Biopharming technique yields cost-effective and environmentally friendly antimicrobial peptides

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Plants engineered to produce therapeutic peptides could provide a cost-effective and sustainable platform for manufacturing drugs.

As a proof of concept, researchers have coaxed a close relative of

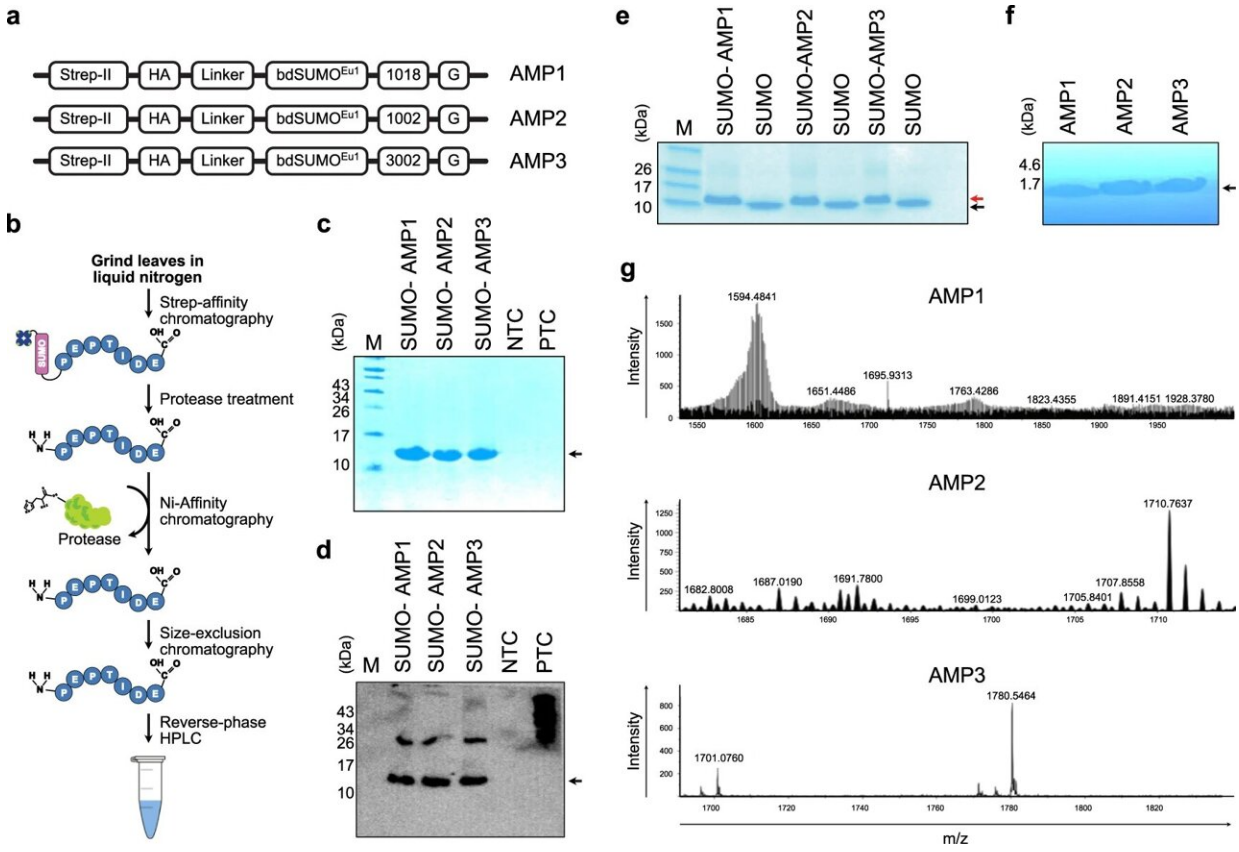
tobacco, Nicotiana benthamiana, to churn out peptides with antibiotic activity against some of the nastiest pathogens known to medicine, as others had done in the past.

But, unlike previous efforts to turn plants into drug-production bioreactors, the scientists also modified their shrubs to express a rat enzyme, called PAM, that enhances the stability and prolongs the activity of antimicrobial peptides.

The resulting plants yielded potent drugs that should cost far less to manufacture than those made via other systems—with the added benefit of offering a more environmentally friendly route to drug assembly.

"These plants can be grown on a massive scale, providing a reliable and cost-effective source of medicines for people around the world," says bioengineering professor Magdy Mahfouz, who led the study.

"We now intend to use this technology to produce a wide range of biologics and therapeutics," adds Shahid Chaudhary, a Ph.D. student in Mahfouz's lab group and the first author of the new report.



Establishment of a SynBio chassis for in planta expression of AMPs. **a** Schematic diagram of the AMP expression cassette for *in planta* expression, using the backbone of the pEAQ-HT vector. Strep-II, high-affinity strep-tag II; HA, human influenza hemagglutinin epitope; linker, flexible GGSGGS linker; bdSUMOEu1, small ubiquitin-related modifier (bdSUMO) from Brachypodium distachyon containing mutations at SUMO-interacting positions; AMP1, AMP2 and AMP3 with a terminal glycine residue. **b**, Flowchart created using Affinity designer (https://affinity.serif.com/en-us/) summarized the plant-based production and purification of biologically active AMPs. The individual plasmids are transformed into Agrobacterium tumefaciens and infiltrated into Nicotiana benthamiana; leaves are harvested at 6 days post infiltration (dpi); total proteins are isolated and applied to Strep-Tactin Superflow resin. Following protease cleavage, His-tagged SENPEuH is removed using Ni-affinity chromatography and isolated AMPs are further purified by size-exclusion chromatography (SEC). Next, the pooled SEC fractions are applied to a reverse-phase high-performance liquid chromatograph (RP-HPLC) for final purification of AMPs. **c** Analysis of the isolated proteins by SDS-PAGE. Strep-II affinity-

captured SUMO-fused AMPs were separated by SDS-PAGE and gels were stained with Coomassie Brilliant blue. **d** Immunoblot confirmation of purified SUMO-fused AMPs. The separated proteins were transferred onto a polyvinylidene difluoride membrane and probed with a monoclonal anti-HA antibody for detection of bdSUMO^{Eu1}-AMPs (~15.5 kDa). Total proteins extracted from non-infiltrated leaves serving as negative control, NTC; HA-tagged protein used as a positive control, PTC. Two independent blots were performed with similar results. Black arrowheads indicate the expected size of protein. **e** Gel shift assay for AMP release. Proteins were separated on a 18% Tricine-SDS gel to detect the release of AMPs peptide (~1.5–1.7 kDa) from the bdSUMOEu1 domain (~14 kDa). Red and black arrowheads indicate the uncleaved and cleaved proteins, respectively. **f** RP-HPLC purification of AMPs. Pooled fractions from SEC were separated on C8 column using acetonitrile gradient. Purified AMPs were separated on a 18% Tricine-SDS gel. Two independent Tricine-SDS-PAGE gels were performed with similar results. **g** Mass analysis of plant-purified AMPs using ESI-MS. The y-axis shows the signal intensity, and the x-axis displays the m/z value of each peptide. Black arrowheads indicate the expected size of peptides. Source data are provided as a Source Data file. Credit: *Nature Communications* (2023). DOI: 10.1038/s41467-023-37003-z

The KAUST research team, which included bioengineers Charlotte Hauser and Samir Hamdan, along with microbiologist Pei-Ying Hong and collaborators from Canada, showed that antimicrobial peptides made in this way could kill several dangerous pathogens, including multiple drug-resistant superbugs responsible for some of the deadliest hospital-acquired infections. The antibiotics also proved harmless to mammalian cells, suggesting that they should be safe for human consumption.

Thinking ahead to eventual deployment of the "biopharming" technique on a massive scale, the researchers showed that their plants were about 3.5-times more efficient at making antibiotics than comparable plants that lack the PAM enzyme modification.

They also added up all the expenses of drug manufacturing and calculated that they could produce 10 milligrams of clinical-grade antimicrobial peptides for less than $0.74 USD—much less than the ~$1000 USD cost of production in commercial companies that chemically synthesize peptides and well below the cost of manufacturing in mammalian cells.

Moreover, plant-based drug manufacturing generates none of the hazardous waste associated with other production platforms, thus making it a much greener option for the pharmaceutical industry.

Mahfouz and his colleagues next plan to make other types of therapeutics in the same way.

"Large-scale industrial production of therapeutic molecules in plants represents a significant step forward in the democratization of medicine," Mahfouz says. "By harnessing the power of molecular biomanufacturing, we can now produce high-quality clinical-grade therapeutics at a fraction of the cost of traditional manufacturing methods."

The study is published in the journal *Nature Communications*.

More information: Shahid Chaudhary et al, Efficient in planta production of amidated antimicrobial peptides that are active against drug-resistant ESKAPE pathogens, *Nature Communications* (2023). DOI: 10.1038/s41467-023-37003-z

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