Table 1. **Bacterial strains**

Strain	Relevant characteristics	Source or reference
W3110	wild type	Laboratory stock
JE7951	*spr-1*	(*7*)
JE7934	*prc*Δ::*neo*	(*7*)
JE7456	*str xyl argG*	Laboratory stock
CAG12072	*zgj-203*::Tn*10*	(*26*)
TOP10	*recA1 araD139* Δ(*araA-leu*) *7697*	Invitrogen
Rev1	*spr-1 nlpI1*	Spontaneously from JE7951
Rev2	*spr-1 nlpI2*	Spontaneously from JE7951
Rev3	*spr-1 nlpI3*	Spontaneously from JE7951
Rev4	*spr-1 nlpI4*	Spontaneously from JE7951
Rev5	*spr-1 nlpI5*	Spontaneously from JE7951
Rev6	*spr-1 nlpI6*	Spontaneously from JE7951
TAR1	*str xyl argG zgj-203*::Tn*10*	CAG12072 (P1) × JE7456
TAR2	*argG zgj-203*::Tn*10*	TAR1 (P1) × W3110
TAR3	*spr-1 argG zgj-203*::Tn*10*	TAR1 (P1) × JE7951
KM354	*recJ* pTP223 *gam bet exo*	(*14*)
MU2	*nlpI*::*cm*	Derived from KM354 by transformation with linearized *nlpI*::*cm* DNA
MU24	*nlpI*::*cm*	MU2 (P1) × W3110
MU25	*nlpI1*	Rev1 (P1) × TAR2
MU26	*nlpI2*	Rev2 (P1) × TAR2
MU27	*nlpI3*	Rev3 (P1) × TAR2
MU124	*spr-1 nlpI*::*cm*	MU2 (P1) × JE7951
MU125	*spr-1 nlpI1*	Rev1 (P1) × TAR3
MU126	*spr-1 nlpI2*	Rev2 (P1) × TAR3
MU127	*spr-1 nlpI3*	Rev3 (P1) × TAR3
PN1	*prc*Δ::*neo nlpI*::*cm*	MU24 (P1) × JE7934
HSB1	*ibpB*::*cm*	Derived from KM354 by transformation with linearized *ibpB*::*cm* DNA
HSB2	*ibpB*::*cm*	HSB1 (P1) × W3110
HSB3	*spr-1 ibpB*::*cm*	HSB1 (P1) × JE7951

(underlined), 5′-TGGGCCATGGAGCCTTTTTTGCG-3′ and 5′-GTCACCATGGGCTGGTCCGATTCTGCC-3′. The amplified DNA was digested with *Nco*I, then inserted into the same site of pBAD *Myc*-His C. To construct pNI6 encoding the hexa-histidine-tagged NlpI with the amino-acid substitution G103D, the mutated *nlpI* in the chromosomal DNA of Rev3 (*spr-1 nlpI3*) was amplified by PCR using the primers described above, and the amplified DNA was digested with the *Nco*I and inserted into the same site of pBAD *Myc*-His C. To construct pNI7 encoding the truncated and hexa-histidine-tagged NlpI, the fragment containing the truncated *nlpI* was amplified by PCR using the forward primer 5′-TGGGCCATGGAGCCTTTTTTGCG-3′ and the reverse primer 5′-GGTCCATGGGGTCCTGGCCC-3′. The amplified DNA was digested with *Nco*I, then inserted into the same site of pBAD *Myc*-His C. pNI8 and pNI9 were constructed in the same way as pNI7 except that 5′-CATCCATGGCCTGGCCCAGG-3′ or 5′-CTTCCATGGGGCCCAGGAGC-3′, respectively, was used as the reverse primer in PCR. To construct pNI10 encoding NlpI, the *nlpI* in the chromosomal DNA of W3110 was amplified by PCR using the forward primer containing the *Nco*I site (underlined) 5′-TGGGCCATGGAGCCTTTTTTGCG-3′ and the reverse primer containing the *Bam*HI site (underlined) 5′-TGGATCCGGCTCAAAGTAGG-3′. The amplified DNA was digested with *Nco*I and *Bam*HI and inserted into the same sites of pTrc99A. pNI12, pNI13, and pNI14 were constructed in the same way as pNI10 except that 5′-CCAGGGATCCTTAGTCCTGGCC-3′, 5′-GTCAGGATCCTACTGGCCCAG-3′, or 5′-ATCTGGATCCTAGCCCAGGAG-3′, respectively, was used as the reverse primer containing the *Bam*HI site (underlined) in PCR. The amplified DNA was digested with *Nco*I and *Bam*HI and then inserted into the same sites of pTrc99A.

Expression and Purification of Hexa-Histidine-Tagged NlpI Protein—E. coli cells were transformed with the recombinant plasmids. The transformants were grown in L broth at 42°C for 4 h, then incubated in L broth containing 0.01% arabinose at 42°C for 4 h for expression of the fusion genes. Cells from 400 ml of medium were collected, washed once with ice-cold 50 mM sodium phosphate buffer (pH 8.0) containing 300 mM NaCl (buffer A), suspended in 4 ml of the buffer A containing 10 mM imidazole and 1% Triton X-100, and disrupted by sonication with cooling by ice-water. The mixture was centrifuged for 15 min at 17,700 ×*g* to obtain a clear lysate. Four milliliters of the supernatant was mixed with 1 ml of nickel-nitrilotriacetic acid (Ni-NTA) resin and incubated with rotation for 2 h at 4°C. The mixture was loaded onto an open column. The column was washed with 4 ml each of buffer A containing 20 mM imidazole and then containing 40 mM imidazole. The bound proteins were eluted with 1.2 ml of buffer A containing 100 mM imidazole, and fractions of 0.4 ml were collected.

Mass Spectrometry Analysis—An electrospray ion trap mass spectrometer (LCQ DECA XP ion trap mass system, Thermo Electron) coupled online with a capillary HPLC (Magic 2002, Michrom BioResources) was used to acquire MS/MS spectra. A 0.2 × 50 mm MAGICMS C18