Chapter 16
The Nature and Function of Carotenoids in the Moderately Halophilic Bacterium *Halobacillus halophilus*

Saskia Köcher and Volker Müller

16.1 Introduction

With more than 750 different molecular structures (Britton et al. 2004) the carotenoids are the most important group of pigments in nature. In contrast to phototrophic organisms for which the presence of carotenoids as photoprotectants is essential, formation of carotenoids is found only in a few heterotrophic microorganisms (Goodwin 1980). However, it is striking that carotenoids are widely distributed in extremophiles. Examples are the thermophilic bacterium *Thermus thermophilus,* which synthesizes zeaxanthin and β-cryptoxanthin glucoside fatty acid esters that help in membrane stabilization (Yokoyama et al. 1995, 1996a), and the psychrotrophic bacterium *Arthrobacter agilis,* which increases its content in C50 bacterioruberin glycosides in response to low temperature (Fong et al. 2001). Bacterioruberin is also the major carotenoid found in the radioresistant bacterium *Rubrobacter radiotolerans* (Saito et al. 1994) and in halophilic archaea of the family *Halobacteriaceae* (Oren 2002). It is striking that saltern ponds are frequently colored orange to deep red. This is due to the presence of mainly the β-carotene-rich alga *Dunaliella salina* (Ben-Amotz and Avron 1990), to C50 carotenoids including bacterioruberin (Straub 1987) produced by halophilic archaea, or the C40 acyl glycoside produced by the extremely halophilic bacterium *Salinibacter ruber* (Lutnæs et al. 2002). Interestingly, also many endospore-forming bacteria isolated from saline environments like salt marshes contain carotenoids, whereas their non-halophilic relatives do not (Turner and Jervis 1963), indicating that carotenoids may play a crucial role in salt adaptation in these organisms.

All these observations suggest an important role and ecological function of carotenoids in marine environments. In this review, we concentrate on the type

S. Köcher • V. Müller (✉)
Molecular Microbiology & Bioenergetics, Institute of Molecular Biosciences, Johann Wolfgang Goethe University Frankfurt, Max-von-Laue-Strasse 9, 60438 Frankfurt am Main, Germany
e-mail: vmueller@bio.uni-frankfurt.de

A. Ventosa et al. (eds.), *Halophiles and Hypersaline Environments*,
DOI 10.1007/978-3-642-20198-1_16,

and function of carotenoids produced by halophilic microorganisms with focus on *Halobacillus halophilus* (formerly *Sporosarcina halophila*). This Gram-positive bacterium is moderately halophilic (Claus et al. 1983; Spring et al. 1996) and can tolerate up to 3.0 M sodium chloride. This osmotic burden is compensated by the accumulation of compatible solutes (Roeßler and Müller 1998, 2001; Müller and Saum 2005; Saum et al. 2006; Saum and Müller 2007, 2008a, b). Like several other halophilic and halotolerant bacteria (Aasen et al. 1969; Duc et al. 2006), *H. halophilus* is pigmented.

16.2 Structure and Biosynthesis of Carotenoids

Carotenoids are isoprenoids containing a characteristic polyene chain of conjugated double bonds. The two general groups of pigments are the hydrocarbons (carotenes) and oxygenated derivatives (xanthophylls). Most of them consist of 40 carbon atoms, but in a few species carotenoids with a variation of the carbon atom number are formed. Especially among non-photosynthetic halophilic bacteria and archaea also carotenoids with a C30 or C50 backbone were identified (Kelly et al. 1970; Marshall and Wilmoth 1981; Takaichi et al. 1997; Krubasik et al. 2001; Köcher et al. 2009). The most prominent example of C50 carotenoids are the straight chain derivatives of α-bacterioruberin (Fig. 16.1 (I)) found in halophilic archaea (Kelly et al. 1970). Other carotenoids found in halophilic organisms, as already mentioned above, are β-carotene (Fig. 16.1 (III)) produced by the alga *Dunaliella salina* or the C40 acyl glycoside salinixanthin (Fig. 16.1 (II)) which is synthesized by the extremely halophilic bacterium *Salinibacter ruber*. But there are also new marine isolates which produce structurally novel and rare carotenoids. These pigments include 2-hydroxyastaxanthin (Fig. 16.1 (IV)) from *Brevundimonas* sp. SD212 (Yokoyama et al. 1996b), saproxanthin (Fig. 16.1 (V)) and myxol (Fig. 16.1 (VI)) from marine strains of the *Flavobacteriaceae* (Shindo et al. 2007), and deoxymyxol 1′-glucoside and 4-ketodeoxymyxol 1′glucoside from *Gordonia terrae* AIST-1 (Takaichi et al. 2008).

Despite the structural diversity of carotenoids, a similar biosynthesis pathway is known for all carotenogenic organisms. The starting point for assembly of the carbon backbone of all carotenoids is the isomerization of the five-carbon compound isopentenyl pyrophosphate (IPP) to its allelic isomer dimethyallyl pyrophosphate (DMAPP) to generate geranyl pyrophosphate (GPP) (Fig. 16.2). This compound undergoes further condensation with IPP to form farnesyl pyrophosphate (FPP) catalyzed by FPP synthase. FPP is the main precursor for all known C30 carotenoids, which is condensed with another FPP molecule to form diapophytoene. For the synthesis of C40, C45 and C50 carotenoids one further addition of IPP to FPP to generate geranylgeranylpyrophosphate (GGPP) is catalyzed by a GGPP synthase. GGPP is then condensed to a second GGPP

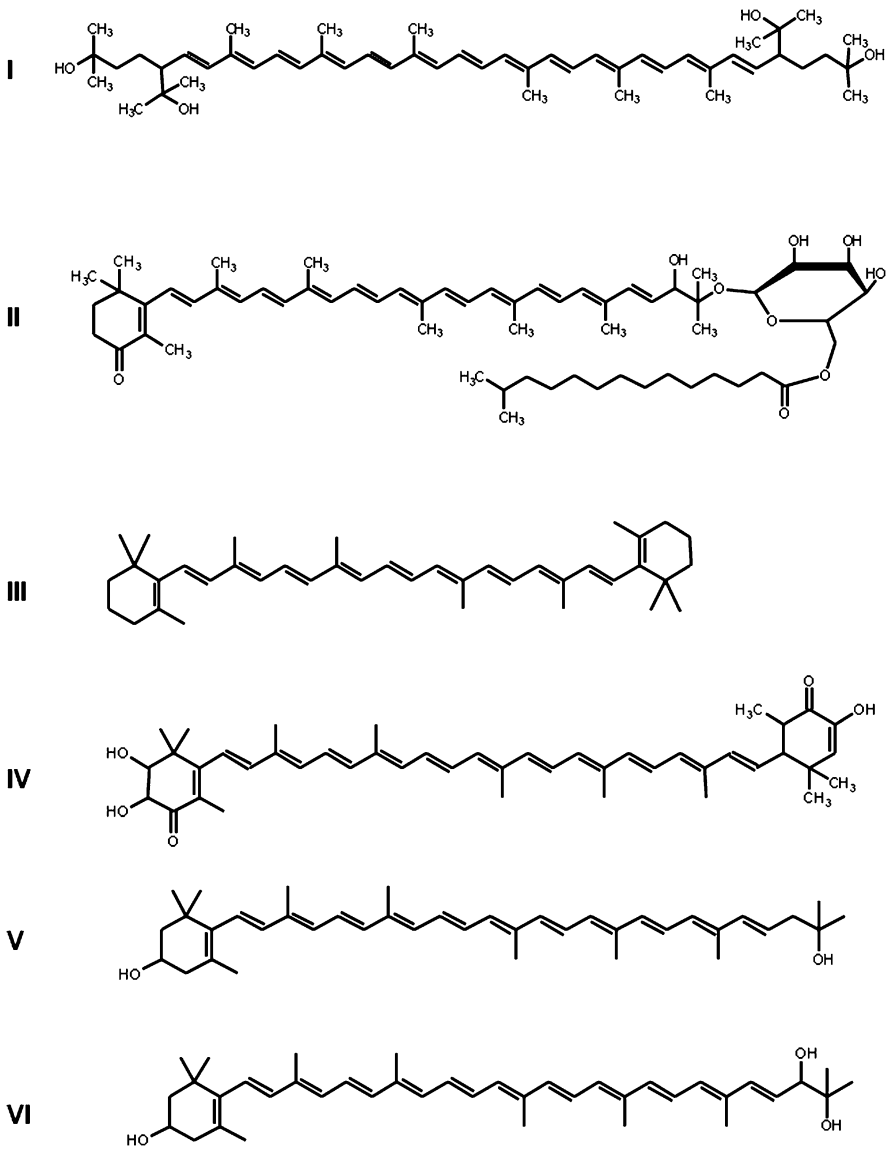

Fig. 16.1 Structure of carotenoids found in halophilic microorganisms. (I) α-bacterioruberin; (II) salinixanthin; (III) β-carotene; (IV) 2-hydroxyastaxanthin; (V) saproxanthin; (VI) myxol

molecule by the action of a phytoene synthase to form the C40 precursor phytoene. Phytoene as well diapophytoene are both colorless but they represent the first step of carotenoid biosynthesis. Next a diapophytoene/phytoene desaturase introduces double bonds into the molecules to yield diapo-/neurosporene, or diapo-/lycopene.

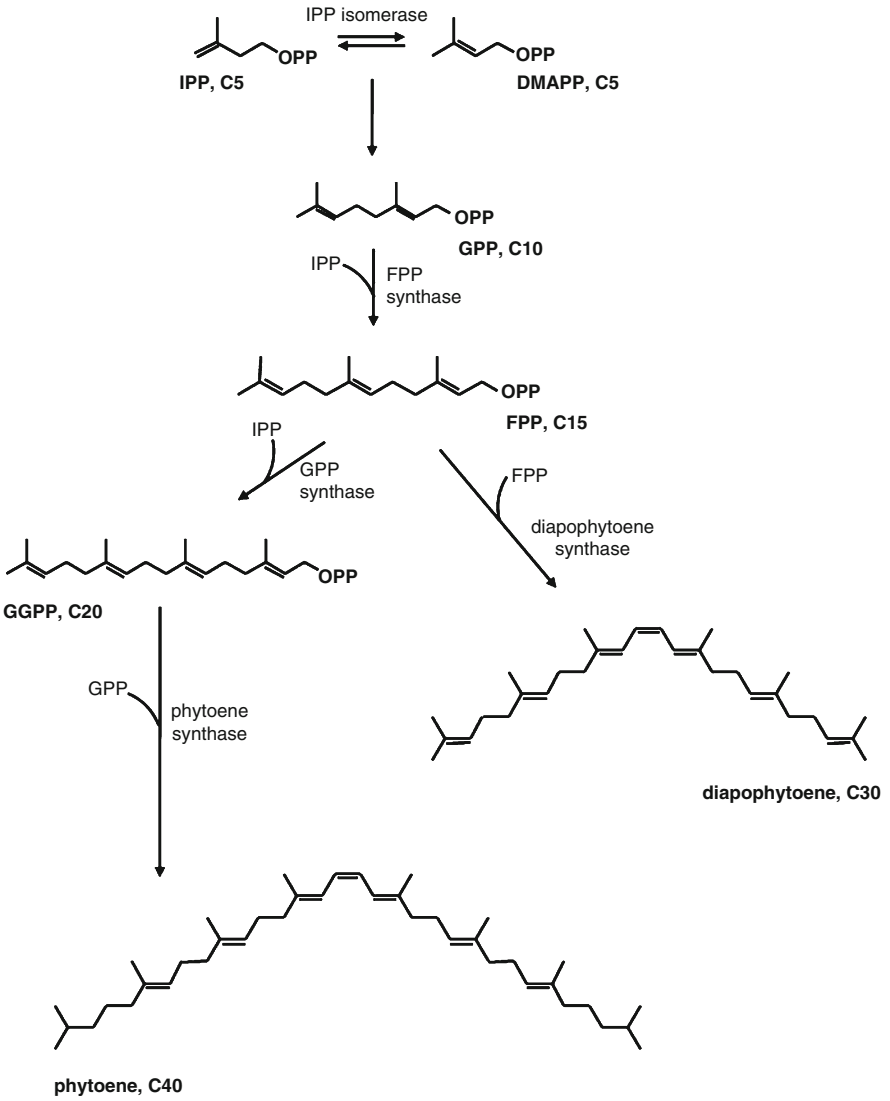

Fig. 16.2 Biosynthetic pathway of C30 and C40, C45 and C50 carotenoids. IPP, C5 (Isopentenyl pyrophosphate); DMAPP, C5 (dimethylallyl pyrophosphate); GPP, C10 (geranyl pyrophosphate); FPP, C15 (farnesyl pyrophosphate); GGPP, C20 (geranylgeranyl pyrophosphate); PP (pyrophosphate)

16.3 Structure of the Carotenoids Produced by *H. halophilus*

A first indication on the nature of the *H. halophilus* pigments was obtained by inhibition of carotene desaturation with diphenylamine (DPA). When cells were grown in the presence of this inhibitor the biosynthesis of colored carotenoids was

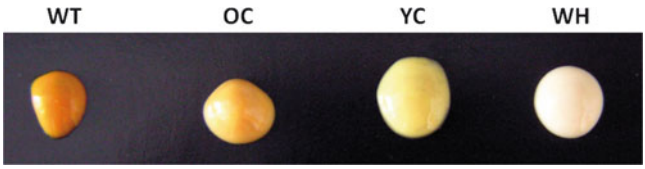

Fig. 16.3 Pigment mutants of *Halobacillus halophilus* generated by chemical mutagenesis with EMS. *WT* wild type; *OC* light orange pigmentation; *YC* yellow pigmentation; *WH* white pigmentation

blocked and they showed a white pigmentation. DPA prevents the insertion of double bonds into phytoene and diapophytoene (Sandmann and Fraser 1993). Biochemical analyses revealed that a C30 carotenoid was accumulated in *H. halophilus* and identified as 15-cis and all-trans 4,4′-diapophytoene (Köcher et al. 2009). The spectra showed the typical maxima at 275, 285 and 295 nm. In addition to inhibitors, pigment mutants are a useful tool to elucidate a carotenogenic pathway. Several mutants of *H. halophilus* were generated by chemical mutagenesis with EMS (ethyl methanesulfonate) (Köcher et al. 2009). They fell into three categories according to their pigmentation: light orange (mutant OC), bright yellow (mutant YC) and white (mutant WH) (Fig. 16.3). The carotenoids which accumulated were separated and identified. In OC, the prominent carotenoid had the same retention time and spectrum as diaponeurosporenoic acid, in WH the only detectable carotenoid was apo- or diapophytoene and in YC only apo-/diaponeurosporene was detectable.

The chemical nature of the carotenoid produced by *H. halophilus* was finally identified by HR-MS and NMR analyses. For this purpose the carotenoids were extracted three times with CH_2Cl_2:MeOH (1:1, [v/v]) and subjected to silica gel chromatography. The main compound was isolated and dissolved in CH_2Cl_2:MeOH (1:1, [v/v]). The carotenoid produced by *H. halophilus* could be identified as a C30 methyl glucosyl-3,4-dehydro-apo-8′-lycopenoate (Osawa et al. 2010) (Fig. 16.4a). This type of carotenoid is also produced by *Planococcus maritimus,* a marine Gram-positive bacterium (Shindo et al. 2008). To get a closer look on the biosynthesis of this new C30 carotenoid we analyzed the structure of the carotenoids of the OC mutant, which are most likely intermediates of the wild type carotenoid. And indeed two novel carotenoids, hydroxy-3,4-dehydro-apo-8′-lycopene and methyl hydroxy-3,4-dehydro-apo-8′-lycopenoate, could be identified (Fig. 16.4b).

16.4 Biosynthesis of a Unique C30 Carotenoid Produced by *H. halophilus*

Inspection of the genome of *H. halophilus* revealed two gene clusters probably involved in carotenoid biosynthesis (Fig. 16.5). Genes *crtNa*, *crtNc,* and *crtM* are arranged in the same orientation. *crtNa* and *crtNc* show an overlap of 3 bp,

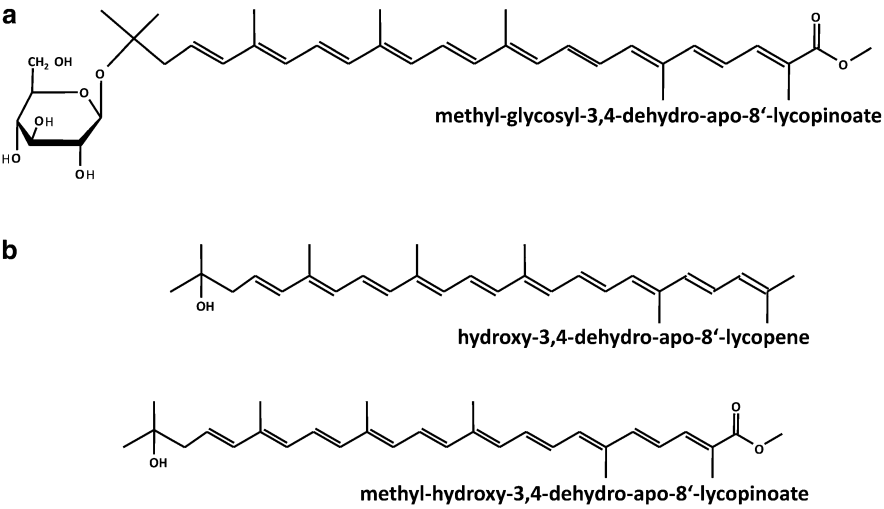

Fig. 16.4 The structure of carotenoids produced by the wild type and the mutant OC of *Halobacillus halophilus*

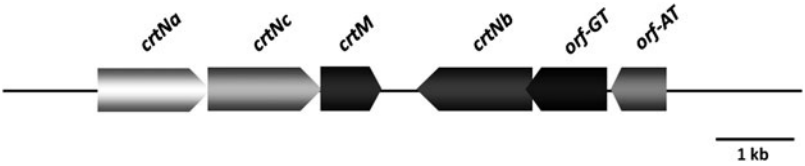

Fig. 16.5 Organization of carotenogenic genes in *Halobacillus halophilus*. *crtNa*: diapophytoene desaturase; *crtNc*: putative diapopyhtoene dehydrogenase; *crtM*: diapopyhtoene synthase; *crtNb*: putative diapolycopine oxidase; *orf-GT*: glucosyl transferase; and orf-AT: acyl transferase

and *crtNc* and *crtM* are separated by only 3 bp. 429 bp downstream of *crtM crtNb*, *orf-GT,* and *orf-AT* build a second cluster in the opposite orientation. They are separated by 21 bp between *orf-GT* and *orf-AT* and overlap 117 bp in the case of *crtNb* and *orf-GT*.

The arrangement of the putative carotenoid biosynthesis genes is indicative of their organization in transcriptional units. To prove the existence of such a polycistronic messenger, mRNA was isolated from *H. halophilus* cells and transcribed into cDNA. This cDNA was then used as a template in a PCR using primers that bridge the intergenic regions between *crtNa* and *crtNc*, between *crtNc* and *crtM*, between *crtNb* and *orf-GT* and between *orf-GT* and *orf-AT*. Products with the expected fragment sizes were obtained indicating that the cDNA originated from two different polycistronic mRNA. This result demonstrates that *crtNa*, *crtNc,* and *crtM* as well as *crtNb*, *orf-GT* and *orf-AT* are part of an operon, respectively.

Three of the genes products, designated CrtNa, CrtNb, and CrtNc, are similar to bacterial carotene desaturases. CrtNa has 48% amino acid identity (similarity 71%)

with that of a CrtN homologue in *Methylomonas* sp 16a. CrtNb had 31% amino acid identity (similarity 54%) with that of a 4,4′-diapolycopene oxidase in *Methylomonas* sp. 16a and 31% amino acid identity (similarity 54%) with the diapophytoene desaturase in *Psychroflexus torquis* ATCC 700755. We could also identify a third homologue of a diapophytoene desaturase. CrtNc is highly similar to the diapophytoene dehydrogenase of *Heliobacillus mobilis* (AAC84034) (55%). CrtM compares best to a putative phytoene/diapophytoene synthase that catalyses the condensation of two molecules of farnesyl-pyrophosphate. The protein of the latter has a length of 301 amino acids and is 55 and 44% identical (similarities, 71% and 61%) to CrtM from *Bacillus* sp. NRRL B-14911 and *Oceanobacillus iheyensis*, respectively. Besides these typical proteins for carotenoid biosynthesis, we could identify two additional proteins that appear to be involved in pigment biosynthesis in *H. halophilus*. The product of *orf-GT* with a deduced length of 369 amino acids is similar to a glycosyl transferase from *Chlorobaculum tepidum* TLS (57% similarity, 35% identity) involved in carotenoid synthesis. *orf-AT* is a putative acyltransferase with a deduced length of 236 amino acids. It is 57% similar (35% identity) to the protein found in *Exiguobacterium sibiricum* 255-15 (Köcher et al. 2009).

The biochemical and genetic data allowed us to postulate a biosynthesis pathway for the carotenoids in *H. halophilus*. It should be pointed out that the identified intermediates as well as methyl glucosyl-3,4-dehydro-apo-8′-lycopenoate are 8′-apo derivatives with asymmetrical arrangement of the methyl groups, unlike the 4,4′-diapo derivatives, which are typically synthesized with two molecules farnesyl diphosphate (Tao et al. 2005; Köcher et al. 2009). In addition, the identification of hydroxyl-3,4-dehydro-apo-8′lycopene without terminal end group oxidation excludes the possibility that apo-8′ products are derived by cleavage of C40 carotenoids at position 8′. Therefore, we propose that the biosynthesis pathway in *H. halophilus* initially starts with apo-8′-phytoene by the condensation of C20 geranylgeranyl pyrophosphate and C10 geranyl pyrophosphate, catalyzed by an apophytoene synthase, encoded by *crtM* (Fig. 16.6). In the next step, apo-8′-lycopene is produced by the action of CrtNa, an apophytoene desaturase. In the later biosynthesis steps towards methyl glucosyl-3,4-dehydro-apo-8′-lycopenoate hydroxyl-3,4-dehydro-apo-8′lycopene is glycosylated and oxidized to the corresponding aldehyde propably catalyzed by an CrtNb-like enzyme as it is described by Tao et al. (2005), and then oxidized with another oxidase, which may be encoded by crtNc.

16.5 Function of Carotenoids in Halophilic Microorganisms

Carotenoids play an important biological role not only in phototrophic but also in heterotrophic organisms. Especially for halophilic microorganisms the diversity of these functions which involve interactions with and without light is essential for surviving in such extreme habitats.

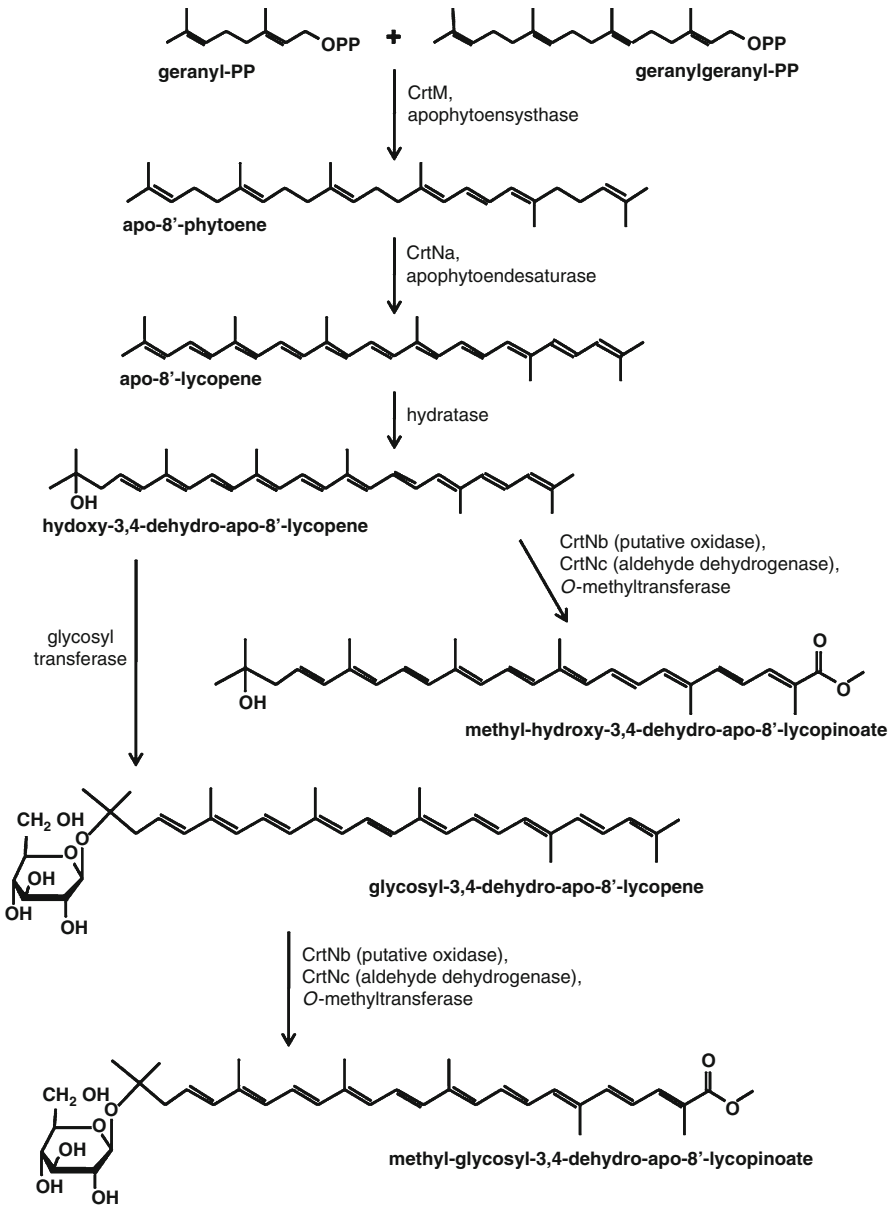

Fig. 16.6 Proposed carotenoid biosynthetic pathway in *Halobacillus halophilus*. Gene products catalyzing the individual reactions are indicated next to the *arrow*

16.5.1 Carotenoids and Light Harvesting

The most prominent function of carotenoids is their contribution to the harvesting of light energy by absorbing light and passing excitation energy on to (bacterio)

chlorophyll, thereby extending the wavelength range of the light that can be harvested. Much simpler retinal-based energy transducers are found in halophilic archaea, bacteriorhodopsin and archaerhodopsin. Absorption of light and the proton transport take place within one protein molecule containing a single retinal chromophore. This protein undergoes a cycle of reactions, resulting in the translocation of a proton from inside the cell to the outside and the generation of a transmembrane potential that is usable for ATP synthesis, ion transport, and cell motility. The reaction is initiated by light-induced isomerization of the chromophore from all-*trans* to 13-*cis* (Mathies et al. 1991) and involves changes in the pK_a values of the buried carboxyl groups (Balashov 2000), and small scale (Lanyi and Schobert 2004) and large-scale (Subramaniam et al. 2002) conformational changes of the protein. The early events involve spectral evolution, which has been interpreted as relaxation through a series of excited states and photoproducts (denoted as H, I, and J) (Sharkov et al. 1985; Gai et al. 1998; Kobayashi et al. 2001; Herbst et al. 2002; Kahan et al. 2007) that leads to the formation of the 13-*cis* photoproduct (Kochendoerfer and Mathies 1995). Another retinal protein, xanthorhodopsin (Balashov et al. 2005), from the cell membrane of the extremely halophilic eubacterium *S. ruber*, showed an unexpected association with a C40 carotenoid, salinixanthin (Lutnæs et al. 2002). This carotenoid was found to undergo large reversible absorption changes when the retinal chromophore was removed and replaced in the protein (Balashov et al. 2005). The carotenoid is bound to the protein in a 1:1 ratio. The action spectrum for proton transport indicated that light absorbed not only by the retinal but also by the carotenoid is utilized for proton transport, with 40% efficiency (Balashov et al. 2005). Subsequent studies using steady-state fluorescence measurements demonstrated that there indeed exists an efficient energy-transfer channel between salinixanthin and retinal (Balashov et al. 2008), making xanthorhodopsin the simplest antenna protein known so far.

16.5.2 Carotenoids as Photoprotectants

High on the list of functions attributed to carotenoids is "photoprotection" against photoxidative damage by quenching singlet oxygen as well as other harmful radicals that are formed when cells are illuminated (Demmig-Adams and Adams 2002). Light energy especially in combination with oxygen, can be very harmful, causing damage to cells via singlet oxygen ($^1O_2$) and oxidizing free radicals. Living organisms have evolved defenses to prevent or minimize this damage. Carotenoids are a major part of this defense and can be effective in a number of ways. Not only photosynthetic organisms have to prevent the formation and damaging effects of $^1O_2$, also in non-photosynthetic organisms, exogenous or endogenous sensitizers (e.g., porphyrin such as protoporphyrin IX and heme) can be excited and cause photooxidative stress by damaging DNA, protein, lipids and other cell components (Sies and Mehlhorn 1986). When cells are illuminated photosensitizer can form the

longer lived, lower energy triplet state. This in turn can undergo energy transfer to oxygen to form the highly reactive singlet oxygen. The triplet energy level of carotenoids is comparatively low, so that these pigments have the ability to accept energy from a triplet state sensitizer or from $^1O_2$, thereby preventing or minimizing damage. The triplet excited state of the carotenoid can then return to the ground state dissipating the energy excess as heat. Thus, the carotenoid acts as a catalyst in the deactivation of $^1O_2$ (Becker et al. 1991). It is known from many studies that light is the environmental factor with the most influence on carotenoid biosynthesis. The best understood examples for non-photosynthetic prokaryotes are the blue light induced carotenoid biosynthesis in *Myxococcus xanthus* (Burchard and Dworkin 1966) and the light induced pigment production in *Streptomyces coelicolor* (Takano et al. 2005). But also for halophilic organisms there is some evidence for the function of carotenoids to prevent photooxidative damage. For *Halobacterium salinarum* it could be shown that white mutants lacking bacterioruberins grown under high light intensities approaching those of full sunlight were outcompeted by the pigmented parent strain (Dundas and Larsen 1963). It has also been shown that bacterioruberin offers protection against ionizing radiation and UV both in vitro, reducing the occurrence of DNA strand breaks (Kottemann et al. 2005) and thymidine degradation (Saito et al. 1997), and in vivo with a decrease in survival of colorless *H. salinarum* mutants lacking bacterioruberin (Shahmohammadi et al. 1998). Also for *H. halophilus* the C30 carotenoid is essential for growth under oxidative stress (Köcher et al. 2009). Growth of non-stressed cells at conditions where the synthesis of colored carotenoids was inhibited was comparable to the non-inhibited culture. However, when oxidative stress was applied by the addition of duroquinone, the culture devoid of colored carotenoids did not grow. This result indicates that the carotenoids produced by *H. halophilus* cope with oxidative stress. This is in accordance with the fact that the main carotenoid produced by *H. halophilus* (methyl glucosyl-3,4-dehydro-apo-8′-lycopenoate) is a very potent antioxidant (Shindo et al. 2008). Also hydroxy-3,4-dehydro-apo-8′-lycopene and methyl hydroxy-3,4-dehydro-apo-8′-lycopenoate, the main carotenoids in the OC mutant, possess a potent antioxidative activity. But they were not as potent as the wild type carotenoid. This may be explained by their reduced hydrophobicity (Shimidzu et al. 1996). Further studies on the antioxidative activities of these carotenoids are in progress.

16.5.3 Carotenoids as Membrane Stabilizers

Membranes in extremophilic prokaryotes often contain polar carotenoids (Anwar et al. 1977; Yokoyama et al. 1995; Chattopadhyay et al. 1997; Jagannadham et al. 2000; Fong et al. 2001). All these organisms live under extreme conditions, and therefore, are dependent of solid membranes as an intact barrier to prevent an uncontrolled penetration of small molecules and ions.

Because of their lipophilic nature carotenoids are located within the hydrophobic inner membrane. The rod-like structure, the presence of polar end groups, and the molecular dimensions of a typical carotenoid, which match the thickness of the bilayer, are directly responsible for the localization and orientation of the molecule within the membrane and for effects on the membrane properties (Gruszecki 1999; Okulski et al. 2000; Sujak et al. 2000). Unpolar carotenoids like β-carotene are entirely lipophilic and remain within the hydrocarbon inner part of the bilayer. Resonance Raman spectroscopy or linear dichroism have indicated an orientation of these pigments with their long axis roughly parallel to the membrane surface (Johanson 1981). In contrast, polar dihydroxycarotenoids like zeaxanthin span across the bilayer with one polar end group associated with each polar surface (Gruszecki and Sielewiesiuk 1990, 1991). By EPR and NMR studies the effect of carotenoids on the structure and dynamics of the lipid membranes was determined (Gruszecki et al. 1999). For the polar carotenoids lutein and zeaxanthin it could be shown that they restrict the molecular motion of lipids and increase the rigidity of the membrane in its fluid state (Subczynski et al. 1992, 1993; Strzalka and Gruszecki 1994). It is concluded, therefore, that the carotenoid acts as a "rivet" mechanically reinforcing and strengthening the bilayer. This idea is supported by studies with *Acholeplasma laidlawii* whose membranes normally contain cholesterol, obtained from his host (Rottem and Markowitz 1979). When no cholesterol is available, the organism begins to synthesize polar carotenoids which take the place of cholesterol in the membranes. As mentioned above the carotenoid molecule is of suitable length to span across the membrane with polar substituents in the end groups associated with the polar outer faces of the bilayer and the hydrocarbon polyene chain in the hydrophobic lipid core. The length of carotenoids produced by the organisms is dependent in the thickness of the membrane of each organism, since it was shown that the incorporation ratio is higher when the molecular length of the carotenoid corresponds to the thickness of the phospholipid bilayer (Lazrak et al. 1987). Glycosylation of carotenoid end groups strengthens the association with the polar head groups of the bilayer. A good example is the acyclic xanthophylls bacterioruberin of *Halobacterium* species, which fits perfectly in the thick diphytanyl lipid membrane of these organisms. The incorporation of bacterioruberins in reconstituted *Halobacterium* lipid membranes was found to greatly increase their rigidity and to decrease their water permeability (Lazrak et al. 1987). For *Haloferax mediterranei*, which grows best in the presence of 1.5–2.5 M NaCl (Rodriguez-Valera et al. 1983), it was shown that with decreasing the NaCl concentrations to 1.0 M in the medium the carotenoid content increased ninefold compared to cells grown in the presence of 3.4 M NaCl (D'Souza et al. 1997). It was suggested, that carotenoids stabilize the membranes, and therefore, prevent the lysis of the cell at such low salt concentrations.

Acknowledgment Work from the authors' laboratory was supported by a fellowship from the Marianne and Dr. Fritz Walter Fisher-Stiftung to Saskia Köcher and by a grant from the Deutsche Forschungsgemeinschaft.

References

Aasen AJ, Francis GW, Liaaen-Jensen S (1969) Bacterial Carotenoids. XXIX. The carotenoids of two yellow halophilic cocci – including a new glycosidic methyl apo-lycopene. Acta Chem Scand 23:4–13

Anwar M, Khan TH, Prebble J, Zagalsky PF (1977) Membrane-bound carotenoid in *Micrococcus luteus* protects naphthoquinone from photodynamic action. Nature 270:538–540

Balashov SP (2000) Protonation reactions and their coupling in bacteriorhodopsin. Biochim Biophys Acta 1460:75–94

Balashov SP, Imasheva ES, Boichenko VA, Anton J, Wang JM, Lanyi JK (2005) Xanthorhodopsin: a proton pump with a light-harvesting carotenoid antenna. Science 309:2061–2064

Balashov SP, Imasheva ES, Wang JM, Lanyi JK (2008) Excitation energy-transfer and the relative orientation of retinal and carotenoid in xanthorhodopsin. Biophys J 95:2402–2414

Becker HGO, Böttcher H, Dietz F, Rehorek D, Roewer G, Schiller K, Timpe H-J (1991) Einführung in die Photochemie. Deutscher Verlag der Wissenschaften, Berlin

Ben-Amotz A, Avron M (1990) The biotechnology of cultivating the halotolerant alga *Dunaliella*. Trends Biotechnol 8:121–126

Britton G, Liaaen-Jensen S, Pfander H (2004) Handbook of carotenoids. Birkhäuser Verlag, Basel

Burchard RP, Dworkin M (1966) Light-induced lysis and carotenogenesis in *Myxococcus xanthus*. J Bacteriol 91:535–545

Chattopadhyay MK, Jagannadham MV, Vairamani M, Shivaji S (1997) Carotenoid pigments of an antarctic psychrotrophic bacterium *Micrococcus roseus*: temperature dependent biosynthesis, structure, and interaction with synthetic membranes. Biochem Biophys Res Commun 239: 85–90

Claus D, Fahmy F, Rolf HJ, Tosunoglu N (1983) *Sporosarcina halophila* sp. nov., an obligate, slightly halophilic bacterium from salt marsh soils. Syst Appl Microbiol 4:496–506

D'Souza SE, Altekar W, D'Souza SF (1997) Adaptive response of *Haloferax mediterranei* to low concentrations of NaCl (<20%) in the growth medium. Arch Microbiol 168:68–71

Demmig-Adams B, Adams WW 3rd (2002) Antioxidants in photosynthesis and human nutrition. Science 298:2149–2153

Duc LH, Fraser PD, Tam NK, Cutting SM (2006) Carotenoids present in halotolerant *Bacillus* spore formers. FEMS Microbiol Lett 255:215–224

Dundas ID, Larsen H (1963) A study on the killing by light of photosensitized cells of *Halobacterium salinarium*. Arch Mikrobiol 46:19–28

Fong NJ, Burgess ML, Barrow KD, Glenn DR (2001) Carotenoid accumulation in the psychrotrophic bacterium *Arthrobacter agilis* in response to thermal and salt stress. Appl Microbiol Biotechnol 56:750–756

Gai F, Hasson KC, McDonald JC, Anfinrud PA (1998) Chemical dynamics in proteins: the photoisomerization of retinal in bacteriorhodopsin. Science 279:1886–1891

Goodwin W (1980) The biochemnistry of carotenoids. Chapman and Hall, London

Gruszecki WI (1999) Carotenoids in membranes. In: Frank HA, Young AJ, Britton G, Cogdell R (eds) The Photochemistry of Carotenoids. Kluwer, Dordrecht, Netherlands, pp 363–379

Gruszecki WI, Sielewiesiuk J (1990) Orientation of xanthophylls in phosphatidylcholine multibilayers. Biochim Biophys Acta 1023:405–412

Gruszecki WI, Sielewiesiuk J (1991) Galactolipid multibilayers modified with xanthophylls: orientational and diffractometric studies. Biochim Biophys Acta 1069:21–26

Gruszecki WI, Sujak A, Strzalka K, Radunz A, Schmid GH (1999) Organisation of xanthophyll-lipid membranes studied by means of specific pigment antisera, spectrophotometry and monomolecular layer technique lutein versus zeaxanthin. Z Naturforsch C 54:517–525

Herbst J, Heyne K, Diller R (2002) Femtosecond infrared spectroscopy of bacteriorhodopsin chromophore isomerization. Science 297:822–825

Jagannadham MV, Chattopadhyay MK, Subbalakshmi C, Vairamani M, Narayanan K, Rao CM, Shivaji S (2000) Carotenoids of an antarctic psychrotolerant bacterium, *Sphingobacterium*

antarcticus, and a mesophilic bacterium, *Sphingobacterium multivorum*. Arch Microbiol 173: 418–424

Johanson CE (1981) Commonalities and differences among reinforcers. NIDA Res Monogr 37: 235–237

Kahan A, Nahmias O, Friedman N, Sheves M, Ruhman S (2007) Following photoinduced dynamics in bacteriorhodopsin with 7-fs impulsive vibrational spectroscopy. J Am Chem Soc 129:537–546

Kelly M, Norgård S, Liaaen-Jensen S (1970) Bacterial carotenoids. 31. C50-carotenoids 5. Carotenoids of *Halobacterium salinarium*, especially bacterioruberin. Acta Chem Scand 24:2169–2182

Kobayashi T, Saito T, Ohtani H (2001) Real-time spectroscopy of transition states in bacteriorhodopsin during retinal isomerization. Nature 414:531–534

Kochendoerfer GG, Mathies RA (1995) Ultrafast spectroscopy of rhodopsins-photochemistry at its best. Isr J Chem 35:211–226

Kottemann M, Kish A, Iloanusi C, Bjork S, DiRuggiero J (2005) Physiological responses of the halophilic archaeon *Halobacterium sp*. strain NRC1 to desiccation and gamma irradiation. Extremophiles 9:219–227

Köcher S, Breitenbach J, Müller V, Sandmann G (2009) Structure, function and biosynthesis of carotenoids in the moderately halophilic bacterium *Halobacillus halophilus*. Arch Microbiol 191: 95–104

Krubasik P, Takaichi S, Maoka T, Kobayashi M, Masamoto K, Sandmann G (2001) Detailed biosynthetic pathway to decaprenoxanthin diglucoside in *Corynebacterium glutamicum* and identification of novel intermediates. Arch Microbiol 176:217–223

Lanyi JK, Schobert B (2004) Local-global conformational coupling in a heptahelical membrane protein: transport mechanism from crystal structures of the nine states in the bacteriorhodopsin photocycle. Biochemistry 43:3–8

Lazrak T, Milon A, Wolff G, Albrecht AM, Miehe M, Ourisson G, Nakatani Y (1987) Comparison of the effects of inserted C40- and C50-terminally dihydroxylated carotenoids on the mechanical properties of various phospholipid vesicles. Biochim Biophys Acta 903:132–141

Lutnæs BF, Oren A, Liaaen-Jensen S (2002) New C(40)-carotenoid acyl glycoside as principal carotenoid in *Salinibacter ruber*, an extremely halophilic eubacterium. J Nat Prod 65: 1340–1343

Marshall JH, Wilmoth GJ (1981) Pigments of *Staphylococcus aureus*, a series of triterpenoid carotenoids. J Bacteriol 147:900–913

Mathies RA, Lin SW, Ames JB, Pollard WT (1991) From femtoseconds to biology: mechanism of bacteriorhodopsin's light-driven proton pump. Annu Rev Biophys Biophys Chem 20:491–518

Müller V, Saum SH (2005) The chloride regulon of *Halobacillus halophilus*: a novel regulatory network for salt perception and signal transduction in bacteria. In: Gunde-Cimerman N, Oren A, Plemenitaš A (eds) Adaptation to life at high salt concentrations in Archaea, Bacteria, and Eukarya. Springer, Dordrecht, pp 303–310

Okulski W, Sujak A, Gruszecki WI (2000) Dipalmitoylphosphatidylcholine membranes modified with zeaxanthin: numeric study of membrane organisation. Biochim Biophys Acta 1509: 216–228

Oren A (2002) Diversity of halophilic microorganisms: environments, phylogeny, physiology, and applications. J Ind Microbiol Biotechnol 28:56–63

Osawa A, Ishii Y, Sasamura N, Morita M, Köcher S, Müller V, Sandmann G, Shindo K (2010) Hydroxy-3,4-dehydro-apo-8′-lycopene and methyl hydroxy-3,4-dehydro-apo-8′-lycopenoate, novel C(30) carotenoids produced by a mutant of marine bacterium *Halobacillus halophilus*. J Antibiot (Tokyo) 63:291–295

Rodriguez-Valera F, Nieto JJ, Ruiz-Berraquero F (1983) Light as an energy source in continuous cultures of bacteriorhodopsin-containing halobacteria. Appl Environ Microbiol 45:868–871

Roeßler M, Müller V (1998) Quantitative and physiological analyses of chloride dependence of growth of *Halobacillus halophilus*. Appl Environ Microbiol 64:3813–3817

Roeßler M, Müller V (2001) Chloride dependence of glycine betaine transport in *Halobacillus halophilus*. FEBS Lett 489:125–128

Rottem S, Markowitz O (1979) Carotenoids acts as reinforcers of the *Acholeplasma laidlawii* lipid bilayer. J Bacteriol 140:944–948

Saito T, Terato H, Yamamoto O (1994) Pigments of *Rubrobacter radiotolerans*. Arch Microbiol 162:414–421

Saito T, Miyabe Y, Ide H, Yamamoto O (1997) Hydroxyl radical scavenging ability of bacterioruberin. Radiat Phys Chem 50:267–269

Sandmann G, Fraser PD (1993) Differential inhibition of phytoene desaturases from diverse origins and analysis of resistant cyanobacterial mutants. Z Naturforsch C 48c:307–311

Saum SH, Müller V (2007) Salinity-dependent switching of osmolyte strategies in a moderately halophilic bacterium: glutamate induces proline biosynthesis in *Halobacillus halophilus*. J Bacteriol 189:6968–6975

Saum SH, Müller V (2008a) Growth phase-dependent switch in osmolyte strategy in a moderate halophile: ectoine is a minor osmolyte but major stationary phase solute in *Halobacillus halophilus*. Environ Microbiol 10:716–726

Saum SH, Müller V (2008b) Regulation of osmoadaptation in the moderate halophile *Halobacillus halophilus*: chloride, glutamate and switching osmolyte strategies. Saline Systems 4:4

Saum SH, Sydow JF, Palm P, Pfeiffer F, Oesterhelt D, Müller V (2006) Biochemical and molecular characterization of the biosynthesis of glutamine and glutamate, two major compatible solutes in the moderately halophilic bacterium *Halobacillus halophilus*. J Bacteriol 188: 6808–6815

Shahmohammadi HR, Asgarani E, Terato H, Saito T, Ohyama Y, Gekko K, Yamamoto O, Ide H (1998) Protective roles of bacterioruberin and intracellular KCl in the resistance of *Halobacterium salinarium* against DNA-damaging agents. J Radiat Res (Tokyo) 39:251–262

Sharkov AV, Matveets Iu A, Chekalin SV, Pakulev AV (1985) Subpicosecond spectroscopy of bacteriorhodopsin. Dokl Akad Nauk SSSR 281:466–470

Shimidzu N, Goto M, Miki W (1996) Carotenoids as singlet oxygen quenchers in marine organisms. Fish Sci 62:134–137

Shindo K, Kikuta K, Suzuki A, Katsuta A, Kasai H, Yasumoto-Hirose M, Matsuo Y, Misawa N, Takaichi S (2007) Rare carotenoids, (3R)-saproxanthin and (3R,2′S)-myxol, isolated from novel marine bacteria (*Flavobacteriaceae*) and their antioxidative activities. Appl Microbiol Biotechnol 74:1350–1357

Shindo K, Endo M, Miyake Y, Wakasugi K, Morritt D, Fraser PD, Kasai H, Misawa N (2008) Methyl glucosyl-3,4-dehydro-apo-8′-lycopenoate, a novel antioxidative glyco-C(30)-carotenoic acid produced by a marine bacterium *Planococcus maritimus*. J Antibiot (Tokyo) 61:729–735

Sies H, Mehlhorn R (1986) Mutagenicity of nitroxide-free radicals. Arch Biochem Biophys 251: 393–396

Spring S, Ludwig W, Marquez MC, Ventosa A, Schleifer K-H (1996) *Halobacillus* gen. nov., with descriptions of *Halobacillus litoralis* sp. nov. and *Halobacilus trueperi* sp. nov., and transfer of *Sporosarcina halophila* to *Halobacillus halophilus* comb. nov. Int J Syst Bacteriol 46:492–496

Straub O (1987) Key of carotenoids. Birkhäuser Verlag, Basel

Strzalka K, Gruszecki WI (1994) Effect of β-carotene on structural and dynamic properties of model phosphatidylcholine membranes. I. An EPR spin label study. Biochim Biophys Acta 1194:138–142

Subczynski WK, Markowska E, Gruszecki WI, Sielewiesiuk J (1992) Effects of polar carotenoids on dimyristoylphosphatidylcholine membranes: a spin-label study. Biochim Biophys Acta 1105:97–108

Subczynski WK, Markowska E, Sielewiesiuk J (1993) Spin-label studies on phosphatidylcholine-polar carotenoid membranes: effects of alkyl-chain length and unsaturation. Biochim Biophys Acta 1150:173–181

Subramaniam S, Hirai T, Henderson R (2002) From structure to mechanism: electron crystallographic studies of bacteriorhodopsin. Philos Transact A Math Phys Eng Sci 360:859–874

Sujak A, Okulski W, Gruszecki WI (2000) Organization of xanthophyll pigments lutein and zeaxanthin in lipid membranes formed with dipalmitoylphosphatidylcholine. Biochim Biophys Acta 1509:255–263

Takaichi S, Inoue K, Akaike M, Kobayashi M, Oh-oka H, Madigan MT (1997) The major carotenoid in all known species of *Heliobacteria* is the C30 carotenoid 4,4′-diaponeurosporene, not neurosporene. Arch Microbiol 168:277–281

Takaichi S, Maoka T, Akimoto N, Carmona ML, Yamaoka Y (2008) Carotenoids in a *Corynebacterineae*, *Gordonia terrae* AIST-1: carotenoid glucosyl mycoloyl esters. Biosci Biotechnol Biochem 72:2615–2622

Takano H, Obitsu S, Beppu T, Ueda K (2005) Light-induced carotenogenesis in *Streptomyces coelicolor* A3(2): identification of an extracytoplasmic function sigma factor that directs photodependent transcription of the carotenoid biosynthesis gene cluster. J Bacteriol 187:1825–1832

Tao L, Schenzle A, Odom JM, Cheng Q (2005) Novel carotenoid oxidase involved in biosynthesis of 4,4′-diapolycopene dialdehyde. Appl Environ Microbiol 71:3294–3301

Turner M, Jervis DI (1963) The distribution of pigmented *Bacillus* species in saltmarsh and other saline and non-saline soils. Nova Hedwig 16:293–298

Yokoyama A, Sandmann G, Hoshino T, Adachi K, Sakai M, Shizuri Y (1995) Thermozeaxanthin, new carotenoid-glycoside-esters from thermophilic eubacterium *Thermus thermophilus*. Tetrahedron Lett 36:4901–4904

Yokoyama A, Shizuri Y, Hoshino T, Sandmann G (1996a) Thermocryptoxanthins: novel intermediates in the carotenoid biosynthetic pathway of *Thermus thermophilus*. Arch Microbiol 165:342–345

Yokoyama A, Miki W, Izumida H, Shizuri Y (1996b) New trihydroxy-keto-carotenoids isolated from an astaxanthin-producing marine bacterium. Biosci Biotechnol Biochem 60:200–203