Engineers of scent

Companies exploring biotech approaches to flavor and fragrance production must navigate challenges in regulations, market dynamics and public perception. Emily Waltz investigates.

The first flavor and fragrance ingredients made with metabolically engineered microorganisms are reaching consumers, and potentially many more such ingredients will follow. Commercial production of biotech-derived vanillin, a compound carrying the characteristic flavor of vanilla, has commenced through a partnership between Reinach, Switzerland–based Evolva and New York-based International Flavors and Fragrances (IFF). Evolva also announced last December that it was acquiring one of its main competitors in the space, San Diego-based Allylix, which brought one of the first biotech-derived ingredients—valencene—to the market. And on the lighter side of biotech, Leavendary, a yeast propagation company in Huntsville, Alabama, modified one of its strains to create green beer as a demonstration project for St. Patrick's Day.

Ingredients made from biotech microbes may find a home among food, beverage and fragrance producers, but adoption will be slow and targeted at niche markets due to the conservative nature of the food and fragrance business, say industry insiders. Along the way, biotech companies in this business should be prepared for a wary eye from investors, and a fight from consumer and environmental watchdogs. "I think we're in a waiting period," says Jay Keasling, a professor of chemical engineering at the University of California, Berkeley, and co-founder of microbial engineering firm Amyris of Emeryville, California. "We're waiting to make sure the companies don't go bust." If some of these early efforts succeed "then I think it could take off," he says.

D. Hurst/Alamy

No more green dye #3. The biotech industry has a solution for providing green beer, a St. Patrick's Day tradition.

Flavors of biotech

Some of the world's most popular flavors and aromas are derived from botanical sources or synthetic processes that are less than ideal. Plant-derived ingredients are subject to weather disruptions, disease and crop competition, and are often produced in regions of geopolitical turmoil. Synthetic routes can involve environmentally unfriendly chemicals and manufacturing processes. These drawbacks have resulted in volatile pricing and availability of many key ingredients, as well as sustainability concerns.

Some coveted aromas and flavors come from rare or endangered plants and have proved impossible to reproduce chemically. Oudh, an oil prized in the Middle East and East Asia for its near-mythical aroma, can only come from a resin produced in the heartwood of a couple of species of fungus-infected agarwood trees. Few wild agarwood trees still exist owing to deforestation, and the tiny amounts of oil produced by farmed agarwood and processed to resemble the wild version just aren't as good, says Trygve Harris, an essential oils consultant and founder of Enfleurage in New York City. Real oudh is off the charts," she says. "But in 2014 there is very, very little now. I don't know a single person who sells it. We don't sell it at Enfleurage either. The same goes for Indian sandalwood. Just gone.

The demand for such prized substances has not gone unnoticed by those in the biotech industry. "There are hundreds or thousands of ingredients that are out in nature that we don't currently get to use because the plant or the animal that makes them isn't farmable," says Neil Goldsmith, co-founder and CEO at Evolva, which has agarwood and sandalwood oils on its long wish list of products it would like to produce. "So for me the challenge is going to be how do we learn to make a large number of these things available to people? How do we go from making one or two products to making hundreds of products?"

Biotech offers an alternative to botanical and synthetic routes. Using fermentation methods, microbes such as yeast and *Escherichia coli* become biofactories that can convert sugar into molecules of interest. Researchers have used both transgenes and synthetic genes to manipulate metabolic pathways in these microbes. Companies are using these techniques to develop not only flavor and fragrance ingredients, but also sweeteners, oils and pharmaceuticals (**Table 1**).

Theoretically, with the right enzyme combination, microbes could be engineered to make any molecule, and this tantalizing possibility has led to a fair amount of enthusiasm. But the scale at which such products can be produced is no minor issue, say researchers. "Hypothetically it's true, you can make anything," says Toni Kutchan, vice president for research at Donald Danforth Plant Science Center in St. Louis. "But the question is, can you make anything on a commercially feasible scale?"

Eight years ago, investors were jumping at the chance to fund companies such as Amyris and S. San Francisco, California–based Solazyme, which were using engineered microbes to produce fuels. But when such companies couldn't demonstrate the titers, rates and yields needed for commercial-scale production, the technology lost its attractiveness on Wall Street, says Keasling. "It's been a really dry period—a really rough period," he says.

Solazyme and Amyris have since expanded their focus from high-volume, low-margin chemicals like biofuels to small-volume, high-margin chemicals, as well as products for the flavor, fragrance and pharmaceuticals industries. In August, large-scale commercial batches of Amyris' first pharmaceutical produced with a biotech microbe—an antimalarial called artemisinin—was delivered for the first time to countries in Africa where malaria is endemic. The company is also working with some of the world's largest flavor and fragrance companies, including IFF, Firmenich of Meyrin, Switzerland, and Givaudan of Vernier, Switzerland. Codexis, an enzyme maker, has similarly shifted its focus from enzymes for cellulosic ethanol to enzymes for pharmaceutical and chemical manufacturing applications, and is also working in the food ingredient market. The company announced in November it had entered into its second partnership with a food industry player.

Enthusiasm from the investment community is likely to remain tempered for a while, says Keasling. "Until we see some companies be

Table 1 Selected companies develop engineering biotech flavors and fragrences

Company	Product areas	Partners
Allylix	Valencene	Acquired by Evolva in December 2014
Amyris	Artemisinin, undisclosed flavor and fragrance ingredients	Firmenich, IFF, Givaudan
Evolva	Vanillin, resveratrol, stevia	IFF, Cargill (Minneapolis)
Ginkgo BioWorks Boston	Rose	Robertet
Isobionics Geleen, The Netherlands	Valencene	DSM (Heerlen, The Netherlands)
Synthetic Genomics La Jolla, California	Omega-3 docosahexanoic acid (DHA) for animal feed	ADM (Chicago)

profitable doing this I don't think there's going to be a lot of enthusiasm from the venture capital community," he says.

A key factor in the success of early biotech flavors and fragrances on the market is whether they can be labeled as "natural" on the consumer products that incorporate them. Consumers' preference for natural products is clearly the trend, say industry insiders, and food and beverage companies are willing to pay a premium for ingredients that will allow them to market their products with that word. "Consumers are moving more toward desiring significantly more transparency in what's in food, and they're asking a lot more questions," says John Hallagan, general counsel for the Flavor and Extract Manufacturers Association (FEMA). "My advice [to biotech companies] would be to very carefully look at the labeling implications of your material because that can have a significant effect on whether it's successful from a business perspective."

Natural-born ingredients

In regulations established by both the US and EU, "fermentation" is included in definitions of ingredients that can be described as natural. However, the US code was written decades ago, before engineered microbes were a consideration. Judges in three different US District Courts in 2013 asked the US Food and Drug Administration (FDA) to clarify its position regarding natural labels on foods made with genetic engineering. The FDA in January 2014 declined to make a determination, citing higher priorities requiring the agency's time. "Because, especially in the foods arena, FDA operates in a world of limited resources, we necessarily must prioritize which issues to address," wrote Leslie Kux, assistant commissioner for policy in a letter to the judges.

Many of the companies developing ingredients from engineered microbes say their products meet the definitions of natural in the US and the EU, and they intend to market them that way. That doesn't sit well with everyone. Michael Hansen, a senior scientist at the Consumers Union in Yonkers, New York, says that although biotech companies' products may legally meet the definition of natural, such ingredients are not what consumers think of as natural. "If you ask members of the public, people think 'natural' is the functional equivalent of 'organic'," he says. In a survey conducted in April 2014 by the Consumer Reports National Research Center, about two-thirds of consumers thought that natural processed foods were made without genetically modified organisms, pesticides or chemicals (even though all three can be and often are involved in manufacturing food labeled "natural" in the US).

Rather than trying to re-educate the public on the various regulatory definitions of natural, Consumers Union petitioned the FDA to ban the label "natural" on all food. "We believe that the use of the natural label on any food currently misleads consumers," the organization wrote in its June 2014 petition. Flavors and fragrances produced with biotech organisms will be included in Consumers Union's quest. "If companies want to produce vanillin or stevia with synthetic biology techniques and call it natural, we will point out to consumers how misleading those claims are," says Hansen.

Indeed, plaintiffs have claimed in a number of lawsuits that food and beverage producers' use of the word natural is misleading. Cargill in 2013 agreed to pay $6.1 million to settle some proposed class action lawsuits against the marketing of its Truvia products, which are marketed as natural sweeteners, even though the labeling "meets all applicable legal and regulatory guidelines" according to the company. Truvia products contain erythritol, a food additive produced from a simple sugar using fermentation of nongenetically modified yeast, and rebaudioside A, which is extracted and purified from the stevia plant.

Sensing the opportunity

How deeply food, beverage and fragrance companies are willing to dive into biotech ingredients will likely be revealed one product at a time. Every flavor niche is different from the next, and each has its own set of market influences, says Kent Swan, vice president of purchasing at flavor and fragrance producer Robertet, headquartered in Grasse, France, who spoke with *Nature Biotechnology* as an industry consultant and not as a representative of his employer. "[Biotech] is something that has been gaining the attention of flavor companies for many years," says Swan, "but there's no one underlying cause for the emergence of the use of the technology in my industry. It depends on the item itself."

Swan says that the markets for each biotech ingredient will be necessarily small—par for the course in the flavor industry. "There are very few truly global approaches to developing flavors," says Swan. Each ingredient has to meet the taste specifications of the food or beverage in which it will be incorporated, has to meet regulations in the country where it is sold and must cater to consumer preferences, which are often regional. "Taste is not necessarily universal," he says.

The role of biotech in the fragrance industry may look a little different from its role in flavor, says Tom Plocek, a managing director at Aroma Chemical Services International in Stahle, Germany. Biotech has many applications, but "the opportunities that make good economic sense are few in number in part because the R&D is so expensive," he says. Biotech products that are successful, however, "have the potential for very large financial returns, and are likely to have a major impact on the fragrance industry."

Swan says his advice to biotech companies is to first get to know the market for the particular ingredient of interest, and to bring in an expert. "The problem is that none of this stuff is in a book," says Swan. "I've seen companies go in a route that from a market perspective is ill advised."

Yeast á l'orange and vanillin bugs

Allylix (now folded into Evolva) may have identified one market in need of a biotech product. The company in 2010 commercialized valencene, a citrus flavor and fragrance ingredient that comes from the peel of the Valencia orange and is used in beverages and chewing gum. Valencene is also a chemical precursor to nootkatone, a grapefruit flavor ingredient.

Valencene has proved tricky to make chemically, so valencene buyers have historically depended on orange extracts, the supply and pricing of which has been disrupted by weather events such as Hurricane Andrew and diseases such as citrus greening. "The orange juice business in general is not healthy," says Swan. Demand for orange juice in the US is huge, but per capita consumption is declining and people

in developing countries are less inclined to pay the high price, he says. "Without a growing orange juice business, they can't possibly hope to meet the need for by-products coming out of it, and valencene is one of those," says Swan.

That's where biotech comes in. "Fermentation is able to provide a stable supply" with stable pricing, says Carolyn Fritz, who was CEO of Allylix until its acquisition. Fritz would not disclose pricing of the company's valencene, but Swan says that it is comparable to its counterpart from the orange. "It's not necessarily a bonanza from a cost-savings perspective," he says. "Possibly over time and as the markets expand they could get there."

Fritz says that to get valencene, her company added a single gene from oranges to a strain of baker's yeast. The molecule that is produced is chemically indistinguishable from the valencene from oranges.

Another relatively simple pathway is that of Evolva's vanillin. The company's product is made using *Saccharomyces cerevisiae* that Evolva's scientists have modified with both synthetic genes and transgenes along the phenylpropanoid pathway. "Vanillin is four main steps off of what yeast does anyway," says Goldsmith at Evolva, who did not disclose additional modification details (*Appl. Environ. Microbiol.* **75**, 2765–2774, 2009).

"Vanillin was a great target," adds Kutchan at Danforth. With the vanillin pathway, "you can kind of piece it together with an enzyme from this organism and one from another and get it to do the few simple transformations you need to make vanillin. There's a huge difference between a small molecule like vanillin and something like morphine or artemisinin where it's not a priori obvious how to get there." She adds, "Evolva is onto something there."

It took Evolva $20 million to move vanillin from start to market, says Goldsmith. The company commercialized it by partnering with IFF, which incorporated it into flavors for its consumer products customers. IFF told *Nature Biotechnology* in March that products containing Evolva's vanillin had reached the market.

Vanillin is the key flavor component in vanilla extracted from the cured pod of the vanilla orchid. Vanillin makes up only about 2% of the vanilla bean by weight, and is among hundreds of flavor components that together give vanilla extract a rich and complex flavor that has not been duplicated technologically, and is sought after by top chefs globally.

Cultivation and curing of vanilla pods is extremely labor intensive, forcing the price of vanilla well above what most of the world can afford. But vanillin, which is 4-hydroxy-3-methoxybenzaldehyde can be made synthetically and through bioconversion processes, and its production has enabled a huge portion of the population to experience the vanilla flavor, albeit an inferior version. About 95% of the 16,000 ton of vanillin produced annually worldwide is made synthetically, largely from guaiacol, a petrochemical, or lignin, a by-product of the paper industry. There are environmental costs, however, including the use of hazardous chemicals in some methods of conversion of guaiacol, and the removal of waste in the lignin route (*Mol. Plant* **8**, 40–57, 2015).

Most of the remaining 5% of vanillin production worldwide is produced through bioconversion of substances such as clove, turmeric and ferulic acid—a market that has emerged in the past ten years. Brussels-based Solvay Group, for example, uses nongenetically modified yeast in a fermentation process that converts ferulic acid found in rice bran to vanillin. Vanillin can be isolated from vanilla, but as vanilla is far more valuable with all of its components intact, only a trivial amount of the world's vanillin production is obtained this way.

All vanillin, regardless of the source, effectively tastes the same. "If I had a series of vanillin samples on the table in front of me, our experts here may be able to pick out the difference—neat—one up against each other," says Kip Gibson, vice president of global category marketing at IFF. "But it would be very, very tiny and by the time [vanillin] is incorporated…at a low percentage into a consumer product, I would find it hard to believe that a consumer would perceive any difference at all." (Vanilla from the bean, with all of its flavor components intact, tastes far different than vanillin, however.)

Despite the lack of difference in taste, the prices of vanillin vary greatly, from $15 for a kilogram of vanillin from guaiacol and lignin, to $800 per kilogram for vanillin from ferulic acid (and about $1,000 for a kilogram of vanillin from vanilla). The reason a food company might pay 50 times more for the same ingredient can be attributed almost exclusively to the legal right to use the word natural on food labels in their target country.

Synthetic vanillin from guaiacol and lignin cannot be described as "natural" on food labels in most regions of the world. But vanillin derived through fermentation of ferulic acid, clove and turmeric can sometimes be described as a natural flavor or some derivation of that, but not as natural vanillin, and not necessarily in the same countries.

Vanillin from clove, for example, is considered a natural flavor in the US but not in the EU. Vanillin from turmeric is seen as natural in parts of Asia Pacific, but not in the EU. Vanillin from ferulic acid can typically be called a natural flavor in both the EU and the US. Making things more complicated, in the US, vanilla flavorings, including vanilla extract, have a special designation known as a federal standard of identity, and the rules for labeling vanilla differ from the rules for labeling other flavors.

The hodgepodge of labeling rules has created gaps in the market that Evolva and IFF say they can fill. The companies say their vanillin meets the definitions of natural in the US, EU and a number of other countries around the world. IFF says it will, at first, price Evolva's vanillin to be competitive with vanillin from ferulic acid, which sells for about $700–800 per kilogram. The goal is to reduce manufacturing costs over time to compete with vanillin from clove, which trades for about $70–100 per kilogram.

"I think vanillin produced via biotechnology will find a home but it will depend on how it's priced and in what country [it is marketed]," says Swan. "Some countries based upon [their labeling] regulations will be very receptive to it, and some will not see the need for it."

Smells like fear

Vanillin has become somewhat of a target for activists who object to biotech-derived ingredients proceeding to the market. Full assessments of potential ecological and societal impacts of such products have not been conducted, and groups concerned about synthetic biology, such as Friends of the Earth (FOE), a global network of more than two million activists, and the Ottawa-based ETC Group, are making an example out of vanillin.

There are few data, for example, on the ecological effects of synthetic or partially synthetic organisms, should they escape into the environment. Indeed, in June 2014 an interdisciplinary group of scientists proposed a research agenda for synthetic biology in an effort to prioritize which questions about the ecological implications of synthetic organisms need to be addressed. The "Synthetic Biology Project" is headed up by the Washington, DC–based Woodrow Wilson International Center for Scholars and the Massachusetts Institute of Technology's program on emerging technologies.

Similarly, there are few data on whether biotech ingredients would increase the demand for sugar or other feedstocks, or the socioenvironmental effects of those demands. "Right now everyone is making assumptions about the feedstocks and volumes of production," says Todd Kuiken, a senior program associate at the Wilson Center. "But there are just not enough data to make generalizations about impact yet."

The fact that ingredients produced using genetically engineered microbes fall outside

Box 1 Self-regulation of the flavor industry

Many of the foods we eat contain ingredients that are "generally recognized as safe" or GRAS. Salt, caffeine and some sweeteners are examples of the thousands of ingredients considered GRAS, allowing manufacturers to put these ingredients in food without making a submission to FDA. A federal law, called the Food Additives Amendment of 1958, allows the manufacturers of food ingredients to determine by themselves whether the ingredients they are producing are GRAS.

The law has come under scrutiny in recent years as there have been increasing demands for transparency on the safety of food ingredients. Public sector researchers and the US Government Accountability Office have recommended that the FDA should minimize the potential for conflicts of interest in companies' GRAS determinations. Under current guidelines, notifying the FDA of a GRAS determination is not required, although many companies voluntarily do it, and some use FDA's green light as a marketing tool. When the agency agrees with a determination, it responds in writing with a "No Questions" letter.

The FDA has rarely disagreed with a company's GRAS determination. There are few, if any, instances of GRAS food ingredients causing a public health hazard, says John Hallagan of FEMA. FEMA has authority, by the same 1958 legislation, to make GRAS determinations of a subset of food ingredients—those that are intended for use as flavorings. Many of the new biotech-derived substances fall within FEMA's purview. Like the FDA process, notifying FEMA is also voluntary, but most companies do it because of the value of the FEMA GRAS designation, says Hallagan. He notes that the safety information FEMA gathers on each GRAS flavoring substance is submitted to the FDA.

Having an industry organization police its members has raised some eyebrows over the years. FEMA lists on its website the measures it has taken to address conflicts of interest in its review process. For example, its standing expert panel cannot be employed by or have consulting relationships with FEMA member companies, and panel members are not told the identity of the company responsible for the GRAS application.

the US Department of Agriculture's authority and don't require environmental review by any federal agency concerns some members of the public. Both vanillin and valencene are substances that have been used as flavors for many decades, and in the US their use as flavors is considered "generally recognized as safe," or GRAS. FEMA (**Box 1**), which oversees GRAS determinations, will review new manufacturing processes for a flavoring if they differ significantly from what has been done in the past, says Hallagan at FEMA. "For a manufacturing process like metabolic engineering, that is being used to produce substances that are already GRAS for flavor use, if there's a significant change in manufacturing, the sponsor comes back for a review," he says. Indeed, Evolva and IFF's vanillin underwent that additional manufacturing review, which involves looking for the presence of contaminants introduced by the new process.

FOE and ETC have pounced on some of the unknowns about metabolically engineered microbes, and added concerns of their own. The groups have publicly campaigned on the claim that Evolva's vanillin "could devastate livelihoods of approximately 200,000 people who are involved with the production of cured vanilla beans per year," according to a FOE fact sheet. But that argument doesn't appear to hold water. Vanillin is a different product than vanilla. "There isn't a connection with vanilla bean farmers," says Kuiken. "This is where I have an issue with their fact sheet. We need to be honest about what we're talking about. Vanillin and vanilla beans are different things."

It is true that vanillin can be extracted from vanilla and is sometimes used to fortify the flavor of vanilla bean, but this is an almost nonexistent market because the bean is far more valuable when left intact. "Vanillin produced from vanilla is an inconsequential category," says Swan. Adds Gibson at IFF: "Why burn up all the beans to make vanillin since you've got alternatives that are already on the market?"

FOE posted a press release online, which was picked up by a couple of major news outlets, claiming that ice cream companies such as Haagen-Dazs "reject extreme genetically engineered vanilla." But that, too, stretches the truth. Hannah Coan, a spokesperson for Nestle, which produces Haagen-Dazs in the US and Canada, says that the company sources its vanilla flavor from vanilla beans produced in Madagascar and doesn't plan to change its source. "Consumers like it so there is no reason to change or consider other alternatives," she says. "[FOE] asked if we would use synthetic vanilla for our Haagen-Dazs brand and we told them we will not. We made no statement overall about synthetic biology—just about one ingredient in one brand. They have chosen to turn this into a press release."

FOE has also been warning the public in its fact sheet that synthetic organisms like Evolva's vanillin "threaten biological diversity if they escape into the environment" and "could become a new class of invasive species or pollutant and disrupt ecosystems." To support these statements, FOE cites Allison Snow, an ecologist at The Ohio State University in Columbus, in a presentation she gave at the July 2010 meeting of the Presidential Commission for the Study of Bioethical Issues in Washington, DC. But Snow says FOE's words are misleading. "I explicitly stated that 'we shouldn't assume a priori that all synthetic organisms will be safe or that all will be dangerous'," she says.

Dana Perls, a lead on food and technology topics at FOE, says she is less concerned about vanillin, and more concerned that it is a "slippery slope" to other, more disruptive products. "This is not just about vanilla," says Perls. "[Evolva's vanillin] sets a dangerous precedent for seeing synthetic biology as natural and for not looking at the full life-cycle analysis."

Evolva has chosen to engage directly with the public and with the activists on health and ecological matters, despite being several steps removed from the consumer. In October the company posted online a consumer-friendly video on fermentation and microbial engineering. "If we're asking people to eat something then we're asking them to trust us so we'd better explain what we're doing," says Goldsmith. "That may not be something the food industry has been famous for in the past." He adds, "I welcome the activist debate. If we can't show people that what we are doing is good to eat and good for the environment and affordable then we don't deserve to be selling a product to them."

But others are shying away from the mess. The Vlaams Instituut voor Biotechnologie, or VIB, in Flanders, Belgium, told *The New York Times* last year that it had sequenced and modified hundreds of beer brewing yeasts, only to leave them sitting in their laboratory's freezer. In that article, Jan Steensels, a microbiologist with the Belgian laboratory, said: "…most brewers and consumers don't want anything to do with them."

Emily Waltz, Nashville, Tennessee