根际促生菌诱导大豆抗大豆胞囊线虫的生化机理

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摘 要:为揭示由根际促生细菌[Sneb207(*Bacillus megaterium*),Sneb482(*Bacillus megaterium*)]诱导大豆抗大豆胞囊线虫(*Heterodera glycines* Ichinohe)的生化机理。使用菌株Sneb207、Sneb482发酵液包衣处理大豆种子,在豆苗三叶期时接种大豆胞囊线虫卵悬液,分别于接种后6、12、18、24、30 d取样,测定大豆根内防御酶系活性(PAL,PPO,POD)、总酚含量和几丁质酶活性的动态变化。结果表明:大豆种子经Sneb207、Sneb482发酵液处理后,根内PAL、PPO、POD活性较对照均表现上升趋势,总酚含量也有所提高。与菌株Sneb482相比,菌株Sneb207表现出对大豆胞囊线虫病更好的诱导抗病潜力。

关键词:根际促生菌;诱导抗性;胞囊线虫;防御酶;几丁质;总酚

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Biochemical Mechanism of Resistance against Soybean Cyst Nematode Induced by Plant Growth Promoting Rhizobacteria in Soybean

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Abstract: It has been a new research focus on biological control that the induction of disease resistance and growth response in plants is elicited by plant growth promoting rhizobacteria(PGPR). This study aimed to examine the biochemical mechanism of the resistance to soybean cyst nematode(*Heterodera glycines* Ichinohe) in soybean induced by PGPR [Sneb207(*Bacillus megaterium*) and Sneb482(*Bacillus megaterium*)]. Seed bacterization with Sneb207 and Sneb482 were utilized in the experiment. The soybean plants were inoculated with eggs of soybean cyst nematode after soybean trefoil stage and the samples of roots were obtained 6, 12, 18, 24 and 30 days later. Activities of plant resistance correlated enzymes including defense enzymes, namely phenylalanine ammonia-lyase (PAL), polyphenoloxidase (PPO), peroxidase (POD) and chitinase were measured, the content of total phenolics was also determined. The results showed that both the activities of PAL, PPO, POD, chitinase and the contents of total phenolics could be increased significantly by seed coating with fermentation liquid of plant growth promoting rhizobacteria (Sneb207, Sneb482) in the soybean roots than those in control. Sneb207 showed greater potential in induced resistance against soybean cyst nematode in the soybean plants than Sneb482.

Key words: PGPR; Induced resistance; *Heterodera glycines*; Defense enzymes; Chitinase; Total phenolics

大豆胞囊线虫病(*Heterodera glycines* Ichinohe)又称大豆根线虫病、黄萎病,俗称“火龙秧子”,是世界大豆生产的重要线虫病害,每年由于大豆胞囊线虫病造成的经济损失可达数十亿美元。在我国主要分布于东北和黄淮海大豆主产区。大豆受其危害后,轻者减产20%~30%,重者可达70%~80%,甚至颗粒无收[1-2]。生产上多使用高毒化学农药来防治大豆胞囊线虫病,既影响人类身体健康,又严重破坏生态环境,不利于农业生产的可持续发展[3]。因此大豆胞囊线虫病的生物防治越来越受到重视,利用细菌作为生物防治因子防治植物病害已成为国内外生物防治研究中的一个热点。根际促生细菌(Plant growth promoting rhizobacteria,简称PGPR)是指一类存在于植物根际,能够促进植物生长、防治病害的细菌[4]。随着对植物根际促生细菌的深入研究,研究者发现它们可以诱发植物产生诱导抗性[5]。孙华等[6]首次发现根际促生巨大芽孢杆菌(*Bacillus megaterium*)Sneb207、Sneb482可诱导大豆产生系统抗性,并证明它们对大豆胞囊线虫病具有良好防治效果,但抗病机理尚不清楚。因此该试验利用具有自主知识产权的根际促生巨大芽孢杆菌Sneb207、Sneb482对大豆进行包衣处理,人工

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接种大豆胞囊线虫,测定细菌对大豆根内防御酶活性、总酚含量及几丁质酶活性的影响,明确大豆根际促生细菌诱导大豆抗大豆胞囊线虫的生化机理,为进一步深入研究其生防机制奠定了基础。

1　材料与方法

1.1　供试材料

1.1.1　*供试菌株*　巨大芽孢杆菌(*Bacillus megaterium*)Sneb207、Sneb482 由沈阳农业大学植物保护学院北方线虫研究所分离获得,具有自主知识产权。

1.1.2　*供试线虫*　大豆胞囊线虫 3 号生理小种,为辽宁地区优势小种。

1.1.3　*供试大豆品种*　感病品种辽豆 15 由辽宁省农科院提供,是辽宁地区主栽品种之一。

1.2　试验方法

1.2.1　*发酵液的制备*　将细菌菌株 Sneb207、Sneb482 接种于 NA 液体培养基上,于 28℃,160 $r \cdot min^{-1}$条件下摇瓶发酵培养 48 h 备用。

1.2.2　*大豆种子处理*　种子消毒:将种子浸入 5% 次氯酸钠溶液(加入 1 滴 Tween 20)7 min,用无菌水冲洗 5 次[7]。处理后的种子分别置于 NA 和 PDA 培养基上培养观察,以验证灭菌效果[8]。

种子包衣方法:用制备的 Sneb207、Sneb482 发酵液以 10%(w/w)种子量进行种子包衣处理。

1.2.3　*大豆胞囊线虫卵悬液的制备*　大豆胞囊线虫卵悬液的制备参照刘维志等[9]的方法。分离大豆胞囊线虫胞囊的土样采自沈阳农业大学北方线虫研究所大豆胞囊线虫 3 号生理小种繁殖圃。采用改良淘洗—过筛法从采取的土样中分离胞囊,在体视镜下挑取新鲜、饱满、成熟、均一的胞囊。胞囊先用 0.5% 次氯酸钠溶液消毒 3 min,再用无菌水冲洗 5 次。将收集的胞囊进行破碎后,用无菌水配制成浓度约为 2 000 卵 $\cdot mL^{-1}$的卵悬液。

1.2.4　*试验设计*　采用温室盆栽试验,试验于 2010 年在沈阳农业大学北方线虫研究所日光温室中进行。设置以下 6 个处理:NA 培养基包衣(CK1)、NA 培养基包衣接种大豆胞囊线虫(CK2)、Sneb207 发酵液包衣(T1)、Sneb207 发酵液包衣接种大豆胞囊线虫(T2)、Sneb482 发酵液包衣(T3)、Sneb482 发酵液包衣接种大豆胞囊线虫(T4),每处理 5 次重复,随机摆放。取无大豆胞囊线虫的菜田土和沙,以 V(沙):V(土)为 1∶2 的比例混匀,在 165℃条件下干热灭菌 3 h。灭菌后土壤装入规格为 18 cm × 18 cm 的塑料钵中,每钵播入 5 粒包衣的大豆种子,待出苗后每钵留 3 株长势一致的豆苗。在豆苗三叶期接种大豆胞囊线虫,每株 2 000 粒卵。分别于接种线虫后 6、12、18、24 和 30 d 取样,将大豆根系用自来水洗净,再用蒸馏水冲洗,放在滤纸上吸干,做好标记,-80℃保存备用。

1.3　测定项目与方法

1.3.1　*防御酶系活性测定*　准确称取 1 g 大豆根系样品,放入预冷的研钵中,加入 1 mL 0.2 $mol \cdot L^{-1}$ pH 8.8 硼酸缓冲液(内含 5 $mmol \cdot L^{-1}$二硫苏糖醇、1 $mmol \cdot L^{-1}$ EDTA、聚乙烯吡咯烷酮 PVP 少许)和 0.2 g 石英砂,于冰浴中充分研磨,转移至预冷的离心管中,用 1.5 mL 上述缓冲液冲洗 2 次,合并倒入 5 mL 离心管中,搅动 20 min 后,10 000 $r \cdot min^{-1}$、4℃离心 20 min,上清液即为酶粗提液。将酶粗提液放入超低温冰箱中(-80℃)冷冻保存待用。苯丙氨酸解氨酶(PAL)活性参照薛应龙[10]的方法测定,多酚氧化酶(PPO)活性参照李靖等[11]的方法测定,过氧化物酶(POD)活性参照张志良[12]的方法测定。

1.3.2　*总酚含量测定*　参照林植芳等[13]的方法,每份材料取 0.5 g 幼根,剪成 2 mm 左右的根段,放入试管中,加入 5 mL 含有 1%(v/v)HCl 的甲醇溶液,提取 2 h,取 1 mL 提取液稀释 50 mL 定容、摇匀,于 280 nm 处测 OD 值。以没食子酸做标准曲线,用回归方程计算出总酚的含量。

总酚含量($mg \cdot g^{-1}$) = [稀释后总酚含量($\mu g \cdot mL^{-1}$) × 稀释倍数 × 提取液体积(mL)]/[样品重量(g) × 1 000]。

1.3.3　*几丁质酶活性测定*　参照 Berger 等[14]的方法提取几丁质酶和制备胶状几丁质。N-乙酰氨基葡萄糖(NAG)标准曲线建立和几丁质酶活性测定参照 Boller 等[15]的方法。根据标准曲线计算反应液中 N-乙酰氨基葡萄糖的含量,以每小时每克鲜组织从胶状几丁质中释放 1 μmol N-乙酰氨基葡萄糖为一个酶活性单位(U)。

2　结果与分析

2.1　大豆根系防御酶系活性变化

2.1.1　*苯丙氨酸解氨酶(PAL)*　苯丙氨酸解氨酶(PAL)的活性变化如图 1 所示。接种大豆胞囊线虫处理较未接种处理 PAL 活性均有增加的趋势,表明苯丙烷类代谢是大豆抗大豆胞囊线虫的代谢途径之一。经根际促生巨大芽孢杆菌 Sneb207、Sneb482 发酵液包衣后接种大豆胞囊线虫,PAL 活性显著高于 NA 培养基包衣接种大豆胞囊线虫。发酵液包衣后接种大豆胞囊线虫 PAL 活性分别于接种后第 6 天和第 18 天 2 次达到高峰,而 NA 培养基包衣接种大豆胞囊线虫只在第 12 天有峰值。其中,Sneb207

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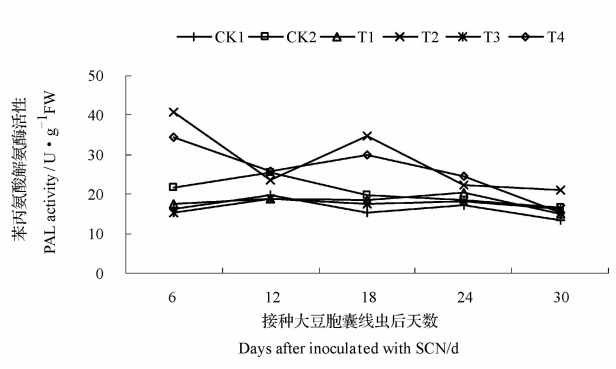

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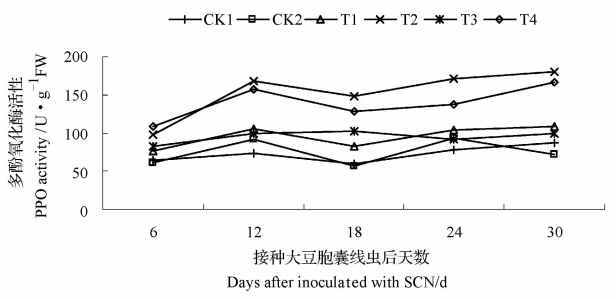

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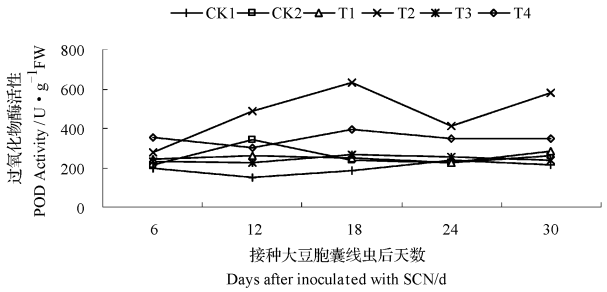

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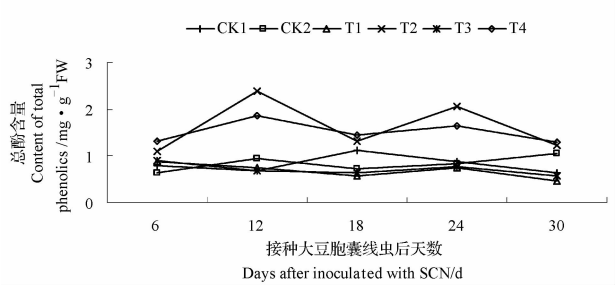

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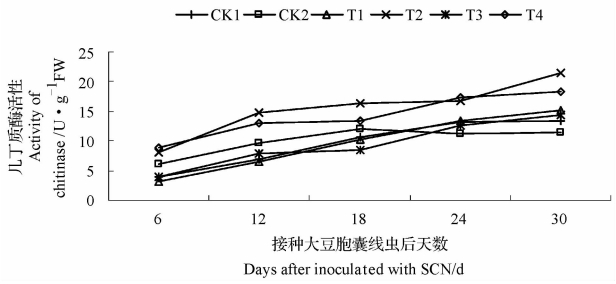

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