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Purple non-sulfur photosynthetic bacteria monitor environmental stresses

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ABSTRACT

Heavy metal ion pollution and oxygen deficiency are major environmental risks for microorganisms in aqueous habitat. The potential of purple non-sulfur photosynthetic bacteria for biomonitoring and bioremediation was assessed by investigating the photosynthetic capacity in heavy metal contaminated environments. Cultures of bacterial strains *Rhodobacter sphaeroides*, *Rhodospirillum rubrum* and *Rubrivivax gelatinosus* were treated with heavy metal ions in micromolar (Hg^{2+}), submillimolar (Cr^{6+}) and millimolar (Pb^{2+}) concentration ranges. Functional assays (flash-induced absorption changes and bacteriochlorophyll fluorescence induction) and electron micrographs were taken to specify the harmful effects of pollution and to correlate to morphological changes of the membrane. The bacterial strains and functional tests showed differentiated responses to environmental stresses, revealing that diverse mechanisms of tolerance and/or resistance are involved. The microorganisms were vulnerable to the prompt effect of Pb^{2+}, showed weak tolerance to Hg^{2+} and proved to be tolerant to Cr^{6+}. The reaction center controlled electron transfer in *Rvx. gelatinosus* demonstrated the highest degree of resistance against heavy metal exposure.

1. Introduction

Microorganisms have to face with and accommodate to several stress factors of either natural or anthropogenic origins in their environment. The scientists have the task to work out useful applications in conservation of the environment including the protection of the biodiversity of aqueous habitats [1–5] and monitor and remediation of pollution in the environment [6–8].

Metal ions of environmental contamination may constitute one of the most important factors of toxicity. Essential metal ions of low concentrations play an integral role in the life processes of microorganisms: they function as catalysts for biochemical reactions, K^+ and Na^+ are required for maintenance of osmotic balance, transition metals like iron, copper, and nickel are involved in redox processes, magnesium and zinc stabilize various enzymes and DNA through electrostatic forces and iron, magnesium, nickel, and cobalt are part of complex molecules with a wide array of functions [9]. However, metals at high concentrations are toxic to microorganisms. The consequence of the metal ion stress is the nonspecific entrapment of the metal ions by binding sites present on the cellular surface (bioadsorption) [10–12] followed by transport through the cell wall and interaction with the metabolic cycle inside the cell [13–15]. The processes are not independent. The metabolic activity reduces the bioadsorption of the metal ions due to increased competition with other cations (e.g. protons) produced by the living cells [16,17] but in several other cases the cells prove to be more efficient in heavy metal binding [18,19]. The essential metals are displaced from their native binding sites or removed from ligand interactions. Nonessential metals bind with greater affinity to thiol-containing groups and oxygen sites than do essential metals [20,21]. Toxicity results from alterations in the conformational structure of nucleic acids, proteins and intracytoplasmic membrane system and in the function of key proteins (e.g. reaction center and antenna in photosynthetic bacteria [19]) and interference with oxidative phosphorylation and osmotic balance [21]. Bacteria can adapt to excess metals through a variety of chromosomal, transposon, and plasmid-mediated resistance systems. A number of different uptake and resistance mechanisms have been identified and reviewed [22–25].

Sensitive and selective detection of toxic chemical compounds and heavy metals is of significant importance for human health and the preservation and conservation of the environment. Microbial biosensors offer considerable advantages: they allow inexpensive and facile detection without complex equipment and

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provide flexibility for various analyses, and pre- and/or post-processes such as purification and separation are not required (see e.g. the microbial luminescence-based biosensors [26]). Heavy metals can be tracked by various spectroscopic methods. Atomic absorption spectroscopy and inductively coupled plasma atomic emission spectroscopy, can offer high sensitivity for Pb^{2+} detection [27]. Recent advances in new materials, particularly in nano- and bio-materials, have opened a new era of analytical techniques. Due to their unique electronic, physical, chemical and mechanical properties, nano- and bio-materials have been explored their extensive applications in electrochemistry [28].

In addition to metal ions, transition between aerobic and anaerobic conditions may create another environmental stress to the organisms. The living cells control the level of expression and the composition of their molecular machinery according to oxygen and redox conditions. This control involves several regulatory systems. However, the chances of survival of the organism would be exposed to high risk if the oxygen partial pressure changed beyond critical levels as a consequence of contamination of physical, chemical or biological origins. For example, its functioning in high oxygen tension could lead to the formation of reactive oxygen species (ROS), in particular singlet oxygen which is highly toxic for the cell.

The quality and quantity of the pollution of the habitat can be exactly determined by a broad variety of sophisticated physical and chemical methods. In some cases, however, biomonitoring systems can be used more directly and demonstratively to characterize the conditions. In this study, purple photosynthetic bacteria will be applied to monitor the properties of the aqueous habitat exposed to major environmental stresses of heavy metal ions and anaerobism. They are very versatile microorganisms and can grow under different conditions. They are capable of growth by aerobic and anaerobic respiration, fermentation, and anoxygenic photosynthesis. The intracytoplasmic membrane (ICM) in *Rba. sphaeroides* adapts to alterations in oxygen tension [29,30]. Due to this versatility, they provide an excellent biomonitoring system by detection of changes of both photosynthesis and membrane development [31]. They act as sponges for the heavy metals accumulated mainly in waterways as a consequence of anthropogenic activities [32,33,19]. Additionally, they have been proved as highly promising candidates for bioremediation [34].

Here, absorption and fluorescence induction of bacteriochlorophylls (BChl) and electrochromic changes of carotenoids in the photosynthetic membrane are used to track the changes of photosynthesis of bacteria exposed to different sorts of contaminations including deoxygenation and Hg^{2+}, Cr^{6+} and Pb^{2+} metal ions among the most toxic and harmful chemical agents in the environment. These methods are timesaving, economical and nondestructive. The BChl fluorescence is a particularly excellent marker of bacterial photosynthesis as a label-free biosensor and has no effect on the environment. It is measured by a low-cost and portable fluorometer [35]. The techniques are sensitive and are able to detect the harmful effect of contamination in the early phase of its development.

2. Materials and methods

2.1. Bacterial strains and growth conditions

The photosynthetic purple bacterium *Rhodospirillum* (*Rps.*) *rubrum* and *Rubrivivax* (*Rvx.*) *gelatinosus* were grown in Siström's medium [36] either in completely filled screw top vessels without oxygen (photoheterotrophic and anaerobic growth), or in half filled Erlenmeyer flasks sparged with a mixture of air and nitrogen provided by an air pump and a N_2 container, respectively (photoheterotrophic and semiaerobic growth). The oxygen-to-nitrogen volumetric ratio of the gas mixture was adjusted by calibrated flow rate meters (rotameters). The oxygen tension balanced with N_2 could be changed between 21% (air) and 0% (anaerobic condition). The medium was inoculated from a dense batch culture (1:100) and was illuminated by tungsten lamps that assured 13 W m^{-2} irradiance on the surface of the vessel as described earlier [37]. For experiments of bleaching and induction (greening) of the ICM under aerobic and anaerobic conditions, respectively, the illuminated culture was sampled for near-IR absorption spectra and BChl fluorescence measurements.

2.2. Chemicals

The cells were harvested at the exponential phase of the growth and bubbled by nitrogen for 15 min before measurements. Variable amounts of $HgCl_2$ (Hg^{2+}), K_2CrO_4 (Cr(VI)) and $Pb(CH_3COO)_2 * 3H_2O$ (Pb(II)-acetate) were added to the bacterial culture for heavy metal ion treatment [7]. These chemicals are highly soluble in aqueous solution under physiological conditions. 10 mM $HgCl_2$, 100 mM K_2CrO_4 and $Pb(CH_3COO)_2 * 3H_2O$ stock solutions were prepared freshly before the experiment. The durations of the Pb(II)-acetate, K_2CrO_4 and $HgCl_2$ treatments were prompt, 4 h and 5 h, respectively. The samples were kept illuminated under anaerobic condition during the treatment.

2.3. Electron microscopy

The bacteria were filtered with high grade filter paper and fixed with 4% glutaraldehyde. The specimens were embedded in Embed812 (EMS, USA) and 70-nm thin sections were prepared with an Ultracut S ultra-microtome (Leica, Austria). After staining with uranyl acetate and lead citrate, the sections were observed with a Phillips CM10 electron microscope (Eindhoven, the Netherlands) equipped with a Mega-view G2 digital camera and iTEM imaging analysis software (Olympus, Münster, Germany).

2.4. Steady-state absorption spectroscopy

The steady-state near infrared absorption spectra of the cells during the growth were recorded at room temperature by a single beam spectrophotometer (Thermo Spectronic Helios). The baselines were corrected for light scattering, and the spectra were decomposed into Gaussian components by least square Marquardt procedure to obtain the band area.

2.5. Flash-induced absorption kinetics

The kinetics of absorption changes of the whole cells induced by Xe flash were detected by a home-constructed spectrophotometer [37]. The electrochromic shift (ECS) of the carotenoids in the photosynthetic membrane were detected at 530 nm wavelength with reference to 510 nm wavelength.

2.6. Induction of BChl fluorescence

The induction of the BChl a fluorescence of intact cells was measured by a home built fluorimeter [35]. The light source was a laser diode (808 nm wavelength and 2 W light power) that produced rectangular shape of illumination and matched the 800 nm absorption band of the LH2 peripheral antenna of the cells. The BChl a fluorescence (centered at 900 nm in mature cells) was detected in the direction perpendicular to the actinic light beam with a near infrared sensitive, large area (diameter 10 mm) and high gain Si-avalanche photodiode (APD; model 394-70-72-581; Advanced