Gene mpl1, activated during mating in Phytophthora infestans: similar to genes encoding pectate lyases

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Abstract Clones of genes activated in mating cultures of A1 and A2 mating-type strains of Phytophthora infestans were isolated using the cDNA-representational difference analysis subtraction method. Clone cET58 was selected based on its accumulation in mating cultures and then was used as a probe to isolate cDNA clone cET58L2 from a cDNA library that was constructed from mycelia grown under mating conditions. Sequence analysis revealed that cET58L2 was 1043 bp long and contained a complete open reading frame of 789 bp. The amino acid sequence of the putative protein was similar to a pectate lyase, PLD, of Fusarium solani f. sp. pisi. The central region of the predicted protein was highly similar to the sequence of other pectate lyases. The gene from which the cDNA clones were derived was designated mpl1. A probe corresponding to the protein-coding region of mpl1 was prepared (probe p58L) for Northern and Southern analyses. The maximum rate of oogonia increase and mpl1 transcript accumulation reached a maximum after 5 days in mating culture. More than 13 genes with sequences similar to that of mpl1 were found in the genome, revealing mpl1 to be a multicopy gene. The mpl1 may be a pectate lyase gene that is activated in P. infestans during mating.

Key words Phytophthora infestans · Mating conditions · Pectate lyase

Introduction

Phytophthora infestans (Mont.) de Bary is a well-known plant pathogenic oomycete fungus that causes late blight on potato and tomato. This heterothallic fungus has two mating types, A1 and A2 (Brasier 1992), that form oospores through sexual reproduction. The oospores are important for dormancy and for genetic crossing. Since the discovery of the A2 mating type in Mexico (Gallegly and Galindo 1958), both mating types have been identified around the world (Deahl et al. 1991; El-Korany et al. 1990; Goodwin et al. 1994; Mosa et al. 1989; Tantius et al. 1986). The recent discovery that oospores form on potato leaves in the field has led to the suggestion that oospores have an important ecological role (Drenth et al. 1994, 1995; Kato et al. 1993).

Previous experiments have demonstrated the existence of oosporogenesis-inducible substances in opposite mating types (Ko 1985; Zaki 1983), and there have been several studies of the chemical and physical stimulation of oosporogenesis (Ann and Ko 1989; Brasier 1971; Ho and Zentmeyer 1977; Reeves and Jackson 1974; Zaki 1983). The biochemical events that drive oosporogenesis have not been defined, although stage-specific gene expression patterns during sexual development have been reported (Akino and Ogoshi 1998; Fabritius et al. 2002). Oosporogenesis of P. infestans can be artificially controlled, as oospores are formed only when isolates of both mating types are present. The aim of this study was to identify gene expression differences between asexual and sexual reproduction of the A1 and A2 strains using representational difference analysis (RDA) (Niwa et al. 1997). Identifying genes expressed during mating is one way to understand the specific reproductive events of this fungus. We designed experiments to detect gene transcripts expressed in A1 and A2 specifically during mating (Akino and Ogoshi 1998). Here, we describe the isolation of cDNA clones derived from a gene that we designated mpl1. Transcriptional regulation of this gene and the predicted amino acid sequence of its product are also presented.
Materials and methods

Phytophthora strains and culture conditions

Japanese P. infestans strains E009 (A1 mating type) and TB201 (A2 mating type) were used for RNA isolation for cDNA-RDA, dot blot analyses, and construction of the mating-specific cDNA library. Strains IB008s (A1) and TB201 were used for RNA isolation for Northern analysis. Strains 98A8 (A1) and TB201 were used for DNA isolation for Southern analysis. All strains were isolated from late blight-diseased leaves of potatoes grown in Japan. The strains were grown in the dark on rye agar medium supplemented with 2% (w/v) sucrose (RS medium) (Caten and Jinks 1968). Agar disks containing E009 and TB201 [cultured on V-8 (Miller 1955) agar medium] were inoculated, placed in contact in the center of a petri dish containing V-8 liquid medium, and incubated for 1 week at 20°C. Oogonia that developed were counted microscopically every day for 12 days. Experiments were repeated six times.

cDNA-RDA and selection of positive clones

Mycelia of E009 and TB201 (incubated on V-8 liquid medium at 20°C for 1 week) were homogenized (Ace Homogenizer AM-5; Nihonseiki Kaisha, Tokyo, Japan) at 10000rpm for 1 min under sterile conditions. The resulting suspensions were used to inoculate individual cultures of single strains on V-8 liquid medium. Mating cultures were generated by mixing equal quantities of two cell suspensions in the same petri dish. These cultures were incubated for 6 days at 20°C. Mycelia from the mating culture (the A1 and A2 mating types in the same culture, 2 g) were collected individually by the same procedure and were ground together in a mortar. RNA was isolated from mycelia of single and mating cultures of V-8 liquid medium, and incubated for 1 week at 20°C. Oogonia formation was monitored every day for 12 days. Experiments were repeated six times.

Isolation and analyses of cDNA clones

A cDNA library of mating culture cells was constructed according to the manufacturers’ instructions (cDNA Synthesis System Plus, Amersham; Uni-Zap XR Cloning Kit, GigaPack III Packaging Extract, Stratagene) from RNA isolated from mycelia of mating cultures as described. cDNA clones were selected using the cET58 probe, the levels of which were shown by Northern analysis to increase under mating conditions. Inserts of isolated clones were subcloned according to the manufacturer’s instructions (Uni-Zap XR Cloning Kit, Stratagene). Fragments of the cloned cDNAs were sequenced as described. For Northern and Southern analyses, a digoxigenin-labeled p58L probe was synthesized as described, using PCR primers 58L1 (5'-GCCAAGCTCAAGAACGTC-3') and 58L2 (5'-TCGTG GACGATGTGGTCT-3'), which amplify a cET58L product from nucleotide 358 to 802.

Mycelia of strain IB008s and TB201 were cultured on V-8 medium, and mating cultures between the two strains were prepared. The oogonia that formed were counted microscopically, and total RNA was isolated every day from each mycelium for 10 days, as described. RNA samples were used for Northern blot analysis with a p58L probe (Boehringer Mannheim GmbH Biochemica 1993). Genomic DNA of strains 98A8 and TB201 was isolated as described by Goodwin et al. (1992). Southern blot analysis was performed using PstI, SalI, and EcoRI (Takara Biochemicals, Tokyo, Japan) and a p58L probe (Shaw 1987).

Results

Oogonia formation

With the mating of E009 and TB201, oogonia were first identified on day 3 in mating cultures. The maximum number of oogonia was found on day 8 (1059 ± 270 oogonia/cm² mycelia, mean ± standard error; n = 6), and the number of