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The effect of growth conditions on the light-harvesting apparatus in *Rhodopseudomonas acidophila*

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Abstract

The detailed effect on the light-harvesting apparatus of three different wild-type strains of *Rhodopseudomonas acidophila* in response to changes in both light-intensity and temperature have been investigated. In all three strains at high light-intensities (160 μmol s m^2 and above) the only LH2 antenna complex synthesised is the B800–850 complex. In strains 7050 and 7750 as the light-intensity is lowered the B800–850 complex is gradually replaced by another type of LH2 the B800–820 complex. However, at no light-intensities studied is this changeover complete when the cells are grown at 30 °C. If however, the light-intensity is lowered at temperatures below 25 °C with strain 7750 there is a complete replacement of the B800–850 complex by the B800–820 complex. At all light-intensities and temperatures tested, strain 10050 only synthesised the B800–850 complex. Strain 7050 also responded to changes in light-intensity by altering its carotenoid composition. At high light-intensity the major carotenoids were rhodopin and rhodopin-glucoside, while at low light-intensities the major ones were rhodopinal and rhodopinal-glucoside. This change in carotenoid content started to occur at rather higher light-intensities than the switchover from B800–850 to B800–820.

Introduction

Rhodopseudomonas acidophila is a purple non-sulphur photosynthetic bacterium that takes its name from the fact that it has an optimal pH for growth at 5.2 (Pfennig 1969). It is an example of a group of purple bacteria which is able to synthesise more than one type of variable (or LH2) light-harvesting complex depending on how the cells are grown (Thornber 1970, Hayashi et al. 1982, Heinemeyer and Schmidt 1983, Cogdell et al. 1983, Angerhofer et al. 1986, Tadros and Waterkamp 1989).

Three wild-type isolates of *Rps. acidophila* (strain 7050, 7750 and 10050) which show different phenotypic responses to changes in growth conditions are currently available in the Göttingen culture collection. Strain 7750 synthesises a typical B800–850 complex when it is grown at high light-intensities (Cogdell et al. 1983, Angerhofer et al. 1986), while at low light-intensities the B800–820 complex predominates. The carotenoid composition of this strain is rather independent of the light-intensity at which the cells are grown (Cogdell et al. 1983). The antenna complexes of strain 7050 show a similar pattern of regulation with respect to light-intensity to strain 7750, however at low light-intensities their carotenoid composition is markedly altered and the cells go from a red-brown colour to a deep purple colour (Heinemeyer and Schmidt 1983, Cogdell et al. 1983, Angerhofer et al. 1986).

Strain 10050 is only able to synthesise the B800–850 complex under any of the various growth conditions that we have tested so far. An additional temperature effect is seen with strain 7750 (R.A. Brunisholz, unpublished observation). If the growth temperature is reduced to between 20–24 °C (the normal growth temperature is 30 °C) then at moderate light-intensities only the B800–820 complex is synthesised. The phenotypic differences between the three strains of *Rps. acidophila* studied in this paper are stable over time.

We are interested in trying to understand the molecular details of how these environmental changes regulate the expression and assembly of these variable antenna complexes. However, in order to dissect out these molecular details we needed a much more precise description of these changes in the light-harvesting apparatus. In this paper, therefore, we present a detailed characterisation of how changes in the light-intensity and temperature at which each of these three wild-type strains are grown, affects the composition of their antenna system. A preliminary report of this study was presented at the IXth International Congress on Photosynthesis (Takaichi et al. 1993).

Materials and methods

Cells of *Rps. acidophila* strains 7750, 7050 and 10050 were grown photoheterotrophically in Pfennig's medium (Pfennig 1969). Special care was needed not to cross-contaminate each strain and so before each growth series was run fresh cultures were grown up from single colonies on agar plates and their phenotypes were checked. The cells were grown at different light-intensities at 30 °C in 100 ml bottles (to minimise shading effects), and at each light-intensity they were adapted to it by allowing them to grow for three separate transfers at that light-intensity and then cells were harvested in mid-log phase (OD 650 nm $\sim 0.5\ cm^{-1}$) after the fourth transfer. A similar growth regime was employed when the cells were grown at different temperatures except that the temperature was controlled by a thermostated water bath. The light-intensity was varied by growing the cells at set distances from a 60 watt incandescent bulb, and was measured with a United Detector Technology 40X light meter.

In order to get reliable absorption spectra of the membranes from the cells grown under the different growth conditions, the cells were harvested by centrifugation, resuspended in 100 mM KCl, 20 mM MES pH 6.8 and disrupted by passage through a French Press at 154 Mpa in the presence of a little DNase and $MgCl_2$ (Cogdell et al. 1983). The broken membranes were then collected by centrifugation as described in Cogdell et al. (1983) and resuspended in 20 mM Tris HCl pH 8.0. Usually the broken membranes were scattering and so these were solubilised with a 1:1 mixture of β-octylglucoside and laurylmaltoside at a Bchl *a*:detergent ratio of 1:35 w/w and this process was without any denaturing effect. Their absorption spectra were then recorded in a Shimadzu UV-2101 PC recording spectrophotometer.

The antenna complexes were quantitatively resolved as judged by the fact that there was very little or no free pigment into LH1/RC 'core' complexes and LH2 complexes by solubilisation in LDAO, followed by separation with sucrose gradient centrifugation as described in Dawkins et al. (1988).

The carotenoid composition of the cells and the isolated antenna complexes was determined by HPLC with a Hewlett Packard system, equipped with a Hewlett Packard 1040A photodiode array detector and a Waters μBondapak C18 column (Takaichi and Shimada 1992). The samples were freeze dried and the pigments were extracted with chloroform–methanol (3:1 v/v). The solution was filtered through a Hewlett Packard membrane filter and then applied to the column.

The separation was achieved with a combination of gradient and isocratic elution with 10 to 0% water in methanol. The carotenoids were monitored at 468 nm and 510 nm and were identified by their retention times and their absorption spectra (Takaichi and Shimada 1992). The following extinction coefficients adapted for this solvent system from Davies (1965), were used, for lycopene, rhodopin and rhodopinol at 468 nm $150\ mM^{-1}\ cm^{-1}$, for spilliroxanthin at 468 nm $105\ mM^{-1}\ cm^{-1}$, for rhodovibrin and

anhydrorhodovirbrin at 510 nm 122 mM^{-1} cm^{-1} and for rhodopinal at 510 nm 125 mM^{-1} cm^{-1} in each case in the HPLC eluant.

Rhodopin-glucoside was purified from strain 10050 and its identity was confirmed by H^1-NMR spectroscopy.

Results

The effect of growth at different light-intensities

Figure 1 shows a typical data set of the effect of growing strain 7050 at different light-intensities at 30 °C on the absorption spectra of its photosynthetic membranes. Two changes are clearly seen, one in the region of the Bchl *a* Qy absorptions in the NIR and one in the visible region where the carotenoids absorb (i.e. between 450 and 550 nm). At the highest light-intensities used in the present study (160 μmol s m^2) the NIR absorption spectrum only shows the presence of a B800–850 complex (the B875 complex is masked by the absorption of the B800–850 complexes). When the light-intensity is reduced there is a switchover to the B800–820 complex as has been previously described (Cogdell et al. 1983). This changeover in antenna types is a gradual process and can be visualised by plotting the ratio of the absorption at 855 nm to that at 800 nm (Fig. 2A). The changeover in antenna types is half-complete at ~40 μmol s m^2. However, it should be pointed out that at no light-intensity that we have tested is the B800–850 complex completely absent with this strain. When the B800–820 complex predominates the presence of the B875 complex is revealed since the spectral overlap between these two complexes is less than with the B800–850 complex. As well as a change in the NIR reflecting a shift in the major type of LH2 complex being synthesised strain 7050 changes its carotenoid composition with light-intensity (Heinemeyer and Schmidt 1983, Cogdell et al. 1983). This can be seen beautifully by eye. At a light-intensity of 160 μmol s m^2 the cells are red/brown in colour, while at light-intensities below 110 μmol s m^2 they are a deep purple colour. The change in carotenoid composition can be seen as the change in shape in the absorption spectra (Fig. 1) in the 450–550 nm region and clearly occurs before the changes in the NIR. This effect will be described in more detail below.

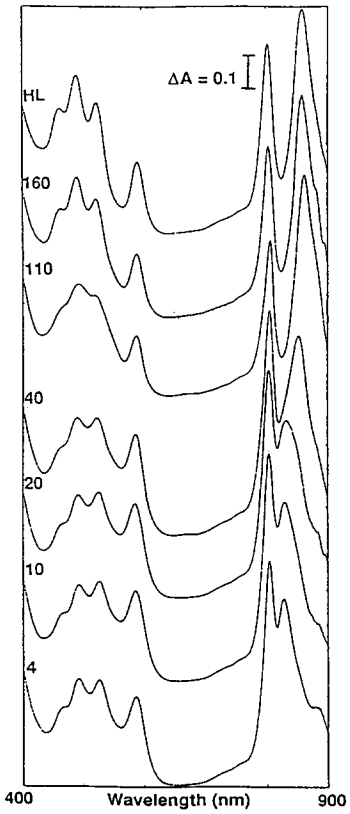

Fig. 1. Spectra of membranes from *Rps. acidophila* 7050 when it is grown at progressively lower light intensities. The spectra presented in this paper all follow the same pattern and have been offset for ease of viewing: the topmost spectrum was obtained when the strain was cultured under 'normal' growth room conditions, i.e., high light (approx. 160 μmol s m^2 at 30 °C) and serves as a benchmark from which any changes can be compared. The second topmost spectra is the first in the series and the conditions have been adjusted as far as possible to simulate the growth room, i.e., unless otherwise stated the top two spectra should be identical. The spectra follow in order from top to bottom; the left-hand numbers indicate the light intensity (μmol s m^2) or temperature (°C) at which that spectrum was obtained.

Membranes were prepared in a minimal volume of 20 mM Tris · HCl (pH 8.0), homogenised and standardised to an $OD_{590} = 25\ cm^{-1}$, prior to solubilisation, as described in the 'Methods'.

Figure 3 shows a typical set of results of the effect of growing strain 7750 at different light-

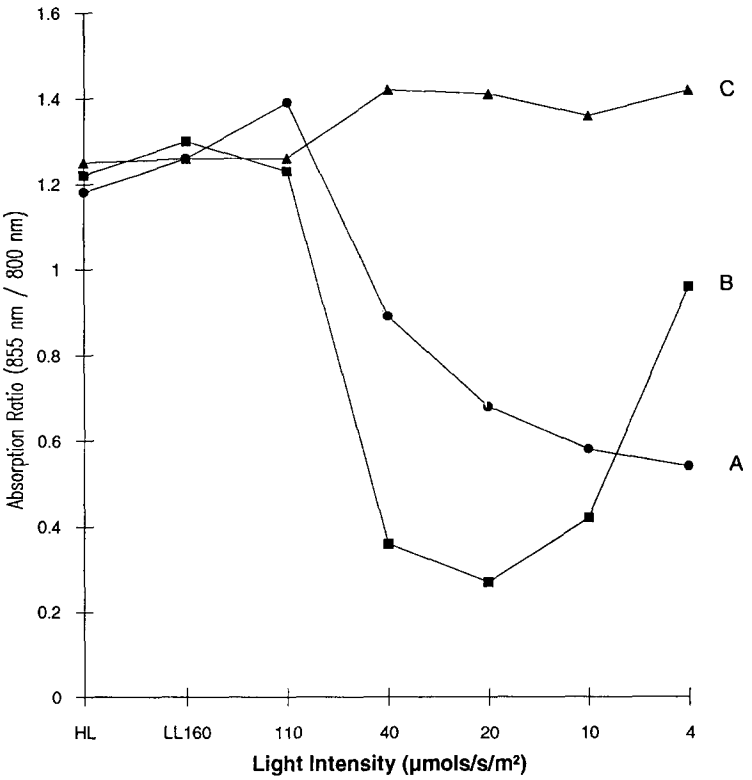

Fig. 2. Plot of the ratio of the 855 nm absorption to that at 800 nm as a function of light intensity. (A) *Rps. acidophila* 7050 (—●—), (B) *Rps. acidophila* 7750 (—■—) and (C) *Rps. acidophila* 10050 (—▲—). The data represent a typical set of results taken from one experiment. The exact changeover points varied slightly between different batches. In previous studies we have characterised the absorption spectra of the isolated antenna complexes from *Rps. acidophila* and the wavelength pair used in this figure was selected as the most appropriate to distinguish simply between the B800–850 and B800–820 complexes.

intensities at 30 °C. Light-intensity only affects this strain in the NIR region of the spectrum. At 160 μmol s m^2 the membranes contain the B800–850 complex (again the presence of the B875 complex is masked) and as the light-intensity is reduced there is a gradual switch over to B800–820 complexes. In our previous work with strain 7750 the changeover to the B800–820 complex (Cogdell et al. 1983) was less dramatic, however, this is probably due to using lower light-intensities here and the fact that in this present study we have fully adapted the cells for three transfers before we collected them for analysis. Interestingly at the lowest light-intensity the B800–850 complex started to reappear. The reason for this remains obscure. Just as with strain 7050 as the switchover between the B800–850 complex and the B800–820 complex occurs so the spectral overlap with the B875 complex is reduced, and the presence of this complex is revealed. Again, the switchover between these two LH2 complexes is clearly seen when the ratio of the absorbances at 800 and 855 nm are plotted against light-intensity (Fig. 2B).

When strain 10050 is grown at different light-intensities at 30 °C, there are no significant changes in the absorption spectra of the photosynthetic membranes (data not shown). At all light-intensities the cells are the same red/brown colour and the B800–850 complex is the only LH2 type synthesised. This is confirmed in Fig. 2C where the ratio of the absorbance at 800 nm to that at 855 nm remains constant at all light-intensities. This is why we use this strain as our source of B800–850 complexes for our crystallographic studies (Papiz et al. 1989), since there is

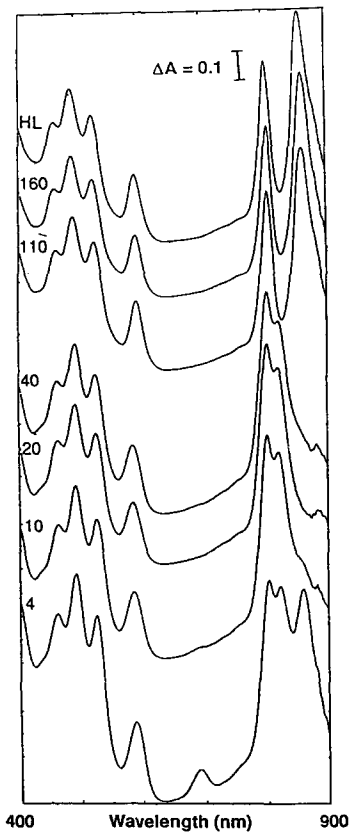

Fig. 3. Spectra of membranes from *Rps. acidophila* 7750 grown at progressively decreased light intensities. Conditions as described in Fig. 1.

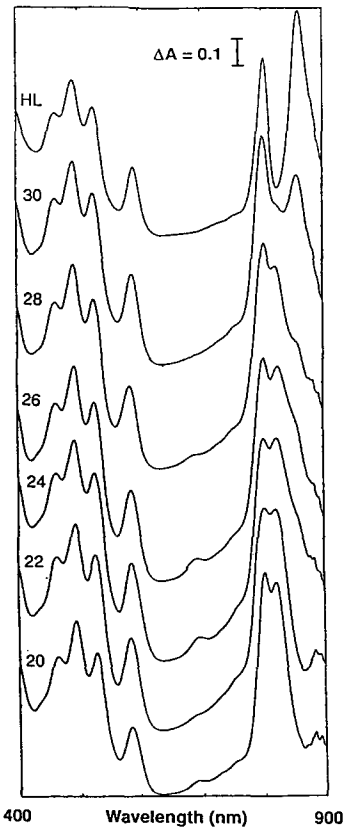

Fig. 4. Spectra of membranes from *Rps. acidophila* 7750 cultured at progressively decreased temperatures (°C) in conjunction with an intermediate light intensity (approx. 50 μmol s m^2). Conditions as described in Fig. 1.

no possibility of contamination with B800–820 complexes.

The effect of growth at different temperatures

An effect of growth temperature on the antenna composition is only seen with strain 7750 and moreover this effect also depends upon the light-intensity at which the cells are grown. At 160 μmol s m^2 temperature has no effect (data not shown), however at 50 μmol s m^2 lowering the temperature causes a dramatic and complete switchover from B800–850 to B800–820 complexes, such that below 24 °C the B800–850 complexes are no longer detectable (Figs. 4 and 5).

The effect of light-intensity upon the carotenoid composition of Rps. Acidophila *and the distribution of carotenoids between the different types of antenna complexes*

Cells of each strain of *Rps. acidophila* were grown at different light-intensities and their carotenoid composition was determined in each case. Again, each strain showed a different phenotypic response.

Strain 7050 shows a large variation in its total carotenoid content with light intensity (Fig. 6). As the light-intensity is reduced the content of rhodopin, rhodopin-glucoside and lycopene falls, while that of rhodopinal and rhodopinal-glucoside rises. This agrees well with the data of

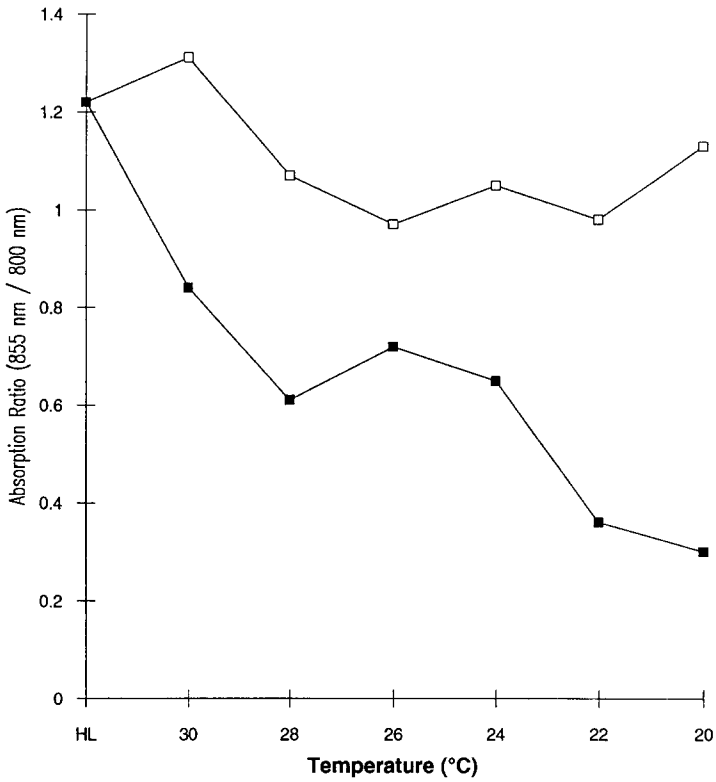

Fig. 5. Plot of the ratio of the 855 nm absorbtion to that at 800 nm for membranes of *Rps. acidophila* 7750 grown at progressively decreased temperatures and at light intensities of approx. 160 μmol s m^2 (—□—) or approx. 50 μmol s m^2 (—■—). The data represent a typical set of results taken from one experiment. The exact absorption ratio value varied slightly between different batches.

Heinemeyer and Schmidt (1983). The oxidation of rhodopin to rhodopinal does affect the chromophoric portion of the molecule and this is why the cells change their colour from red/brown to deep purple when they are grown at different light-intensities.

We investigated these changes further to see in which antenna complexes they occurred. Therefore for strain 7050 at each light-intensity their photosynthetic unit was solubilised and fractionated into B875-RC 'core' complexes and LH2 complexes, the data are summarised in Table 1. In this presentation we have not attempted to separate the B800–850 complexes from the B800–820 complexes since we do not yet have methods which will achieve this quantitatively. The LH1 and the LH2 complexes have different carotenoid compositions. At 'high light' LH1 mainly binds spirilloxanthin and anhydrorhodovibrin, while at 'low light' lycopene and rhodopin predominate. At all light-intensities the content of glucosides is very small. At 'high light' the LH2 complexes mainly contain rhodopin, rhodopin-glucoside and lycopene, then as the light-intensity is reduced the lycopene content remains high, but the rhodopin and rhodopin-glucoside are replaced by rhodopinal-glucoside.

Figure 7 shows the major carotenoids found in cells of strain 7750 grown at light-intensities of (a) 160 μmol s m^2 and (b) 10 μmol s m^2. The major change is a switch from rhodopin-glucoside at 'high light' to 'rhodopin' at 'low light'. These data are summarised in the histogram shown in Fig. 7, which is an average of three separate determinations. The change from rhodopin-glucoside to rhodopin does not affect the chromophoric portion of the molecule and this is why cells of strain 7750 do not change their colour when they are grown at different light-intensities.

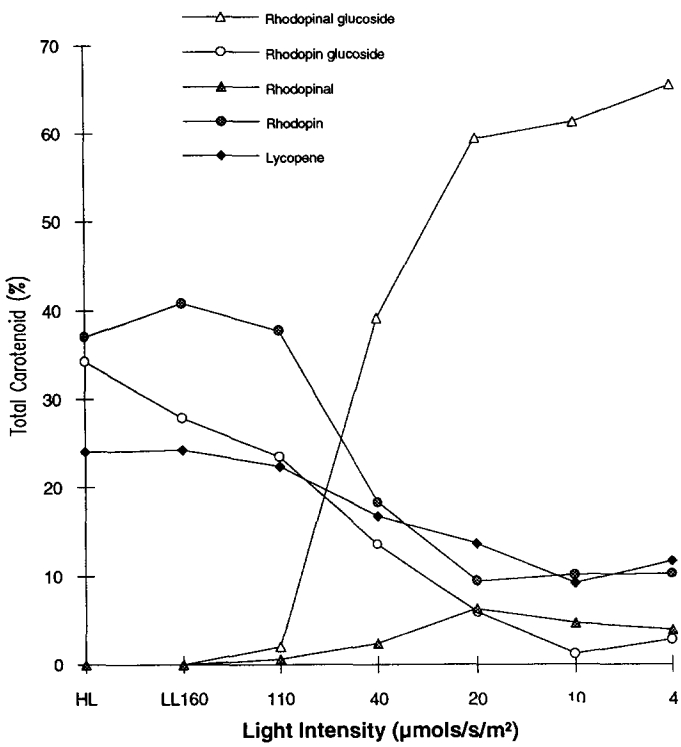

Fig. 6. Effect of light intensity on total carotenoid composition of cells of *Rps. acidophila* 7050. The data represent a typical set of results attained by HPLC analysis from one experiment; the exact percentage of each carotenoid present varied slightly between each batch.

Table 1. Effect of light intensity on the percentage carotenoid composition in the LH1 and LH2 antenna complexes from *Rps. acidophila* 7050. The exact numbers varied slightly by a few per cent from batch to batch but the major trends are seen consistently

	Light intensity (μmol s m^2)											
	LH1						LH2					
Carotenoid	160	110	75	45	35	10	160	110	75	45	35	10
Lycopene	8	9	7	5	9	60	26	47	36	23	28	48
Rhodopin	7	6	8	40	44	22	36	17	16	15	15	4
Rhodopinol									1	3	4	2
Rhodopinal									1	4	4	3
Dihydromethoxylycopene						10						1
Anhydrorhodovibrin (AHRV)	31	43	28	23	24	2	4	5	2	1	1	
Rhodovibrin	5	6	3	7	6							
Spirilloxanthin	48	35	50	14	12							
Rhodopin glucoside	1	1	2	11	5	5	31	16	11	12	10	3
Rodopinol glucoside								3	5	7	3	2
Rhodopinal glucoside							3	12	29	34	34	35
Total rhodopin	8	7	10	51	49	27	67	33	27	27	25	7
Total rhodopinal							3	12	30	38	38	38
Absorption ratio (855 nm/800 nm)							1.24	0.78	0.69	0.53	0.51	0.39

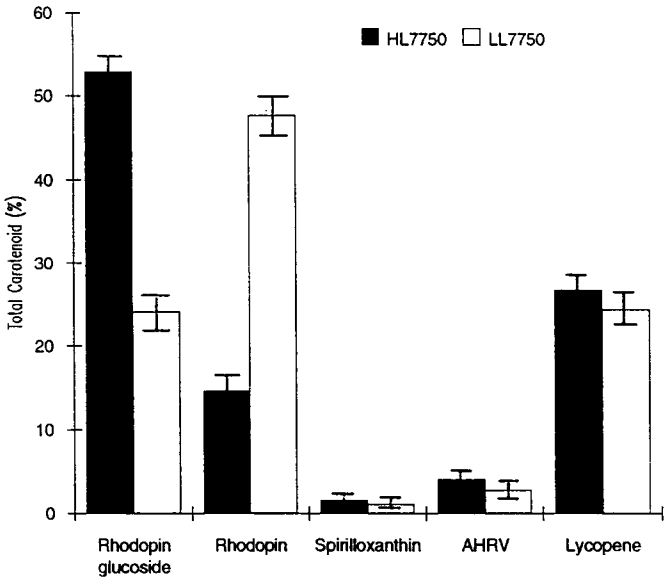

Fig. 7. Comparison of the total carotenoid composition of *Rps. acidophila* 7750 grown at high (160 μmol s m^2) and low (10 μmol s m^2) light intensities. Full details of the chromatography are given in the 'Methods'.

The carotenoid content of strain 10050 does not change with growth at different light-intensities (data not shown). In this case rhodopin-glucoside is the main carotenoid and accounts for more than 80% of the carotenoids present at all light-intensities tested.

Discussion

Once cells of *Rps. acidophila* are growing photosynthetically, anaerobically in the light, then clearly the light-intensity at which they are grown is the major influence on the composition of their photosynthetic unit (Cogdell et al. 1983, Angerhofer et al. 1986). However, as we have confirmed in detail here the exact nature of the response depends upon the phenotype of the strain under study. Strain 10050 behaves very much in the same way as the well-studied examples of *Rhodobacter sphaeroides* and *Rhodobacter capsulatus* (Aagaard and Sistrom, 1972, Schumacher and Drews 1979), where only the B800–850 type of LH2 complex can be synthesised. With strain 7750 light-intensity is able to regulate the ratio of B800–850 to B800–820 synthesis. In this case at the lower light-intensities reduction of the temperature to below 24 °C also completely switches off the production of the B800–850 complex. We have been able to show that in strain 7750 light-intensity is the major regulating influence and that the low temperature effect only occurs below light-intensities of ~50 μmol s m^2. Strain 7050 is also able to respond to light-intensity by regulating the ratio of B800–850 to B800–820. This strain, however, is unique in that its carotenoid composition is light-regulated as well (Heinemeyer and Schmidt 1983).

Our major aim for this study was to try to define the physiological response of these strains to growth and different light-intensities and temperatures in order to be able to use molecular methods to start to understand the molecular details of this regulatory process. A simple view of the differences between strain 10050 to the other two strains, could be that 10050 does not have the gene for the B800–820 complex. This is not the case, in an unpublished study we have shown that each strain studied here contains both types of structural genes, and so these phenotypic differences must reflect changes in the regulatory processes.

It is interesting to ask what benefits strain 7050 and 7750 may gain by the ability to synthesise the B800–820 complex at low light-intensities.

One answer may have been revealed by the recent study of Deinum et al. (1991). These authors showed that in the presence of the B800–820 complex absorbed light-energy was more effectively restricted to the reaction centres and that this could make light-harvesting more efficient under these conditions.

Acknowledgements

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