BIOFUELS

Engineered Superbugs Boost Hopes Of Turning Seaweed Into Fuel

In the search for renewable fuels, there's no perfect solution. Biofuels can be readily made from corn starch and sugar cane, but they take land and resources away from food crops. Feedstocks such as switchgrass and wood sidestep that problem—but they are hamstrung by a molecule called lignin, which makes it harder to extract the sugars that ferment into ethanol.

Enter seaweed: It has no lignin and requires no land, fresh water, or fertilizer. Several countries have pilot programs for generating biofuels from seaweed. But there's a hitch: About a third of the sugars in seaweed take the form of alginate, a complex polymer that industrial microbes can't convert into ethanol.

On page 308, researchers led by Yasuo Yoshikuni of Bio Architecture Lab (BAL), a 4-year-old biotech company in Berkeley, California, describe a strain of *Escherichia coli* that they have genetically engineered to break down and ferment alginate and all the other major sugars in seaweed into ethanol. "This is very impressive work," says James Liao, a metabolic engineer at the University of California (UC), Los Angeles. But experts wonder whether enough seaweed can be harvested to make it a significant contributor to petroleum independence or even a lower-carbon economy. "Is this a game-changer? Probably not," says molecular biologist Stephen Mayfield of UC San Diego. "But it's a step in the right direction."

Yoshikuni's team started by giving *E. coli* a gene that enabled it to break down alginate, a polymer of uronic acids, into short fragments called oligomers. The gene—for an enzyme called alginate lyase (Aly)—came from the marine bacterium *Pseudoalteromonas sp.* To beef up the modified bug's alginate-busting ability, the researchers tacked on a so-called autotransporter molecule that forced the cells to secrete all the Aly they made.

Next, the researchers had to modify *E. coli* so that it could take up the alginate oligomers floating nearby. The plan was to install a cellular transport system for the oligomers, but first they had to find one. "It wasn't an easy process," Yoshikuni says. Their clue was pectin, another kind of polymer abundant in fruit peels and other plant materials. Because the genes behind the pectin transport system are known, the team could search for similar genes in a genomics database. Bingo; they found that the bacterium *Vibrio splendidus* 12B01 carries a stretch of DNA similar to that for pectin transport and bearing genes for alginate-degrading enzymes.

But the researchers weren't sure which genes in this stretch they needed because many of their exact functions were a mystery. Turning to natural selection for help, they created thousands of random snippets of *V. splendidus* 12B01's genome and tucked them individually into *E. coli* cells. Then they dropped cells into a thin alginate-oligomer broth to see which ones could use it as food. One 13-gene snippet allowed the *E. coli* to thrive.

The team then deleted individual genes to figure out their functions. Additionally, the team sought and introduced other *V. splendidus* genes that might function in alginate metabolism. The resulting *E. coli* strain was able to take up all the available alginate oligomers and further break them down into even simpler components. Thanks to the added DNA, the *E. coli* could also convert them into molecules, such as pyruvate, used to make chemical building blocks. Finally, the researchers added a pathway—borrowed from *Zymomonas mobilis*, a bacterium originally isolated from fermented cane juice—that turns the pyruvate into ethanol.

When the fully engineered *E. coli* was fed a slurry of the common brown seaweed called kombu (*Saccharina japonica*), the cells fermented the brew up to a concentration of 5% ethanol—comparable to the benchmark for yeast-based fermentation of woody biomass. It's also more than twice as good as existing fermentation methods for seaweed and can produce more than 80% of the theoretical maximum yield of ethanol from sugars in seaweed. "This is a big step," says Richard Sayre of Los Alamos National Laboratory in New Mexico. As with other microbial systems, BAL's *E. coli* could be modified to create other fuels and valuable chemicals.

A big question, especially for the United States, is where to get enough seaweed. "It's cultivation at the scale that we need—that is a major hurdle," says Guri Roesijadi of Pacific Northwest National Laboratory (PNNL) in Sequim, Washington. In a 2010 report on seaweed for the Department of Energy, he and other PNNL researchers calculated that replacing 1% of the U.S. gasoline supply with ethanol would require growing seaweed over nearly 11,000 square kilometers. Several other countries grow seaweed for food, animal feed, fertilizer, or polymers, but there is almost no such farming in U.S. waters.

Seaweed supply directly affects two other key factors: cost and sustainability. If refiners have to haul seaweed long distances, that will raise the cost of seaweed-based ethanol and give it a larger carbon footprint, which in turn can reduce tax breaks and other subsidies from the federal government. "This technology will be competitive only in locations where very large amounts of seaweed are readily available, such as coastal areas," predicts Michael Henson, a chemical engineer at the University of Massachusetts, Amherst. BAL's CEO, Daniel Trunfio, says the company has contracted a life-cycle analysis to show exactly how competitive and "green" its process is; results should be available later this year.

–ERIK STOKSTAD

Fuel line? Refiners will need a cheap supply of seaweed to make it a feasible biofuel.

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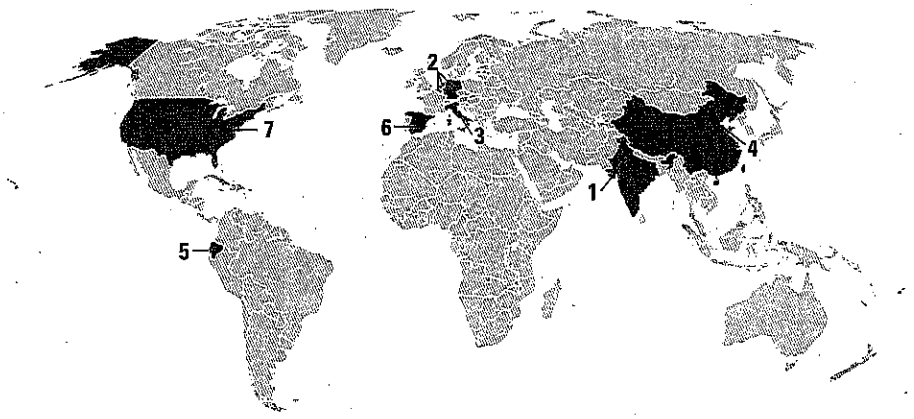
AROUND THE WORLD



India **1**

Country Marks Year Without Polio

The country celebrates a hard-won victory in its fight against polio: As of 13 January, India appears to have gone 1 year without a single case of the disease.

Since the Global Polio Eradication Initiative (GPEI) began in 1988, polio cases worldwide have dropped by more than 99%. The disease has remained in a few strongholds, including India, Afghanistan, Pakistan, and Nigeria, where wild poliovirus transmission has never been interrupted. And for years, India reported more cases than any other country. But cases there dropped from 741 in 2009 to zero in 2011, thanks to the redoubled efforts of India, the GPEI partners, and a recent infusion of support from the Bill and Melinda Gates Foundation.

India's achievement is a major boost to the beleaguered GPEI, which has spent more than $8 billion over the past 23 years trying to rid the world of the disease. Still, success is not assured. More than 600 cases of polio were recorded worldwide last year, with an alarming rise in neighboring Pakistan, which poses a huge threat to India's fragile success.
http://scim.ag/IndiaPolio

Germany, the Netherlands, and Belgium **2**

New Virus Spreads to Dozens Of Farms in Three Countries

The Schmallenberg virus, a newly discovered pathogen infecting cattle, sheep, and goats, has now been found at 86 farms across Germany, the Netherlands, and Belgium, animal health officials have reported—and the number is rapidly climbing. The virus, named after the German town from which the first positive tissue samples came last November (*Science*, 2 December 2011, p. 1186), causes drastically reduced milk production as well as fetal malformations and stillbirths.

Infections likely began during the summer and autumn of last year, but fetuses exposed to the virus in the womb are only now being born, meaning that cases seen so far could just be the first wave. "This is a serious threat to animal health in Europe,"

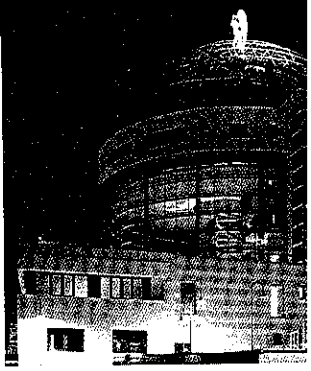
says Wim van der Poel of the Dutch Central Veterinary Institute in Lelystad.

A team at the Friedrich Loeffler Institute, a German federal animal health lab, has isolated the virus and has sequenced its genome. Experts say that it's unlikely that Schmallenberg can cause human disease, but urge monitoring of those who come into contact with infected animals.
http://scim.ag/Schmallenberg

Milan, Italy **3**

Health Mogul Nabs Bid For Research Center

A long-running Italian drama takes another twist: The imperiled hospital and research center run by the San Raffaele del Monte Tabor Foundation may have found a buyer. But it's not the Vatican Bank as many expected. On 10 January, health industry entrepreneur Giuseppe Rotelli won the bidding for the Milan center, which specializes



in gene therapy and molecular
Last year, San Raffaele had
that it was facing a €1.5 billion
fall that media reports suggeste
July suicide of center vice pres
Cal. In October 2011, a bankru
ruling paved the way for the Va
assume leadership. But in a sur
Rotelli outbid the Vatican City-
institute, promising to take on
€500 million of debt and to in
€405 million.
Although the agreement isn
San Raffaele scientists have ex
doubts about Rotelli's support
research. In a statement, the er
addressed those worries, sayin
priority "is the protection and
opment of the professional ski
involved." **http://scim.ag/GRot**

Beijing **4**

Ministry Moves Against Unauthorized Stem Cell

China's Ministry of Health ha
that it will crack down on unp
treatments. Representatives fr
try and the State Food and Dr
tion will inspect approved ste
and hospitals while rooting ou
ones—desperate mainlanders
tourists have fueled to a boom
ics. A hold will also be put on
als of stem cell clinics until 1
spokesman Deng Haihua said

NOTED

>The German chemical co
will move the headquarter
science division from Limbu
many, to Raleigh, North C
cials announced 16 Janua
also shut down two more r
ties in Europe. European c
farmers are leery of geneti
crops, officials said, so the
no longer develop crops for

AGRICULTURE

What Next for Agriculture After Durban?

Despite obstacles in the UN climate talks, modest progress and opportunities for scientific input on agriculture arose.

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Global agriculture must produce more food to feed a growing population. Yet scientific assessments point to climate change as a growing threat to agricultural yields and food security (*1*–*4*). Recent droughts and floods in the Horn of Africa, Russia, Pakistan, and Australia affected food production and prices. The Intergovernmental Panel on Climate Change predicts that the frequency of such extreme weather events will increase (*5*), which, when combined with poverty, weak governance, conflict, and poor market access, can result in hunger and famine. At the same time, agriculture exacerbates climate change when greenhouse gases (GHGs) are released by land clearing, inappropriate fertilizer use, and other practices (*6*).

Alternative agricultural practices, tailored to different regions, show promise for reducing net GHG emissions and maintaining or improving yields despite extreme weather (*7*). In Niger, agroforestry on 5 million hectares has benefited >1.25 million households, sequestered carbon, and produced an extra 500,000 metric tons of grain per year (*8*). In Denmark, agricultural emissions have been reduced by 28%, while productivity increased (*9*).

Agriculture, the FCCC, and Durban

Despite growing support for an integrated approach to agricultural adaptation to, and mitigation of, climate change, financial and policy actions have been slow to materialize in most countries and at the global level, including the United Nations Framework Convention on Climate Change (FCCC). At the 15th FCCC Conference of the Parties (COP-15) in Copenhagen, negotiators developed text on agriculture, but no agreement was reached. In the lead-up to COP-17 in Durban in late 2011, political momentum grew for a work program on agricultural adaptation and mitigation within the FCCC's Subsidiary Body for Scientific and Technological Advice (SBSTA). This included a common position by African Ministers (*10*), the scientific Wageningen Statement (*11*), a joint letter from the United Nations and other agencies (*12*), and public statements by South African President Jacob Zuma and former UN Secretary-General Kofi Annan.

COP-17 produced the "Durban Platform for Enhanced Action" (*13*), which commits parties to reach a legal framework for reducing global emissions by 2015. The only specific agreement on agriculture was to consider adopting a framework for sectoral actions, which could include agriculture, and for the SBSTA to "exchange views on agriculture," with a 5 March deadline for parties and observers to provide evidence (*13*). This modest progress, without adoption of a formal work program on agriculture, can be attributed to the following issues:

•Views on inclusion of agriculture depend on the degree to which agriculture features in national economies. Countries vary in their vulnerability to climate change, their GHG emissions from agriculture, and their opportunities to reduce emissions from changes in agricultural practice. Forested nations that may benefit from Reducing Emissions from Deforestation and Forest Degradation (REDD+) policies may see the inclusion of agriculture as delaying or competing for climate finance.

•Actions agreed in Durban were in the mitigation track of FCCC negotiations, which are separate from adaptation discussions. This obscures opportunities for agriculture, which can deliver benefits for both, and has led to concern that the focus on agricultural adaptation—a priority for developing countries—will be reduced. Others worry that inclusion of agriculture under the mitigation track could lead to mandatory commitments and/or that possible mechanisms (e.g., carbon trading) will not benefit smallholder farmers. Some countries do not welcome potential restrictions on conversion of land to agricultural use. Export-focused agricultural producers worry that mitigation measures for agriculture could restrict trade from "high-emission agriculture."

•Some negotiators are concerned that technical challenges (e.g., carbon monitoring by millions of farmers and pastoralists) are too great to develop agriculture agreements.

In general, higher-income countries, farmers' organizations, UN and agricultural agencies, and some nongovernmental organizations (NGOs) supported a SBSTA work program on agricultural adaptation and mitigation. Other nations, primarily low and middle income, supported by a different set of NGOs, resisted a work program and called for emphasis on agricultural adaptation to climate change.

What Now on Agriculture?

The Commission on Sustainable Agriculture and Climate Change was set up in early 2011 to synthesize evidence into policy actions to help achieve a food-secure world in the face of climate change. The Commission encouraged policy action inside the FCCC, as well as through other global processes (e.g., the UN Conference on Sustainable Development and the G-20) and bilateral, national, public-private, and "bottom-up" initiatives (*14*). Seven priority actions were identified:

1. Integrate food security and sustainable agriculture into global and national policies, including adaptation and mitigation;

2. Increase global investment in sustainable agriculture and food systems;

3. Sustainably intensify agricultural production while reducing emissions and other environmental impacts;

4. Target programs and policies to assist vulnerable populations;

5. Reshape food access and consumption to ensure that basic nutritional needs are met and to foster healthy and sustainable eating habits;

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