Microbiology Focus

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Fluka Analytical

Staphylococcus aureus in the Focus

Milk — a possible source of *Staphylococcus aureus* contamination

Staphylococcus aureus in the Focus

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Staphylococcus aureus is frequently a part of our skin flora but is also a cause of a broad range of illnesses. Current studies report a remarkable increase of _Methicillin Resistant Staphylococcus aureus_ (MRSA) over recent years.

Staphylococci may be airborne and can occur in both animals and humans, in sewage, water, milk, or food, and on environmental surfaces or food equipment. The main issue of *S. aureus* presented by food poisoning is the production of several relatively heat stable exotoxins. The exotoxins can be categorized into three groups: the superantigens, the exfoliative toxins along with a group of other toxins which act on the cell membranes, and the biocomponent toxins. *S. aureus* is also still one of the five most common causes of nosocomial infections, often causing postsurgical wound infections. Consequently, it poses a major concern in hospitals, especially in regard to MRSA, methicillin-resistant *Staphylococcus aureus*.

The name *Staphylococcus aureus* comes from the Greek words "staphylé," meaning a bunch of grapes, "coccus," which means round-shaped, and "aureus," for golden, because most colonies have a characteristic orange-yellow coloring on the traditionally-used agar plates; in addition, *S. aureus* are responsible for the golden-yellow pus indicating infection. Actually, under a microscope they often appear as grouped cocci resembling a cluster of grapes. They are relatives to the lactic acid bacteria, and the fermentation of these facultative anaerobes pathogen ends as well at lactic acid . They can grow in the temperature range of 15-45 °C and in a medium with up to 15% sodium chloride.

A wide range of media employing selectivity and biochemical differentiation systems may be used for the detection and identification of *S. aureus* **(Table 3)**. Also, chromogenic media such as HiCrome™ Aureus Agar (Fluka 05662), HiCrome™ MeReSa Agar (Fluka 90923) and Phenolphthalein Phosphate Agar (Fluka 68879) are available and used for detection and enumeration. Lithium chloride, sodium azide, tellurite and sulphamezatine may serve as selective agents in a medium.

There are diverse biochemical characteristics for the confirmation and identification of *S. aureus*. A flowchart of one of the most common and easiest identification pathways appears in **Figure 2**. As usual, the recommended starting point is to check the shape of the bacteria under the microscope and the gram coloration. The second step is a catalase test, followed by any of a diverse range of other individual tests or media that are available **(Tables 1, 2 and 3)**. An immunological test such as the Staphylo Monotec test kit Plus (Fluka 50448) is also often used for confirmation. This kit has been evaluated for clinical specimens and food material. With this kit, coagulase, protein A and capsular Polysaccharide (serotype 5) on *S. aureus* can be detected in one step, which gives a highly reliable result.

Figure 1: Petri plate with *S. aureus* (haemolytic), *Moraxella catarrhalis*

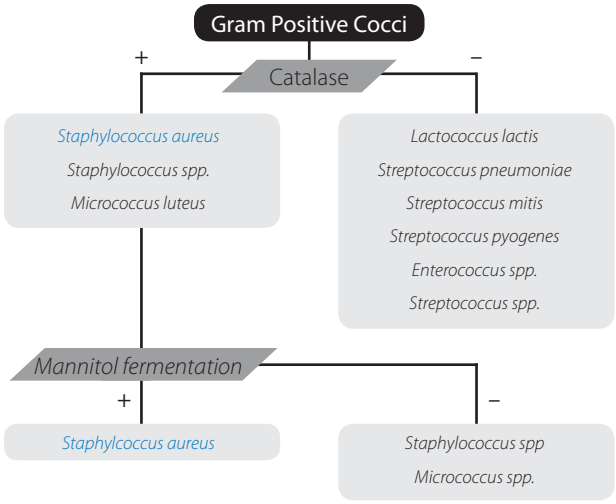
Table 1: Biochemical characteristics of *S. aureus*

Characteristic properties	Result	Tests
Higher peptidoglycan and lower lipid content in cell wall	+	Gram colouration
Catalase	+	H_2O_2 test
Facultative anaerobe	+	TSA Deeps
Oxidase	-	
Coagulase	+ (97%)	Cloating with fibrinogen
Protein A	+ (95%)	Immunological
Capsular Polysaccharide (serotype 5)	+	Immunological
Lecithinase	+	Egg yolk-lecithinase reaction (e.g. Baird Parker Agar)
Reduction of tellurite	+	Reduction to tellurium (e.g. Baird Parker Agar)
DNase	+	DNase test
Mannitol fermentation	+	
β-Hemolysis	+ (mostly)	Blood Agar
Phosphatase	+	Phenolphthalein Phosphate Agar
Proteolytic enzymes	+	Nutrient Gelatin

Table 2: Tests for Detection and Identification of *S. aureus*

Kits & Tests	Cat. No.
Catalase Test (Hydrogen peroxide 3%)	88597
Coagulase Test (Slide; see figure 4 & 5)	75832
Coagulase Test (Tubes)	74226
Gram Staining Kit	77730
Mannitol disks	94438
Oxidase Reagent acc. Gaby-Hadley A + Oxidase Reagent acc. Gaby-Hadley B	07345 + 07817
Oxidase Reagent acc. Gordon-McLeod	18502
Oxidase Strips	40560
Oxidase Test	70439
Staphylo Monotec test kit Plus	50448

Figure 2: ID flowchart for *S. aureus* (Bergey's Manual)



MRSA:

S. aureus is an invasive pathogen that can cause disease in almost any tissue or organ in the human body, primarily in compromised individuals. In the past, staphylococcal infections were treated using penicillin, but over the years this pathogen developed resistance to penicillin by building penicillinase. Methicillin was the next drug of choice as it is not cleaved by the penicillinase. While methicillin is very effective in treating most *Staphylococcus* infections, some strains have developed resistance to methicillin by production of penicillin binding protein and can no longer be killed by this antibiotic. These resistant bacteria are called Methicillin Resistant *Staphylococcus aureus* (MRSA) (1).

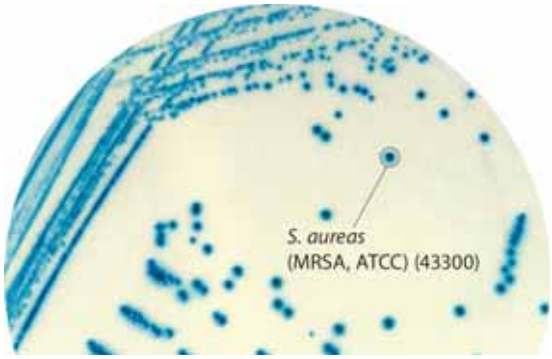
Today there are many innovative solutions to detect MRSA. Sigma-Aldrich strongly supports the microbiologist with a selective chromogenic HiCrome MeReSa Agar (Fluka 90923) for detection of MRSA from clinical isolates and other samples. The proprietary chromogenic mixture incorporated in the medium is specifically cleaved by *S. aureus* to give bluish green colonies **(Figure 5)** on this medium and can be clearly differentiated from other species. The medium is made selective for MRSA by the addition of methicillin.

Table 3: Media for detection and identification of *S. aureus* (*not sold in USA)

Identification Media	Cat. No.	Testing features
Baird Parker Agar* Supplements: Egg-Yolk Tellurite Emulsion (Fluka 75208) or RPF Supplement (Fluka 05939)	11705	Detection of lipolytic and proteolytic activity, ability to reduce tellurite to metallic tellurium (EN-ISO 6888-1: 1999); with RPF Supplement the coagulase activity and the ability to reduce tellurite is detected (EN-ISO 6888-2:2000)
Blood Agar Supplement: defibrinated blood	70133	Detection of β-hemolysis
Blood Agar No. 2 Supplement: defibrinated blood	B1676	Detection of β-hemolysis
Bromo Thymol Blue (B.T.B.) Lactose Agar	B3676	Differentiated by their ability to grow at a high pH and in the presence of bromo thymol blue (golden yellow colonies)
CLED Agar	55420	Detection of lactose fermentation
Deoxyribonuclease Test Agar	30787 / 70136	Detection of deoxyribonuclease activity
DNase Test Agar with Toluidine Blue	D2560	Detection of deoxyribonuclease activity
HiCrome™ Aureus Agar Base* Supplement: Egg-Yolk Tellurite Emulsion (Fluka 75208)	05662	Testing for ability to reduce tellurite to metallic tellurium and detection of lipase and protease by chromogenic substrate; brown-black colonies
HiCrome™ MeReSa Agar Base* Supplement: MRSA Selective Supplement (Fluka 51387)	90923	Detection by chromogenic subtrate mixture specifically cleaved by *S. aureus*; selective to MRSA; MRSA give bluish green colonies
China Blue Lactose Agar*	22520	Detection of lactose fermentation
Mannitol Salt Agar	63567	Detection of mannitol fermentation in high sodium chloride concentration
Nutrient Gelatin	70151	Detection of gelatin-liquefying (proteolytic enzymes)
Phenolphthalein Phosphate Agar	68879	Phosphatase detection; pink-red colonies
Spirit Blue Agar Supplement: Lipase Substrate (see data sheet)	S4306	Detection and enumeration of lipolytic activity
Staphylococcus Agar*	70193	Detection of salt tolerance, pigmentation, D-mannitol utilization and gelatin liquefaction
Tributyrin Agar* Supplement: Neutral tributyrin (Fluka 91010)	91015	Detection and enumeration of lipolytic activity
Vogel-Johnson Agar Supplements: Potassium Tellurite 1% (Fluka 17774)	70195	Checking for ability to reduce tellurite to tellurium and ability to ferment mannitol

Figure 3 & 4: Coagulase slide test

Figure 5: HiCrome MeReSa Agar (Fluka 90923) with *S. aureus*



Microorganisms as defined CFU on Discs

By Jvo Siegrist, Product Manager Microbiology…. *ivo.siegrist@sial.com*

Figure 1: Organisms on membrane filter placed on an agar plate

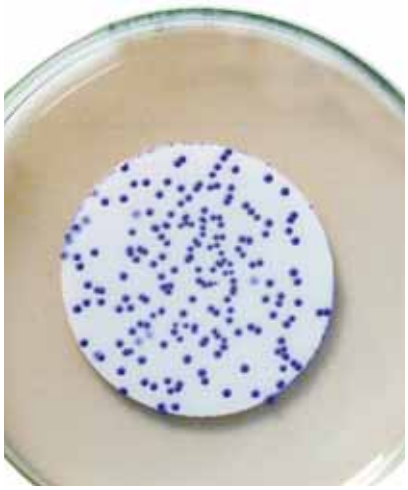


Figure 2: HiCrome™ Rapid Coliform Broth (Fluka 51489; left = positive reaction with coliforms; right = negative control; under UV light *E. coli* could be confirmed)

It is a microbiologist's dream to be able to just simply start with a microbiological analysis or experiment and circumvent the necessary steps for maintaining the microorganisms.

In the real world, the practical microbiologist will find help from RapidCheck™ which is a disc with certified test strains! RapidCheck discs contain viable microorganisms in a certified quantity (ISO 17025), produced under reproducible conditions (ISO Guide 34) with strains obtained from ATCC and NCTC. The discs consist of bacteria or fungi in a solid water soluble matrix. Microorganisms in this form are very stable for at least one year and are in a viable stage (no lag phase or recovery time).

Applications

- As reference strains in routine quality control, for food, beverage, water and environmental testing
- For method development and validations
- As cultures used in research studies
- For educational purposes

Strains

The strains are all derived from ATCC® and NCTC and are characterized from these cell culture collections.

Preparation:

Rehydrate the disc with a common phosphate buffer, or place the disc onto a solid or into a liquid medium. The rehydration process takes approximately 10 minutes. On solid media, the disc forms a droplet that can be spread with a loop. Liquid media may simply be shaken to dissolve the disc. The discs can be rehydrated in as little as 100 µL of water or added into larger volumes, e.g. 100 mL, for general water testing methods (MF, MTF, Quanti-Tray, etc). It is also possible to add the disc to the media for pour plate techniques.

Benefits:

Reproducibility:

- Produced under ISO Guide 34
- Certified under ISO 17025
- Supplied with a comprehensive Certificate of Analysis reporting the colony forming units (cfu)
- Strains are derived from ATCC and NCTC
- Between-disc reproducibility of 3% at levels of 100 cfu.

Stability:

- Very stable - minimum expiry date of one year stored at 4 °C
- No change of the cfu
- Stability could be significantly extended at -20 °C, as long as the tubes are sealed
- Stable at ambient temperatures for shipping

Save time & costs:

- No maintenance of stock cultures
- No or minimal dilution of control samples
- No recovery time or pre-enrichment step necessary
- Easy to use
- Low price / good value

Packaging

One pack contains 10 discs; each disc is in an individual vial. The screw-cap vial has a special seal and contains silica gel as a desiccant. Each package is accompanied by a comprehensive certificate of analysis.

Figure 4: RapidCheck box, vials and discs

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Table 1: Available certified test strains

RapidCheck™ Test Strains	Origin	Strain No.	CFU	Cat. No.
Aspergillus niger	ATCC	16404™	80	RQC15003
Bacillus subtilis	ATCC	6633™	10000	RQC02258
Bacillus subtilis	ATCC	6633™	80	RQC16003
Candida albicans	ATCC	10321™	80	RQC14003
Clostridium perfringens	NCTC	10240	30	RQC02351
Clostridium perfringens	NCTC	10240	500	RQC20106
Clostridium sporogenes	ATCC	19404™	80	RQC19003
Enterobacter aerogenes	ATCC	13048™	50	RQC01652
Enterobacter aerogenes	ATCC	13048™	10	RQC01657
Enterobacter aerogenes	ATCC	13048™	20	RQC01655
Enterococcus cloacae	ATCC	35030™	50	RQC21102
Enterococcus faecalis	ATCC	19433™	500	RQC01776
Enterococcus faecalis	ATCC	19433™	200	RQC01775
Enterococcus faecalis	ATCC	19433™	50	RQC01772
Enterococcus faecalis	ATCC	19433™	1000	RQC01777
Escherichia coli	ATCC	8739™	80	RQC11003
Escherichia coli	ATCC	11775™	1000	RQC01707
Escherichia coli	ATCC	11775™	200	RQC01705
Escherichia coli	ATCC	11775™	50	RQC01702
Heterotrophic Organisms			100	RQC02504
Legionella pneumophilia	ATCC	12821™	50000	RQC02008
Listeria monocytogenes	ATCC	19115™	30	RQC01901
Pseudomonas aeruginosa	ATCC	9027™	1000	RQC12007
Pseudomonas aeruginosa	ATCC	9027™	100	RQC02204
Pseudomonas aeruginosa	ATCC	9027™	200	RQC12005
Pseudomonas aeruginosa	ATCC	9027™	30	RQC02201
Pseudomonas aeruginosa	ATCC	9027™	50	RQC12002
Legionella bozemanii	NCTC	11368	50000	RQC02908
Salmonella enterica subsp. Enterica serovar Abony	NCTC	6017	80	RQC18003
Salmonella enterica subsp. Enterica serovar Typhimurium	ATCC	13301™	50	RQC17002
Salmonella goldcoast	NCTC	13175	30	RQC02302
Staphylococcus aureus susp. Aureus	ATCC	6538™	50	RQC13002

Help us expand the product range! Please email us at *RTC@RT-Corp.com* to ask for new strains and different concentration ranges.

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"Tough Guys" - Microbes in Alpine Ecosystems

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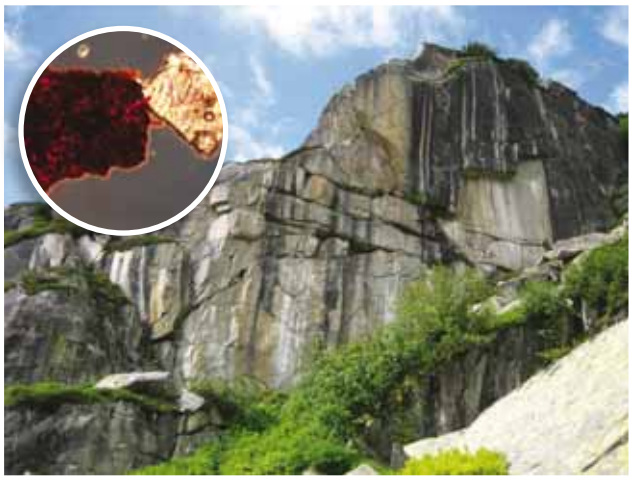
High mountain ecosystems such as the Swiss Alps offer diverse unique locations where microbes thrive under – from our point of view – inhospitable environmental conditions. Occurring in many places as "blooms" they are visible even to the "naked eye".

It is a fact that microorganisms in natural environments are often exposed to harsh or "extreme" conditions such as high or low temperatures, pH values, and salinities as well as nutrient limitations or high UV radiation. However, there is no need to visit distant places such as Antarctica or remote deserts to find these extremophilic microorganisms. These "tough guys" might live right in your neighborhood! In fact, in many alpine environments they find optimal conditions for living.

Making a living "on the rocks"

It is commonly observed that rock surfaces in mountain areas are sometimes covered by dark lines or bands. These are called "Tintenstriche" (meaning "ink lines" or "ink smears") and represent rich communities of mainly cyanobacteria. In certain periods of the year, "Tintenstrich" communities are exposed not only to extreme dryness, high UV radiation (especially at altitudes above 1500 m.a.s.l.), and enormous (not only seasonal) temperature variations of -30 °C to 50 °C or higher, but also fluctuations during the course of a day. Particular metabolic features such as the formation of slimes or capsules (consisting of extracellular polymeric substances) or dark pigments (e.g., scytonemin) allow them to withstand dry conditions and protect them from UV. Depending on the proportions of various pigments, cyanobacteria can appear blue, red, green, or even black, such as in these colonies. "Tintenstrich" communities have been described in detail by the Swiss botanist and hydrobiologist Otto Jaag (1900-1978). In many cases, communities are dominated by the genera *Gloeocapsa* and *Scytonema*. The photosynthetic organisms secrete a variety of metabolites which enhance not only the biological weathering of the rocks and the mobilization of minerals, but also serve as nutrients for heterotrophs. In addition to these organisms, green algae *(Gloeocystis, Pleurococcus, Stichococcus)* might also be part of the microbial communities. "Tintenstrich" communities are not only found on natural rocks but are also common on stone walls, buildings, monuments, and statues.

Figure 1: Rocks near Göscheneralp (canton of Uri, Switzerland) covered by black "Tintenstrich" microbial communities. Insert: Scraped crust material seen through the fluorescence microscope (100x magnification) causing red chlorophyll autofluorescence and making individual cyanobacterial cells in the crust visible.



"Watermelon snow"

In early summer, when some snow fields are still left in the Alps at higher altitudes, patches of red-colored snow can commonly be observed. Blooms of motile, single cell, green algae, *Chlamydomonas nivalis*, are responsible for this phenomenon. Cryobiontic algae can be found worldwide in polar or alpine regions. They live around the freezing point at the snow-air interface in the pores of ice crystal and have to be metabolically adapted to diurnal cycles of freezing and thawing. The red color of the alga is a reaction to stress presumably induced by high light intensities and nutrient limitations. As a metabolic response and survival mechanism, robust cysts are formed, characterized by their high content of carotinoids, leading to the red color of the snow. As in "Tintenstrich" communities, pigments act as a sunscreen. In green or brownish snow, other *Chlamydomonas* species can occur in addition to *Chorella, Mycacanthococcus, Raphidemona* and *Scotiella*. Until today, more than 350 algal species have been described in snow and ice, sometimes in densities of 106 per cm^2 snow surface.

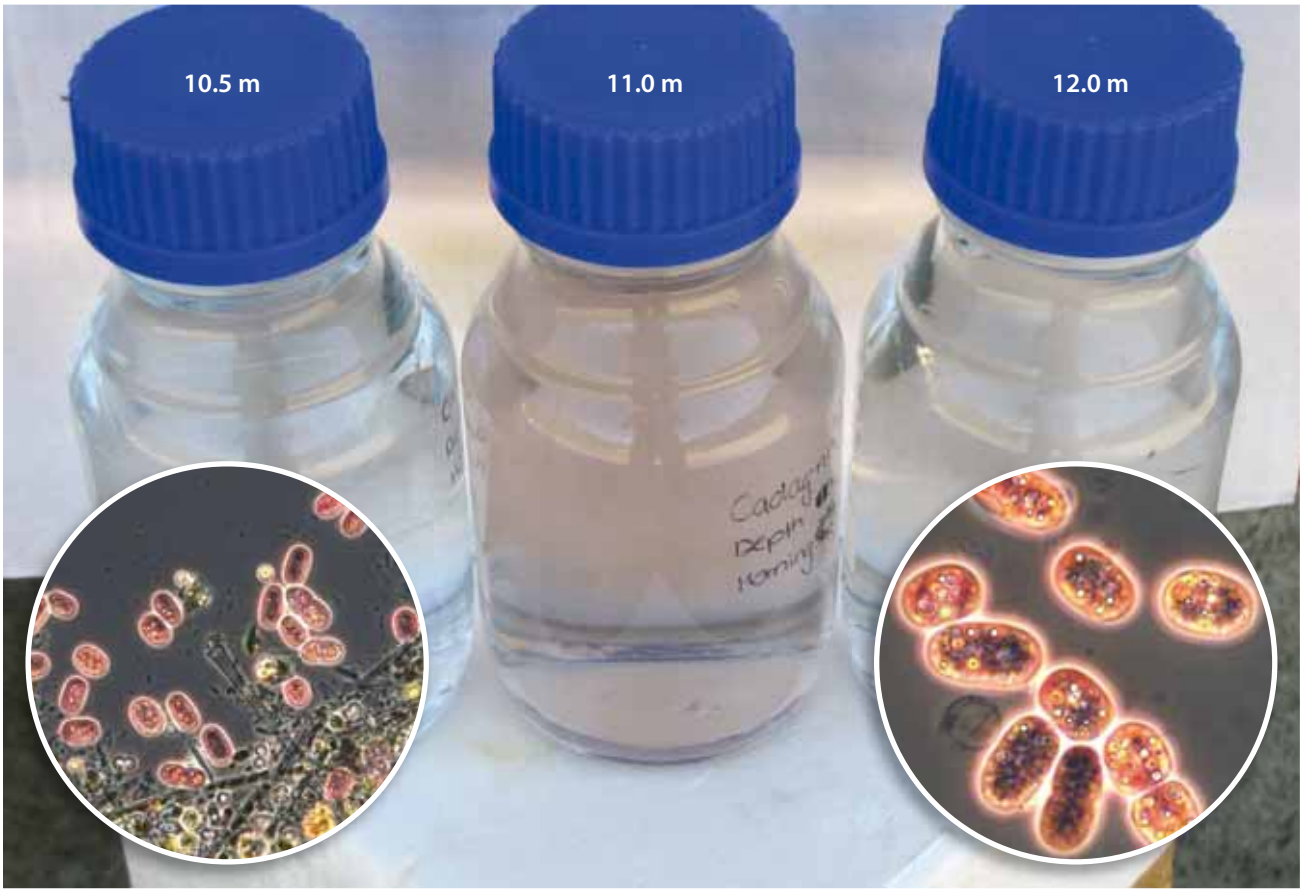
Figure 2: Patches of red snow during melting in early summer near Passo del Sole (canton of Ticino, Switzerland). Inserts: Close-up of red snow (right); microscopic view of red cysts of *Chlamydomonas nivalis* with some mineral constituents (left; 400x magnification).

"Underwater" bacterial blooms in meromictic Lake Cadagno

Located in the Gotthard massif of the central Swiss Alps, the Piora Valley is the site of a unique aquatic ecosystem. Lake Cadagno (1923 m a.s.l.) is a rare example of crenogenic meromixis. In contrast to most freshwater lakes, meromictic lakes do not show a seasonal turnover of the water column. As a result, the water body of Lake Cadagno is permanently stratified due to a natural geological phenomenon. The oxygenated top water body (epilimnion, mixolimion) is supplied by surface waters, whereas underwater springs supply the lower water layers (hypoliminion, monimolimnion) with salt rich, dense water. These sublacustrine springs supply dissolved mineral salts (calcium, magnesium, sulfate, carbonate) originating from dolomite minerals. As a consequence, oxygen is completely depleted in the hypolimnion. In the transition zone, at a depth of 11 to 13 m between the oxic epilimnion and the anoxic reduced hypolimnion, a stable, dense, and species-rich community of anaerobic phototrophic bacteria has been developed– among them *Chromatium okenii*. These phototophs can be seen due to the reddish-brownish color of the water. They thrive between the two layers, where they find ideal conditions for their development. However, these communities have to cope with low light intensities (only 0.5 to 5% of surface light) and high concentrations of sulfide. During anoxygenic photosynthesis, sulfide is oxidized to elemental sulfur and stored as tiny sulfur droplets inside the cell. *Chromatium okenii* is the largest member of the purple phototrophic community, with cell sizes of more than 10 µm. Cells can easily be identified under the microscope, although their biomass share is only a few percent. The most important members regarding biomass are green sulfur bacteria such as *Chlorobium clathratiforme*. The habitat of these phototrophic communities is in a spatial range of the water column where chemical gradients of e.g. oxygen, sulfate, sulfide, ammonium, and redox potential are changing within a scale of centimeters, requiring motility and the ability to respond quickly to changing environmental conditions.

Over all – although sometimes considered as inhospitable from our point of view– alpine ecosystems provide environmental conditions for "blooming" microbial communities which can be easily observed even with the "naked eye".

Figure 3: Water samples from Lake Cadagno (canton of Ticino, Switzerland) collected at different depths showing a distinct layer of phototrophic microorganisms at 11 m. Inserts: Diverse phototrophic community mainly consisting of purple and green sulfur bacteria, cyanobacteria, and diatoms, (left; 400x magnification); close-up of cells of *Chromatium okenii*, the largest member in the group of sulfur bacteria, with intracellular sulfur droplets (right; 1000x magnification).



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