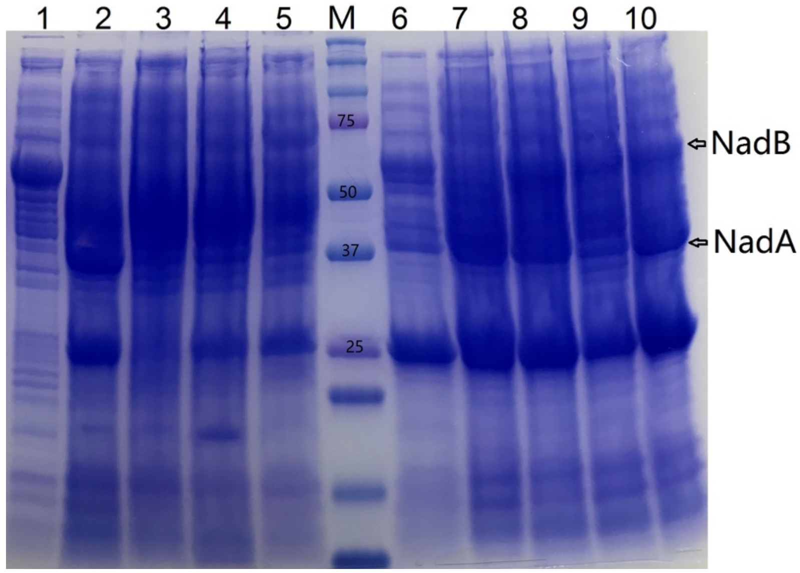

Fig. S1. SDS-PAGE analysis of soluble protein. FZ763 was used as the host strain, 20 µL sample was loaded unless otherwise specified. From left to right: 1, pFZGNB14 [*E. coli* NadB (5 µL)]; 2, pFZGNB34 (*E. coli* NadA); 3, pTrc99a- St*nadB* [*Sulfolobus tokodaii* NadB (StNadB)]; 4, pFZGNB183 [*Pseudomonas putida* KT2440 NadB (PpNadB)]; 5, pFZGNB43[*Bacillus subtilis* NadA (BsNadA)]; M: Bio-Rad dual color standards (5 µL); 6, FZ763/pFZGNB190 (7 µL); 7, FZ763/pFZGNB189; 8, FZ763/pFZGNB193; 9, FZ763/pFZGNB207; 10, FZ763/pFZGNB190. Numbers on the marker indicates the protein molecular weight (kDa)

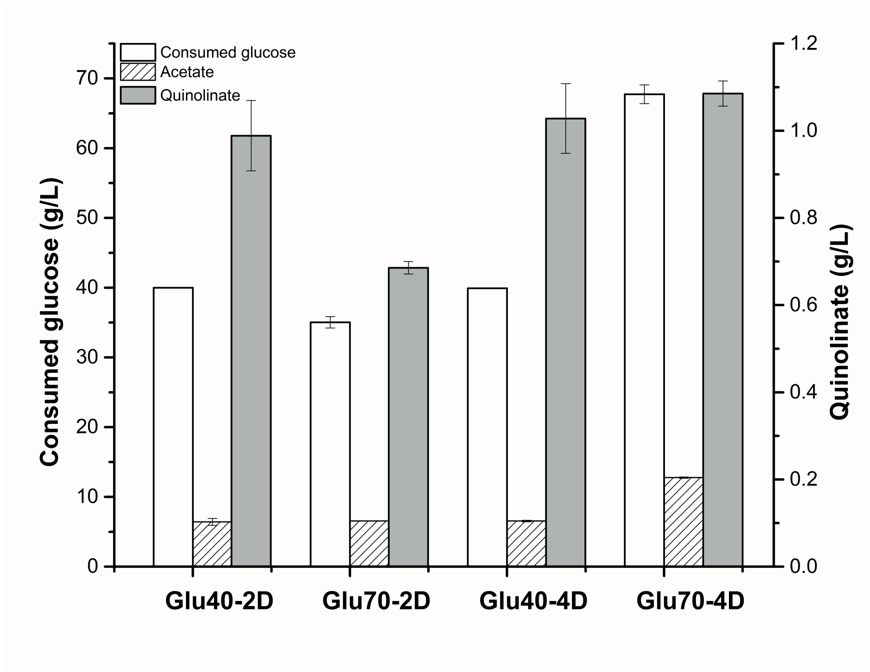

Fig. S2. The effect of glucose concentration on quinolinate production in FZ763/pFZGNB190. Aerobic cultures were performed at 37 ^{o}C, 350 rpm. Values are the average of three replicates with error bars indicating standard deviation. Glu40/70 indicates fermentation with 40 or 70 g/L glucose and the -2D or -4D indicates the culture was incubated for 2 or 4 days.

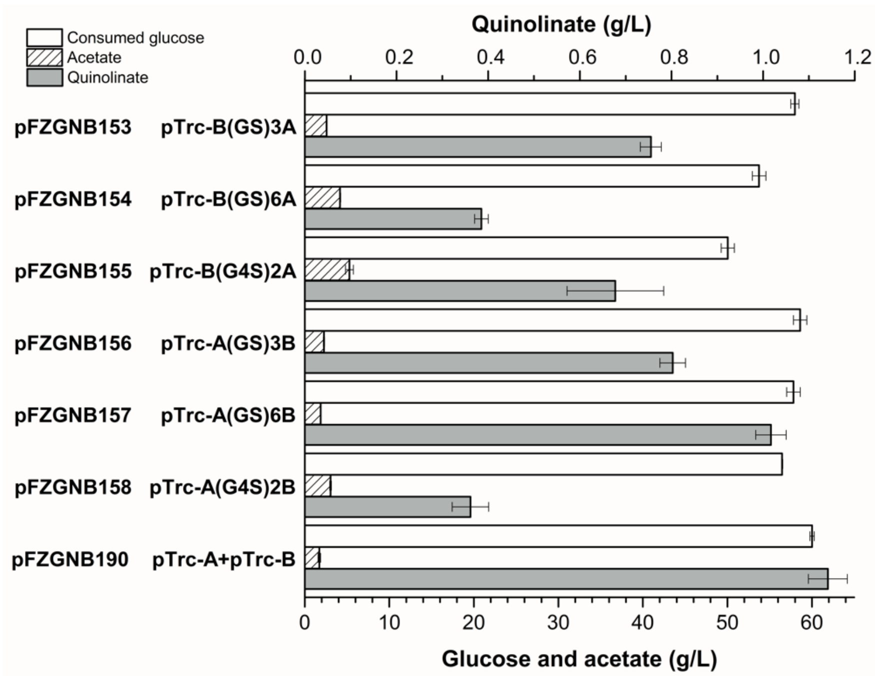

Fig. S3. The effect of NadA-NadB fusion proteins on quinolinic acid production in FZ763. Aerobic cultures were performed at 37 ºC, 350 rpm for 4 days. NadA-NadB fusion protein with different linkers and different orders were over-expressed under the control of pTrc promoter in pFZGNB33. Values are the average of three replicates with error bars indicating standard deviation.

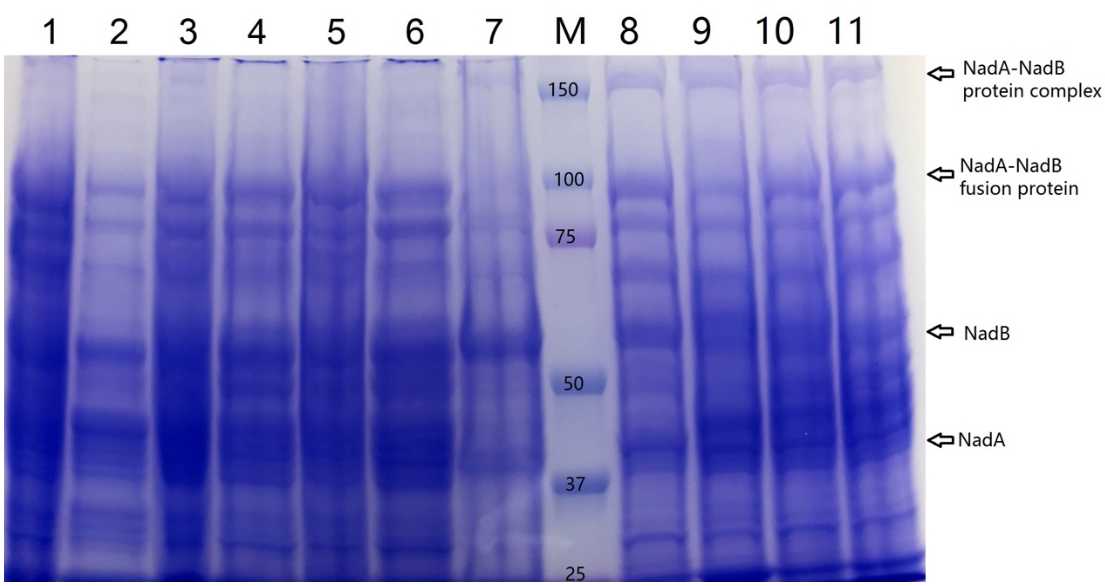

Fig. S4. Non-reducing SDS-PAGE analysis of soluble protein in FZ763 harboring

different plasmids. 20 μL sample was loaded otherwise specified. From left to right: 1, pFZGNB153; 2, pFZGNB154; 3, pFZGNB155; 4, pFZGNB156; 5, pFZGNB157; 6, pFZGNB158; 7, pFZGNB190 (7 μL); M: Bio-Rad dual color standards (5 μL); 8, pFZGNB232 (15 μL); 9, pFZGNB233 (25 μL); 10, pFZGNB234 (15 μL); 11, pFZGNB235 (15 μL). Numbers on the marker indicates the protein molecular weight (kDa), the approximate protein location was also labled based on the protein molecular weight. Non-reducing SDS-PAGE indicates no reductant (Dithiothreitol or 2-mercaptoethanol) was added to the loading buffer.

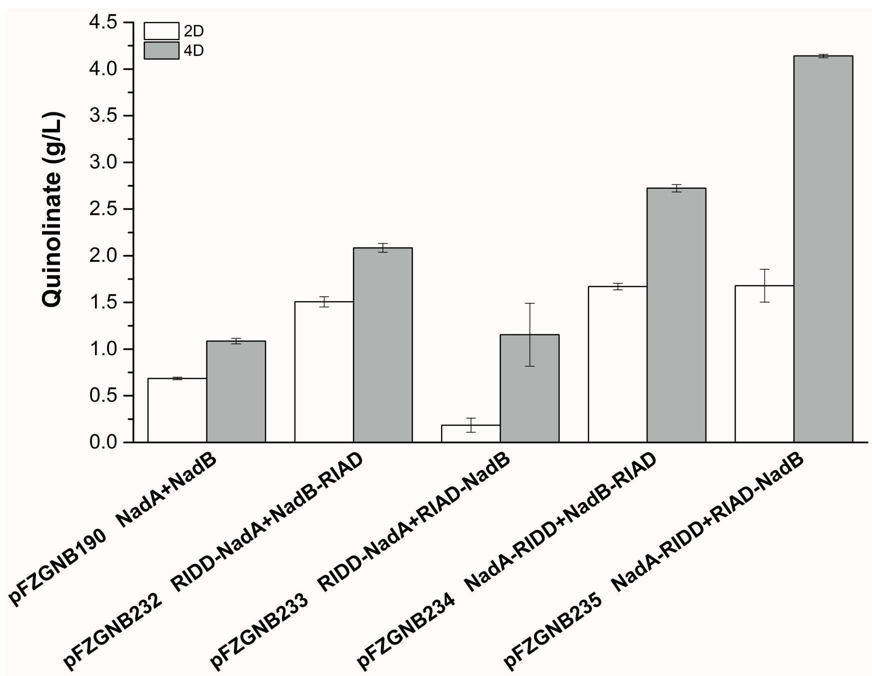

Fig. S5. The effect of NadA-NadB enzyme complex on quinolinic acid production in FZ763 with 70 g/L glucose. Aerobic cultures were performed at 37 ^{o}C, 350 rpm for 4 days. NadA-RIDD and NadB-RIAD fusion proteins were over-expressed under the control of their pTrc promoter in pFZGNB227, NadA-NadB enzyme complex was assembled with the help of peptides, RIAD and RIDD. Values are the average of three replicates with error bars indicating standard deviation.

Table S1. Plasmids and strains used in this study.

	Description	Source
plasmids		
pHL413K	Amp^R of pTrc99a was replaced with Km^R, and *pycA* gene	(Thakker et al., 2011)
pKK313	S8D mutant *Pepc* from *sorghum*, Amp^R	(Wang et al., 1992)
pFZGNB16	BglII was introduced 193 bp before pTrc promoter of pTrc99a, Amp^R	This study
pFZGNB33	BglII was introduced 193 bp before pTrc promoter, Amp^R of pTrc99a was replaced by Km^R from pHL413K, Km^R	This study
pFZGNB34	*nadA* was inserted into pFZGNB33 between EcoRI and BamHI	This study
pFZGNB35	*nadB* was inserted into pFZGNB33 between NcoI and BamHI	This study
pFZGNB36	*aspC* was inserted into pFZGNB33 between EcoRI and BamHI	This study
pFZGNB37	pFZGNB33 with *nadA-nadB-aspC*	This study
pFZGNB38	pFZGNB33 with *nadA-aspC-nadB*	This study
pFZGNB39	pFZGNB33 with *nadB-aspC-nadA*	This study
pFZGNB40	pFZGNB33 with *nadB-nadA-aspC*	This study
pFZGNB41	pFZGNB33 with *aspC-nadA-nadB*	This study
pFZGNB42	pFZGNB33 with *aspC-nadB-nadA*	This study

pFZGNB43	*nadA* from *Bacillus subtilis* with an extra DNA fragment coding an HisTag at C-terminal (Bs*nadA*-HisTag) was cloned into pFZGNB33 between EcoRI and XbaI	This study
pFZGNB44	pTargetT contains N_{20} specific for *ptsG*, Sm^R	This study
pFZGNB46	Upstream and downstream homologous arms of *ptsG* was inserted into pFZGNB44, for *ptsG* deletion, Sm^R	This study
pFZGNB50	pTrc-*pepc* from pKK313 was inserted into pFZGNB46 between homologous arms, for replacement of *ptsG* with pTrc-*pepc*, Sm^R	This study
pFZGNB63	pTargetT contains N_{20} specific for *tpiA*, upstream and downstream homologous arms of *tpiA*, Sm^R, for *tpiA* deletion	This study
pFZGNB65	pTargetT contains N_{20} specific for *sucA*, upstream and downstream homologous arms of suc*AB*, Sm^R, for *sucAB* deletion	This study
pFZGNB74	pTargetT contains N_{20} specific for *lpd*, upstream and downstream homologous arms of *lpd*, Sm^R, for *lpd* deletion	This study
pFZGNB75	pTargetT contains N_{20} specific for *ackA*, upstream and downstream homologous arms of *ackA*, Sm^R, for *ackA* deletion	This study
pFZGNB76	pTargetT contains N_{20} specific for *ackA*, upstream and downstream homologous arms of *pta-ackA*, Sm^R, for *pta-ackA* deletion	This study

pFZGNB117	pTrc-*pepc* from pKK313 was inserted into pFZGNB75 between homologous arms, for replacement of *ackA* with pTrc-*pepc*, Sm^R	This study
pFZGNB118	pTrc-*pepc* from pKK313 was inserted into pFZGNB76 between homologous arms, for replacement of *pta-ackA* with pTrc-*pepc*, Sm^R	This study
pFZGNB153	Gene encoding NadB-$(GlySer)_3$-NadA under the control of pTrc promoter in pFZGNB33	This study
pFZGNB154	Gene encoding NadB-$(GlySer)_6$-NadA under the control of pTrc promoter in pFZGNB33	This study
pFZGNB155	Gene encoding NadB-$(Gly_4Ser)_2$-NadA under the control of pTrc promoter in pFZGNB33	This study
pFZGNB156	Gene encoding NadA-$(GlySer)_3$-NadB under the control of pTrc promoter in pFZGNB33	This study
pFZGNB157	Gene encoding NadA-$(GlySer)_6$-NadB under the control of pTrc promoter in pFZGNB33	This study
pFZGNB158	Gene encoding NadA-$(Gly_4Ser)_2$-NadB under the control of pTrc promoter in pFZGNB33	This study
pFZGNB183	*nadB* from *Pseudomonas putida* KT2440 (Pp*nadB*) was inserted into pFZGNB33 between BamHI and XbaI	This study
pFZGNB33-staspO	*nadB* (aspO) from *Sulfolobus tokodaii* (St*nadB*) was inserted into pFZGNB33 between NcoI and BamHI	This study
pFZGNB189	pFZGNB33 with pTrc-*nadA* and pTrc-St*nadB*	This study

pFZGNB190	pFZGNB33 with pTrc-*nadA* and pTrc-*nadB*	This study
pFZGNB193	pFZGNB33 with pTrc-*nadA* and pTrc-Pp*nadB*	This study
pFZGNB204	pFZGNB33 with pTrc-*nadA*, pTrc-*nadB* and pTrc-*aspC*	This study
pFZGNB207	pFZGNB33 with pTrc-Bs*nadA* and pTrc-*nadB*	This study
RIDD	DNA coding RIDD with linker was synthesized and inserted into pUC57	This study
RIAD	DNA coding RIAD with linker was synthesized and inserted into pUC57	This study
pFZGNB227	A SpeI was introduced into pFZGNB33 in front of pTrc promoter, Km^R	This study
pFZGNB228	pFZGNB227 with pTrc-*ridd-nadA*	This study
pFZGNB229	pFZGNB227 with pTrc-*nadB-riad*	This study
pFZGNB230	pFZGNB227 with pTrc-*riad-nadB*	This study
pFZGNB231	pFZGNB227 with pTrc-*nadA-ridd*	This study
pFZGNB232	pFZGNB227 with pTrc-*ridd-nadA* and pTrc-*nadB-riad*	This study
pFZGNB233	pFZGNB227 with pTrc-*ridd-nadA* and pTrc-*riad-nadB*	This study
pFZGNB234	pFZGNB227 with pTrc-*nadA-ridd* and pTrc-*nadB-riad*	This study
pFZGNB235	pFZGNB227 with pTrc-*nadA-ridd* and pTrc-*riad-nadB*	This study
Strains		
MG1655	*E. coli* K12 MG1655, *F⁻ lambda⁻ ilvG*- *rfb*-50 *rph*-1	
FZ700	MG1655 Δ*nadC*	This study
FZ703	MG1655 Δ*nadC,* Δ*nadR*	This study
FZ723	MG1655 Δ*nadC,* Δ*nadR,* Δ*ptsG*	This study

FZ734	MG1655 Δ*nadC*, Δ*nadR*, *ptsG::pTrc-pepc*	This study
FZ738	FZ734 Δ*tpiA*	This study
FZ740	FZ734 Δ*sucAB*	This study
FZ741	FZ734 Δ*poxB*	This study
FZ742	FZ734 Δ*lpd*	This study
FZ756	FZ734 Δ*poxB::pTrc-pepc*	This study
FZ757	FZ734 Δ*poxB*, Δ*ackA*	This study
FZ763	FZ734 Δ*poxB*, *ackA::pTrc-pepc*	This study

Table S2. Acetate accumulated in engineered strains

	MG1655	FZ700	FZ703	FZ723	FZ734
1 day	18.0 ± 0.3	7.2 ± 0.1	14.1 ± 0.3	4.8 ± 0.5	4.6 ± 0.1
3 day	34.7 ± 0.4	30.4 ± 1.9	29.0 ± 0.5	18.8 ± 0.2	18.4 ± 0.5
5 day	38.8 ± 1.2	34.2 ± 1.1	33.9 ± 0.3	26.1 ± 0.1	25.8 ± .5
7 day	42.2 ± 0.9	36.0 ± .4	37.0 ± 0.4	32.0 ± 0.6	32.5 ± 0.5
nadC	+	-	-	-	-
nadR	+	+	-	-	-
ptsG	+	+	+	-	-
pTrc-pepc	-	-	-	-	+

Plasmid pFZGNB42 (pTrc-*aspC-nadB-nadA*) was introduced into the tested strains to check their performance, aerobic cultures were performed at 37 ºC, 350 rpm. The numbers indicate acetate concentration (g/L, average of three replicates with error bars indicating standard deviation).

Table S3. DNA sequences used in this study

>pFZGNB33 partial sequence

GCGCAACGCAATTAATGTGAGTTAGCGCGAATAGATCTGGTTTGACAGCTTATCATC
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GCTGTGCAGGTCGTAAATCACTGCATAATTCGTGTCGCTCAAGGCGCACTCCCGTTC
TGGATAATGTTTTTTGCGCCGACATCATAACGGTTCTGGCAAATATTCTGAAATGAG
CTGTTGACAATTAATCATCCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAA
TTTCACACAGGAAACAGACCATGGAATTCGAGCTCGGTACCCGGGGATCCTCTAGA
GTCGACCTGCAGGCATGCAAGCTTAGCTTGCAGTGGGCTTACATGGCGATAGCTAGA
CTGGGCGGTTTTATGGACAGCAAGCGAACCGGA

The special features are highlighted BglII, pTrc promoter, MCS

>pFZGNB227 partial sequence

GCGCAACGCAATTAATGTGAGTTAGCGCGAATAGATCTGGTTTGACAGCTTATCATC
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GCTGTGCAGGTCGTAAATCACTGCATAATTCGTGTCGCTCAAGGCGCACTCCCGTTC
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GAGTCGACCTGCAGGCATGCAAGCTTAGCTTGCAGTGGGCTTACATGGCGATAGCTA
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The special features are highlighted BglII, SpeI, pTrc promoter, MCS

> BsnadA-HisTag sequence

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>stnadB sequence

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>PpnadB sequence

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TCGAGCTGCGCAACCTGGCGCAAGTGGCCGAGCTGATGATCCTGTCAGCCATGCAG
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>optimized RIDD with linker sequence

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GAGTGCGAACTGTACGTTCAGAAACACAACATCCAGGCGCTGCTGAAAGATAGCAT
CGTTCAACTGTGTACCGCACGTCCGGAACGTCCGATGGCATTTCTGCGCGAATACTT
CGAACGCCTGGAAAAAGAAGAAGCGAAAGGTGGCGGTGGCTCAGGTGGCGGTGGT
TCAGGTGGTGGTGGTTGTGGT

>optimized RIAD with linker sequence

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GGAACAATACGCGAATCAGCTGGCGGATCAGATTATCAAAGAAGCGACCGAAGGCT
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