Supplementary table 1. Commonly used expression, peptide release and purification systems of antimicrobial peptides produced in *E.coli*

Expression			
Direct expression	Non-carrier bearing expression	• Large size and aggregation prone antimicrobial peptides are eligible • His-tag is usually used for purification, and can block toxicity of antimicrobial peptides o Refolding is time-consuming and may not guarantee recovery of activity	
Fusion expression	Solubility-enhancing carriers	GST (26.9 kDa)	• Rapid purification of the GST-fused protein from crude lysate • Blocks toxicity to the host cell o GST was highly susceptible to proteolytic degradation in several cases o Reduced yield due to the large size of the GST due to low peptide-to-carrier ratio
		Thioredoxin (11.8 kDa)	• High yield due to high peptide-to-carrier ratio • Chaperone-like activity can promote correct disulfide bond formation • Blocks toxicity to the host cell and prevents proteolytic degradation of peptides o Affinity tag such as His-tag is usually used for purification
		SUMO (11.2 kDa)	• High yield due to high peptide-to-carrier ratio • Improves folding by the attachment of the carrier • Peptide release by SUMO protease is both efficient and highly accurate, leaving no additional amino acids • Blocks toxicity to the host cell and prevents proteolytic degradation of peptides o Requires additional affinity tag such as His for purification
	Aggregation-promoting carriers	PurF fragment (16.3 kDa)/ PaP3.30 (17.6 kDa)/Ketosteroid isomerase (13.5 kDa)/ TAF12 domain (8.4 kDa)	• Rapid purification of fused peptides through insoluble expression • Blocks toxicity to the host cell and prevents proteolytic degradation of peptides o Requires refolding to restore activity
	Self-cleavable carriers	Intein (51.5 or 17.3 kDa) and N^{pro} systems	• Exogenous proteases or chemicals are not required to remove tag • Facile purification of target proteins o Reduced yield due to large size of intein/chitin domain o Uncontrolled autocleavage of the intein fusions could cause toxicity to host o Requires extensive dilution and long incubation time for N^{pro} system, and possible incomplete cleavage
	Secretion signals	Signal peptide	• Eligible expression of cysteine-rich antimicrobial peptides in periplasmic space where disulfide bond formation is favored o Possible secretion of the peptide to culture media can be toxic to host

• advantages o disadvantages

<table>
<tr><th colspan="4">Peptide release</th></tr>
<tr><td rowspan="3">Chemical</td><td>CNBr</td><td>Met ↓</td><td>o Limited use of chemical reagents due to methionine presence within the target peptide
o Increased complexity and reduced yield of purification due to methionine presence within the target peptide
o Side chain modification by reaction buffers such as 70% formic acid</td></tr>
<tr><td>Formic acid</td><td>Asp ↓ Pro</td><td rowspan="2">• Increased cleavage accuracy in comparison to CNBr due to residue pair recognition
o Esterification of serine and threonine residues by formic acid
o Modification of Asn or Gln to their hydroxamic acid forms by hydroxylamine</td></tr>
<tr><td>Hydroxylamine</td><td>Asn ↓ Gly</td></tr>
<tr><td rowspan="5">Enzyme</td><td>Enterokinase</td><td>AspAspAspAspLys ↓</td><td rowspan="3">o More expensive and more sensitive to pH and chaotropes in comparison to SUMO and TEV proteases</td></tr>
<tr><td>Factor Xa</td><td>IleGluGlyArg ↓</td></tr>
<tr><td>Thrombin</td><td>LeuValProArg ↓ GlySer</td></tr>
<tr><td>SUMO protease</td><td>SUMO ↓</td><td rowspan="2">• Cleaved target peptide maintains native N-terminus, without unwanted amino acid attached because the SUMO protease recognize SUMO’s tertiary structure, rather than linear sequence
• Like the SUMO protease, TEV protease also can generate native N-terminus in many cases
• Urea-resistant property of the enzymes enables cleavage of peptides from the carriers in the presence of a small amount of urea which prevents protein aggregation and loosens compact structures
• Both enzymes can be manufactured in large quantities with relative ease compared to enterokinase, factor Xa and thrombin</td></tr>
<tr><td>TEV protease</td><td>GluAsnLeuTyrPheGln ↓ (Gly/Ser)</td></tr>
<tr><th colspan="4">Purification</th></tr>
<tr><td colspan="4">• Released peptides are usually purified by high performance liquid chromatography (HPLC)
• Liberated peptides can also be more efficiently purified using cation-exchange chromatography due to the high PI values of antimicrobial peptides compared to carrier proteins</td></tr>
</table>

• advantages o disadvantages

References

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3. Malakhov MP, Mattern MR, Malakhova OA, Drinker M, Weeks SD, Butt TR. SUMO fusions and SUMO-specific protease for efficient expression and purification of proteins. J Struct Funct Genomics. 2004;5:75–86.