Bacterial Chatter

How patterns reveal clues about bacteria's chemical communication

By RICHARD LIPKIN

Among nature's intriguing phenomena, the appearance of similar structures in different arenas of life offers endless fascination.

That treelike branches sprout in human lungs, that dendritelike tendrils spread through cooling crystals, that fractal patterns turn up in bones and shells no longer occasion surprise. Such similarities appear over and over again in the material world and animal kingdom.

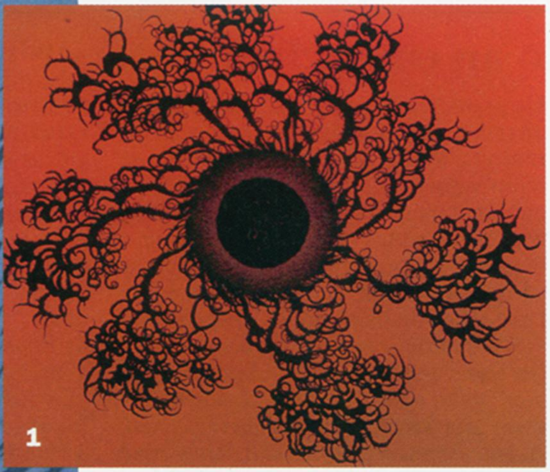
So the fact that bacterial colonies arrange themselves in snowflakelike clusters under certain conditions isn't particularly startling. The question is what purpose these shapes may serve. Could these elegant patterns actually reveal something about how bacteria communicate with one another?

Indeed, they may. Wielding analytical tools normally reserved for physicists and materials scientists, a few researchers are examining the intricate patterns that colonies of bacteria form when starvation threatens. Those patterns may hold clues to how bacteria disseminate information throughout a colony.

Beyond that, the work challenges the tenet of genetics that mutations occur randomly. It suggests instead that a bacterial colony may direct its genetic changes in response to its changing environment.

For more than a decade, a small community of microbiologists has been investigating the ways in which genetic variations and adaptations in bacteria may result from interactions with each other and the environment.

"Some classes of genetic changes in bacteria occur only under certain circumstances," says James A. Shapiro, a microbiologist at the University of Chicago. "When a bacterium moves an element in its genome, the process isn't really ran-



Complex patterns emerge in hungry colonies of Bacillus subtilis. The bacteria yield chiral (1), vortex (2, 3), snowflake (4), and branching (5) structures.

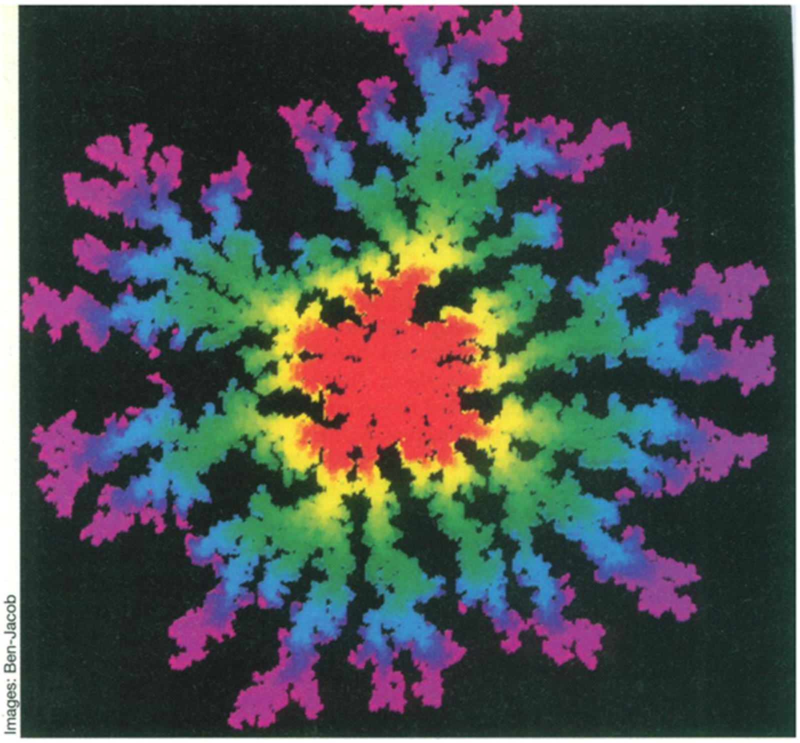
dom." The bacterium brings into play a biochemical mechanism that is regulated and controlled, he says.

"When mutations show up in response to new conditions, it's as if the bacteria are operating an internal genetic engineering system. How they alter the genome depends on what they're responding to," Shapiro says. "Microbiologists working with vaccine cultures have known about this for a long time. Yet the idea of adaptive mutations flies in the face of a lot of orthodox genetics, which is why it's considered controversial."

Physicist Eshel Ben-Jacob of Tel-Aviv University in Israel concurs. "We know that colonies of bacteria tend to form networks. Individual bacteria communicate with each other and with their environment through chemical signaling. It has also been shown that mutations arise in bacteria in response to their surroundings. The bacteria's genomes interact with each other and with what's around them."

A relationship exists between the unusual fractal patterns that often appear in bacterial colonies and the manner in which they signal each other chemically, Ben-Jacob suggests. The elaborate branchings and spirals, visible to the naked eye, hint at a chemical mechanism for feedback and control of the colony.

"We believe that new mutations in bacteria arise not from random changes that just happen to be useful for survival, but as a result of a calculation of the colony as a network," Ben-Jacob says. "We've shown this by exposing bacterial colonies as a whole . . . to selective pressures. The genetic mutations that appear suggest that the colony is responding as a whole, not just as randomly mutating bacteria."



Images: Ben-Jacob

***A** computer simulation shows how structure forms over time as modeled* B. subtilis *spreads out from the center.*

Six years ago, physicist Mitsugu Matsushita of Chuo University in Tokyo and his colleagues observed that under certain conditions, bacterial colonies formed unusual snowflake patterns. Reporting in the November 1989 Journal of the Physical Society of Japan, Matsushita noted that *Bacillus subtilis* grown on dry, nutrient-poor agar plates tends to fan out into patterns that strongly resemble a fractal pattern seen in nonliving systems, called a diffusion-limited aggregation (DLA). For instance, when deposited on a material, zinc can yield snowflakelike forms.

Researchers had studied DLA patterns in nonbiological materials but had not associated those patterns with organisms. Matsushita speculated that nutrient concentrations in the bacteria's growth medium might have something to do with the colony's DLA-like shape.

Impressed with these observations, Ben-Jacob and his colleagues set out to detail the mechanisms used by bacteria to generate such provocative complex forms. To accomplish this, they took the unorthodox approach of bringing methods from physics and materials science to bear on living bacterial colonies.

Not only have Ben-Jacob's efforts yielded intriguing results, they have shown how importing ideas from the physical sciences can stimulate new thinking about a biological problem.

"If one thinks of a bacterial colony as a network that processes information, then one is led to questions about how the network as a whole learns from its environment," Ben-Jacob says. "How does the signal system, or feedback mechanism, work?"

To investigate this, Ben-Jacob and his colleagues have spent nearly 5 years studying thousands of cultures of *Bacillus subtilis*. Ultimately, they aim to organize and categorize bacterial patterns to show how a colony uses chemical signals to integrate and disseminate new information about its environment.

So far, his team has confirmed a correlation between the nutrients in the bacteria's environment and the complexity of their networks.

To cope with unfavorable conditions, it seems, bacteria behave cooperatively. And some of that behavior, the researchers maintain, indeed resembles "nonequilibrium" growth processes seen in nonliving materials — for instance, the means by which snowflake shapes form during the cooling of crystals or during chemical deposition.

Ben-Jacob's group developed a simple model to explain how and why bacterial colonies generate distinctive, complex patterns. The model describes how clusters of bacteria, called "random walkers," move. The clusters navigate by responding to changes in nutrient concentration, and they communicate among themselves through "chemotactic feedback," a form of chemical signaling. Each colony has about 10,000 walkers; each walker contains 1,000 bacteria.

By decoding the rules that bacterial clusters follow to form complex shapes, the researchers can explain each colony's response to poor growth conditions and its mode of self-organization.

A nutrient-rich growth medium tends to produce compact, bulky colonies; a poor environment leads to fine, spindly colonies. Struggling for food appears to force the bacteria to spread themselves into a thinner, leaner, more efficient network. "As environmental conditions grow more hostile, the colony requires a higher level of cooperation to achieve the same level of efficiency," Ben-Jacob says. "The bacteria must devise a method of nonlocal communication to transfer information throughout the colony."

Ben-Jacob contends that chemotactic signals facilitate this communication. Such signals would guide bacteria toward or away from areas rich or poor in nutrients. Individually, each bacterium responds to something it senses and excretes a chemical alerting others to what it has detected. Those signals may relay a message as simple as "come closer" or "stay away."

In this model of bacterial behavior, the interplay of two primitive chemical signals — an attractant and a repellent, adjusted to a specific threshold — accounts for the complex shape of the networks the bacteria spawn in response to starvation. By modulating the ways in which the bacteria signal each other in the model, the researchers can show how and why colonies self-organized to adopt the observed complex patterns.

For instance, slight adjustments in variables — the availability of nutrients, the ease with which bacteria can spread on the culture surface, the threshold of the chemical signals, their rate of transmission — prompt colonies to adopt different shapes. Pattern features such as

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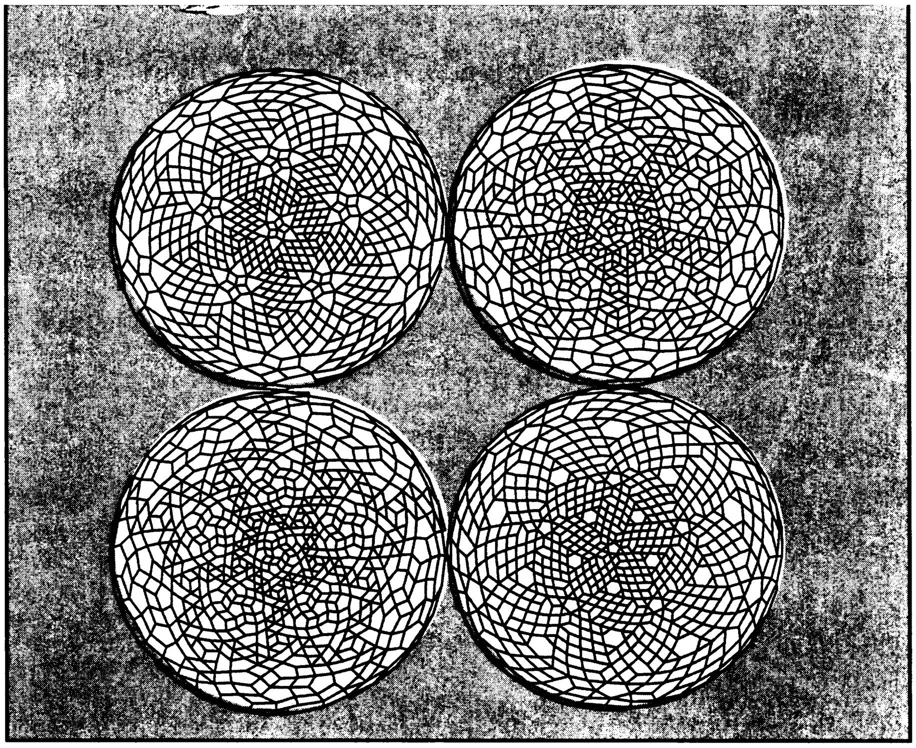

The best icosahedral packings now known of 782, 792, 800 and, 810 points.

Labs aren't being used for anything else.

As they explore possible arrangements, the programs automatically notify the team whenever they identify a superior arrangement of points. They also enter the coordinates of these points in the appropriate table.

In most instances, the Codemart team can't claim that it has found the very best geometrical arrangement of points on a sphere to meet a certain criterion. Mathematical proofs that such patterns are truly optimal are few and far between. But in nearly all cases, the team holds the record for the best *known* arrangement, and the programs keep improving on these results.

The Codemart effort has already generated tables far more extensive than any available elsewhere. The new electronic archive includes packings of up to 130 points on spheres in three, four, and five dimensions, coverings of points on spheres in three dimensions, and many special cases.

The team expects eventually to publish their tables in a book. "But in view of the considerable recent interest in these problems, we are making these tables available before the book is completed," the researchers say.

The Codemart catalog is accessible via electronic mail at *netlib@research.att.com.* One can find out what is available by sending to this address a message containing a line such as "send index for att/math/sloane/packings."

At present, the tables include only the coordinates of the points in any particular arrangement. Sloane and his colleagues are now considering the possibility of adding pictures of some of the examples.

At the same time, "we're still searching, and we're continually widening our scope," Sloane remarks. "The programs are running day and night." ☐

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branching, diffusing, tip splitting, and dendrite forming emerge. Moreover, Ben-Jacob's group reports in the Feb. 16 NATURE, the modeled patterns correlate with forms grown in petri dishes.

"This work is very provocative," says Albert Libchaber, a physicist at Rockefeller University. "Fractal analysis is a tool that can give you some intuitions about the cause of a phenomenon. Of course, identical patterns can have different physical causes. So the pattern itself does not determine a phenomenon's origin. But an *evolving* pattern can tell you that something interesting may be going on."

Ben-Jacob argues that patterns visible to the naked eye often convey meaningful clues about molecular processes the unaided eye cannot see. "There is an interplay between information at the macroscopic level and the microscopic level in bacterial colonies similar to what we see in nonliving systems that are changing and evolving," he says.

Using the metaphor of information processors to describe bacterial communication within colonies, Ben-Jacob advocates viewing each bacterium's genome as an "adaptive cybernetic unit." Those units, he asserts, may be influenced by the chemotactic signals generated through the colony's structure. This, he maintains, helps account for the survival of mutations that benefit the colony as a whole.

"There's strong evidence now that in many bacterial systems, genetic changes result from many kinds of chemical regulation and control," Shapiro says. "Mutation is a biochemical process which does not just occur because of random insults to DNA. Biochemical complexes come into play. It would be surprising, in fact, if we found that as conditions change, genomes didn't change."

"We know that bacterial colonies can alter themselves dramatically in response to changing conditions," Shapiro adds. "An area of investigation now is to understand how specific, meaningful, and useful those changes are. This lies at the heart of studies of adaptive mutations."

Shapiro says other findings support Ben-Jacob's models. "His work fits into a trend away from a view of bacteria as isolated, autonomous, and relatively unaware of things around them. Instead, we're seeing that bacteria constantly pick up chemical signals."

As for the usefulness of applying ideas from materials science to biology, Herbert Levine, a physicist at the University of California, San Diego, says that such interdisciplinary techniques often spark clever insights.

"One reason to try to understand nonliving chemical systems undergoing changes is that many scientists think it will lead to useful explanations of how biological systems form structure," Levine says. "If we understand nonliving systems well enough, then maybe we can use the same ideas to make predictions about biological systems."

H. Eugene Stanley, a materials scientist at Boston University, agrees. "I think Ben-Jacob is correct to take seriously what the eye tells us about bacteria," he says. "Given that the bacterial colonies look so much like patterns we already know about from materials science, either the resemblance is fortuitous or it's telling us something valuable. I suspect the resemblance is a powerful clue," he observes.

Shapiro, for one, welcomes the influx of physicists into the biological arena.

"Biologists can learn from people in other fields with different analytical techniques," he says. The synergism will benefit everyone. The physicists will be forced to give up their dogmas of understanding a whole organism by understanding a small piece of it, and the biologists will have to give up some dogmas about genetics." ☐