

氏名	Hamid Sadeghi Garmaroodi
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学位論文の題目	Studies on the accumulation of <i>PDAI</i> conditionally dispensable chromosome in the genome of plant pathogenic fungus <i>Nectria haematococca</i> (植物病原菌 <i>Nectria haematococca</i> のゲノム中における <i>PDAI</i> CD染色体の蓄積に関する研究)
論文審査委員	准教授 多賀 正節 教授 鎌田 堯 教授 高橋 卓

学位論文内容の要旨

Certain strains of a plant-pathogenic ascomycete *Nectria haematococca* mating population VI (MPVI) contain supernumerary chromosomes called conditionally dispensable chromosome (CDCs) in their genomes. CDC is essential for this fungus to survive as a plant pathogen because it harbors functional genes that can confer higher fitness to the host strains to survive in specific habitats such as host plants and rhizosphere. Among CDCs in MPVI, 1.6-Mb *PDAI* CDC has been intensively studied molecular biologically and the genes specific to this chromosome such as *PEP* cluster including *PDAI* pisatin demethylation gene and several other virulence genes have been identified. Furthermore, DNA sequencing of the whole chromosome is almost completed. Thus, *PDAI* CDC represents the best-analyzed CD chromosome in fungi. Nevertheless, biological properties of *PDAI* CDC are poorly known. In this thesis, using 1.6 M *PDAI* CDC as a model, we attempted to accumulate CDC in the fungal genome artificially. Dosage effects of the duplicated CDCs on the phenotypes and meiotic behavior were also analyzed.

The strategy we used in this study for accumulating *PDAI* CDC was to exploit protoplast fusion. In the first step of this study, protocols for performing PEG-mediated and electric protoplast fusion were established by examining optimal conditions and parameters. Strains to be used for fusion were also produced by transformation with drug resistant genes as selectable genetic markers. Then, the two transformants containing one *PDAI* CDC and marked by either hygromycin B or geneticin resistant genes were protoplast-fused and analyzed for the number of CD and phenotypes of the fusants. Molecular (PCR and PFGE-Southern) and cytological (mitotic metaphase chromosome counting and fluorescence *in situ* hybridization) analyses showed that most fusants contained two CDCs from both parents and were haploid resulting from the deletion of an extra set(s) of A chromosomes in the fused nuclei. Besides fusants with two CDs, one fusant with four CDCs in the background of haploid set of A chromosomes was also obtained by PEG-mediated protoplast fusion using *nit* mutants made from fusants each containing two CDCs. Fusants with multiple copies of CD showed significantly higher virulence and homoserine-utilizing ability compared with their parents, indicating that multiplication of CDC has positive dosage effects on the phenotypes. As for the maintenance of CDCs in the genome, two CDCs were maintained rather stably under non-selective condition. Finally, inheritance mode of *PDAI* CD was analyzed by crossing with fusants. It seemed that *PDAI* CDC behaves in Mendelian manner in crosses between the strains with and without CD, but the number of CD in the genome could affect the fertility.

Consequently, we succeeded in artificial accumulation of *PDAI* CDC in the genome of *N. haematococca*. Interesting features of fungal CDC such as multiplication in the genome and its dosage effects were uncovered for the first time in this study by combining protoplast fusion and cytological and molecular biological techniques. This study was also the first for artificial addition of specific chromosome to the haploid genome in the filamentous fungi. The information and protocols presented in this study will contribute the advancement of fungal genetics as well as to plant pathology.

論文審査結果の要旨

本論文は植物病原性の子のう菌 *Nectria haematococca* において発見された *PDAI* conditionally dispensable (CD) chromosome と呼ばれる過剰染色体について、主に細胞遺伝学的な立場から研究を行ったものである。*PDAI* CD染色体上には、宿主植物であるエンドウに対する病原性遺伝子やエンドウが根圏に分泌する化合物を栄養源として利用する遺伝子が座乗しており、本染色体は菌類の病原性獲得機構のモデルとして盛んに分子遺伝学的な解析が行われている。しかし、染色体レベルでの性質に関する研究例はなく、本論文はその嚆矢である。

著者は、まず *PDAI* CD染色体のゲノム中での蓄積に焦点を当て、プロトプラスト細胞融合法と *N. haematococca* の倍数性に関する特質を利用することによって半数体ゲノム中に本染色体のみを倍加させることに成功した。また、倍加した染色体は体細胞ゲノム中ではかなり安定的に保持されることを実証し、表現型に及ぼす量的効果として病原性やエンドウの根圏分泌物質であるホモセリンの利用能が向上することを見出した。さらに、有性生殖における本染色体の遺伝様式を解析し、染色体不分離によって減数分裂産物である子のう胞子の核中に本染色体が蓄積することを発見した。これらは *PDAI* CD染色体のみならず糸状菌の細胞遺伝学全般においても新発見であり、一連の成果はこの分野のトップジャーナルである *Molecular Plant-Microbe Interactions* 誌に掲載されて国際的に高い評価を受けた。

以上、本論文は方法及び知見の両面で菌類の染色体研究に貢献するところ大であり、博士号の授与に値すると判定する。