

分子水平上解析落叶松枯梢病扩散蔓延规律

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摘要: 【目的】明确落叶松枯梢病的扩散蔓延规律以及传播过程中其致病菌-落叶松新壳梭孢 (*Neofusicoccum laricinum*) 的遗传分化情况, 对落叶松枯梢病的防治具有重要意义。【方法】对来自全国多省份多样地的落叶松枯梢病样品的病健交界处采用表面消毒法进行病原菌的分离, 结合形态学与分子生物学对其进行鉴定。柯赫氏法则验证代表性菌株致病性, 观察不同的培养基、碳源、氮源、pH、光照等条件下新壳梭孢菌的生长情况以分析其生物学特性。采用三代测序技术对代表性菌株进行基因组测序及其余地理小种基因组重测序, 基于多基因组 SNP 数据对落叶松新壳梭孢遗传多样性及地源信息分析, 确定适合落叶松新壳梭孢地理小种鉴定的 SNP 高多态性靶 DNA 片段及 DNA 条形码等, 最终系统全面的分析落叶松枯梢病的传播规律及遗传特性。【结果】2022-2023 年共完成来自辽宁, 吉林, 黑龙江, 内蒙古, 河北, 甘肃、山东, 7 个省份, 101 个林场样品的采集。结合形态学与分子生物学, 已从黑龙江省, 吉林省及内蒙古自治区的 17 个林场中分离鉴定出 62 株新壳梭孢菌 (*N. laricinum*), 其中黑龙江省与吉林省各有 7 个林场。分别选择来自黑龙江齐齐哈尔的 HLJ-QQHE-4 和吉林图们的 JL-TM-2 的两株菌在落叶松小枝上进行致病性检测, 接种 3 d 时接种点处出现了退绿现象, 15 d 时, 小枝完全干枯下垂, 愈加确定所分离病原菌为落叶松枯梢病病原。生物学特性研究发现: 在碱性条件下并添加可溶性淀粉 30 g/L, 硫酸铵 2g /L, 连续光照, 新壳梭孢菌的生长状态最优。Hi-C 组装结果显示黑龙江省克东县爱华林场 HLJ-QQHE-4 菌株的基因组有 14 条染色体, GC 含量为 53.34%, 基因组大小为 42.47 Mb, 仅包含 1 个小 Gap, 测序质量较高, 编码蛋白的总基因为 11275 个。进化分析表明该菌与同一单系群的小新壳梭孢菌 (*N. parnum*) 进化关系最近, 进一步证明此菌株的分类地位。【结论】已成熟掌握该病害的鉴别及病原菌分离技术, 分析发现吉林与黑龙江的发病面积较大。基因组测序为该菌遗传特性的研究提供参考, 筛选出的最适生长条件, 也为该菌后期的培养与研究奠定基础。为完成从分子水平解析落叶松枯梢病的扩散蔓延规律, 后续试验还在进行中。

Molecular level analysis of the diffusion and spread of larch wilt disease

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Abstract: 【Objective】 Clarifying the diffusion and spread patterns of larch wilt disease, as well as the genetic differentiation of its pathogenic pathogen, *Neofusicoccum laricinum*, during the transmission process, is of great significance for the prevention and control of larch wilt disease. 【Method】 The surface disinfection method was used to isolate the pathogenic fungal at the disease-health junction of samples of Larch wilt disease from multiple provinces and diverse regions across the country, and combined with morphology and molecular biology, they were identified. Koch's rule verifies the pathogenicity of representative strains and observes the growth of *N. laricinum* under different conditions, such as culture medium, carbon source, nitrogen source, pH, and light, to analyze its biological characteristics. Using third-generation sequencing technology to sequence the genomes of representative strains and resequencing the genomes of other geographical races based on multi-genome SNP data, analyze the genetic diversity and geographic information of *Larix gmelinii*. Determine SNP-high polymorphism target DNA fragments and DNA barcodes suitable for the identification of geographical races of *Larix gmelinii*. Finally, systematically and comprehensively analyze the transmission law and genetic characteristics of *Larix gmelinii* wilt disease. 【Result】 From 2022 to 2023, a total of 101 tree farm samples from 7 provinces, including Liaoning, Jilin, Heilongjiang, Inner Mongolia, Hebei, Gansu, and Shandong, were collected. Combining morphology and molecular biology, 62 strains of *N. laricinum* have been isolated and identified from 17 forest farms in Heilongjiang Province, Jilin Province, and Inner Mongolia Autonomous Region, with 7 forest farms in Heilongjiang Province and Jilin Province each. Two fungal strains, HLJ-QQQHE-4 from Qiqihar, Heilongjiang, and JL-TM-2 from Tumen, Jilin, were selected for pathogenicity testing on larch twigs. After 3 days of inoculation, there was a phenomenon of chlorosis at the inoculation site. After 15 days, the twigs completely withered and drooped, further confirming that the isolated pathogen was the pathogen of larch shoot blight. Research on biological characteristics has found that under alkaline conditions, with the addition of 30 g/L soluble starch and 2 g/L ammonium sulfate and continuous illumination, the growth state of *N. laricinum* is optimal. The Hi-C assembly results showed that the genome of the HLJ-QQQHE-4 strain from Aihua Forest Farm in Kedong County, Heilongjiang Province, has 14 chromosomes, a GC content of 53.34%, a genome size of 42.47 Mb, and only one small gap. The sequencing quality is high, with a total of 11275 genes encoding the protein. Evolutionary analysis shows that this strain has the closest evolutionary relationship with the same monophyletic group as *N. parvum*, further proving its taxonomic status. 【Conclusion】 The identification and pathogen isolation techniques for this disease have matured and been mastered. Analysis has found that Jilin and Heilongjiang have a large incidence area. Genome sequencing provides a reference for the study of the genetic characteristics of this fungal. The optimal growth conditions selected also lay the foundation for the later cultivation and research of this fungal. To complete the molecular-level analysis of the diffusion and spread of larch wilt disease, subsequent experiments are still ongoing.