

Discovery of novel fluorochromes for use in plant studies

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Plant cells store neutral lipids such as triacylglycerols in distinct cytosolic organelles called oil bodies (OBs) (also referred to as lipid bodies/droplets, spherosomes or oleosomes). Although initially thought to be found only in oleogenic seeds and fruits, where they serve as fuel for the growth and development of seedlings prior to photosynthetic establishment, in recent years, studies have shown that OBs are quite ubiquitous. Their role extends from being static depots for carbon storage to stress response, lipid homeostasis, pathogen resistance, hormone metabolism and signaling and a specialized role in anther development. Plant OBs similar to their counterparts in yeast and mammals, are highly dynamic. Proteomic analyses have revealed that, there are a host of proteins which reside on the surface of these OBs and the exact content is changing under varying conditions. But a lot still remains to be uncovered in the area of OB protein and lipid composition as well as OB transport, mechanism of protein targeting, assembly and regulation. Live cell analysis is thus required to unravel the dynamic regulation of this important organelle.

Live-cell imaging offers a unique opportunity for investigating OB regulation. A large collection of imaging tools based on fluorescence is currently available. Varying colors of photostable genetically-encoded fluorescent proteins can be used in multiplexed tracking of protein remodeling on OBs. Photo-switchable fluorescent proteins as well as fluorescent timers allow quantitative assessment of OB protein dynamics. Fluorescent sensors, such as those based on fluorescence resonance energy transfer (FRET), can be applied to follow protein conformational changes or protein-protein interactions relevant to OBs. These tools, combined with the use of reliable OB markers, represent a versatile scheme for investigating OB biology. However, commercially available live cell dyes are limiting in their ability to penetrate cell walls and those that are permeable, in addition to other drawbacks such as photostability (BODIPY) and broad emission range (Nile Red), also restrict multicolor imaging, as most fluoresce in the green to red region of the visible spectrum.

In the present study, we report new fluorochromes as markers for OB in living plant cells. The fluorochromes, which are thalidomide analogs were in-house synthesized and were tested on live plant suspension cultured cells at various concentrations. The spectral emission range for the fluorochromes was identified and cell viability assays were also performed. We could also observe that the OBs remain highly mobile after staining with these fluorochromes, suggesting that the mobility dynamics were not affected significantly. We expect that these new chemicals will provide a novel approach for microscopy analyses of OBs in live plant cells.

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Bacterial symbionts enhance photo-fermentative hydrogen evolution of *Chlamydomonas* algae

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Chlamydomonas reinhardtii represents a well-established algae model system for biohydrogen generation. Two major methods are known for sustained *Chlamydomonas*-based hydrogen evolution. Hydrogen production using sulfur-deprived photoheterotrophic cultures of *Ch. reinhardtii* is the most widespread approach. Sulfur deprivation leads to complete anoxia via the inactivation of the photosynthetic system, thus, the extremely oxygen-sensitive algal Fe-hydrogenase will be able to become functional. Another algal hydrogen evolution approach is possible through dark fermentation. In dark and anaerobic conditions algae can catabolize endogenous carbohydrates or secondary metabolites generating organic acids, ethanol, CO₂ and H₂. The vast majority of algal hydrogen evolution studies were conducted using pure algae cultures. Our knowledge on the exploitability of algal-bacterial consortia in biohydrogen production is fairly short. Biohydrogen production capacity and growth rate of *Ch. reinhardtii* cc849 or the transgenic strain Iba co-cultured with *Bradyrhizobium japonicum* has been investigated. The sulfur-deprivation method together with *B. japonicum* inoculation resulted in enhanced rate of the oxygen consumption in the cultures, increased growth rate of algae and significantly improved hydrogen evolution rate. Our investigations aimed the elucidation of the nature and dynamics of algal hydrogen evolution observed in various algal-bacterial interactions.

The green algae *Chlamydomonas* sp. strain 549 was investigated for its hydrogen-evolution capability in algal-bacterial mixed cultures. Stable bacterial contaminations were identified during algae cultivation, the symbionts belonged to various genera, mostly *Brevundimonas* sp., *Rhodococcus* sp. and *Leifsonia* sp. All natural symbiotic partners enhanced fermentative algal hydrogen production. This phenomenon was not limited for the natural associations, increased algal hydrogen evolution was achieved by simple artificial algae-bacterium communities as well. Designed algal-bacterial co-cultures were tested in hydrogen evolution experiments, the highest hydrogen yield was obtained