**Supplementary data**

**Figure S1.** Growth profiles of the recombinant *L. plantarum* WCFS1 strains harboring AmyL (A) and AmyA (B) secretion plasmids with different signal peptides in 200 mL of MRS containing 5 μg/mL erythromycin at 37°C. Values given are the average values from at least two independent experiments and the error bars indicate the standard deviation.

**Figure S2.** SDS-PAGE analysis of cell-free supernatant and lysate of *L. plantarum* WCFS1 strains at 9 h after induction overexpressing the ~100 kDa α-amylase encoded by *amyL* (A) and the ~50 kDa α-amylase encoded by *amyA* (B). The arrows indicate the bands of α-amylases, M denotes the Precision protein ladder (Bio-Rad). Non-induced strains harbouring the plasmid pLp\_spAmyA\_AmyL and pLp\_spAmyA\_AmyA were representatives for non-induced conditions in (A) and (B), respectively.

**Tables**

**Table S1.** Primers used for cloning in this study

|  |  |  |
| --- | --- | --- |
| **Primers** | **Primer sequences (5'→3')** | **Restriction site underlined** |
| AmyL\_SalI\_Fw | GGCGGAGTCGACGATAGTTATACGACATCAACTGATGAC | *Sal*I |
| AmyL\_EcoRI\_Rv | AGTAGTGAATTCTTACGAAGTGCTTGATGTGC | *Eco*RI |
| 401\_spAmyA\_EcoRI\_Fw | GATAAGAATTCGGTACCCCGGGTTCGAA | *Eco*RI |
| 401\_spAmyA\_SalI\_Rv | GATTAGTCGACACTAGCCGCTTGAGCAACTTGTTTAGA | *Sal*I |

**Table S2.** Strains and plasmids used in this study

|  |  |  |
| --- | --- | --- |
| **Strains and plasmids** | **Characteristics** | **References/Sources** |
| **Strains** |  |  |
| *E. coli* NEB5α | Host strain | New England Biolabs (Frankfurt am Main, Germany) |
| *L. plantarum* WCFS1 | Host, protein expression strain | Kleerebezem *et al.* (2003) |
| *L. plantarum* S21 | Native strain | Kanpiengjai *et al.* (2014) |
| **Plasmids** |  |  |
| pSIP401 | *spp*-based expression vector with inducible promoter PsppA; ErmR | Sørvig *et al.* (2003) |
| pSIP409 | *spp-*based expression vector with inducible promoter PsppQ, ErmR | Sørvig *et al.* (2005) |
| *pSIP401 derivatives* |  |  |
| pLp\_2145s\_AmyA | *amyA* fused to Lp\_2145 | Mathiesen *et al.* (2009) |
| pLp\_3050s\_AmyA | *amyA* fused to Lp\_3050 | Mathiesen *et al.* (2008) |
| pLp\_0373s\_AmyA | *amyA* fused to Lp\_0373 | Mathiesen *et al.* (2008) |
| pLp\_spAmyA\_AmyA | *amyA* with its native signal peptide (SP\_AmyA) | Mathiesen *et al.* (2008) |
| pLp\_2145s\_AmyL | *amyL* fused to Lp\_2145 | This study |
| pLp\_3050s\_AmyL | *amyL* fused to Lp\_3050 | This study |
| pLp\_0373s\_AmyL | *amyL* fused to Lp\_0373 | This study |
| pLp\_spAmyA\_AmyL | *amyL* fused to signal peptide of AmyA (SP\_AmyA) | This study |
| *pSIP409 derivative* |  |  |
| pLp\_AmyL7 | *amyL* with native signal peptide (SP\_AmyL) | Kanpiengjai *et al.* (2015b) |

Table S3. Sequences of the signal peptides used in this study.

|  |  |
| --- | --- |
| **Signal peptides** | **Amino acid sequences of the signal peptides**  (including 2 amino acids downstream of cleavage site)a |
| SP\_AmyA | MKKKKSFWLVSFLVIVASVFFISFGFSNHSKQVAQA🡫AS |
| SP\_AmyL | MKKKKSFWLVSFLVIVASVFFISFGLSNHSNQVAQA🡫DS |
| Lp\_2145  Lp\_3050 | MKKINKLMILGMLVFGVTGATMINPEMTTAAHA🡫SA  MKKFNFKTMLLLVLASCVFGVVVNVTTSLGPQTAITAQA🡫SK |
| Lp\_0373 | MYTENTGKHHRNGLPVWLLPLLVVISFWGVSQNIMVVDA🡫SS |

aThe arrows 🡫 represent the cleave sites of the signal peptides as determined by SignalP-5.0 (www.cbs.dtu.dk/services/SignalP/) (Armenteros *et al.*, 2019)