细菌线虫的总数分别是空白处理的 14.8 倍和 8.9 倍,并且主要是 *Protorhabditis* sp. 线虫的增加。

关键词:食细菌线虫;原位富集培养;猪粪;稻草

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Soil bacterial-feeding nematodes enrichment culturing *in situ*

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Abstract: Soil mixed with either pig manure or rice straw was placed in a mesh bag (1mm or 5μm), and then surrounded by an outer layer of unamended soil, to generate with greater populations of bacterial-feeding nematodes, compared to a normal field soil. The results showed that addition of pig manure and rice straw significantly increased the nematode populations. The increased nematodes were able to migrate through the 1mm mesh into the outer soil, thus giving greater populations than in soil surrounding the 5μm mesh, which nematodes cannot migrate through. After 28 and 35 days incubation respectively, the outer soil contained a 9.1-times increase of nematodes in the pig manure treatment and an 8.9-times in the rice straw treatment, compared to soil with no added substrate. While the nematode abundance in the outer soils surrounding the 5μm mesh was not significantly different from the no-substrate treatment. The increased nematodes were mainly bacterial-feeders (primarily *Protorhabditis* sp.), accounting for 98.2% of total nematodes in the pig manure treatment and 90.5% in the rice straw treatment at the end of the incubation. And the total abundance of bacterial-feeding nematodes was increased by 14.8 times and 8.9 times than no-substrate treatment respectively in pig manure treatment and

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the rice straw treatment.

Key Words: bacterial-feeding nematode; enrichment culturing *in situ*; pig manure; rice straw

线虫是土壤中最为丰富的后生动物,据估计其数量约有 7.6×10^5 条/m^2 (沙漠)到 2.9×10^7 条/ m^2 (混交阔叶林)[1,2]。土壤中的线虫大约有 20% ~50% 为食细菌线虫,而在一些微生物活性强的区域 (如根际),甚至可占 90% ~99% [3,4]。土壤食细菌线虫因其个体小、世代短、代谢活性高,可通过其自身代谢周转释放出有效养分,并且土壤食细菌线虫对微生物的选择性取食能够改变土壤微生物的群落结构,提高微生物活性,加快微生物周转,从而能够促进土壤氮素矿化,并促进植物生长[5~9]。因此,自 Aderson 和 Colemen 等人率先利用微宇宙 (microcosm),研究食细菌线虫与微生物的相互作用及其对土壤生态系统能量传递和养分动态的影响[10,11]以来,该领域受到广泛关注。

利用线虫进行微宇宙土培试验时,通常是采取向土壤中直接接种线虫的方式。所接种的线虫一般是通过在培养基上富化培养获得[2],这就带来如下几个问题:(1)这些富化培养的线虫可能并不一定是该土壤的土著优势种群;(2)在接种线虫的时候会将微生物同时接种到土壤中;(3)接种的线虫可能有一部分会死亡。

本试验的目的就是希望通过原位培养,在添加有机物料促进线虫繁殖的同时,配以不同孔径的网袋允许或限制线虫的迁移,从而能够获得土著食细菌线虫大量富集的土壤及对照土壤,为今后进一步开展线虫试验提供更为理想的富集培养方法以获得试验材料。

1　材料与方法

1.1　试验材料

1.1.1　供试土壤

土壤采自南京雨花台区板桥镇长江南岸冲积地的潮土 (美国制土壤质地分类为砂质壤土),种植制度为稻麦轮作。土壤取样深度为 0 ~20cm,鲜土采集后,剔除石块、大中型土壤动物及根茬等残体,备用。土壤有机碳含量为 9.20 g C/kg,全氮 0.89 g N /kg,NH_4^+-N 6.84 μg/g,NO_3^--N 2.39 μg/g,pH 为 6.32。

1.1.2　供试基质

猪粪 (C/N 比为 18.6)和稻草 (C/N 比为 64.7)晒干磨细过 2mm 筛,备用。

1.2　试验设计

试验采用盆钵培养,首先在 1mm 或 5μm 尼龙网袋内装入 400g 混合了 14g 基质 (猪粪或稻草)的新鲜土壤,或直接装入 400g 新鲜土壤 (空白对照,处理见表 1)。将装好土壤的网袋放在盆钵中央,外层装入 650g 新鲜土壤。土壤装盆后,调节土壤含水量到田间持水量,并于 20℃ 培养。对于添加基质的处理,网袋内层土壤由于混合了基质,土壤线虫得到大量富化,并且内层富化的线虫能够穿过 1mm 网袋迁移到外层土壤,而 5μm 的网袋则阻止了线虫的迁移 (一般食细菌线虫的幼虫体大于 5μm,并且成虫虫体小于 1mm)。每周对添加基质的 1mm 网袋处理 (SM1 和 SR1)各破坏性采样 3 个盆钵,检测内层、外层土壤线虫数量,直到外层线虫数量达到预期要求时结束培养 (本次试验选择外层线虫数达到原土中线虫数量的 6 倍以上)。培养结束时,检测各个处理盆钵外层土壤线虫数量,并进行功能类群鉴定。

表 1　试验处理*

Table 1　The treatments of the experiment

处理编号 Codes of the treatments	内层 Inner of the pot	网袋 Mesh	外层 Outer of the pot
SM1	土壤 + 猪粪 Soil + pig manure	1mm	土壤 Soil
SM5	土壤 + 猪粪 Soil + pig manure	5μm	土壤 Soil
SR1	土壤 + 稻草 Soil + rice straw	1mm	土壤 Soil
SR5	土壤 + 稻草 Soil + rice straw	5μm	土壤 Soil
S	土壤 Soil	1mm	土壤 Soil

* 下同 the same below

1.3 线虫鉴定

线虫分离采用浅盘法[3]，称取 20g 土样，室温下分离 48h 后，收集线虫悬液，对所有线虫在体视镜下进行记数。线虫记数后在 60℃下杀线 2min，并保存在 4% 的福尔马林中[4]。每个样本随机对 100 条线虫进行鉴定，分为：(1)食细菌(bacterial-feeding)；(2)食真菌(fungal-feeding)；(3)植食(plant - feeding)；(4)杂食/捕食线虫(omnivorous/predators)4 个功能类群[5,16]。对于线虫数少于 100 条的样本，对所有线虫进行鉴定划分。仅在培养结束时，对外层土壤作此分析。

1.4 数据分析

数据统计采用 SPSS 统计软件。

2 结果与讨论

2.1 线虫数量动态变化

添加猪粪和稻草显著($P < 0.05$)促进了线虫的繁殖，在添加猪粪和稻草的 1mm 网袋处理(SM1 和 SR1)中，在第 7 天，内层线虫数量就已经显著增加，并且从第 14 天开始，SM1 处理外层线虫也快速增加，但 SR1 处理外层线虫的增加相比 SM1 处理显得较为缓慢(图 1)。外层线虫的增加主要是由于内层添加基质增加的线虫向外层的迁移。De Guiran 等[7]发现向土壤中添加猪粪后 24h 就检测到线虫数量的增加，Fu 等[8]在向土壤中添加玉米秸秆后，在第 8 天采样也发现了线虫数量的显著增加。而线虫在土壤中迁移非常迅速[9]，尤其是由添加基质区域向外迁移[10]，因为在这些区域线虫大量繁殖，彼此对空间以及资源不断地竞争[11]。比较两种基质，猪粪对线虫繁殖的促进作用优于稻草。在培养结束时(猪粪处理是培养 28d，稻草处理 35d)，猪粪处理外层的线虫数要高于稻草处理(图 1)，各自分别是新鲜土样起始线虫数的 10.4 倍和 6.8 倍(新鲜土样起始线虫数为 11.67 条线虫/g 干土)。这可能和猪粪的分解更快有关，因为猪粪的 C :N 比较小(见材料和方法)。线虫的繁殖，尤其是食细菌线虫的繁殖，和有机物料的分解速度直接相关[12]。

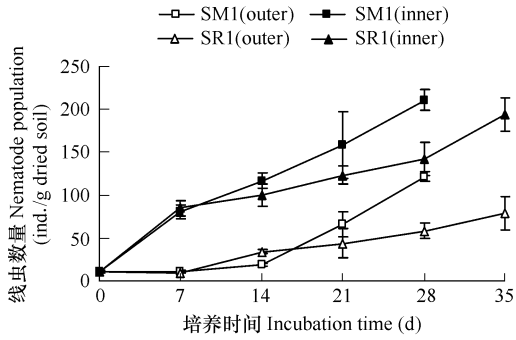

图 1 添加猪粪或稻草 1mm 网袋处理线虫数量动态变化

Fig. 1 Dynamic of nematode populations in soils amended with pig manure or rice straw contained within a 1mm diameter mesh and in surrounding soil

2.2 线虫种群变化

对于添加基质的 5μm 网袋处理(SM5 和 SR5)，外层的线虫数与空白处理(S)比较，虽然有所增加，但未达到显著水平，但它们与各自对应的 1mm 网袋处理相比，线虫数却显著地减少了($P < 0.05$)(表 2)，这主要是由于网袋内层富化的线虫无法通过 5μm 网袋向外层迁移(线虫的幼虫体大于 5μm)。而对于 SM1 和 SR1 处理，如前所述，添加猪粪和稻草促进线虫繁殖并向外层迁移，在培养结束时，外层线虫数分别是空白处理的 9.1 和 5.9 倍。添加猪粪和稻草促进线虫繁殖并向外层迁移的同时，还改变了外层线虫的营养结构组成：主要是显著增加了食细菌线虫数量并显著减少了其他营养类群线虫数量。SM1 处理的食细菌线虫比例达到 98.2%，SR1 处理的食细菌线虫比例达到 90.5%，食细菌线虫的比例分别比未加基质处理(S)提高了 1.6 倍和 1.5 倍，而两个处理食细菌线虫的总数则又分别是 S 处理的 14.8 倍和 8.9 倍，并且其中主要是 *Protorhabditis* sp 线虫的增加。SM1 处理的食细菌线虫比例高于 SR1 处理，但未达到显著水平。

这种添加有机物质对线虫种群的促进作用和许多研究结果一致。Griffiths 等人发现：添加家禽粪显著促进了线虫种群并且主要是促进了食细菌线虫的繁殖[12]。Villenave 等人通过田间试验发现畜禽肥料促进线虫种群增加了 75%[13]。Opperman 等人在田间和室内试验中都发现，将牛粪添加到不同的土壤中都促进了线虫种群的增长，并且这种增长主要是由于添加牛粪区域自由生活的食细菌线虫的繁殖[14]。向土壤中添加基质后，线虫种群结构的这种变化主要是由于基质的分解释放养分，促进细菌的生长及活性，从而促进了食细菌线

虫的繁殖,并且食细菌线虫的繁殖又能进一步促进细菌的活性,加快对基质的分解[2,25]。

表 2　培养结束时,各处理外层土壤的线虫种群(线虫/g 干土)以及各类群的百分比(%)

Table 2　Nematode abundance (individuals/g dried soil) and proportion (%) ($n = 3$) of individual taxa in outer soils at the end of the incubation

项目 Item	处理 Treatments				
	SM1	SM5	SR1	SR5	S
线虫总数 Abundance (nd./g dried soil)	121.45a	24.16c	79.31b	17.40cd	13.33cd
食细菌线虫比例 Bacterial-feeders (%)	98.2a	75.6b	90.5a	62.1c	60.3c
Cephalobus	2.3	3.8	8.6	15.9	16.3
Eucephalobus	0	0.4	1.5	7.9	3.2
Protorhabditis	93.2	68.5	80.2	23.2	24.2
Rhabditis	2.7	2.9	0.2	15.1	16.6
食真菌线虫比例 Fungal-feeders (%)	1.3d	13.2bc	5.6cd	20.3b	20.8b
Aphelenchoides	1.3	13.2	5.5	20.1	18.6
Aphelenchus	0	0	0.1	0.2	2.2
植食线虫比例 Plant-feeders (%)	0.5c	7.9b	2.6c	11.5ab	13.2a
Tylenchus	0.1	1.8	0.5	2.3	2.6
Tylenchorhynchus	0.4	5.3	2.1	9.2	6.4
Tetylenchus	0	0.8	0	0	4.2
杂食/捕食线虫比例 Omnivores/Predators (%)	0	3.3ab	1.3b	6.1a	5.7a
Mononchus	0	2.5	1.3	4.2	4.8
Dorylaimus	0	0.8	0	1.9	0.9

同一行内字母不同表示有显著性差异($P < 0.05$) Values with the various letters in each row means there are significant difference ($P < 0.05$).

3　结论

以往利用线虫进行土培试验时,通常是采取向土壤中直接接种从培养基上富化培养获得的线虫的方式,但这种方式存在一些问题:接种的线虫可能不是土著优势种群,或接种线虫时会将微生物同时接种到土壤中,并且接种的线虫可能有一部分会死亡,这些都会对研究结果产生一定的影响。本试验通过用两种不同孔径的网袋将盆钵分成内外两层,内层添加基质促进土著食细菌线虫的大量繁殖,内层富化繁殖的食细菌线虫通过 1mm 网袋迁移到外层,而同时 5μm 网袋阻止了线虫的迁移,从而获得土著食细菌线虫大量富集的土壤及对照土壤。研究结果为进一步开展食细菌线虫土培试验提供了获得试验材料的更理想的线虫原位富集培养方法。

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