

Unusual autofluorescence characteristic of cultured red-rain cells

Godfrey Louis*^a and A. Santhosh Kumar^b

^aDepartment of Physics, Cochin University of Science & Technology, Cochin - 682022, Kerala, India.

^bSchool of Pure & Applied Physics, Mahatma Gandhi University, Kottayam-686560, Kerala, India.

ABSTRACT

The red cells found in the red rain in Kerala, India are now considered as a possible case of extraterrestrial life form. These cells can undergo rapid replication even at an extreme high temperature of 300 deg C. They can also be cultured in diverse unconventional chemical substrates. The molecular composition of these cells is yet to be identified. This paper reports the unusual autofluorescence characteristic of the cultured red rain cells. A spectrofluorimetric study has been performed to investigate this, which shows a systematic shift of the fluorescence emission peak wavelength as the excitation wavelength is increased. Conventional biomolecules are not known to have this property. Details of this investigation and the results are discussed.

Keywords: red rain of Kerala, red rain cells, Extraterrestrial life, extremophilic, autofluorescence, Intrinsic fluorescence

1. INTRODUCTION

1.1 Red rain phenomenon

In the red rain phenomenon that occurred in the southern Indian State of Kerala during July to September 2001 at least 50 tons of red colored particles resembling biological cells fell through the rain. During the period more than 100 cases of isolated red rain occurred over a wide area of 300 kilometers in Kerala. Louis & Kumar¹ conducted a detailed analysis of this phenomenon. On the basis of geographical and time distribution pattern of the red rain fall, they reported that these red colored biological cells are possibly from an extraterrestrial source such as from cometary fragments which disintegrated in the upper atmosphere. While atmospheric fragmentation of an incoming cometary meteor was considered as the reason for the observed geographical distribution, slow settling of the micrometer sized cells into the rain clouds from the upper atmosphere was proposed as the reason for the time distribution pattern of the red rain cases. In an earlier paper² they have also argued that the Kerala red rain could be a case of cometary panspermia. Considering the characteristics of the red rain phenomenon it was also shown that a terrestrial origin for the cells are unlikely. Considering the highly localized nature of the red rain cases a distant desert origin for the cells was ruled out.

1.2 Red rain cells

The scanning electron microscopy study¹ revealed that the red rain cells are 4-10 microns in size and many cells were having a collapsed surface indicating a fluid interior. Transmission electron microscopy revealed that the cell had thick cell wall and were having no nucleus. Optical microscopy with dyed cells also indicated absence of a nucleus but revealed the presence of the thick cell wall. Elemental analysis using EDAX showed that the major elements present in the cell are carbon and oxygen confirming the organic nature of the cells. Minor amount of silicon, Fe and Al were also detected in the cell. The cells were found to be very stable for years and no decay of the cells could be found when stored in the original rainwater at room temperature without any preservative. On the basis of a spectrofluorimeter study using ethidium bromide dye it was also reported that DNA is absent in these cells.

1.3 High temperature replication of red rain cells

In another study³ to culture this microbe Louis and Kumar reported that it was optimally replicating at an extreme high temperature of 300 deg C in hydrothermal condition and could metabolize inorganic and organic compounds including hydrocarbons. Reproduction process of this new organism was identified as a special kind of multiple fission process and the original red rain cells were identified as the resting spores of this microbe. These findings now rules out the possibility that these cells are common algal or fungal cells. Considering the ability of this organism to replicate at extreme high temperature of 300 deg C and the fact that ordinary biomolecules cannot stand this temperature, Louis and

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Kumar proposed that these cells possibly represent a new kind of biology. It was also speculated that these cells might have alternate type of biomolecules, which could withstand extreme high temperatures. However the biomolecular constituents of these cells are yet to be identified.

1.4 Biomolecular Autofluorescence

Fluorescence microscopy and fluorescence spectral studies are widely used in biology both for microscopic imaging of biological structures and for identification and quantification of biomolecules. Many biological macromolecules such as some proteins and enzymes have intrinsic fluorescence or autofluorescence and others are made fluorescent by tagging with specific extrinsic probe molecules. Protein fluorescence is widely used to study a variety of structural information, such as the extent of rotational freedom, the exposure of amino acid side chains to quenchers, and intramolecular distances⁴. Green fluorescent protein (GFP) is used for monitoring gene expression and protein localization in both prokaryotes and eukaryotes⁵. While the presence of proteins or other conventional biomolecules has not been confirmed in the high temperature cultured red rain microbes they exhibit strong intrinsic fluorescence or autofluorescence over a wide range of excitation wavelengths than conventional microbes. According to Kasha's rule the fluorescence emission peak should remain fixed and should be independent of the excitation wavelength. But the red rain cells show an unusual type of autofluorescence where the fluorescence emission peak is showing a shift with a change in excitation wavelength. The details of this unusual autofluorescence are reported below.

2. METHODOLOGY

The sample used for the present fluorescence study, was cultured at 300deg C under hydrothermal condition in a specially fabricated steel pressure vessel, which can withstand high temperature and pressure. The nutrient used was 500 mg of glycine added with 15 ml of distilled water. The same was taken in a culture bottle, which was placed inside the pressure vessel and heated at 300 deg C for about 30 minutes after seeding with red rain microbes using a wire loop. Replication of the cells is indicated by the turbidity of the solution, which now contain millions of suspended cells. A drop of this culture can now be examined under microscope to see the individual cells. The culture experiment was also repeated for a different nutrient medium, which uses 250 µL of Cedar wood oil added with 15ml of distilled water.

The autofluorescence of the cultured red rain cells was visualized in an Olympus fluorescence microscope (BX51) with epi-illumination. Various combinations of excitation and emission filters were employed. The Fluorescence spectra of the cultured red rain cells were recorded using Shimadzu spectrofluorimeter (RF-5301 PC, Shimadzu). Emission wavelength was scanned from 300 nm to 900 nm for constant excitation wavelengths of 10 nm intervals.

3. RESULTS AND DISCUSSION

3.1 Fluorescence microscopy

Microscopic examination of the culture medium after culturing at 300 deg C shows a large population of viable cells suspended in the medium. Figure 1 shows the bright field image of the red rain microbes cultured at 300 deg C. The cells are colorless coccoidal and have a size variation of about 1-5 microns. The autofluorescence was detected in the cultured red rain cells for blue, green and red region and are shown in figures 2-4. Blue emission was observed when the sample is excited with UV light through wideband excitation filter (BP330-385), and an emission filter 420 nm (BA420). Green autofluorescence was detected when the sample is excited with a wide band Blue excitation filter (BP460-490), and an emission filter 510 nm (BA510-IF). Similarly red autofluorescence was observed when the sample is excited with a wide band Green excitation filter BP510-550, and an emission filter 590 nm (BA590). The intensity of the emitted fluorescence is high in the green emission.

3.2 Spectrofluorimetric study

In order to study the fluorescence emission characteristics of the cultured red rain cells under different excitation wavelengths the spectra were recorded using a spectrofluorimeter. The fluorescence spectra of the cultured red rain cell are shown in fig 5 and 6. Figure 5 shows the emission spectra for the excitation wavelength region 250-360 nm

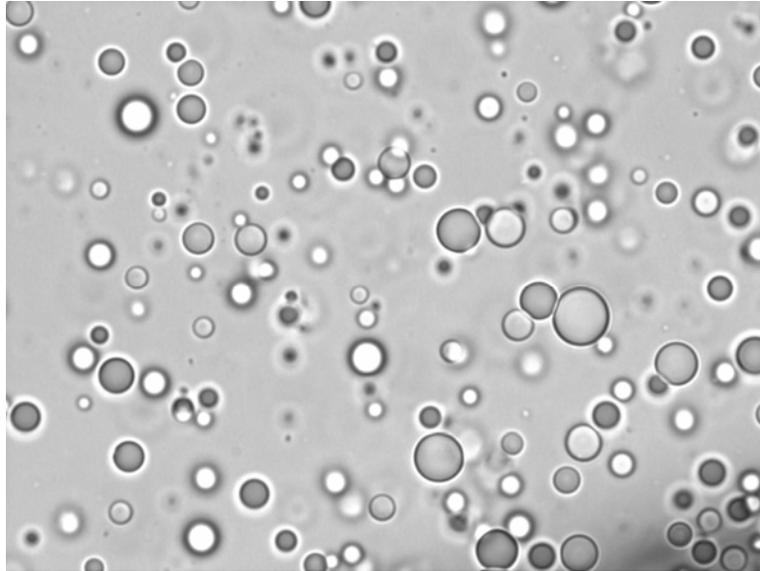


Fig. 1. Photomicrograph of the cultured cells of the red rain microbe. Cells have size ranging from approximately 1 to 5 micrometers. Cells were cultured in aqueous medium containing cedar wood oil as nutrient at 300 deg C in hydrothermal condition. Cells cultured using glycine as nutrient also have the same appearance.



Fig. 2. Fluorescence microscopy image of the cultured cells of the red rain microbe under UV light excitation giving blue fluorescence.

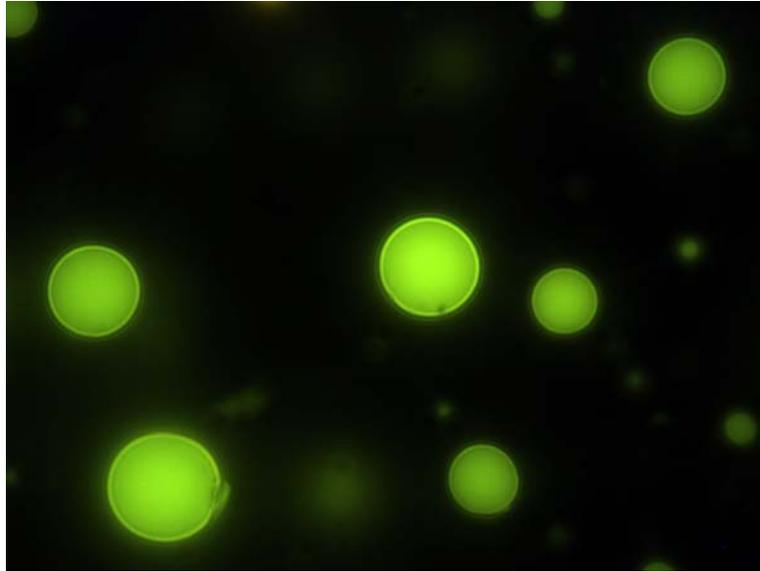


Fig. 3. Fluorescence microscopy image of the cultured cells of the red rain microbe under blue light excitation giving green fluorescence.

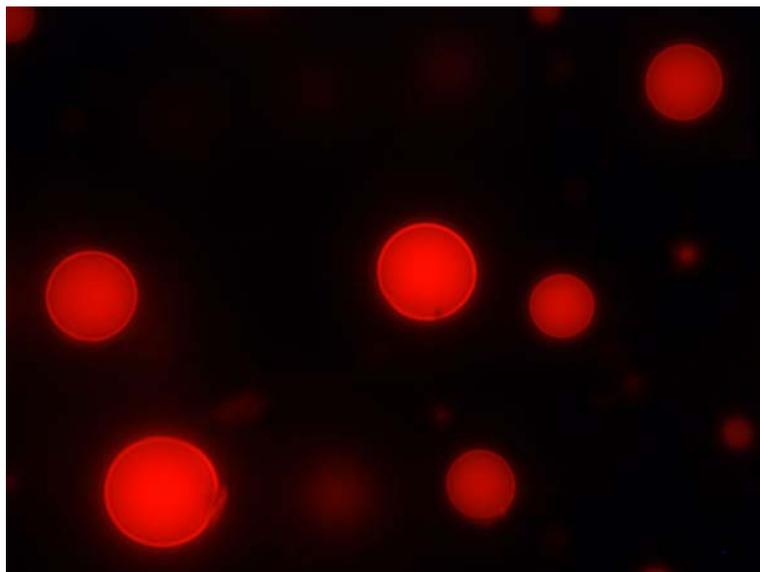


Fig. 4. Fluorescence microscopy image of the cultured cells of the red rain microbe under green light excitation giving red fluorescence.

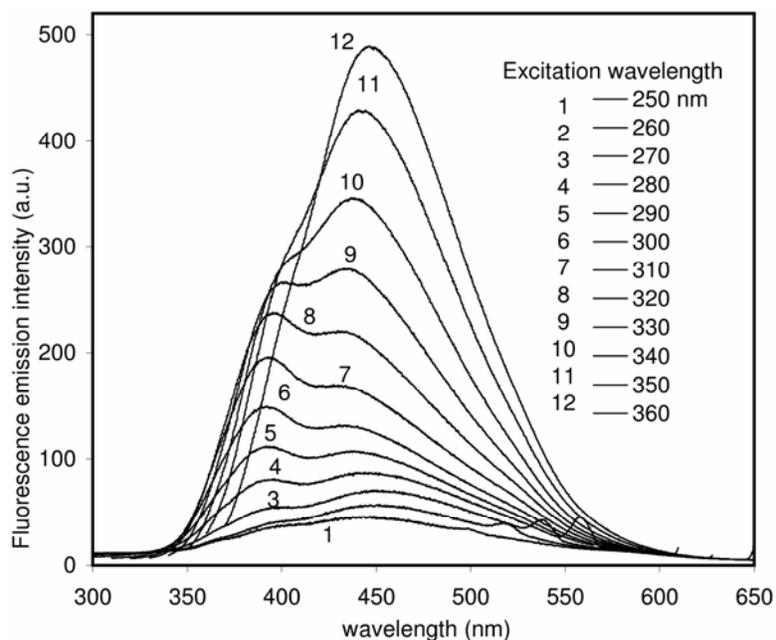


Fig. 5. Fluorescence emission spectrums of an aqueous suspension of the cells for different excitation wavelengths ranging from 250 to 360 nm. The curves show that in this region of excitation wavelengths there is no significant systematic shift of the emission peak with excitation wavelength. These cells were cultured at high temperature using glycine as nutrient.

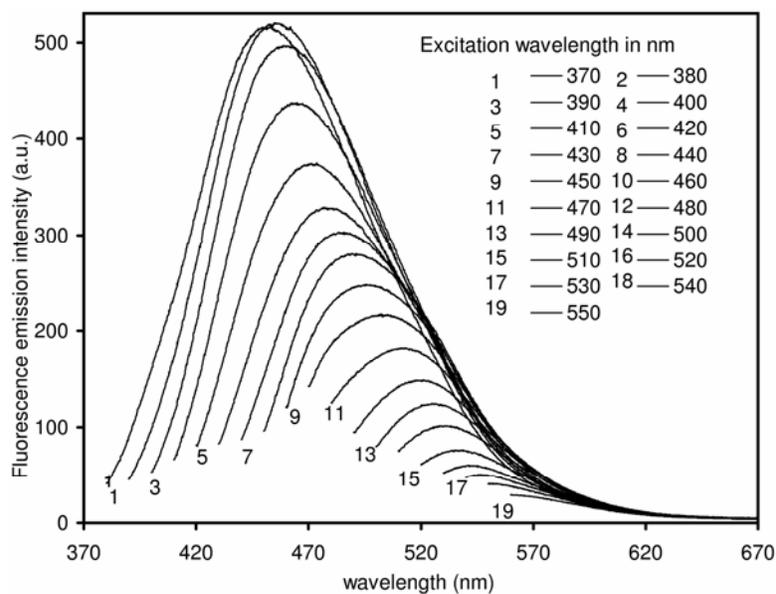


Fig. 6. Fluorescence emission spectrums of an aqueous suspension of the cells for different excitation wavelengths ranging from 370 to 550 nm. The curves show a systematic shift of the emission peak with excitation wavelength. These cells were cultured at high temperature using glycine as nutrient.

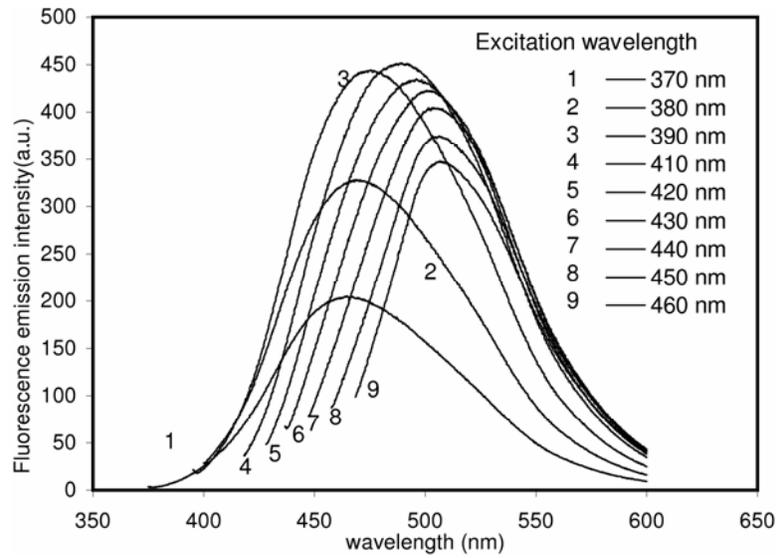


Fig. 7. Fluorescence emission spectra of an aqueous suspension of cells for different excitation wavelengths ranging from 370 to 460 nm. The curves show a systematic shift of the emission peak with excitation wavelength. These cells were cultured at high temperature using cedar wood oil as nutrient.

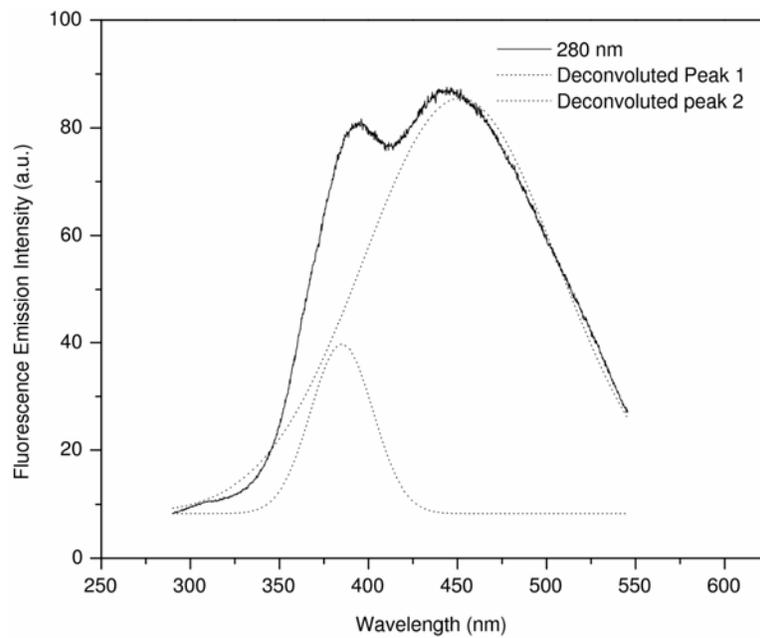


Fig. 8. Fluorescence emission spectrum of cells for an excitation wavelength of 280 nm along with the corresponding deconvoluted peaks. The deconvoluted peaks are at 385nm and 450nm.

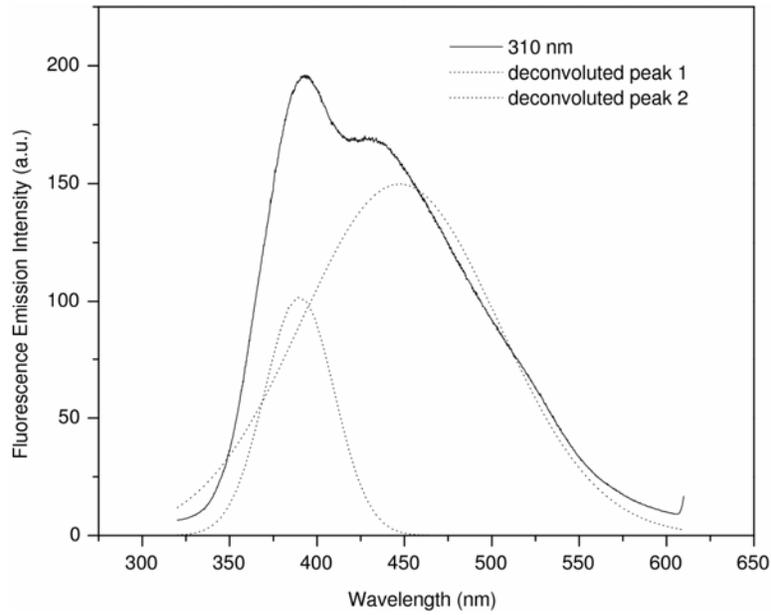


Fig. 9. Fluorescence emission spectrum of cells for an excitation wavelength of 310 nm along with the corresponding deconvoluted peaks. The deconvoluted peaks are at 385nm and 450nm.

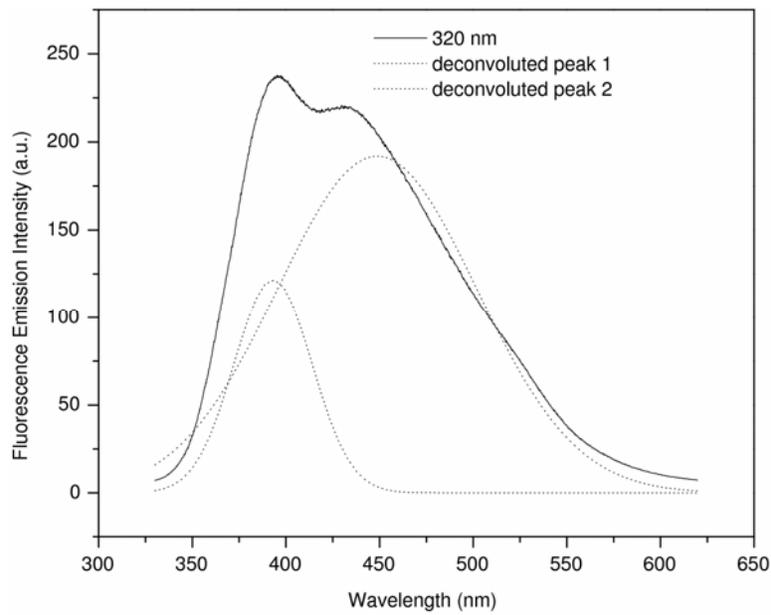


Fig. 10. Fluorescence emission spectrum of cells for an excitation wavelength of 320 nm along with the corresponding deconvoluted peaks. The deconvoluted peaks are at 385nm and 450nm.

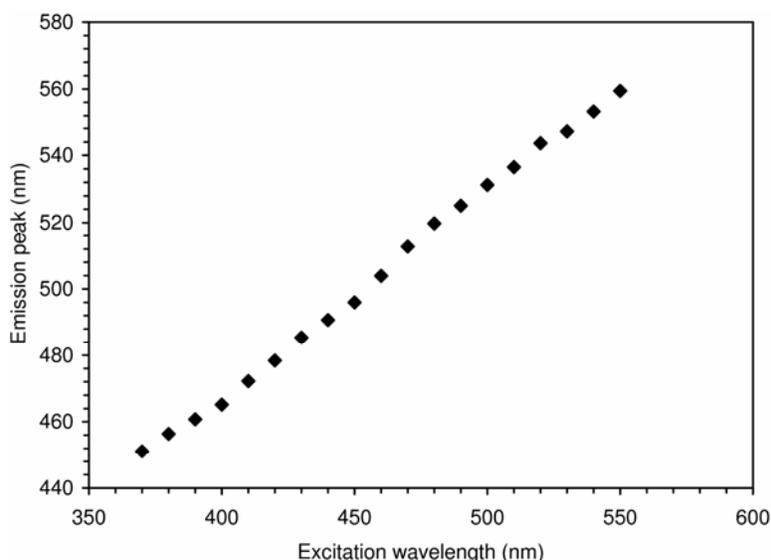


Fig. 11. Excitation wavelength dependent shift of fluorescence emission peak. Figure shows the almost linear shift of the fluorescent emission peak for the cells as the excitation wavelength is changed from 370 to 550 nm. This shift is unusual and apparently violates Kasha's Rule, which requires the emission peak to remain constant. This plot is derived from figure 6.

where as figure 6 show the spectra in the excitation wavelength region 370-550 nm. Excitation dependant emission peaks are clearly observed for the cells in the 370 - 550 nm excitation range (Figure 6). Cells grown with cedar wood oil nutrient also show the excitation wavelength dependent emission peaks and this result is shown in figure 7. In the UV excitation region 270-340 nm two broad emission peaks are observed which are overlapped and found at 385 nm and at 450 nm respectively. Hence these spectrums were de-convoluted using Gaussian de-convolution method and the resulted spectrum are shown in figures 8-10 for the excitation wavelengths 280, 310 and 320 nm respectively. The deconvoluted curves clearly show the presence of two emission peaks at 385 nm and at 450 nm. Considering figure 6 it can be seen that it is the 450 nm peak, which starts shifting to longer wavelengths as the excitation is increased beyond 370 nm. Figure 11 clearly illustrates this behaviour and shows that this shift is almost linearly dependent on the excitation wavelength. The emission maximum in the fluorescence spectrum occurred at 460 nm when excited with 380 nm for the whole spectral region studied. Emission intensity is observed to be decreasing towards the red end of the spectrum.

3.3 Discussion and conclusion

Results of the fluorescence microscopy and the spectrofluorimetric study clearly shows that the cultured red rain cells have intrinsic fluorescence over wide excitation wavelengths. The observed excitation wavelength dependent emission peak shifting is an unusual result, which is against the Kasha's rule. This appears to be a unique property of the red rain microbes. Conventional biomolecules or organisms are not known to have this kind of unusual autofluorescence and hence the presence of new kind of biomolecules can be inferred in the red rain microbes. Special kind of energy level structure and relaxation processes in these new biomolecules can possibly explain the violation of Kasha's rule.

Organisms replicating at 300 deg C and showing this kind of autofluorescence are currently unknown to exist on earth yet several thousand kilograms of these cells came down through the red rain, which is again an indication supporting the view that these cells are possibly extraterrestrial.

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