**Table S1** Strains and plasmids used in this study

| Strain or plasmid | Relevant characteristics | Reference or source |
| --- | --- | --- |
| Strains |  |  |
| *Escherichia coli* |  |  |
| DH5 | *supE44 lacU169*(80dlacZΔM15)*hsdR17 recA1 endA1 gyrA96Δthi relA1* | (Woodcock et al. 1989) |
| BL21(DE3) | F- *ompT hsdSB*  (*rB*- *mB*-)*gal dcm lacY1*(DE3) | Transgen |
| BL21(DE3)-*ndpB* | BL21(DE3) transformed with pET28a-*ndpB*, Kanr | This study |
| BL21(DE3)-*ndpD*-C | BL21(DE3) transformed with pET28a-*ndpD*-C, Kanr | This study |
| BL21(DE3)-*ndpD*-N | BL21(DE3) transformed with pET28a-*ndpD*-N, Kanr | This study |
| Origami B(DE3) | F- *ompT* *hsdSB*(*rB*- *mB*-) *gal* *dcm* *lacY1* *ahpC* (DE3) *gor522*::Tn*10* (TcR) *trx*B::kan | Transgen |
| OrigamiB(DE3)-*ndpB* | OrigamiB(DE3) transformed with pET22b-*ndpB*, Kanr, Tcr, Ampr | This study |
| WM3064 | Donor strain for conjugation, 2,6-diaminopimelic  acid auxotroph: *thrB1*004 *pro* *thi* *rpsL hsdS*  *lacZ*ΔM15 RP4-1360 Δ(*araBAD*)*567*  Δ*dapA*1341::[*erm pir*(wt)] | (Dehio and Meyer 1997; Saltikov and Newman 2003) |
| *Sphingomonas* species |  |  |
| TY | Wild type, nicotine-degrading strain, G-, Ampr, Kans, Tcs | This study |
| TYΔ*ndpAL* | TY mutant with *ndpAL* gene replaced by kanamycin resistance gene from plasposon pTnMod-Okm, Ampr, Kanr | This study |
| TYΔ*ndpB* | TY mutant with *ndpB* gene replaced by kanamycin resistance gene from plasposon pTnMod-Okm, Ampr, Kanr | This study |
| TYΔ*ndpC* | TY mutant with *ndpC* gene replaced by kanamycin resistance gene from plasposon pTnMod-Okm, Ampr, Kanr | This study |
| TYΔ*ndpD* | TY mutant with *ndpD* gene replaced by kanamycin resistance gene from plasposon pTnMod-Okm, Ampr, Kanr | This study |
| TYΔ*ndpAL*（pRK415-*ndpAL*） | *ndpAL* gene was complemented by pRK415-*ndpAL* in TYΔ*ndpAL*, Ampr, Kanr, Tcr | This study |
| TYΔ*ndpB*（pRK415-*ndpB*） | *ndpB* gene was complemented by pRK415-*ndpB* in TYΔ*ndpB*, Ampr, Kanr, Tcr | This study |
| TYΔ*ndpC*（pRK415-*ndpC*） | *ndpC* gene was complemented by pRK415-*ndpC* in TYΔ*ndpC*, Ampr, Kanr, Tcr | This study |
| TYΔ*ndpD*（pRK415-*ndpD*） | *ndpD* gene was complemented by pRK415-*ndpD* in TYΔ*ndpD*, Ampr, Kanr, Tcr | This study |
| TYΔ*ndpB*-*ndpBhi*s | TYΔ*ndpB* transformed with pRK415-*ndpBhis*, TcR | This study |
| *Sphingomonas aquatilis* JSS7T | Wild type, non-nicotine-degrading strain, G-, Tcs | (Lee et al. 2001) |
| *Sphingomonas*-*ndpA* | *Sphingomonas aquatilis* transformed with pRK415-*ndpA*, Tcr | This study |
| *Sphingomonas*-*ndpC* | *Sphingomonas aquatilis* transformed with pRK415-*ndpC*, Tcr | This study |
| *Sphingomonas*-*ndpD* | *Sphingomonas aquatilis* transformed with pRK415-*ndpD*, Tcr | This study |
| *Pseudomonas putida* species |  |  |
| KT2440 | Metabolically versatile saprophytic soil bacterium | (Nelson et al. 2002) |
| KT-*ndpA* | KT2440 transformed with pRK415-*ndpA*, Tcr | This study |
| KT-*ndpAplus* | KT2440 transformed with pRK415-*ndpAplus*, Tcr | This study |
| KT-*ndpB* | KT2440 transformed with pRK415-*ndpB*, Tcr | This study |
| KT-*ndpC* | KT2440 transformed with pRK415-*ndpC*, Tcr | This study |
| KT-*ndpD* | KT2440 transformed with pRK415-*ndpD*, Tcr | This study |
| Plasmids |  |  |
| pTnMod-Okm | Source of kanamycin resistance gene | (Dennis and Zylstra 1998) |
| pEX18Tc | Gene knockout vector, oriT+, sacB+, Tcr | (Hoang et al. 1998) |
| pEX18Tc-*ndpAL* | *ndpAL* gene knockout vector containing two DNA fragments homologous to the upstream and downstream regions of the *ndpAL* and kanamycin resistance gene from pTnMod-Okm | This study |
| pEX18Tc-*ndpB* | *ndpB* gene knockout vector containing two DNA fragments homologous to the upstream and downstream regions of the *ndpB* and kanamycin resistance gene from pTnMod-Okm | This study |
| pEX18Tc-*ndpC* | *ndpC* gene knockout vector containing two DNA fragments homologous to the upstream and downstream regions of the *ndpC* and kanamycin resistance gene from pTnMod-Okm | This study |
| pEX18Tc-*ndpD* | *ndpD* gene knockout vector containing two DNA fragments homologous to the upstream and downstream regions of the *ndpD* and kanamycin resistance gene from pTnMod-Okm | This study |
| pRK415 | Broad host range vector , Tcr | (Keen et al. 1988) |
| pRK415-*ndpAL* | *ndpAL* gene complementation vector by fusing *ndpAL* into the *Hin*d III-*EcoR* I restriction site of pRK415 | This study |
| pRK415-*ndpA* | Heterologous expression vector with *ndpA* insert into the *Hin*d III-*Eco*R I restriction site of pRK415 |  |
| pRK415-*npdAplus* | Heterologous expression vector by adding an extended segment of 223 bp in 5’ of *ndpA* compared with pRK415-*ndpA* |  |
| pRK415-*ndpB* | *ndpB* gene complementation vector by fusing *ndpB* into the *Hin*d III-*EcoR* I restriction site of pRK415 | This study |
| pRK415-*ndpBhis* | *ndpB* gene with a 6-Histag insert into the *Hin*d III-*EcoR* I restriction site of pRK415 | This study |
| pRK415-*ndpC* | *ndpC* gene complementation vector by fusing *ndpC* into the *Hin*d III-*EcoR* I restriction site of pRK415 | This study |
| pRK415-*ndpD* | *ndpD* gene complementation vector by fusing *ndpD* into the *Hin*d III-*EcoR* I restriction site of pRK415 | This study |
| pET-28a(+) | Expression vector, Kanr, C/N-terminal His•Tag/thrombin/T7•Tag, T7 *lac* promoter, T7 transcription start, f1 origin, *lacI* | Novagen |
| pET28a-*ndpB* | Expression vector for *ndpB* with C-terminal His•Tag by cloning *ndpB* into the *Nco* I-*Hin*d III restriction site | This study |
| pET28a-*ndpD*-C | Expression vector for *ndpD* with C-terminal His•Tag by cloning *ndpD* into the *Nco* I-*Hin*d III restriction site | This study |
| pET28a-*ndpD*-N | Expression vector for *ndpD* with N-terminal His•Tag by cloning *ndpD* into the *Nde* I- *Xho* I restriction site | This study |
| pET-22b(+) | Expression vector, Ampr, C-terminal His•Tag, T7 *lac* promoter, *pelB* signal sequence | Novagen |
| pET22b-*ndpB* | Expression vector for *ndpB* with C-terminal His•Tag by cloning *ndpB* into the *Nco* I-*Hin*d III restriction site | This study |

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