
Chaotropes comprise a diverse set of structurally disruptive compounds that includes ethanol, urea, fructose, salts such as magnesium chloride, and aromatic agents such as phenol. They work by interfering with noncovalent bonds and find use in labs during purifications and analyses of macromolecules.

At lower temperatures, because chaotropes disrupt noncovalent interactions among macromolecules, their presence tends to enliven metabolic activities that would otherwise remain sluggish. For example, sea-ice algae withstand −20°C and very high levels of sodium chloride, according to microbial ecologist Graham Underwood of the University of Essex in Essex, England.

Cold can be a double whammy for prokaryotes, Hallsworth says. Although high solute concentrations keep water from freezing once it reaches 0°C, high solute itself is exceptionally stressful to microbes. Chaotropes, however, can loosen cellular structures that ordinarily stiffen with falling temperatures. “I wondered whether and how such cells would be able to use the solute activities of environmental substances or their own metabolites to enhance their metabolic activities at otherwise prohibitively low temperatures, he says.

Hallsworth, Underwood, and their collaborators screened cold-tolerant algae, fungi, and bacteria for solute tolerance, screened a set of 161 solute-tolerant fungi for low-temperature tolerance, and then cultured the microbes with the highest combined solute-and-low-temperature tolerance on growth media supplemented either with chaotropes or with their functional opposites, macromolecule-stabilizing compounds, called kosmotropes.

At temperatures near 0°C, microbial colonies on media containing kosmotropes stopped growing, whereas media with chaotropes enhanced microbial growth and survival. “Microbial cells may preferentially synthesize and accumulate chaotropic metabolites . . . to retain activity in the cold,” Hallsworth adds. Further, spores treated with chaotropes outsurvived untreated spores when held at −80°C.

The study breaks new ground, says Rocco Mancinelli of the National Aeronautics and Space Administration. “The environments in which organisms live are extremely important in defining the limits in which they can survive, compared with standard laboratory studies.” Moreover, he adds, changes in environments can drastically change organisms within them, a generalization that carries major implications for carbon and nitrogen cycling on Earth.

David C. Holzman
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Siderophores Shed Light on the “Great Plate Count Anomaly”

Missing siderophores may account for why microbiologists can culture only about 1% of the microorganisms that they collect from diverse environments, according to Kim Lewis of Northeastern University and his collaborators there and at nearby Harvard Medical School, both in Boston, Mass. Without siderophores to bind iron for them, these microorganisms fail to grow in the lab despite being bathed in nutrients. If this explanation holds up, it should enable microbiologists to overcome what some of them call the “great plate count anomaly” and to learn a great deal more about countless recalcitrant species that were set aside as “nongrowers.” The work appeared in the March 26, 2010 Chemistry & Biology.
Uncultured microorganisms from marine biofilms can be coaxed to grow when they are exposed to natural marine sediment or other bacterial species from the same environment. The research team suspected that some type of natural diffusible molecule stimulates this syntrophy among marine microbes. The researchers inoculated the uncultured isolate *Marinibacter polysiphonae* KLE1104 with strains of *Escherichia coli* containing deletions for various growth factors to identify metabolites that promote growth. From among three likely candidates—the siderophore enterobactin, the universal quorum sensing factor AI-2, and an autoinducer indole—only the strain missing enterobactin fails to induce growth. “We had no idea what the growth factor would be, and we got lucky using our knockout collection of *E. coli* mutants,” says study leader Kim Lewis, director of Northeastern’s Antimicrobial Discovery Center.

Lewis’ team also discovered that another strain, *Micrococcus luteus* KLE1011, is a potent natural helper that promotes the growth of *M. polysiphonae* KLE1104 and other uncultured strains in the laboratory. They purified five novel siderophores from media collected from cultures of *M. luteus* KLE1011. These newly identified siderophores belong to the desferrioxamine class of iron-binding agents, and they all have flanking acyl side chains that make them very hydrophobic.

Their unusual chemical structure “probably helps them stay within the biofilm, and they do not easily leak out into the open ocean,” Lewis says. Moreover, the five new siderophores individually induce the growth of uncultured strains, including *Cyclobacterium, Sulfitobacter, and Bacteroidetes*, show distinctly different patterns of siderophore dependence. Some siderophores universally induce growth, whereas others act more specifically. The results suggest that siderophores show wide variations in their preference for aiding growth of uncultured isolates.

The multitude of uncultured microbes likely will require other yet-undiscovered factors to stimulate growth. Nonetheless, siderophores are a first important step towards solving the 100-year-old mystery of the great plate count anomaly. “It gives us a tool to access biodiversity that has been hidden from us,” says Lewis, thus allowing exploration of unknown bacterial metabolites, such as much-needed novel antibiotics.

“Improving our ability to culture difficult microorganisms promises to increase our understanding of them and verify that bacteria detected indirectly using gene sequencing techniques are truly present in particular environments,” says Garth James, medical projects manager at the Center for Biofilm Engineering at Montana State University, Bozeman. For example, 16S rRNA data indicate that chronic wounds contain diverse bacteria that cannot be cultured. “It would not surprise me,” says Garth, “to find siderophore-based syntrophy in wounds and across the human microbiome.”

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**Highlights from 2010 ASM General Meeting**

**Synthetic, Transplanted Genome Directs New Host Cell**

A synthetic and slightly streamlined version of the *Mycoplasma mycoides* genome, consisting of about 1.08 million base pairs, worked fine and took over the genetic controls after being transplanted into a similar but distinct host cell, *Mycoplasma capricolum*, according to Clyde Hutchison from the J. Craig Venter Institute, San Diego, Calif. He spoke during the colloquium “Engineering a Better Bacterium,” held during the 110th ASM General Meeting last May in San Diego, Calif.

The synthetic genome, which omits 14 “dispensable” genes that are found in the natural version of the *M. mycoides* genome, also carries slight changes, or “watermarks,” to make it readily distinguishable from that native version, Hutchison says. Assembled in step-wise fashion, the gargantuan piece of DNA is eased into the recipient cell with assistance from polyethylene glycol (PEG) in a step that is nearly as inefficient as it is mechanistically inscrutable. PEG “may cause cell membranes to fuse,” he says, “but we don’t know how to study this step.” Thus, the genome “transplantation” from one cell into another remains a “rare event.”

Nonetheless, the recipient cells containing the synthetic genome are “happy,” and even grow slightly “faster than the reference strain,” Hutchison continues. Having cells unquestionably under control of synthetic DNA speaks to the “main interest” of the Venter group, namely to try to understand what genes are essential for life and, down the road, develop a model to predict how “subsets of genes affect cell behavior.” Eventually, the research group would like to do away with recipient host cells and