

解蛋白芽孢杆菌 *Bacillus proteolyticus* 提高银杉抗落针病的生防机制研究

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摘要: 【目的】银杉 *Cathaya argyrophylla* [Chun et Kuang.] 是中国一级重点保护野生植物, 其数量稀少, 具有很高的保护和科研价值。2022 年 6 月, 在贵州省大沙河国家级自然保护区, 发现约 30% 的银杉出现落针病 ($n=200$)。这种病害严重制约了银杉的生长, 干扰了银杉幼苗的野外回归, 加剧了其濒危的处境。为明确银杉落针病的病原菌, 寻找该病害有针对性的防治方法。【方法】我们收集患落针病的银杉针叶, 采取组织分离法分离和纯化病原菌, 利用柯赫氏法则验证病原菌的致病性, 并通过形态学和分子生物学的方法对病原菌进行鉴定。采用组织分离法分离健康银杉针叶的内生细菌, 通过体外双重培养筛选对银杉落针病具有抑菌效果的生防菌, 并通过在银杉幼苗上喷施生防菌的方式, 检测其生防效果, 并对生防菌提高银杉抗落针病的机制进行了初步的研究。【结果】结果表明, 银杉落针病的病原体为新壳梭孢菌 (*Neofusicoccum parvum*); 从银杉针叶内分离得到 1 株细菌 X6-1 (*Bacillus proteolyticus*) 在体外对 *N. parvum* 具有较好的抑菌活性, 对落针病的防治效果达到 59.13%; 菌株 X6-1 对银杉抗病生理和叶际微生物群落组成的影响结果表明, 与单独接种病原菌处理相比, 接种 *B. proteolyticus* 能够减轻因病原体入侵对银杉造成的细胞膜损伤及针叶组织结构的破坏, 提高银杉光合作用、JA、GA、ABA、IAA 激素含量和抗氧化酶活性。菌株 X6-1 还可以改变叶际微生物群落的组成, 特别是促进了部分有益微生物的富集, 降低了 *Neofusicoccum* spp. 等病原菌的丰度。通过转录组分析发现, 接种 *B. proteolyticus* 诱导了银杉 8890 个差异基因 (1604 个基因显著上调, 7286 个基因显著下调) 的表达, 这些差异基因主要参与光合作用、核糖体、糖酵解、谷胱甘肽和三羧酸循环等代谢通路。【结论】综上, 我们揭示了应用银杉内生细菌可以调节银杉幼苗的生理反应、改变其叶际微生物组的组成和影响基因表达等方式提高银杉对落针病的抗性。

关键词: 银杉; 落针病; 芽孢杆菌; 叶际微生物; 生理途径; 生物防治

Study on the Biological Control Mechanism of *Bacillus proteolyticus* to improve the resistance of Needle Cast of *Cathaya argyrophylla*

Abstract: 【Objective】 *Cathaya argyrophylla* [Chun et Kuang.] is a key wild plant under first-degree protection in China. As a unique relict plant in China, *C. argyrophylla* is rare in

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number and has high protection and scientific research value. In June 2022, needle cast was observed in approximately 30% of *C. argyrophylla* ($n=200$) at Dashahe National Nature Reserve in Daozhen County, Guizhou, China. This disease severely restricts the growth of *C. argyrophylla*, interferes with the return of *C. argyrophylla* seedlings to the wild, and exacerbates its endangered situation. To identify the pathogen of Needle Cast of *C. argyrophylla* and to find targeted control methods for this disease. **【Method】** We collected *C. argyrophylla* needles afflicted with Needle Cast. We then employed the tissue isolation method to isolate and purify the pathogen, corroborated its pathogenicity through the application of Koch's postulates, and identified the pathogen through the utilisation of morphological and molecular biological techniques. Tissue isolation method was employed to isolate endophytic bacteria from healthy *C. argyrophylla* needles. An in vitro double culture was utilised to screen the biocontrol bacteria with an inhibitory effect on *C. argyrophylla* needles disease. The biocontrol effect was evaluated by spraying the biocontrol bacteria on the *C. argyrophylla* seedlings. A preliminary study was conducted to elucidate the mechanism of the biocontrol bacteria in improving the resistance of *C. argyrophylla* to needles disease. **【Result】** The results showed that the pathogen of Needle Cast of *C. argyrophylla* was *Neofusicoccum parvum*; A strain of the bacterium X6-1 (*Bacillus proteolyticus*) was isolated from within the needles of *C. argyrophylla*, which could secrete protease and β -glucanase and had a better bacterial inhibitory activity against *N. parvum* in vitro, and could achieve 59.13% of the efficacy against Needle Cast of *C. argyrophylla* seedlings; The results of the effects of strain X6-1 on the physiological system and the composition of the phyllosphere microbiome community of *C. argyrophylla* showed that the inoculation of *B. proteolyticus* was able to alleviate the damage to cell membranes and the destruction of the needle tissue structure due to the invasion of pathogens, and to increase the photosynthesis, the content of the hormones JA, GA, ABA, and IAA, and the activity of antioxidant enzymes of *C. argyrophylla*, as compared with the treatment of the pathogenic bacteria alone. Furthermore, the application of Strain X6-1 resulted in alterations to the composition of the phyllosphere microbiome community. This was evidenced by an increase in the abundance of beneficial microorganisms and a reduction in the abundance of *Neofusicoccum* spp. By transcriptome analysis, inoculation with *B. proteolyticus* induced the expression of 8,890 differential genes (1,604 genes significantly up-regulated and 7,286 genes significantly down-regulated), which were mainly involved in metabolic pathways such as photosynthesis, ribosomes, Glycolysis / Gluconeogenesis、Glutathione metabolism and TCA cycle. **【Conclusion】** In conclusion, we revealed that the application of endophytic bacteria associated with *C. argyrophylla* seedlings can regulate the physiological response of *C. argyrophylla* seedlings and change the composition of the phyllosphere microbiome, so as to inhibit the occurrence of needle fall disease in *C. argyrophylla* seedlings.

Key words: *C. argyrophylla*, Needle Cast, *Bacillus*, phyllosphere microbiome, physiological pathways, biological control.