Introduction of a leptomycin-sensitive mutation into *Fusarium graminearum*

**Keywords**
Crm1; *Fusarium graminearum*; leptomycin B; nuclear export; TRI6 trichothecene regulator

Leptomycin B (LMB)-sensitive strains of *Fusarium graminearum* were generated. An attempt to detect TRI6 in the nucleus of LMB-treated cells was unsuccessful.

LMB is a secondary metabolite of a strain of *Streptomyces*. The binding of LMB to chromosomal region maintenance 1 protein (CRM1) abolishes the association of this exportin with the nuclear export signal, and inhibits nuclear export of proteins in eukaryotes\(^1\),\(^2\).

In an LMB-sensitive fission yeast, *Schizosaccharomyces pombe*, a single amino acid exchange of Cys-529 to Ser in the central conserved region of CRM1 (Fig. 1A) confers a high resistance to LMB, suggesting that the Cys-529 residue is critical for LMB binding\(^3\).

Unlike higher eukaryotes and *S. pombe*, *Saccharomyces cerevisiae* and *Aspergillus nidulans* are highly resistant to LMB. In their CRM1 orthologues, a Cys residue essential for LMB binding is substituted for a Thr residue (Fig. 1A). Sequence analysis of the *F. graminearum* CRM1 orthologue (hereafter referred to as *FgCrm1*) (FGSG_10894) indicates that *FgCRM1* contains an LMB-insensitive Thr residue (Fig. 1A). Indeed, most fungi appear to be resistant to LMB because the Cys residue is similarly substituted for a Thr residue in their orthologues.

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**Fig. 1** Construction and analysis of *F. graminearum* LMB-sensitive mutants.

(A) Alignment of CRM1 orthologues. The National Center for Biotechnology Information (NCBI) gi numbers of each protein are as follows: *H. sapiens*, 5541867; *S. pombe*, 6137058; *S. cerevisiae*, 1323393; *C. albicans*, 7658229; *N. crassa*, 157072056; *A. nidulans*, 259488259; *M. oryzae*, 351648865; *F. graminearum*, 558867781; *P. rubens*, 255939554; *R. delemar*, 384486812; *C. immitis*, 119184944.

(B) Schematic diagram showing our strategy to generate the *FgCrm1* T527C mutants. Transformants were selected with 50 µg/ml of geneticin.

(C) LMB-sensitivity assay of the *FgCrm1* T527C mutants (CRM1 T527C #1 and #2). The assay was done using YG agar plates containing 100 nM LMB. Growth of the mutants was significantly impaired in the presence of LMB.
Despite conservation of the LMB-insensitive Thr residue, the wild-type strain of *Magnaporthe oryzae* (formerly *Magnaporthe grisea*) was described previously as an organism highly sensitive to LMB. With regard to *A. nidulans*, an LMB-sensitive strain was generated by introducing a *TS25C* mutation into a *CRM1* orthologue, *crmA*, by homologous recombination. We thus examined the sensitivity of wild-type *F. graminearum* to LBM and, if resistant, whether the corresponding *TS27C* mutation (*FgCrm1* *TS27C*) drastically increased LMB-sensitivity.

*F. graminearum* JCM 9873 was transformed with a *TS27C* mutation vector pNeo-*FgCrm1* *TS27C* (Supplementary Fig. 1 and Fig. 1B), and the resulting mutants used for the LMB-sensitivity assay. Although the parental strain and ectopic mutant were resistant to 100 nM LMB, the *FgCrm1* *TS27C* mutants were highly sensitive to the drug on YG agar plate [0.5% (w/v) Difco™ yeast extract, 2% (w/v) glucose] (Fig. 1C). Growth of the *FgCrm1* *TS27C* mutants in *YS_60* liquid culture was inhibited weakly with 1 nM LMB, while hyphae treated with 5 nM LMB were viable but growth was impaired severely (data not shown). Thus, restriction of membrane permeability and/or extrusion of the drug appeared not to be significant in *F. graminearum*, and LMB-sensitivity could be attained easily by simply introducing a *TS27C* mutation into *FgCrm1*.

LMB sensitivity is useful in investigating whether the regulation of transcriptional activity is dependent on export of the transcription factor in question. For example, rapid export of *AreA*, a GATA transcription factor that is involved in nitrogen utilization in response to ammonium supplementation, was shown to be mediated by the rapid nuclear exit of *AreA* using the *crmA* *TS25C* mutant of *A. nidulans*. To examine whether previous unsuccessful attempts to detect *TRI6*-EGFP in the nucleus were due to the rapid loss of *FgCrm1*-mediated nuclear export of *TRI6*, the *TS27C* mutation vector was introduced into a marker-free transgenic strain carrying a *TRI6*-EGFP fusion gene at the native *TRI6* locus (our unpublished material). The resulting transformant was cultured in trichothecene-inducing *YS_60* medium for 48 h, and then nuclear export inhibited with 5 nM LMB. However, in contrast to *A. nidulans* *AreA*, no accumulation of green fluorescence was observed in the nucleus (data not shown). Thus, *TRI6*-EGFP may emit only extremely faint fluorescence below detection limit and/or may not be exported out of the nucleus by the CRM1/exportin pathway.

The LMB sensitive strain generated in this study does not carry a hygromycin B resistance marker gene, a versatile tool for the genetic manipulation of filamentous fungi. The system established here may be used for functional analysis of other tagged transcription factors in *F. graminearum*.  

Yuichi Nakajima1, Kazuyuki Maeda1, Shuichi Ohsa1, Kyoko Kanamaru1, Tetsuo Kobayashi1, Makoto Kimura1

1Department of Biological Mechanisms and Functions, Graduate school of Bioagricultural Sciences, Nagoya University, Furo-cho, Chikusa-ku, Nagoya, Aichi 464-8601, Japan
2Department of Agriculture, Graduate School of Agriculture, Meiji University, 1-1-1 Higashimita, Kawasaki, Kanagawa 214-8571, Japan

**Correspondence**

Yuichi Nakajima
E-mail: nakajima.yuichi@a.mbox.nagoya-u.ac.jp

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**Supplementary Materials**

Supplementary materials may be found in the online version of this article: Supplementary Fig. 1 Structure and sequence of a *TS27C* mutation vector pNeo-*FgCrm1* *TS27C*.

**References**


Supplementary Fig 1  Structure and sequence of a T527C mutation vector pNeo-FgCrm1_{T527C}.