

Figure S1. The effect of single EAA omission on the growth of LAB strains in an artificial medium. Changes in OD 570 nm of each bacterium in different media, each with a single EAA omission, for culturing over the indicated time. Strain 1: *Lactiplantibacillus plantarum* (ATCC 8014); Strain 2: *Lactobacillus delbrueckii* (ATCC 9649); Strain 3: *Lactocaseibacillus rhamnosus* (ATCC 7469a); Strain 4: *Lactocaseibacillus casei* (ATCC 393); and Strain 5: *Lactocaseibacillus paracasei* subsp. *Paracasei* (ATCC 25302). *Lactobacillus delbrueckii* (ATCC 11842) was excluded from this experiment due to its inability to survive in the artificial medium supplemented with 20 proteinogenic AAs. The symbol \triangle indicates that a specific compound was omitted from the artificial medium. Error bars show SD. Data represent the mean \pm SD from 3 independent experiments performed in triplicate. Statistical significance was assessed using a one-way ANOVA followed by Tukey's multiple comparisons test, with the following significance thresholds: ns (not significant) for $P > 0.05$, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$.

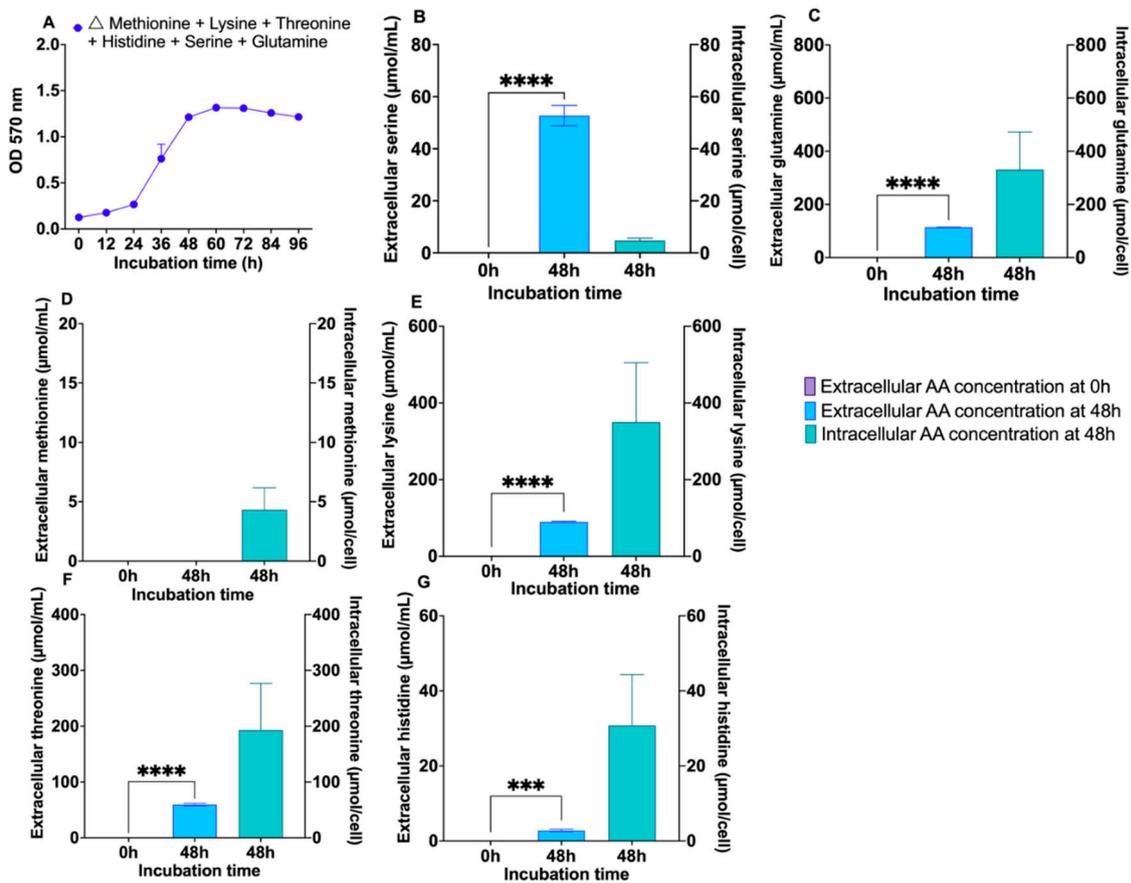


Figure S2. The effect of AA deprivation on growth and intracellular and extracellular AA concentration in *L. plantarum*.

In this assay, *L. plantarum* cell pellets were harvested after 48 hours of culture in an artificial medium lacking the 6 AAs, rather than in MRS broth, and were then reintroduced into the same 6 AA-excluded medium for further culturing. (A) Growth curves of *L. plantarum* in an artificial medium deficient in 6 AAs. (B-G) A quantitative comparison of the extracellular concentrations of AAs at 0 hours (prior to inoculation with *L. plantarum*) and 48 hours post-inoculation, alongside the analysis of intracellular concentrations of AAs at 48 hours. Error bars show SD. All of the experiments were repeated in triplicate. Significance was calculated by unpaired Student's t test; not significant for $P > 0.05$, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$.

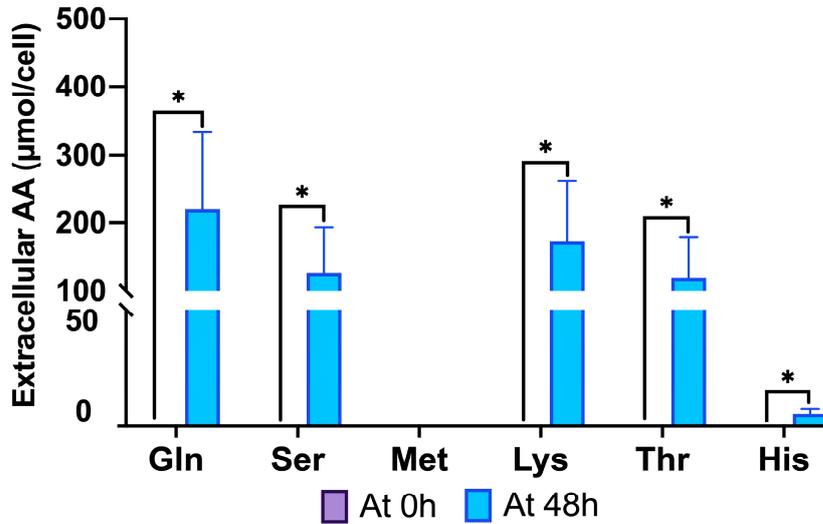


Figure S3. The effect of AA deprivation on extracellular AA concentrations in *L. plantarum*. A quantitative comparison of extracellular AA concentrations was conducted at 0 hours (prior to inoculation with *L. plantarum*) and 48 hours post-inoculation. The extracellular AA concentrations were normalized to the colony counts in the culture medium samples. Error bars show SD. All of the experiments were repeated in triplicate. Significance was calculated by unpaired Student's t-test; ns (not significant) for $P > 0.05$, $*P < 0.05$. Gln (glutamine), Ser (serine), Met (methionine), Lys (lysine), Thr (threonine), His (histidine).

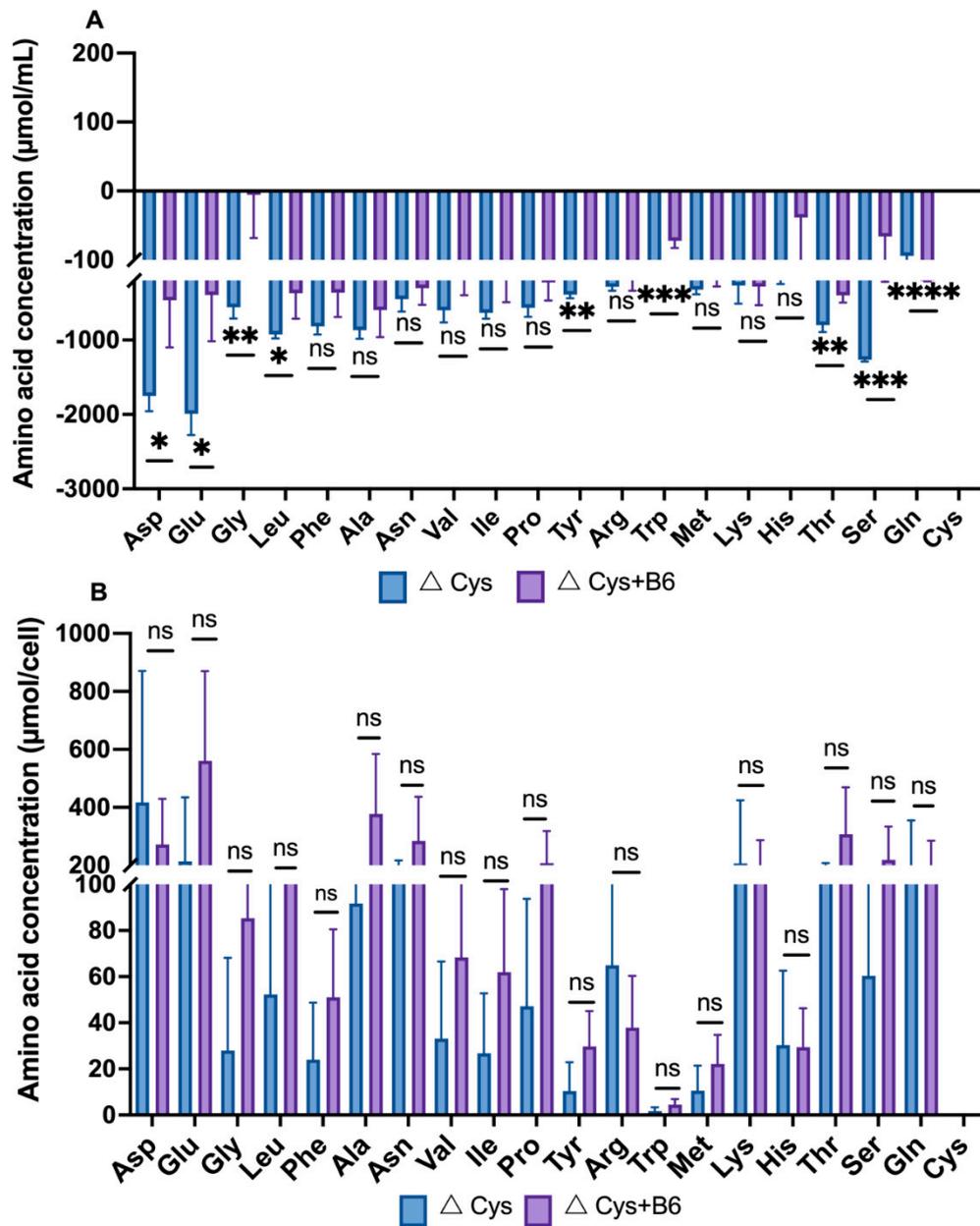


Figure S4.

The effect of cysteine and vitamin B6 deprivation on AA concentration in *L. plantarum*. Comparative analysis of change in extracellular (A) and intracellular AA concentrations (B) in two cysteine-deficient artificial media, one supplemented with vitamin B6 and the other without (related to Figure 6B). Error bars show SD. All of the experiments were repeated in triplicate. Significance was calculated by unpaired Student's t test; ns (not significant) for $P > 0.05$, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$. Ala (alanine), Arg (arginine), Asn (asparagine), Asp (aspartic acid), Cys (cysteine), Gln (glutamine), Glu (glutamic acid), Gly (glycine), His (histidine), Ile (isoleucine), Leu (leucine), Lys (lysine), Met (methionine), Phe (phenylalanine), Pro (proline), Ser (serine), Thr (threonine), Trp (tryptophan), Tyr (tyrosine), Val (valine).