liquid solution are decreased by a factor of ~16 as compared to that of pure acetonitrile as a result of the absorption of the laser excitation and Raman light by the coal liquid. The background intensity observed in Fig. 1b is similar to that found for the high-purity ultraviolet-grade acetonitrile (Fig. 1a). Indeed, no fluorescence is observed even with excitation at shorter wavelengths (down to 220 nm). The lack of fluorescence interference in the Raman spectrum of this complex coal liquid sample is consistent with all our ultraviolet Raman studies of samples excited below 260 nm; significant fluorescence interference has not been observed for any sample yet examined.

The reason for this initially surprising result is that the smallest conjugated polyene or aromatic molecule to show significant fluorescence from its first excited singlet state is benzene, which shows fluorescence at a wavelength >260 nm. Larger aromatics show absorption bands below 260 nm but excitation into these bands results in fast internal conversion of this energy into the lowest energy singlet or triplet excited state, and fluorescence or phosphorescence occurs at much longer wavelengths. Other molecules or functional groups that have their lowest singlet states below 250 nm, such as butadiene and hexatriene, show extremely low fluorescence quantum yields (*7*). Thus, it appears that few if any organic compounds will show fluorescence in the spectral region below 260 nm. The obvious conclusion is that ultraviolet resonance Raman measurements will show little fluorescence interference as compared to conventional visible-wavelength Raman measurements. Thus, it is possible, perhaps likely, that complex samples such as coal liquids, biological samples, and complex organic reactions can be best studied by excitation in the ultraviolet; the only obvious complicating factor in ultraviolet Raman measurements is photochemical decomposition. However, photochemical decomposition did not pose a major problem for our aromatic amino acid studies (*8*) or for studies of benzene derivatives (*9*) or for our polycyclic aromatic hydrocarbon studies (*4*) or for excitation profile studies in the $n \rightarrow \pi^*$ transition of the carbonyl group in acetone (*10*). Thus, ultraviolet resonance Raman spectroscopy appears to be a uniquely sensitive and selective new technique for characterizing highly complex systems. These systems are obviously most tractable when a technique is used with high selectivity for one or a limited number of the species present. In the case of resonance Raman spectroscopy, the molecular selectivity can be tuned by changing the excitation wavelength.

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A Novel Photosynthetic Purple Bacterium Isolated from a Yellowstone Hot Spring

Abstract. *A thermophilic photosynthetic purple bacterium was isolated from the waters of a hot spring in Yellowstone National Park, Wyoming. The organism differs from all known purple bacteria in that it grows optimally at a temperature of about 50°C. The isolate contains bacteriochlorophyll a and grows autotrophically, oxidizing sulfide to elemental sulfur which is then stored as globules inside the cell. These properties indicate that the phototroph is a member of the Chromatiaceae (purple sulfur bacteria).*

A variety of photosynthetic prokaryotes inhabit thermal springs whose water temperatures may be as high as 73°C (*1*, *2*). The only photosynthetic bacterium that has been isolated from thermal environments is *Chloroflexus aurantiacus*, a filamentous bacterium that shares physiological and biochemical properties with both the purple and green anoxygenic phototrophs (*3–6*). The composition and location of pigments in *Chloroflexus*, however, more closely resemble those of the green bacteria, and the organism is generally considered to be a member of this group (*3–7*). Purple sulfur bacteria growing in thermal waters have been reported in a number of field observations (*2*, *8*, *9*), but the characteristics of pure cultures of these phototrophs have not been reported. I now describe the general properties of a truly thermophilic purple bacterium.

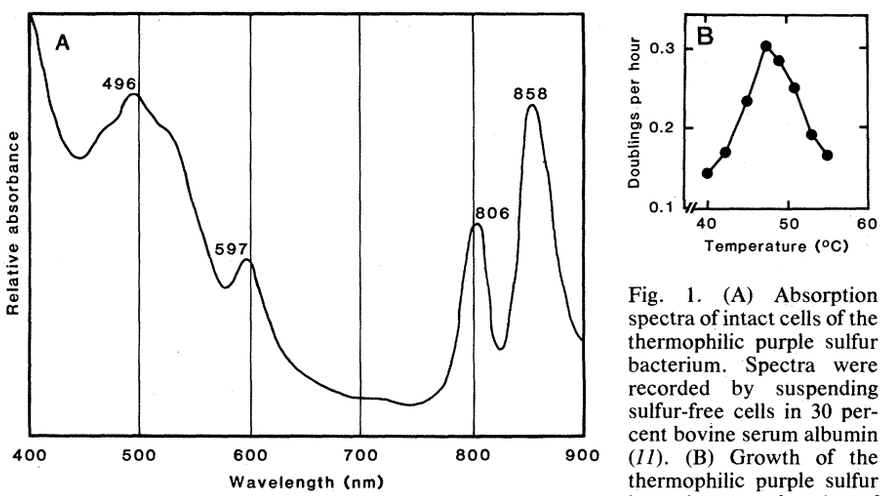

Fig. 1. (A) Absorption spectra of intact cells of the thermophilic purple sulfur bacterium. Spectra were recorded by suspending sulfur-free cells in 30 percent bovine serum albumin (*11*). (B) Growth of the thermophilic purple sulfur bacterium as a function of temperature. Cells were grown in the medium described (*16*). Cultures (250-ml bottles incubated in a water bath) were illuminated with 4300 lux of incandescent light. Cell suspensions were removed anaerobically at intervals with a sterile sampling device (*17*), and the number of cells was determined by direct microscopic counts with a Petroff-Hauser counting chamber. Growth rates (doublings per hour) were calculated from semilogarithmic plots of cell numbers against time.

Table 1. Differential properties of the most thermophilic representative of each group of prokaryotic phototrophs.

Organism	Type of bacteria	Type of photosynthesis	Major chlorophyll pigment	Temperatures (°C)		Reference
				Upper limit	Growth optimum	
Synechococcus lividus	Cyano-	Oxygenic	Chlorophyll a	72	63 to 67	(*18*)
Chloroflexus aurantiacus	Green	Anoxygenic	Bacteriochlorophyll c_s	65 to 70	55	(*1, 3, 4*)
Chromatium MC	Purple	Anoxygenic	Bacteriochlorophyll a	57 to 58	48 to 50	

Enrichment cultures for photosynthetic bacteria with a medium containing 0.05 percent (weight by volume) of sulfide and acetate (*p*H 7) were incubated anaerobically at 52°C and resulted in a dark red pigmented culture containing highly motile, rod-shaped bacteria. The inoculum for these enrichments was obtained from a reddish bacterial mat embedded in the carbonate sinter of a sulfide thermal spring (~45°C) located in the Upper Terrace area of Mammoth Hot Springs, Yellowstone National Park, Wyoming. No cyanobacteria were found in the water. From the original liquid enrichment, pure cultures of the bacterium were obtained by successive application of the agar shake-culture technique (*10*).

Absorption spectra (Fig. 1A) of intact cells (*11*) of the new organism showed maxima at 858, 806, and 597 nm, which is typical of bacteriochlorophyll a (*7*). Spectra of organic solvent extracts (*12*) of cells showed a major peak at 770 nm (data not shown), which clearly identifies the chlorophyll pigment as bacteriochlorophyll a (*12*). Absorption maxima of intact cells at 496 nm and the presence of shoulders at 526 and 468 nm (Fig. 1A) indicated the presence of carotenoids of the spirilloxanthin series (*7*). The organism was capable of autotrophic growth in a mineral salts medium containing up to 5 m*M* sulfide as the photosynthetic electron donor and carbon dioxide–bicarbonate as the sole source of carbon. The minimum generation time for cultures of the new organism was 3.3 hours (Fig. 1B); this generation time was obtained only at an incubation temperature of 48 to 50°C. The upper temperature limit for growth was approximately 58°C. Growth of the organism was accompanied by the oxidation of sulfide, and globules of elemental sulfur were stored within the cells (Fig. 2). Since storage of sulfur is a major taxonomic criterion for photosynthetic bacteria of the family Chromatiaceae (*7*), this thermophilic phototroph therefore represents a new species of the genus *Chromatium*.

A remarkable property of this organism (which I now refer to as *Chromatium* strain MC) is its thermophilic character. The isolation of *Chromatium* MC in pure culture represents the first report of a purple bacterium capable of growth above 50°C (*10*) and indicates that, among prokaryotic phototrophs, the thermophilic phenotype is not restricted to members of the green and cyanobacterial groups (Table 1). The maximum growth temperature of about 57°C determined experimentally for *Chromatium* MC is in good agreement with field observations of organisms similar to *Chromatium* in thermal springs in Yellowstone and Oregon at temperatures below 60°C (*2, 9*). Although *Chromatium* MC is not as tolerant to such temperatures as *Chloroflexus* or the cyanobacterium *Synechococcus lividus* (see Table 1), the optimum temperature for the thermophilic *Chromatium* is at least 20°C above that for *Chromatium vinosum* strain D (*13*), the species that bears the closest morphological resemblance to the thermophilic strain. Comparisons of strains D and MC, however, have revealed a number of other differences. Unlike *Chromatium* D (*13*), strain MC has an obligate requirement for sulfide and is unable to grow phototrophically with organic compounds as sole sources of carbon [certain other species of *Chromatium*, most notably the large cell species such as *C. okenii*, also require sulfide for growth (*7*)]. In addition, the major carotenoid of strain MC is not spirilloxanthin or rhodopsin, as is the case in various strains of *C. vinosum* (*14*). Instead its major carotenoid is rhodovibrin [or a compound similar to rhodovibrin (*15*)], which is present only in trace amounts in *Chromatium* D and is totally absent from other strains of *C. vinosum* (*14*).

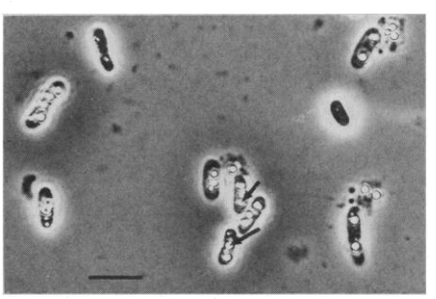

Fig. 2. Phase-contrast photomicrograph of cells of *Chromatium* MC. Cells were grown at 48°C in the medium described (*16*). Arrows indicate intracellular sulfur globules. Scale bar is 2 μm.

The characteristics of strain MC indicate that it is phylogenetically related to mesophilic *Chromatium* species but that it represents an evolutionary branch that has adapted to a thermal environment. Although only a single strain has been obtained in pure culture thus far, thermophilic chromatia may be present in other thermal regions. For example, enrichment cultures established in my laboratory with mat material collected from a New Mexico hot spring (47° to 48°C) have also yielded thermophilic purple sulfur bacteria that resemble *Chromatium* MC. Thermophilic purple bacteria may therefore have an ecological effect as primary producers in nonacidic hot springs containing sulfide, especially in springs where the sulfide concentration prevents the development of cyanobacteria. The discovery of *Chromatium* MC should make available a source of heat-stable pigment-protein complexes and membranes from a purple bacterium; such material has been available only from the taxonomically distinct green photosynthetic bacterium *Chloroflexus* (*5, 6*).

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Observed Ozone Response to Variations in Solar Ultraviolet Radiation

Abstract. *During the winter of 1979, the solar ultraviolet irradiance varied with a period of 13.5 days and an amplitude of 1 percent. The zonal mean ozone values in the tropics varied with the solar irradiance, with an amplitude of 0.25 to 0.60 percent. This observation agrees with earlier calculations, although the response may be overestimated. These results imply changes in ozone at an altitude of 48 kilometers of up to 12 percent over an 11-year solar cycle. Interpretation of ozone changes in the upper stratosphere will require measurements of solar ultraviolet radiation at wavelengths near 200 nanometers.*

The composition of the stratosphere, including the concentration of ozone (O_3), is controlled by photochemical processes that are driven by solar radiation (*1*). Humphreys suggested (*2*) that variations in the solar output could modify the O_3 content of the stratosphere. Numerous photochemical calculations, including those carried out recently (*3, 4*), have confirmed this prediction. Many investigators (*5*) have used observational data to search for relationships between solar parameters and amounts of O_3, but the results have been controversial and not always convincing, usually because the measurements have been relatively limited in number, noisy, or subject to long-term drift or to interference by other geophysical phenomena. We report here the clearest and most quantitative relationship between solar ultraviolet radiation and stratospheric O_3 thus far.

The O_3 response to solar radiation results from the reaction

$$O_2 + h\nu \rightarrow O + O$$

at a wavelength $\lambda \leq 242$ nm, which is followed by the reaction

$$O + O_2 + M \rightarrow O_3 + M$$

The destruction of odd oxygen ($O + O_3$) is less sensitive to ultraviolet changes (*1*). Thus, we expect an increase in solar ultraviolet radiation for $\lambda < 242$ nm to cause an increase in O_3.

The Limb Infrared Monitor of the Stratosphere (LIMS) experiment operated on the Nimbus 7 spacecraft from 25 October 1978 to 28 May 1979. It measured the infrared emitted by the earth's atmosphere at the limb, from which vertical distributions of temperature, O_3, and three other trace gases were derived (*6*). The individual O_3 profiles appear to be accurate to 10 percent and—more important here—precise to ≤5 percent (*7*). The Solar Backscatter Ultraviolet Experiment (SBUV) also flew on Nimbus 7, measuring the solar spectrum and its variation (*8*).

The LIMS profiles were derived every 4° of latitude, from 64°S to 84°N. We then analyzed the data at these latitudes into daily values for the zonal mean, plus the amplitudes and phases of the first six harmonics around the latitude circle, using a Kalman filter method (*9*). We have looked for solar effects on the zonal mean O_3 values.

It has long been recognized that rotation of active solar regions should lead to a modulation of ultraviolet solar irradiance. Measurements by Heath and his co-workers (*10*) have shown that the relative amplitude of this modulation is a stable function of λ, and so changes at one λ can be related to those at another. We have used SBUV measurements at 205 nm as an indicator of the variations between 190 and 220 nm (*3*) to which atmospheric O_3 is most sensitive. Comparison could be made to the sum of variations at all wavelengths, weighted by their calculated photochemical efficiency, but this would introduce model dependence which we wished to avoid.

Beginning in early 1979, the solar (*S*) measurements show several oscillations with a period of ~13.5 days, due to two groups of strong solar-active regions about 180° solar longitude apart and the sun's 27-day rotation (*10*). These data are shown in the lower panel of Fig. 1a.

The LIMS zonally averaged O_3 for the equator at 5 mbar (~36 km altitude) is also shown as a function of time in the upper panel of Fig. 1a. The largest variations are due to seasonal effects, which obscure ultraviolet-related effects.

Since the sun provides an oscillating signal of narrow bandwidth, we used statistical spectral methods to look for an O_3 response in the same frequency range. A power-spectral analysis of the *S* data (one point per day for the 139 days from 10 January to 28 May) shows a large peak at a frequency near 0.074 day^{-1}, or a period of 13.5 days, as expected. Similar analysis of the O_3 data for a range of altitudes and latitudes shows the power predominantly at low frequencies but with a distinct peak in the frequency range 0.06 to 0.075 day^{-1}.

Cross spectra between the two series were calculated (*11*) and smoothed with a Hamming window five modes wide. These spectra invariably showed significant peaks in the squared coherence (C^2) at the 13.5-day period. Almost all $C^2 > 0.5$ occurred at and above the altitude of the 10-mbar levels, with the largest single region between 24°S and 24°N. This is not surprising, since traveling waves (*12*) in mid-latitudes, which are unrelated to solar effects, would be expected to have considerable power of the same frequencies. The subsequent discussion focuses on this tropical region. Because atmospheric variability is a source of noise whereas the true O_3 response to the ultraviolet variation should be quite similar at all tropical latitudes, we averaged the C^2 spectra for each pressure level. The values for 5 mbar are shown by the solid line in Fig. 1b. Cross spectra were also calculated between *S* and a white-noise sequence. The range of values is shown by the dashed lines in Fig. 1b. The peak at 13.5 days is 0.68, several standard deviations above a random effect. Almost all other frequencies are within or close to the range of random effects. These significant peaks at the several pressure levels indicate a strong connection between ultraviolet variation and O_3 response.

The phase lag ϕ between the 13.5-day O_3 and *S* variations was also estimated. It is less than 1 day above 40 km, whereas below that level O_3 lags *S* by intervals that increase with increasing pressure. Theoretically, ϕ (in days) = $13.5/2\pi \tan^{-1}(2\pi\tau/13.5)$, where the response time to the perturbation τ is half the