Photosynthesis Without the Organisms: The Bacterial Chromatophores

Emiliano Altamura, Fabio Mavelli, Francesco Milano and Massimo Trotta

Abstract Anoxygenic photosynthetic bacteria are an extremely old form of life that inhabits planet Earth since approximately 3 Gya. They represent the model system for the far more complex photosynthetic organisms appeared later in time, i.e. cyanobacteria, algae and plants, and capable of performing the oxygenic photosynthesis. In this chapter we wish to present a short review of the cell architecture of one specific phototrophic bacterium, namely *Rhodobacter sphaeroides*, devoting particular attention to cytoplasmic membrane and its invagination originating under anoxygenic conditions and exposure to light. The structure and enzyme organization of the so-called chromatophores of two strains of *Rhodobacter sphaeroides* are presented, along with the isolation and purification procedures.

Keyword Photosynthetic bacteria

1 Introduction

Aliens landing on Earth will immediately realise that virtually the sole energy source for our planet is solar radiation. Next, they will realize that photosynthetic organisms are able to transduce solar radiation in other forms of energy readily viable for all life forms and, consequentially, sustain life on Earth. Their attention will be drawn toward awesome organisms like trees, bushes, or grass fields but will hardly find any interests in the small and often disregarded ecological niches where photosynthetic bacteria live and prosper.

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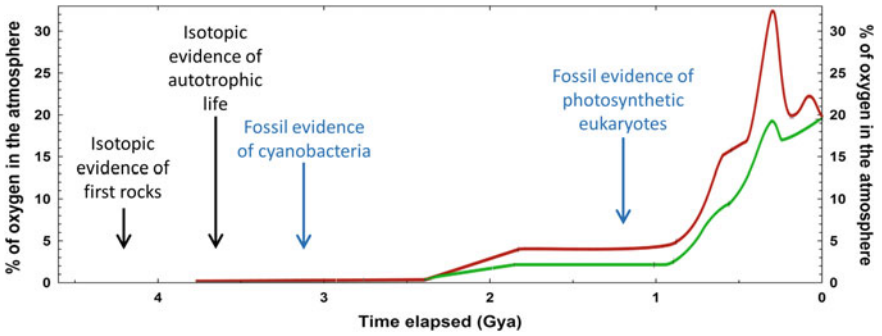

Fig. 1 The evolution of oxygen content in the atmosphere since the formation of planet Earth obtained by isotopic evidence. *Red* and *green lines* represent the higher and lower estimation of Oxygen concentration. Figure adapted from [3]

Photosynthetic bacteria "thrive in the anaerobic part of aquatic environments; from moist and muddy soils to ditches, pools, ponds, lake, sulphur springs, and marine habitats" [1, 2]. What a pity for an alien to miss them, since they will not discover some colourful spots and, most of all, they will not learn how photosynthesis works in anoxygenic conditions, i.e. the environmental conditions present on planet Earth in the first billion or so years after its formation [3, 4] (see Fig. 1).

Photosynthetic bacteria live in anaerobic environment and hence make no use of oxygen during the synthesis of the constituents of their bacterial cell. The overall chemical equation for the photosynthetic reaction taking place in anoxygenic bacteria was described by Van Niel [5] in the form:

$$CO_2 + 2H_2A + \text{Light Energy} \rightarrow [CH_2O] \ + \ 2A \ + \ H_2O \qquad (1)$$

where H_2A represents any electron donor molecule able to reduce carbon dioxide and eventually fix it. The above equation is quite general and remains valid for oxygenic photosynthetic organisms; in the latter case A represents oxygen.

2 Classification of Photosynthetic Bacteria

Photosynthetic bacteria are classified on the bases of their distinctive features in five main families, according to Fig. 2.

The first branching of the scheme deals with the electron donor species of Eq. 1: photosynthesis in cyanobacteria is based on two large enzymatic complexes called Photosystem I and II; the latter includes the so-called oxygen evolving complex, a protein subunit were electrons withdrawn from water are used in the bacteria metabolism. The overall reaction produces oxygen as by-product. The appearance of these microorganisms on Earth is responsible for the dramatic modification of the planet atmosphere shown in Fig. 1 and, as consequence, for the emergence of all oxygen-based life forms.

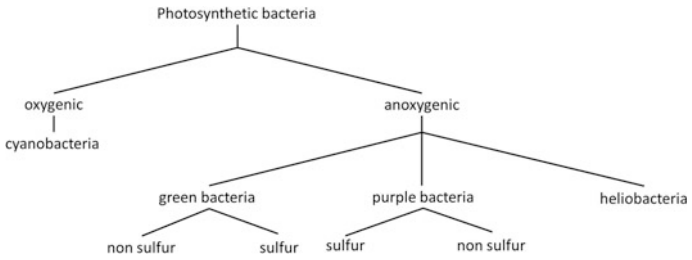

Fig. 2 Classification of the photosynthetic bacteria

Green phototrophs are gram-negative bacteria and fall in two categories; the first collects the nonmotile and obligate anaerobe collectively known as Chlorobiaceae. They depend on the presence of light and hydrogen sulphide to develop. Hydrogen sulphide is reduced to sulphate or, depending on the intensity of light and on the sulphide concentration, to sulphur that collects in small granules outside the bacterial cells. The second category is represented by the Chloroflexaceae, filamentous gliding bacteria, able to thrive under anoxygenic conditions, performing photosynthesis mostly using organic compounds as electron donor, and under oxygenic conditions. Both categories have a photosynthetic apparatus related to the photosystem I of the cyanobacteria.

Purple bacteria are gram-negative motile species and are classified according to their ability to oxidize sulphur to sulphate. In particular, all purple sulphur bacteria do show such capability, while the purple non sulphur photosynthetic bacteria cannot withdraw electrons from sulphur [6]. As result, purple non sulphur photosynthetic bacteria either directly oxidize sulphide to sulphate or, if sulphide reduction produces elementary sulphur, the latter is deposited outside the cells and left unused. Purple non sulphur photosynthetic bacteria exhibit a notable range of morphological, biochemical and metabolic diversity. Purple bacteria develop a photosynthetic apparatus related to the photosystem II of the cyanobacteria.

The first member in the group of Heliobacteria was identified in the soil of a garden in the campus of Indiana University, Bloominton in 1983 [7]. Remarkably this newly discovered *Halobacterium chlorum* belongs to the class of gram-positive bacteria, differently to all other phototrophs. Subsequently several other members of the Heliobacteria were identified and cultured in several laboratories. Heliobacteria grow photosynthetically over a limited range of organic compounds and present a photosynthetic apparatus related to the photosystem I of the cyanobacteria.

3 The Wild Type of the Bacterium *Rhodobacter sphaeroides*

The purple non sulphur bacteria *Rhodobacter* (*R.*) *sphaeroides*, main actor of the present article, is taxonomically placed in the α subgroup of Proteobacteria and exhibits a very versatile metabolic capability, being able to grow

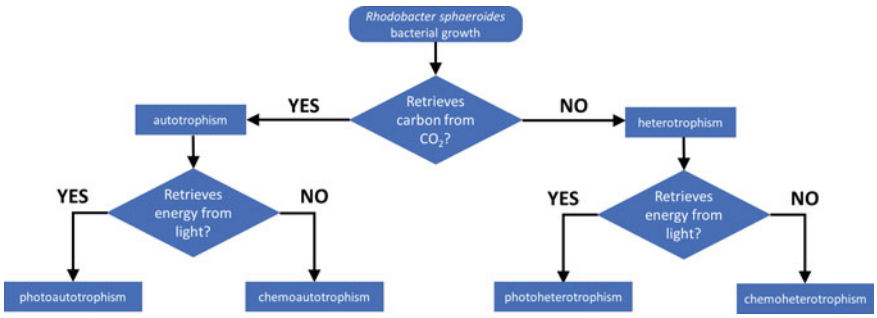

Fig. 3 A flowchart description of the metabolic versatility of the purple non sulphur bacterium *Rhodobacter sphaeroides*

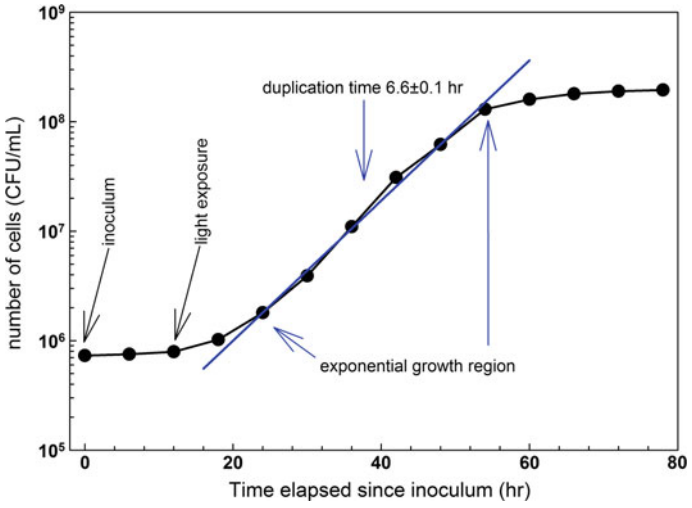

Fig. 4 Growth curve for a culture of *R. sphaeroides* wild type exposed to 10^4 lx in a closed system. Redrawn from [8]. The duplication time of the strain R26 (see text) is twice larger

photoautotrophically, photoheterotrophically, chemoautotrophically, and chemoheterotrophically according to the scheme in Fig. 3.

Rhodobacter sphaeroides is able to use a variety of electron donors and carbon sources during its photoheterotropic growth, namely acetate, citrate, ethanol, fructose, fumarate, gluconate, glucose, glycerol, lactate, malate, mannitol, pyruvate, succinate, tartrate, yeast extract.

Cell culture of the wild type strain of *R. sphaeroides* under illumination and kept in anoxygenic conditions grow photosynthetically. A typical culture requires an initial inoculum followed by a dark adaptation time that cells will use to consume all oxygen present in the liquid medium by respiration. Six to twelve hours after the inoculum, the cell culture is exposed to light (in the range between 1500 and 65,000 lx) and the photosynthetic growth initiates, entering in the exponential growth phase (see Fig. 4).

Photosynthetically grown cultures assume a deep red colour because of the presence of carotenoids molecules, are rod-shaped and their dimensions are 0.5–0.8 μm in width and 1–2 μm in length [9]. The cellular architecture shows the presence of several components:

- An external cell wall responsible for the maintaining the cellular shape and to ensure osmotic protection to the inner cell. The thickness of the cell wall ranges between 5–6 nm [10];
- An internal membrane that separates the periplasm from the cytoplasm. The thickness of inner membrane ranges between 7–8 nm [10].
- The intracytoplasmic membranes (ICM), invaginations of the membrane that forms exclusively under illumination and in anaerobic conditions and that contains the entire photosynthetic apparatus. These ICM can be obtained mechanically isolated from the cell membrane. Under opportune conditions, isolated ICM form closed spherical vesicles and can be purified to high degree. These small vesicles were firstly isolated and identified in 1952 and named chromatophores [11, 12] as they appear strongly coloured due to the presence of the photosynthetic pigments bacteriochlorophylls and carotenoids.
- A single flagellum medially located externally to the cell that allows to swim and change direction [13, 14]. A video showing swimming cells of *R. sphaeroides* is available at https://www.youtube.com/watch?v=wcJIK7EP7UE.

4 The Energy Conversion Apparatus

R. sphaeroides is capable to grow chemoheterotrophically in presence of oxygen. Reducing the partial pressure of O_2 to less than 3% (compared to 21% of the atmosphere) in the culture vessel and illuminating, imposes the transition toward photosynthetic metabolism, triggering the invagination of the cytoplasm membrane, to form the ICM, and the consequent synthesis and assembly of the photosynthetic apparatus. The yield in bacterial biomass is rather high, reaching 4 g of wet cells per litre of liquid medium. Once the mature photosynthetic growth conditions are reached, the cascade of reactions starting with the absorption of light and eventually ending with the synthesis of the energy-rich adenosine triphosphate (ATP) will be responsible for powering the entire cellular metabolism. The photon-to-ATP conversion apparatus is mainly based on a cluster of four main proteins sketched in Fig. 5:

- The light harvesting complexes (LHC), a set of two transmembrane multi-subunit proteins organized in circular shape, responsible of the light harvesting and its subsequent transfer to the following enzymatic complex;
- The photosynthetic reaction center (RC), a three-subunit transmembrane enzymatic complex, that functions as the photochemical core where photons are used to reduce ubiquinone-10 (coenzyme Q) into ubiquinol withdrawing electron from the redox protein cytochrome c_2 (cyt c_2) present in the periplasm. As consequence of these reactions, protons are sequestered from the cytoplasm and stored into the membrane;

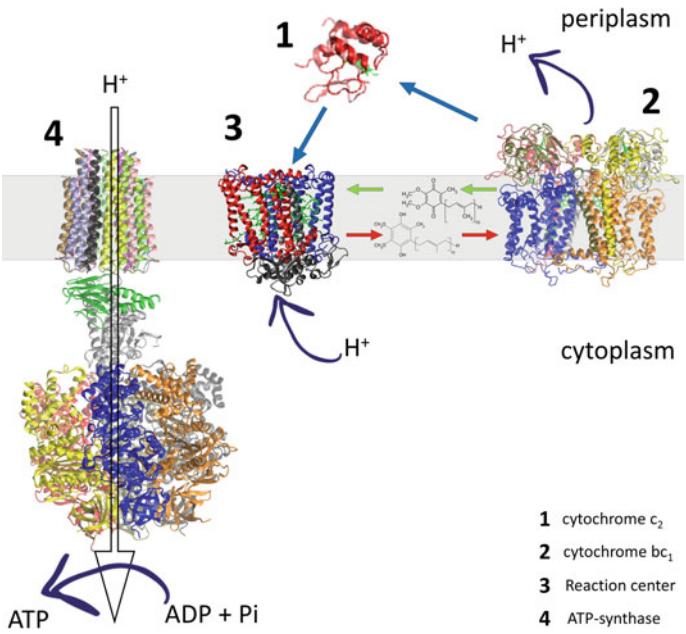

Fig. 5 Schematic representation of the main components of the photosynthetic and proton translocating apparats of a purple non sulphur bacterium. Sitting in the center of the IC membrane are shown the molecule of ubiquinone-10 and ubiquinol-10. Enzymes and molecules are drawn not in scale. Red arrows indicate the release of ubiquinol by the RC and its uptake by the cytochrome bc_1. *Green arrows* indicate the release of ubiquinone by the cytochrome and the subsequent uptake by the RC. *Blue arrows* indicate the transfer of one electron from the cytochrome bc_1 to the oxidized cyt c_2, and from the reduced cyt c_2 to the RC. For sake of clarity, the light harvesting complexes are omitted. See also [15, 16]

- The ubiquinol-cytochrome c oxidoreductase (cyt bc_1), a multi-subunit membrane enzyme, which uses the ubiquinol molecule produced by the RC to oxidize the cyt c_2. During this reactions protons are released into the periplasm;
- The net ΔpH established between periplasm and cytoplasm will be eventually used to drive the ATP-synthase, a multi-subunit enzyme having a large membrane and a soluble portions, which translocates protons back from the periplasm to the cytoplasm and synthesizes ATP from ADP and inorganic phosphate.

5 The Chromatophores

The contemporary exposure to anaerobic conditions and to light triggers the invagination of the cytoplasmic membrane and the development of the photosynthetic apparatus, which is exclusively located into the ICM. The isolation of the

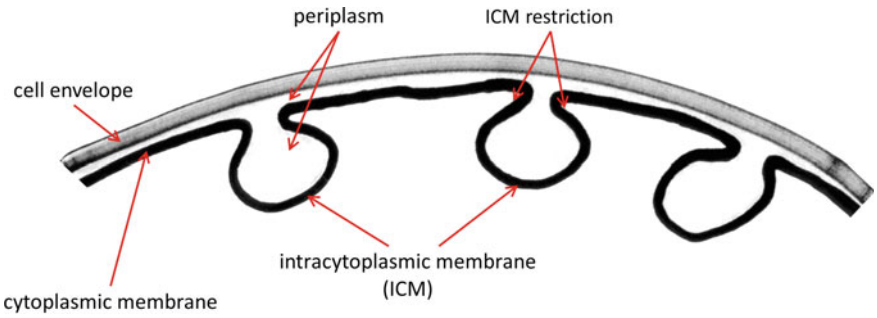

Fig. 6 Representation of a portion of the cell from *R. sphaeroides*

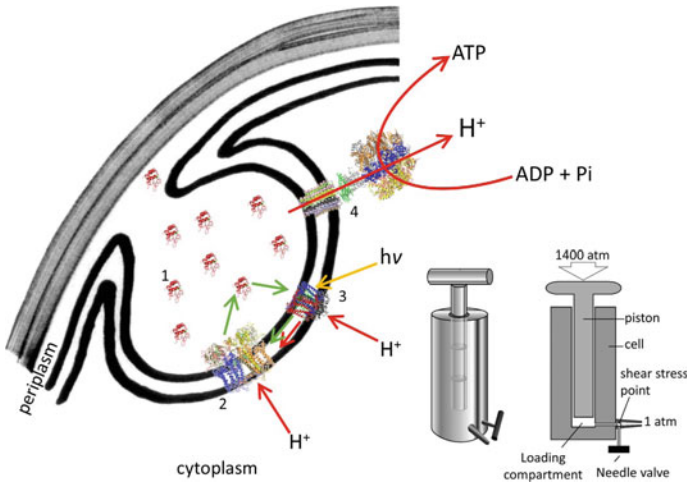

Fig. 7 Zoom of a single ICM. The orientation of the enzymatic complexes illustrated in Fig. 5 is shown. Cyt c_2 is confined in the periplasm, while the synthesis of ATP takes place in the cytoplasm. Numeration of enzymes is coincident to Fig. 5. In the inset is shown the French Pressure Cell used to disrupt the cell wall of photosynthetic bacteria retaining the activity of enzymes and producing chromatophores. This press consists of a cell with a central bore on the *top* and a *smaller* bore at the bottom. The bore at the *bottom* is sealed with a needle valve. The cell suspension is poured in the upper bore with the lower one closed. A piston is than inserted and a pressure of 1400 atm is applied. When the needle valve is opened, the suspension is squeezed through and experience a shear stress and a sudden decompressions. Bacterial cells are efficiently disrupted and the biological material retains full activity if all operations are performed at temperature lower than 4 °C

ICM requires a method to disrupt the bacterial cell wall that is accomplished mechanically by the use of a French Pressure Cell Press (see inset Fig. 7).

Luckily enough, the use of the cell press ensures the full activity of the enzymes present in the bacteria and, in the specific case of *R. sphaeroides*, results in the closure of the ICM that detach from the membrane and reorganize in closed vesicle,

the chromatophores. The sealing process most probably takes place at the neck of the invagination (see Fig. 6) [11, 12].

Upon cells disruption, a fraction of the cyt c_2 is lost while another portion is trapped within the chromatophores, which could be considered as the cytoplasm of the vesicle (Fig. 7). On the opposite, the ATP-synthase will synthesize ATP in the open solution, which can be considered the periplasm of the chromatophores. For these reasons, the energy transduction and proton translocation in chromatophores are often considered as inverted compared to the intact whole cell [17].

The chromatophores have a diameter varying from 60–100 nm and an internal volume of roughly 1.2 μL per mg of protein [17]. The overall content in reaction centers depends on the actual size of the vesicle; for vesicles having a diameter of 60 nm, Saphon et al. found an average of 20 reaction center and 2–3 ATP-synthase per chromatophore [18]. Both enzymes are fully active [19, 20].

The purification procedure of chromatophores present in literature requires 7 steps and is shown in Fig. 8.

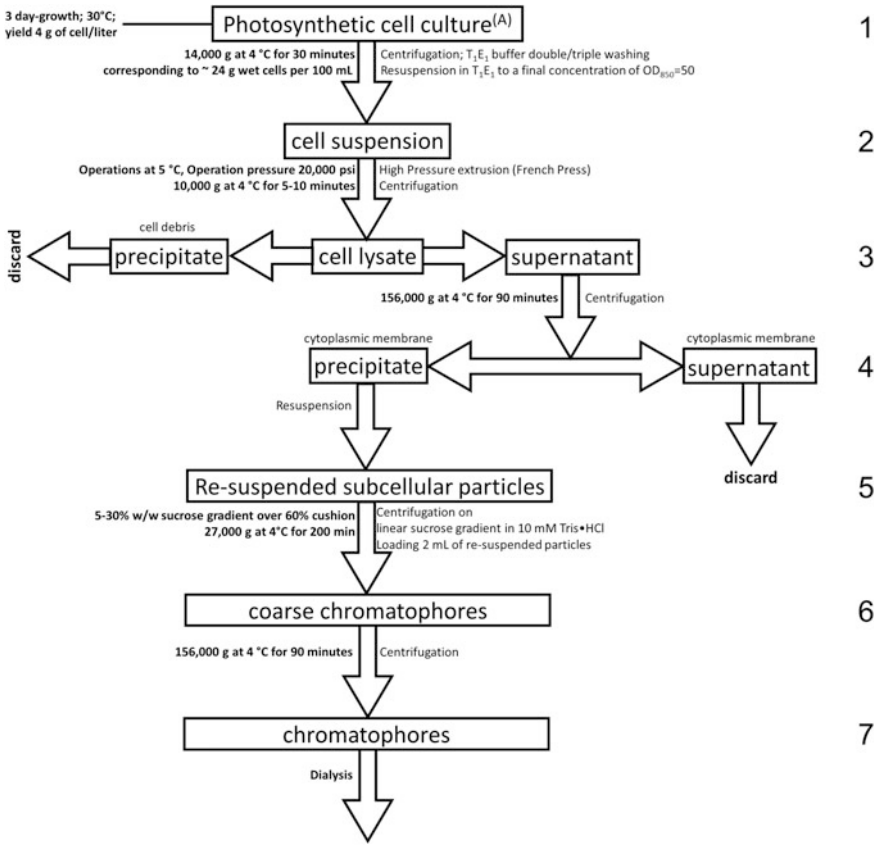

Fig. 8 The procedure for preparation and purification of chromatophores

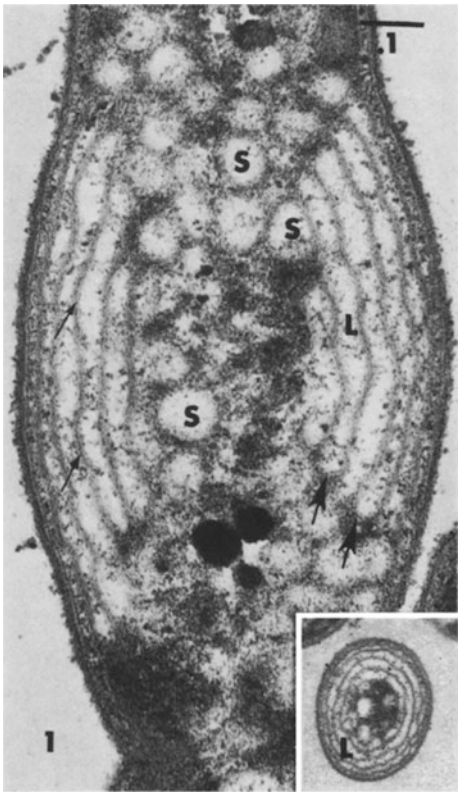

Fig. 9 Stained thin-section of a cell form R. sphaeroides R26 examined under microscope with a ×99,000 magnification. Two kinds of intracyoplasmic membranes are visible, the spherical (**S**) ones and the lamellar (**L**) ones. The measuring bar corresponds to 0.1 μm. The inset shows a 27,000 magnification factor. Reproduced from [21] with permission

6 The Strain R26 of *R. Sphaeroides*

A green coloured carotenoidless mutant of *R. sphaeroides* named R26 is also deeply investigated as model system in bacterial photosynthesis. Not much is available in literature on the ICM organization in this strain. Lommen and Takemoto [21] showed that, differently from the wild type described above, the ICM of *R. sphaeroides* R26 are not homogenously developed, but form a inhomogeneous assembly of round and lamellar investigations, as shown in Fig. 9.

7 Conclusions

Purified chromatophores represent a very intriguing system to be used in almost in-vivo investigations, including the photochemistry of the reaction center and the synthesis of ATP by ATP-synthase. Chromatophores offer to chase the possibility of isolate the sole and entire photosynthetic apparatus, getting rid of all the

"obnoxious" biological components not involved in the process of light transduction. The short review here presented cannot be considered in any way exhaustive. State-of-the-art investigations on the structure of ICM and on the architecture of the enzymatic organization within the chromatophores are present in literature [10, 22].

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