Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Figure S1: DNA fragments and nucleic acid electrophoresis in the construction of the strain. Table S1: Bacterial strains and plasmids used in this study. Table S2: Oligonucleotides used in this study. Figure S2: Evolutionary tree of amino acid transporter gene in *Bacillus licheniformis* from NCBI. Figure S3: Cell growth in minimal medium with free L-Asp as the only carbon and nitrogen source.

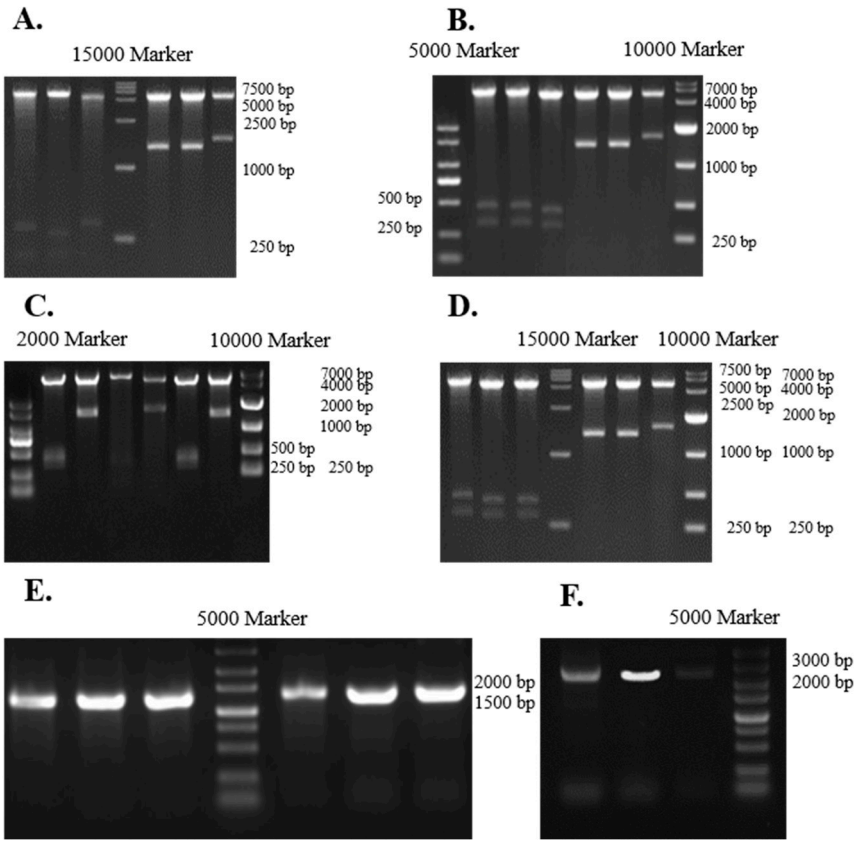

Figure S1: DNA fragments and nucleic acid electrophoresis in the construction of the strain. (A) (B) (C) The nucleic acid electrophoretogram of the fragments obtained after double-enzyme digestion of the overexpressed protein particles. The overexpression of genes *ydgF*, *yvbW*, and *yveA* was mediated by different promoters in *B. licheniformis*. (A) The overexpression of the three genes mediated by promoter *PmtlA*; (B) The overexpression of the three genes mediated by promoter *Pshuttle09*; (C) The overexpression of gene *ydgF* mediated by promoter *Pshuttle09-XBS*; (D) Nucleic acid electrophoresis of gene fragments of the expressed protein on the plasmid and PCR amplified promoter fragments; (E) (F) The nucleic acid electrophoretogram when verifying whether three genes are knocked out separately in *B. licheni-formis CICIM B1391*; (E) The left exchange and right exchange of the three single knockout strains; (F) The double ex-change of the three single knockout strains.

Table S1. Bacterial strains and plasmids used in this study

Strain and plasmid	Genotype or phenotype	Reference
Plasmid		
pHY300-PLK	*E. coli*/*Bacillus* shuttle vector, Ap^R/Tet^R	TaKaRa
pMD19T-simple	*E. coli* cloning vector, Ap^R	TaKaRa
pNZT1	The delivery vector of replication thermosensitive and rolling circle amplification, Em^R	Laboratory construct
pNZTT	pNZT1 inset a tetracycline gene for Tet^r at Not	Laboratory construct
pMF	Promoter P*mtlA* mediated protein YdgF overexpression	This work
pS09F	Promoter P*shuttle09* mediated protein YdgF overexpression	This work
pS09XF	Promoter P*shuttle09-XBS* mediated protein YdgF overexpression	This work
pMW	Promoter P*mtlA* mediated protein YvbW overexpression	This work
pS09W	Promoter P*shuttle09* mediated protein YvbW overexpression	This work
pS09XW	Promoter P*shuttle09-XBS* mediated protein YvbW overexpression	This work
pMA	Promoter P*mtlA* mediated protein YvbA overexpression	This work
pS09A	Promoter P*shuttle09* mediated protein YvbA overexpression	This work
pS09XA	Promoter P*shuttle09-XBS* mediated protein YvbA overexpression	This work
19T-*ydgf*	pMD19T-simple with the *ydgF* fragment and its homology arm	This work
19T-*yvbw*	pMD19T-simple with the *yvbW* fragment and its homology arm	This work
19T-*yvea*	pMD19T-simple with the *yveA* fragment and its homology arm	This work
19T-FFKF	pMD19T-simple with the deletion cassette of *ydgf*	This work
19T-WFKF	pMD19T-simple with the deletion cassette of *yvbw*	This work
19T-AFKF	pMD19T-simple with the deletion cassette of *yvea*	This work

pNZTT-FFKF	Knock-out vector pNZTT with the deletion cassette of *ydgF*	This work
pNZTT-WFKF	Knock-out vector pNZTT with the deletion cassette of *yvbw*	This work
pNZTT-AFKF	Knock-out vector pNZTT with the deletion cassette of *yvea*	This work
Strains		
E. coli JM109	F′, traD36, proAB + lacIq, Δ(lacZ), M15/Δ (lac-proAB), gln V44, e14–, gyrA96, recA1, relA1, endA1, thi, hsdR17 (CICIM B0012)	CICIM-CU
Bacillus licheniformis CICIM B1391	Wild type (CICIM B1391)	CICIM-CU
BldF	*Bacillus licheniformis* CICIM B1391, knockout of *ydgF gene*， Kan^r	This work
BldW	*Bacillus licheniformis* CICIM B1391, knockout of *yvbW gene*， Kan^r	This work
BldA	*Bacillus licheniformis* CICIM B1391, knockout of *yveA gene*， Kan^r	This work
BlpMF	*Bacillus licheniformis* CICIM B1391, harboring pMF	This work
BlpS09F	*Bacillus licheniformis* CICIM B1391, harboring pS09F	This work
BlpS09XF	*Bacillus licheniformis* CICIM B1391, harboring pS09XF	This work
BlpMW	*Bacillus licheniformis* CICIM B1391, harboring pMW	This work
BlpS09W	*Bacillus licheniformis* CICIM B1391, harboring pS09W	This work
BlpS09XW	*Bacillus licheniformis* CICIM B1391, harboring pS09XW	This work
BlpMA	*Bacillus licheniformis* CICIM B1391, harboring pMA	This work
BlpS09A	*Bacillus licheniformis* CICIM B1391, harboring pS09A	This work
BlpS09XA	*Bacillus licheniformis* CICIM B1391, harboring pS09XA	This work
BlpHY	*Bacillus licheniformis* CICIM B1391, harboring pHY300-PLK	This work
BldFpHY	*Bacillus licheniformis* CICIM B1391, d*ydgF,* harboring pHY300-PLK	This work
BldWpHY	*Bacillus licheniformis* CICIM B1391, d*yvbW,* harboring pHY300-PLK	This work
BldApHY	*Bacillus licheniformis* CICIM B1391, d*yveA,* harboring pHY300-PLK	This work
BldFpS09F	*Bacillus licheniformis* CICIM B1391, d*ydgF,* harboring	This work

	pS09F	
BldWpS09W	*Bacillus licheniformis* CICIM B1391, d*yvbw,* harboring pS09W	This work
BldApS09A	*Bacillus licheniformis* CICIM B1391, d*yveA,* harboring pS09A	This work

[1] Ap^R, ampicillin resistance; Tet^R, tetracycline resistance; Kan^R, kanamycin resistance; Em^R, erythromycin resistance

[2] CICIM-CU: Culture and Information Center of Industrial Microorganisms of China Universities

Table S2. Oligonucleotides used in this study

Primers	Sequence
yvbW-F	CGGGGTACCCCGATGGAGAAAGACATGCAGAAGCTCG
yveA-F	CGGGGTACCCCGTTGTCAAATCAGGGCAATTTCAAAA
yveA-R	GCGTCGACGTCTTTAAACGGTTTCTTCTTTTTTCTCA
ydgF-F	CGGGGTACCCCGGTGACAGAAGATGTAACTGACAACG
ydgF-R	GGAAGATCTTCCCTACACTTGCTGTGACTTAGGTTT
Kan-M-F	GAGCTGTTTGAATATGCAGGCAAATGGCGTAATATTCGTG
Kan-M-R	TTCTACGATAAGGGCACAAATCGCATCGTGGAACGTTT
*ydgF*L-*Xho*I-F	CCCAAGCTTGGGTCATCATCCGCTTGCCGT
*ydgF*R-*Hin*dIII-R	CCGCTCGAGCCTCGGGCCTCCCGTTTTT
FFRT-*Kpn*I-Kan-F	CGGGGTACCCCGGAAGTTCCTATTCCGAAGTTCCTATTCT CTAGAAAGTATAGGAACTTCGGC
FFRT-*Sal*I-Kan-R	GCGTCGACGTCTTGGCCATAGCGGCCGCGGAAGTTCCTAT ACTTTCTAGAGAATAGGAACTTCGGAATAGGAACTTCCA C
*ydgF-Kpn*I-R	GCGTCGACGTCTTGGCCATAGCGGCCGCGAAACCTAAGT CACAGCAAGTG
*ydgF-Sal*I-F	CGGGGTACCCCGCAGTTACATCTTCTGTCAC
ydgF-L-F	GCCAGCCGACGTATTCACAAGAACA
ydgF-L-R	TTTTGTTCAGCTTTCGTTCCTCCTA
*yvbW*L-*Xho*I-F	CCGCTCGAGCGGAGCATAATCCCTCCCGAACCGATGC
*yvbW*R-*Sal*I-R	GCGTCGACGTCTTCCCTTGTATTTTCCAATGGGAAAGG
WFRT-*Nhe*I-Kan-F	CTAGCTAGCTAGATCGAAGTTCCTATTCCGAAGTTCCTATT CTCTAGAAAGTATAGGAACTTCGGC
WFRT-*Eco*RI-Kan-R	CCGGAATTCCGGATCGAAGTTCCTATACTTTCTAGAGAAT AGGAACTTCGGAATAGGAACTTCCAC
*yvbW-Nhe*I-R	CTAGCTAGCTAGCGTCCGCTTCTTAAAAAAAGGCTTG
*yvbW-Eco*RI-F	CCGGAATTCCGGCTCCATCTATGTTCACTTCCTCTAG
yvbW-L-F	ACATTGCCGGACGGCTTAAAATACTGGCGGAGTTC
yvbW-L-R	CGGTTCACACTCTATCATCGTTAAAATATTTTGTAAAGT
*yveA*L-*Xho*I-F	CCGCTCGAGCGGATTGAATGCCGGTGTACCGCTTGTT
*yveA*R-*Pst*I-R	TGCACTGCAGTGCACTTGGAACGGGATGCTTCCCAAACA
AFRT-*Kpn*I-Kan-F	CGGGGTACCCCGATCGAAGTTCCTATTCCGAAGTTCCTAT TCTCTAGAAAGTATAGGAACTTCGGC
AFRT-*Sal*I-Kan-R	GCGTCGACGTCTTATCGAAGTTCCTATACTTTCTAGAGAAT

	AGGAACTTCGGAATAGGAACTTCC
*yveA-Kpn*I-R	CGGGGTACCCCGGACAAATCACTTCCTCCTTTTTAAT
*yveA-Sal*I-F	GCGTCGACGTCTTTAAATAAGAATCCCCCGCGTATGGA
yveA-L-F	CCGGAATTTTATCATATGTGCATCGAAGCATTCAG
yveA-L-R	ACCTTGATACTAATGATGTGTCCTTCACACAATAA

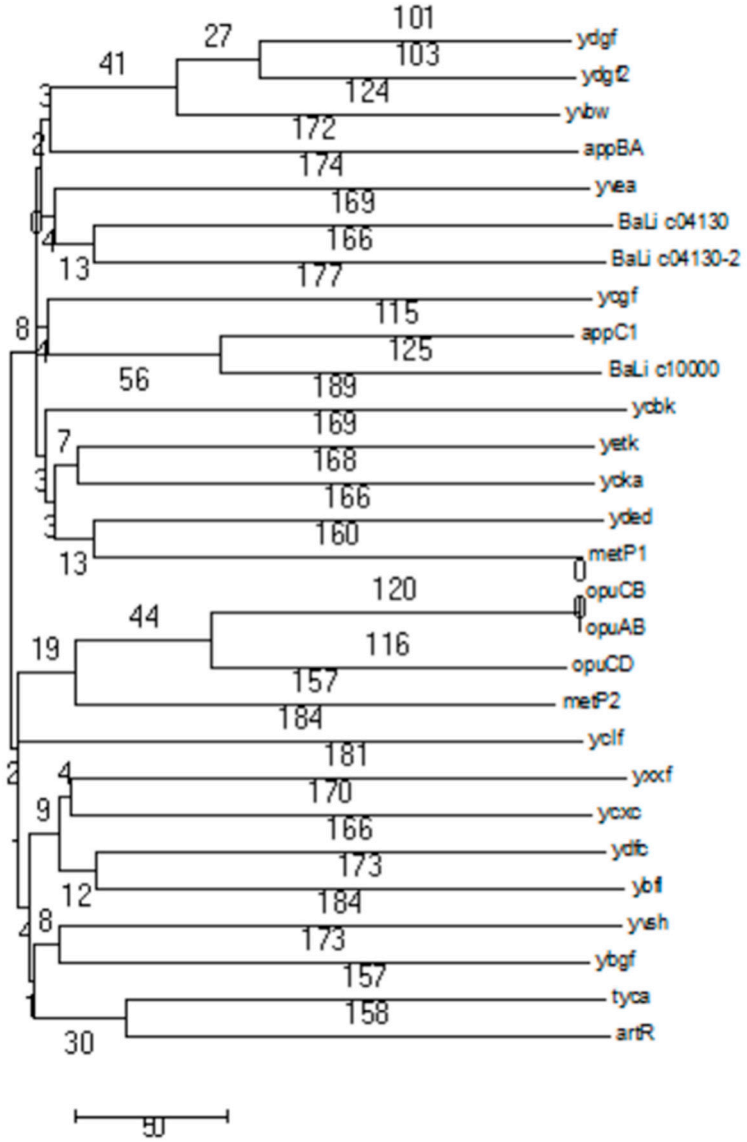

Figure S2: Evolutionary tree of amino acid transporter gene in *Bacillus licheniformis* from NCBI.

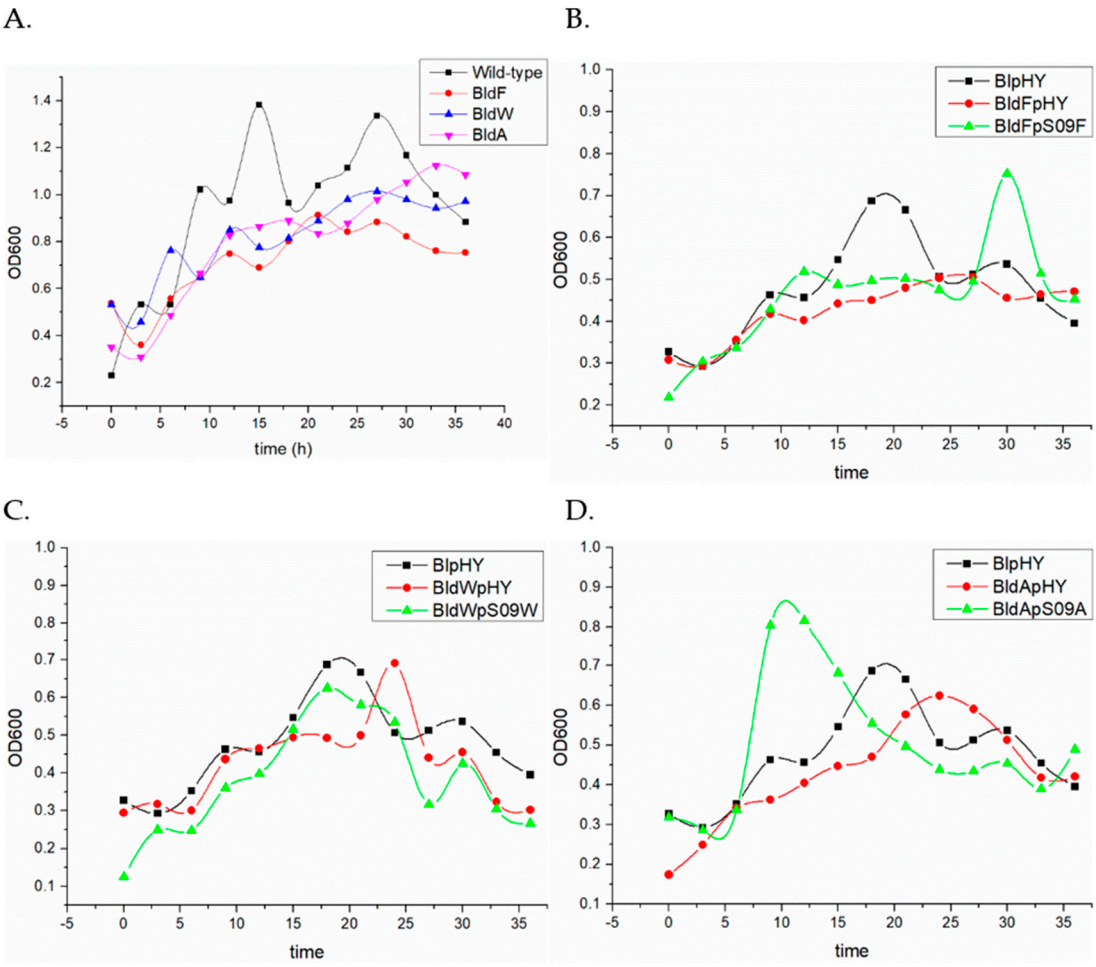

Figure S3: Cell growth in minimal medium with free L-Asp as the only carbon and nitrogen source. (A) The growth curve of wild-type strain (solid square), strain BldF (solid circle), strain BldW (solid regular triangle) and strain BldA (solid inverted triangle); (B) (C) (D) Strains BlpHY (solid squares), transporter gene single knockout strains with empty plasmids (solid circles) and transporter gene single knockout strains with P*shuttle09* promoter mediated transporter gene overexpression plasmids, which means the transporter gene is supplemented and expressed in the strains with single knock-out transporter gene (solid equi-lateral triangle); (B) Strain BldFpS09F; (C) Strain BldWpS09W; (D) Strain BldApS09A.