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Analysis of the promoter region of the gene *LIP1* encoding triglyceride lipase from *Fusarium graminearum*

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Abstract

Triglyceride lipases catalyze the reversible degradation of glycerol esters with long-chain fatty acids into fatty acids and glycerol. *In silico* analysis of 5′-end flanking sequence of the gene *LIP1* encoding a triglyceride lipase from the wheat head blight pathogen *Fusarium graminearum* revealed the presence of several *cis*-regulatory elements. To delineate the function of these regulatory elements, we constructed a series of deletion mutants in the *LIP1* promoter region fused to the open reading frame of a green fluorescent protein (GFP) and assayed the promoter activity. Analysis of GFP expression levels in mutants indicated that a 563-bp promoter sequence was sufficient to drive the expression of *LIP1* and regulatory elements responsible for the gene induction were located within the 563—372 bp region. To further investigate the regulatory elements, putative *cis*-acting elements spanned within the 563—372 bp region were mutated using a targeted mutagenesis approach. A CCAAT box, a CreA binding site, and a fatty acid responsive element (FARE) were identified and confirmed to be required for the basal expression of *LIP1*, glucose suppression and fatty acid induction, respectively.

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1. Introduction

Many microorganisms prefer easily metabolizable substrates over less readily metabolizable substrates as the carbon source. One of the mechanisms exploited by these microorganisms to achieve this preferential utilization is repression of the synthesis of enzymes related to the catabolism of the alternative carbon sources by a metabolic process known as catabolite or glucose repression (Ruijter and Visser 1997). Many fungal genes encoding cell wall-degrading enzymes are subject to glucose repression, e.g., a cutinase gene from *Fusarium solani* f. sp. *pisi* (Kämper et al. 1994), xylanase (Zeilinger et al. 1996) and cellulase (Zeilinger et al. 1998) genes from *Trichoderma reesei*, a pectinase gene from *Colletotrichum lindemuthianum* (Herbert et al. 2002), and a xylanase gene from *Hypocrea jecorina* (Rauscher et al. 2006).

Expression regulation of fungal lipases is of particular interest due to the usage of these lipases in industrial processes and the fact that filamentous fungi are amiable hosts for the production of homologous and heterologous lipases (Verdoes et al. 1995; Gouka et al. 1997). The expression of an extracellular lipase gene *NhL1* from *Nectria haematococca* was induced by olive oil but repressed by glucose (Nasser-Eddine et al. 2001). Similarly, a lipase gene from *Botrytis cinerea* was induced by wax and free fatty acids extracted from grape berry cuticle but repressed by cutin hydrolysates and glucose (Reis et al. 2005). From our previous study (Feng et al. 2005), a triglyceride lipase gene (*LIP1*) was identified from *Fusarium graminearum*, the causal agent of *Fusarium* head blight of wheat and other cereals. The importance of *LIP1* in fungal utilization of saturated fatty acids was clarified through targeted gene disruption. Northern blot analysis indicated that the expression of *LIP1* was strongly induced by wheat germ oil (WGO) and saturated fatty acids, whereas repressed by unsaturated fatty acids and sugars, such as glucose. These observations implied that the expression of *LIP1* was under the control of a finely regulated promoter, which was responsible for the substrate-specific regulation of *LIP1* expression.

Since substrate specific induction and repression of *LIP1* suggested that expression control at the transcriptional level was critical for *LIP1* expression, promoter deletion and targeted mutagenesis were conducted to elucidate the *cis*-acting elements implicated in transcription and to provide an insight into the underlying mechanisms. Such a study can render information on the spatio-temporal interaction between the fungus and the wheat plant. In addition, clarifying the specific induction or repression of the *LIP1* promoter may provide the possibility of the utilization of this promoter for industrial use. Furthermore, a finely controlled promoter can be used in studies of molecular and functional characterization of essential genes, for which direct gene mutagenesis may not be possible.

2. Materials and methods

2.1. Chemicals and standard techniques

All chemicals were purchased from Fisher Scientific (Ottawa, ON, Canada) unless otherwise specified. Restriction enzymes and PCR kits, including Taq polymerase, reaction buffer and deoxynucleoside triphosphates (dNTPs), were purchased from New England Biolabs (Ipswich, MA). PCR primers (Table 1) were synthesized by Integrated DNA Technologies (Coralville, IA). Molecular techniques, if not specified, were performed according to the protocols described by Sambrook and Russell (2001). The accessibility of all websites listed in this paper was verified on the day of submission.

2.2. Fungal material

The wild-type *F. graminearum* strain PH-1 was obtained from the University of Kansas Medical Center (Kansas City, KS). The fungus was routinely maintained in Petri dishes containing potato dextrose agar. For long-term storage, a spore suspension in 15% glycerol was stored at −80 °C.

2.3. Genetic transformation of *F. graminearum*

Polyethylene glycol (PEG)-mediated protoplast transformation was performed following the method of Feng et al. (2005) with one modification. Two vectors, *LIP1* promoter-GFP fusion vector and hygromycin resistance vector pSTU1, were used simultaneously to transform *F. graminearum* (co-transformation). Hygromycin-resistant transformants were isolated and verified by PCR using primers specific to the 5′ end of the *LIP1* promoter fragment (primers 1—5, Table 1) and the 3′-end of *GFP* coding region (primer 22, Table 1), respectively.

2.4. Database search and computational analysis

The 5′-upstream sequence of *LIP1* was obtained from the *F. graminearum*