Table 1 | Selected ingredients derived from fermented microbes

Compound	Developers	Use
Vanillin	Evolva	Flavor; carries the characteristic taste of vanilla
Rebaudioside M or D	Cargill, Amyris	Sweetener, key compounds in stevia
Nootkatone	Evolva, Isobionics, Oxford Biotrans	Pest control, flavor and fragrance; carries the characteristic scent and taste of grapefruit
Cannabinoids	Lavvan, Amyris	Wellness, beauty, pharmaceutical and recreational industries
Squalane	Amyris	Beauty products and vaccine adjuvant
β-Bisabolene	Isobionics	Flavor, fragrance and skin care; carries a flowery, citrus odor

these organisms into little biofactories by modifying their genome to produce key enzymes to manipulate metabolic pathways involved in producing intermediates to nootkatone. The engineered microbes are then grown via a fermentation process using sugar as a feedstock (*Nat. Biotechnol.* **33**, 329–332, 2015).

Evolva accomplished nootkatone production via a semisynthetic process by adding a single gene from thistle to the yeast species *Saccharomyces cerevisiae*. The engineered yeast produces valencene, a chemical intermediate to nootkatone. Valencene is then subjected to chemical oxidation and purification steps, yielding over 99% pure nootkatone with a chemical structure identical to that of the extract, according to Evolva. Because Evolva uses a chemical conversion step and catalysts, the company's nootkatone compound cannot be marketed to US consumers as a 'natural' ingredient.

Even so, the fact that nootkatone is found natively in food may make it more appealing to consumers who are looking for an alternative to DEET and other common, chemically synthesized bug spray ingredients. "It smells nicer and it works just as well, and because it's a food additive you can eat it," says Addesso. "I feel like it's going to have a slightly more favorable adoption for people who are concerned about using DEET, whether they feel it's not safe or they don't like the smell," she says. Nootkatone's mode of action, while not fully understood, may be different from that of DEET and other pest control ingredients. That would make it ideal in geographical regions where insects are developing resistance to common insecticides.

Evolva will market the ingredient under the name "NootkaShield" and will partner with companies large and small to incorporate it into product formulations. Each of these formulations will require a separate EPA registration. "Some of the formulations we'd love to see down the road are things such as a lotion repellent that can be sprayed on clothes or rubbed onto the skin in the morning and it would stay there through the day and repel ticks" and other insects, says Ben Beard, deputy director of the division of vector-borne diseases at the CDC. "Or a soft soap or a shampoo that could actually kill ticks would be very helpful."

Oliver Walker, CEO of Evolva, says that in addition to the use of nootkatone on people, he sees a market for it as a protectant for cattle against bovine ticks. Nootkatone as an insecticide for crops and other agricultural applications "is probably a long shot," he says. "All those chemicals are dirt cheap."

The initial discovery and patents for nootkatone from microbes arose from the work of the CDC. The agency in 2014 licensed the technology to Allylix, which was acquired by Evolva the same year. Two years later, the CDC and Evolva established a research agreement to conduct safety and efficacy trials using nootkatone as a repellent and pesticide against ticks and mosquitoes. In 2017, after the Zika virus outbreak, Evolva received $8.35 million through a contract with the CDC's sister agency, the Biomedical Advanced Research and Development Authority (BARDA), to advance the development of nootkatone for the protection against mosquito-borne diseases.

At least two other companies — Isobionics in Geleen, the Netherlands, and Oxford Biotrans in Milton Park, UK — have been working on the production of nootkatone from the fermentation of microbes. Both companies have thus far targeted the flavor and fragrance industries. State-sponsored Chinese researchers have also genetically modified yeast to produce nootkatone.

"If we can find other plant compounds that are really effective that can be created cheaply this way, it might open the door for new biofriendly, environmentally friendly insecticides and repellents," says Addesso. "The trajectory in the pesticide world has been to make things safer. If you can make things like this that [people] can eat and is also effective, you want to move in that direction." ❐

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Published online: 3 December 2020
https://doi.org/10.1038/s41587-020-00760-z

DARPins stack up as anti-COVID-19 agents

Molecular Partners and Novartis are teaming up to test whether biologic alternatives to antibodies called DARPins can help control COVID-19. DARPins — designed ankyrin repeat proteins — are protein scaffolds a tenth the size of monoclonal antibodies. Like other protein-scaffold biologics, DARPins promise benefits over conventional antibodies. By stringing DARPins together, for example, Molecular Partners can quickly design and screen multispecific candidates. In the case of the COVID-19, the company selected DARPin modules with high target affinity for SARS-CoV-2 and joined them with peptide linkers to form trispecific candidates. "We really own the space of multi-specificity. Mono-, bi-, tri- and tetraspecific compounds — for us it's really all the same," says Molecular Partners CEO Patrick Amstutz. The resulting multi-DARPins can bind more than one epitope at once. In contrast, the over ten monoclonal antibody therapeutics in clinical testing for SARS-CoV-2 target a single site on the spike protein used by the virus to bind and enter host cells. Because mutations at this site might lead to viral escape, some antibody developers have turned to antibody cocktails to get around this possible limitation. These combinations, however, bring optimization, manufacturing and regulatory challenges of their own. Instead, Molecular Partners' lead multi-DARPins for treating COVID-19 bind different epitopes on the spike protein. The MP0420 molecule binds three epitopes on the spike's receptor-binding domain (RBD); MP0423 targets one epitope on the spike's RBD, one on its S1 N-terminal domain and another on its S2 domain.

A phase 1 trial of MP0420 started in November. Novartis paid $22 million up front, acquired $44 million in equity and committed up to $165 million in future milestone payments to collaborate on the development of these biologics for COVID-19. Earlier in the year, the US Food and Drug Administration rejected Molecular Partners, AbbVie and Allergan's VEGF-targeting DARPin abicipar pegol, for wet age-related macular degeneration, citing concerns with intraocular inflammation.

Published online: 3 December 2020
https://doi.org/10.1038/s41587-020-00771-w