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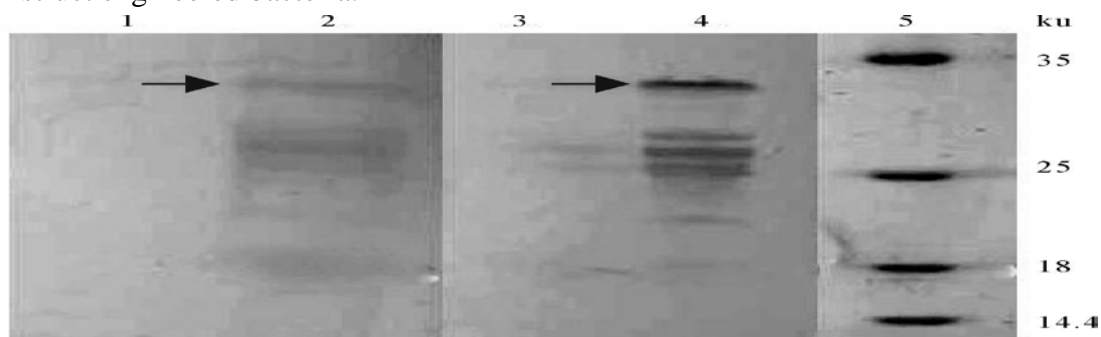
(139) Construction of Engineering Bacterium Expressing Flagellin of *Pseudomonas fluorescens* and its Toxicity to *Pinus thunbergii* in Vivo

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A constitute and secreting expressing plasmid pUC18ompA was constructed. The gene *fliC* encoding flagellin of *Pseudomonas fluorescens* Pf-5 was cloned into this plasmid to construct pUC18ompA-*fliC*. The plasmid was transformed into *E. coli* BL21 (DE3) to construct engineered bacteria.



1 supernatant of *E. coli* BL21 (DE3); 2 supernatant of the engineered bacteria; 3 Proteins of *E. coli* BL21 (DE3); 4 Proteins of the engineered bacteria; 5 Marks of the standard proteins

Fig. 1 Western blotting of the proteins in the engineered bacteria

Bacterium-free seedlings of *Pinus thunbergii* were inoculated with a mixture of the engineered bacteria and the aseptic pine wood nematodes (*Bursaphelenchus xylophilus*) to determine its pathogenicity. The results of inoculation showed that inoculation with a mixture of engineered bacteria and aseptic pine wood nematodes also caused wilt of pine seedlings to some extent. The important role of flagellin played in vivo in pathological process was further verified.

Table 1. The wilting rate of the inoculated seedlings and re-isolation of the nematodes and bacteria

Treatment	wilted seedlings within 7 days	Re-isolation of the inoculated bacteria		Re-isolation of the inoculated nematodes	
		Frequency	Species	Frequency	Species
Bx	20/20	20/20	<i>P. fluorescens</i> etc.	20/20	<i>B. xylophilus</i>
ABx	0/20	0/20	-	20/20	<i>B. xylophilus</i>
ABx+ <i>E. coli</i>	0/20	20/20	<i>E. coli</i> BL21 (DE3)	16/20	<i>B. xylophilus</i>
ABx - EB	12/20	20/20	the engineered bacteria	20/20	<i>B. xylophilus</i>
ABx+ Pf	16/20	16/20	<i>P. fluorescens</i>	16/20	<i>B. xylophilus</i>
<i>E. coli</i>	0/20	0/20	-	0/20	-
Pf	0/20	0/20	-	0/20	-
EB	0/20	0/20	-	0/20	-
CK	0/20	0/20	-	0/20	-

Bx: non-sterilized nematodes; ABx: Sterilized nematodes; *E. coli*: *E. coli* BL.21 (DE3); EB: the engineered bacteria; ABx+ *E. coli*: the mixture of sterilized nematodes and *E. coli* BL.21 (DE3); ABx+EB: the mixture of sterilized nematodes and the engineered bacteria; Pf: *Pseudomonas fluorescens*; ABx+Pf: the mixture of sterilized nematodes and *Pseudomonas fluorescens*; Ck: sterilized water control; "-": neither bacteria no nematodes were re-isolated.