Supplementary Material

1 Supplementary Experimental Procedures

Construction of plasmids and strains.

Phusion DNA polymerase and restriction enzymes (Thermo Scientific) were used in plasmid constructions (primers are listed in Table S2). Plasmids were verified by sequencing.

To construct the *dnaA* allele that will encode either pseudo-phosphorylated or phospho-ablative protein, site-directed mutagenesis was conducted by using corresponding primers (Table S2) and pET28a-DnaA$_{His6}$ as a template. Mutagenized sequence were obtained by PCR amplification and ligation into StuI and XhoI cut pET28-DnaA$_{His6}$ plasmid.

For construction of *attφC31* attachment site integrative vector, harbouring different *dnaA* allele, pSET152 plasmid was digested with EcoRV and XbaI enzymes. The ca. 500 bp fragment of chromosomal DNA, localized upstream of the *dnaA* gene and partially within the ORF (reaching the first natural BamHI restriction site within *dnaA* gene), was amplified with primers flanked with XbaI and BamHI restriction site. The remaining *dnaA* coding sequence was obtained by digestion of pET28a-DnaA$_{His6}$ WT/T486D vector with XhoI enzyme and filling in the 5' ends, followed by partial digestion with BamHI, resulting in 1900 bp fragment. Obtained DNA fragments were ligated, resulting in pSET-*dnaA*$_{His6}$ WT/T486D/T486A integrative vector. An attempt to delete the native *dnaA* gene was made by generating apramycin-resistance cassette, derived from pIJ773, with primers dnaA_del_FW and dnaA_del_RV (Table S2). The *dnaA* gene on cosmid StH18 (John Innes Centre, Norwich) was replaced with obtained cassette. The modified cosmid was used for PCR targeting protocol as described elsewhere[1].

For construction of AK120 *ΔafsK* mutant, apramycin-resistance cassette, derived from pIJ773, was generated with primers afsK_del_FW and afsK_del_RV (Table S2). The *afsK* gene present on cosmid St6F11 (John Innes Centre, Norwich) was replaced with obtained cassette and PCR targeting protocol was used as described elsewhere[1] on AK120 strain.

2 Supplementary Figures and Tables

2.1 Supplementary Figures

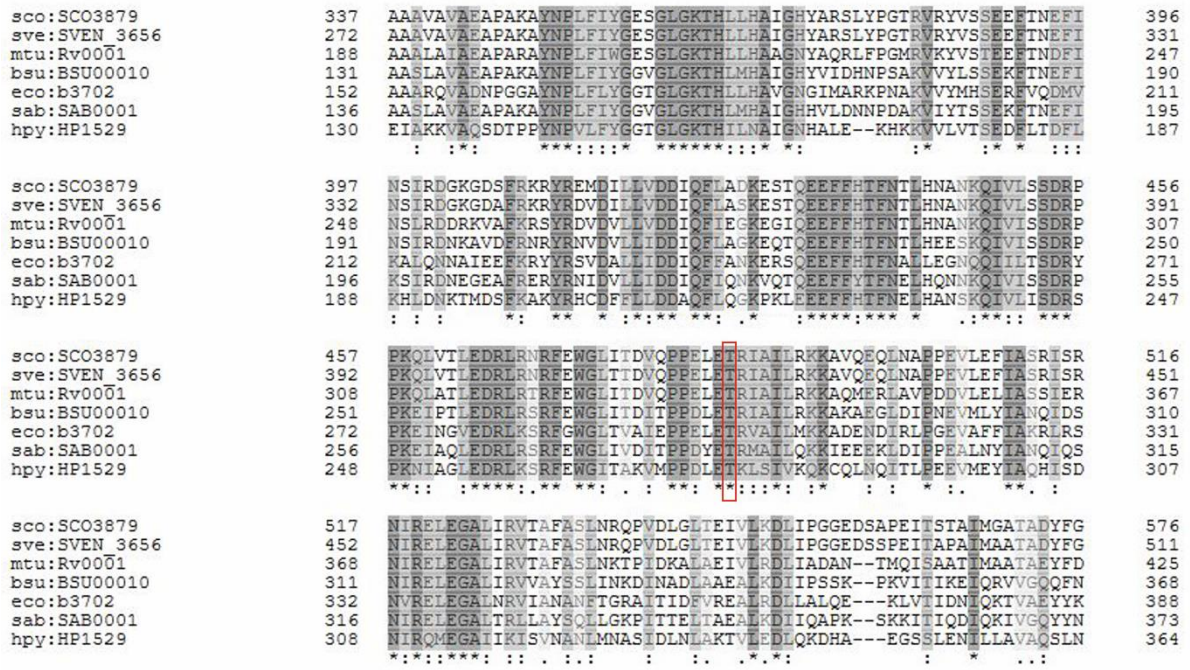

Fig. S1. High conservation of the DnaA domain III from various bacterial species.

Alignment of multiple DnaA domain III sequences from closely and distantly related bacterial species: *Streptomyces coelicolor*, *Streptomyces venezuelae*, *Mycobacterium tuberculosis*, *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* and *Helicobacter pylori* (indicated as sco:SCO3879, sve:SVEN_3656, mtu:Rv0001, bsu:BSU00010, eco:b3702, sab:SAB0001 and hpy:HP1529, respectively). Identical residues are marked by dark grey background and asterisk, similar residues are marked by light gray background and dot or colon. Numbers correspond to the position of given residue within protein sequence. *S. coelicolor* threonine 486 position and corresponding residues in other DnaA proteins are marked by red rectangle. Sequence alignment was made with Clustal Omega software[2]. Protein sequence data was obtained from UniProt database.

2.2 Supplementary Tables

Table S1. Bacterial strains and plasmids.

S. coelicolor

Strain	**Genotype**	**Source**
M600	Prototrophic, SCP1$^-$, SCP2$^-$	laboratory stock
M145	Prototrophic, SCP1$^-$, SCP2$^-$	
M1101	M600 *ΔafsK::aac(3)-IV*	Hempel *et. al*, 2012; Prof. Mark Buttner
M1102	M600 *ΔramC::aac(3)-IV*	
M1103	M600 *Δ1468::aac(3)-IV*	
M1104	M600 *Δ2244::aac(3)-IV*	
M1105	M600 *Δ3102::aac(3)-IV*	
M1106	M600 *Δ3820-3821::aac(3)-IV*	
M1107	M600 *Δ4487-4488::aac(3)-IV*	
M1108	M600 *Δ4507::aac(3)-IV*	
AK120	M145 *dnaN-EGFP* (in frame)	Prof. Dagmara Jakimowicz and dr Agnieszka Kois-Ostrowska, laboratory stock
AK120 *ΔafsK::aac(3)-IV*	M145 *dnaN-EGFP* (in frame); *ΔafsK::aac(3)-IV*	this work
AK120 *tipAØ*	M145 *dnaN-EGFP* (in frame); *attB$_{\varphi C31}$::tipAØ*	
AK120 *tipA afsK*	M145 *dnaN-EGFP* (in frame); *attB$_{\varphi C31}$::tipA afsK*	
M1101 pKF256	M600 *ΔafsK::aac(3)-IV; attB$_{\varphi BT1}$::afsK*	
del-DnaA	M145 *attB$_{\varphi C31}$::dnaA$_{His6}$* WT; *ΔdnaA* (in frame)	
DnaA$_{His6}$ WT	M145 *attB$_{\varphi C31}$::dnaA$_{His6}$* WT	
DnaA$_{His6}$ T486D	M145 *attB$_{\varphi C31}$::dnaA$_{His6}$* T486D	

Table S1 continued.

E. coli

Strain	**Genotype**	**Source**
DH5α	*F⁻ endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG purB20 φ80dlacZΔM15 Δ(lacZYA-argF)U169, hsdR17($r_K^-m_K^+$), λ^-*	laboratory stock
ET pUZ8002	*am-13::Tn9 dcm-6 hsdM, pUZ8002, Kan^r*	
BL21(DE3)	*B F⁻ ompT gal dcm lon hsdS$_B$($r_B^-m_B^-$) λ(DE3 [lacI lacUV5-T7p07 ind1 sam7 nin5]) [malB$^+$]$_{K-12}$(λ^S)*	
BL21	*B F⁻ ompT gal dcm lon hsdS$_B$($r_B^-m_B^-$)[malB$^+$]$_{K-12}$(λ^S)*	

Plasmids

Plasmid	**General description**	**Source**
pET28a-DnaA$_{His6}$	pET28a induced expression vector carrying *dnaA$_{His6}$* gene	laboratory stock
pIJ6902	vector integrating into *S. coelicolor* chromosomal φC31 attachment site; carries thiostrepton-inducible *tipA* promoter	laboratory stock
pKF 592	pIJ6902 derivative, carries thiostrepton inducible *afsK* gene	Hempel *et. al*, 2012
pSET152	vector integrating into *S. coelicolor* chromosomal φC31 attachment site	laboratory stock
pSET152-DnaA$_{His6}$ WT/T486D	vector integrating into *S. coelicolor* chromosomal φC31 attachment site; carries *dnaA* allele	this work
pIJ773	source of the *aac(3)IV* (apramycin) resistance cassette	laboratory stock

Table S2. Oligonucleotide primers used in this study.

Primer	Sequence 5' - 3'	General description
StuI_dnaA_FW	TCGCCGAGGCACCCGCCA	Primers used in site-directed mutagenesis of *dnaA*$_{His6}$ encoding pET28a plasmid.
XhoI_dnaA_RV	GTGGTGCTCGAGTGCGGC	
dnaA_T486D_UP	GCCGCCGGAGCTGGAGGACCGCATCGCGATCCTGC	
dnaA_T486D_DN	GCAGGATCGCGATGCGGTCCTCCAGCTCCGGCGGC	
dnaA_del_FW	TTGGAGCAACTTCTCGGAGAGGGCCGCGGGCAGGGCGTCCATATGATTCCGGGGATCCGTCGACC	Primers used for generation of *dnaA*:: *aac(3)-IV* disruption cassete.
dnaA_del_RV	GGGTCCCGGAGGCGCCTTCGTGACCGGTGGGGTCGGTCGCATATGTGTAGGCTGGAGCTGCTTC	
dnaA_pprom_FW	GGATCCAGTGCTCGTCCTTCGAC	Primers used for amplification of ~500 bp fragment of chromosomal DNA, localized upstream of the *dnaA* gene and partially within the ORF (see Construction of plasmid and strains in Supplementary procedures).
dnaA_pprom_RV	TCTAGAGATGTCATGACCCGACCGGGAT	
afsK_del_FW	GTGGTGGATCAGCTGACGCAGCACGATCCGCGGCGGATCATTCCGGGGATCCGTCGACC	Primers used for generation of *afsK*:: *aac(3)-IV* disruption cassete.
afsK_del_RV	TCACGTCGTACGGGCGGTCCCCGTGCCCTTCTCCGCGTCTGTAGGCTGGAGCTGCTTC	
afsK_FW	GGATCCGTGGTGGATCAGCTGACGCA	Primers used for amplification of *afsK* gene
afsK_RV	TCACGTCGTACGGGCGGT	
oriC_Bf2_IR700	CCTCGCACGTCCCCGCGTCTC	Primers used for generation of near-infrared labelled *oriC* fragment.
oriC_Br7_IR700	GGCATGTGGAGAAGCTGGTGATC	

References

1. Gust B, Challis GL, Fowler K, Kieser T, Chater KF (2003) PCR-targeted Streptomyces gene replacement identifies a protein domain needed for biosynthesis of the se quiterpene soil odor geosmin. Proc Natl Acad Sci USA *100,* 1541–1546.

2. Sievers F, Wilm A, Dineen DG, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Söding J, Thompson JD, Higgins DG (2011). Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Molecular Systems Biology 7:539 doi:10.1038/msb.2011.75