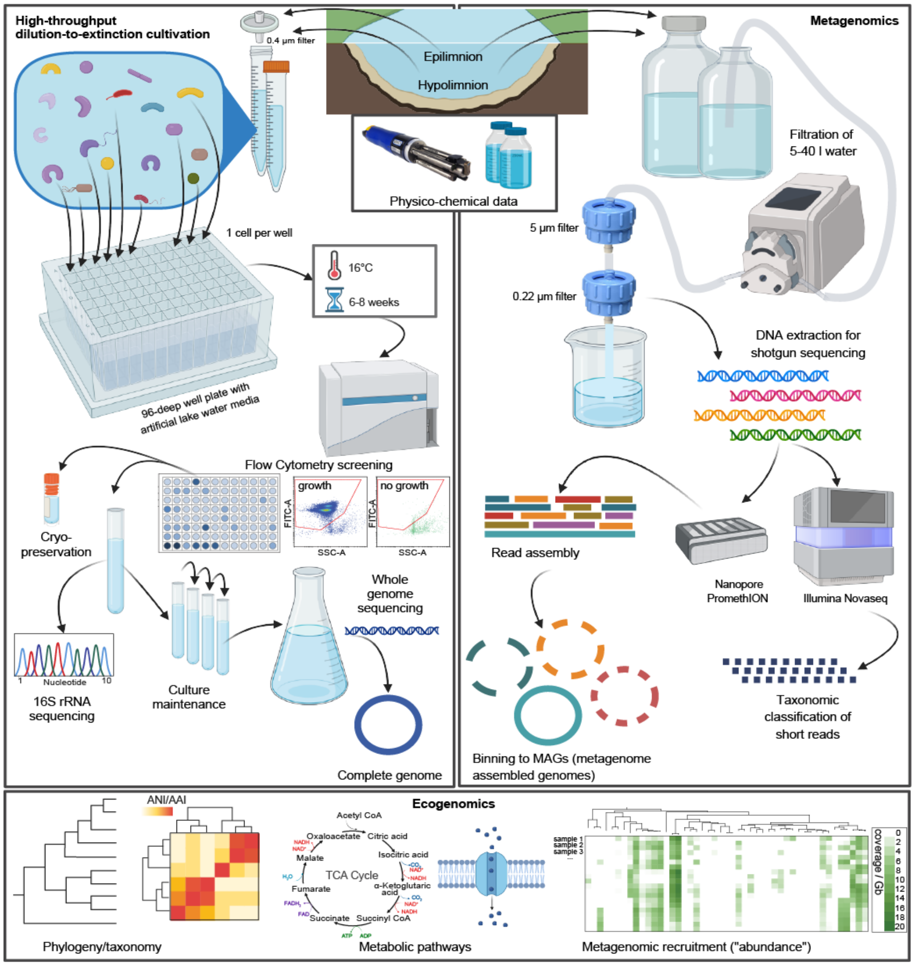
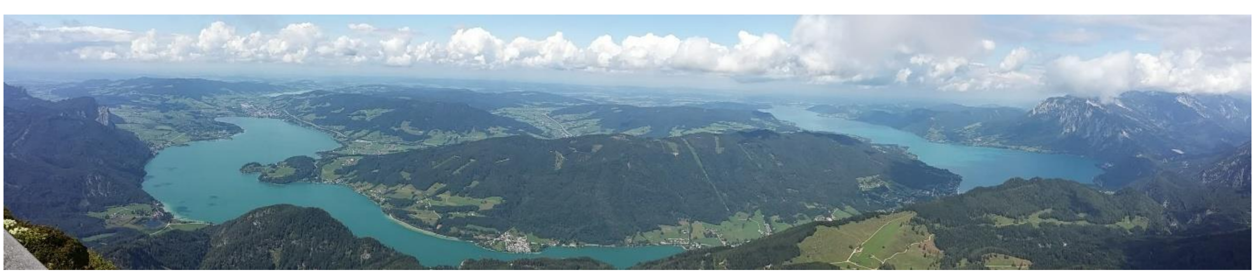
Researchers cultivate hundreds of abundant freshwater microbes, with many slowly growing oligotrophs

A recent study in Nature Communications *marks a significant advance in microbiology by bringing a large collection of previously uncultured freshwater bacteria into culture, organisms long known to be highly abundant in nature but rarely represented in public culture collections.*



Workflow for the isolation of aquatic bacteria (left side), metagenomic sequencing (right side), and ecogenomic analyses (bottom).

Until now, most microbial cultivation efforts focused on fast-growing organisms that grow in nutrient-rich media. This has left many of the most abundant aquatic microbes, slow-growing oligotrophs that are adapted to low nutrient conditions, largely unstudied. The new research, led by scientists at the Biology Centre of the Czech Academy of Sciences, fills that gap by successfully cultivating **627 pure strains** from 72 genera, across 14 lakes in Central Europe.



Mondsee and Attersee (Austria), two of the sampled lakes. Image credit: M. Salcher

Cultivating the Uncultivated
The international team led by Michaela Salcher, head of the Laboratory of Microbial Cultivation and Ecogenomics in České Budějovice, in cooperation with Bettina Sonntag from the University of Innsbruck (Research Dept. for Limnology, Mondsee), used a high-throughput dilution-to-extinction approach with custom-designed media that mimic natural lake water with low nutrient concentrations, supporting growth of oligotrophs while avoiding overgrowth by fast-growing competitors. This method yielded a rich diversity of cultured microbes, including strains from **15 of the 30 most abundant freshwater bacterial genera**, representing up to **72% of genera** detected in environmental samples (average 40%) and encompassing key lineages widespread across global freshwater systems.

The newly established culture collection includes many so far uncultivated taxa from classical freshwater phyla (e.g., Actinomycetota, Pseudomonadota, Verrucomicrobiota, Bacteroidota), and the first free-living freshwater representative of Armatimonadota. Among the cultivated strains are many of the most abundant freshwater bacterial genera, including representatives from the freshwater SAR11 genus *Fontibacterium*, the methylotrophic *Methylopumilus*, and freshwater specialists such as *Planktophila*. These taxa are notoriously underrepresented in public repositories because of their slow and unstable growth and unknown growth requirements.

From Lakes to a Global Context
Metagenomic sequencing of the same lake samples revealed that the cultured strains closely match metagenome-assembled genomes (MAGs), confirming the relevance of the cultures to natural microbial communities. The researchers also analyzed over 460 publicly available freshwater metagenomes from six continents, confirming that many of these taxa are **widespread globally**.

Lago Maggiore (Italy) and Lake Milada (Czechia), two lakes from where many new microbes were isolated. Image credit: M. Salcher

Genomic Novelty and Metabolic Insights
Whole-genome sequencing of 87 of the cultured strains revealed **two new families, nine new genera and 41 novel species**. The sequenced genomes provide insights into ecological adaptations to freshwater environments, such as:

- **Genome streamlining:** Small genome sizes leading to energy conservation and reduced metabolic flexibility are common in highly abundant taxa like *Fontibacterium* and *Planktophila*, which are adapted to low-nutrient (oligotrophic) environments.
- **Light-harvesting capabilities**: Many strains use proton-pumping rhodopsins or aerobic anoxygenic phototrophy to harvest light energy.
- **Diverse metabolic traits**: From sulfur and nitrogen metabolism to vitamin biosynthesis and carbohydrate degradation, the culture collection spans a wide array of ecological functions. While most strains lack biosynthetic pathways for several vitamins such as cobalamin (vitamin B_{12}), some strains appear capable of supplementing the surrounding freshwater communities with these important vitamins.

Left: Preparation for dilution-to-extinction isolation with 96-well plates filled with artificial lake water medium. Right: Part of the culture collection grown in 15 ml tubes. Image credit: M. Salcher

Why It Matters

The new cultures fill a major gap in microbial research by providing access to **model organisms** that are abundant in nature but rarely cultivated in the lab. This enables controlled experiments to answer fundamental ecological and evolutionary questions: How do these microbes interact? What are their growth limitations? How might environmental change affect them?

The new strain collection and genomes are publicly available, offering a foundational resource for researchers exploring microbial roles in carbon and nutrient cycling, water quality, ecosystem resilience, and more.

Original paper: Salcher MM, Layoun P, Fernandes C, Chiriac M-C, Bulzu P-A, Ghai R, Shabarova T, Lanta V, Callieri C, Sonntag B, Posch T, Lepori F, Znachor P, Haber M (2025) Bringing the uncultivated microbial majority of freshwater ecosystems into culture. *Nature Communications* 16:7971. Doi:10.1038/s41467-025-63266-9

Behind the paper blog: https://go.nature.com/47PdiJ5

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