Biosciences, Biotechnology Research Asia Vol. **6**(1), 81-85 (2009)

Biological and microbial origin: A comparison of the morphology of red rain samples from India and UK

SULAIMAN ALI ALHARBI

Department of Botany and Microbiology, College of Science,
King Saud University, P.O. Box 2455 Riyadh - 11451 (Saudi Arabia).

(Received: March 10, 2009; Accepted: April 12, 2009)

ABSTRACT

Large quantities of red rain fell in parts of India in 2003. It has been variously described as fat globules, sand, and red blood cells and the alga *Trentepohlia*. Claims have also been made that red rain is cellular, but lacks DNA and that it has an extraterrestrial origin. A red rain event also recently occurred in the UK, thereby providing an opportunity to study red rain from two distinct sources. Here the similarities and differences between the cells occurring in the red rain events are discussed. The results show that different types of red rain exist.

Key words: Red rain, astrobiology, aerobiology, exobiology.

INTRODUCTION

Between July and September, 2001 an unusual red rain fell on the State of Kerala in India. This phenomenon appeared in various newspapers and other media (*Nature*, 2001) and is currently carried by several websites (Surendran, 2001). Claims were made (Hoyle & Wickramasinghe, 1999; Louis and Kumar, 2006) that red rain is biological in nature, is relatively rich in silicon, but lacks DNA. As the result of these, and some other properties of red rain, these authors suggested that red rain may have an extraterrestrial origin (Louis and Kumar, 2006). Veerabuthiran and Satyanarayana (2003) and Satyanarayana *et al.* (2004) claimed that the dust generated from desert areas of the west Asian countries was a possible cause of the observed coloured rain.

Another red rain event occurred in the UK in 2007, providing the opportunity to compare the two types of biological material found in the two types of red rain, and determine if they comprise the same material, or differ substantially from one another.

MATERIAL AND METHODS

Scanning electron microscopy

The cells were air dried using a dryer directly onto an aluminium stub and examined using an environmental scanning electron microscope.

Staining with DNA probes

Red rain cells were re-suspended in Milli-Q purified, distilled water and a 1ml sample was sonicated on ice for 8x20 seconds at amplitude of 15 microns using a Sanyo Soniprep 150 (MSE). Ten second cooling intervals ensured that the sample was not overheated. Approximately 1 in 10 cells were lysed. The samples were subjected to a further 6 periods of sonication at the same amplitude, each lasting 30 seconds with a 10 second cooling interval. SYBR Green II was diluted 1:10 in 50mM Tris-HCl buffer, pH 8.0and examined using a fluorescent light microscope.

RESULTS AND DISCUSSION

The two types of rain are substantially

different with regard to their morphologies. Both types of rain are clearly red when viewed with the naked eye, although the UK rain is somewhat more purple in colour than the Indian version (Fig.1).

Under the light microscope, the two rains are again seen to be substantially different (Fig.2a,b) The Indian red rain is approximately 10µm in diameter, compared to the much UK rain cells, which is approximately 20µm in diameter. The UK red rain is also spheroid compared to the more ovate morphology of the Indian red rain. Both cells appear to have contained within them, smaller, or daughter, cells, The UK red rain is also noticeably a richer mix of organisms, bacteria, algae and fungi, than is the Indian red rain, which is less heavily contaminated.

Some uniformed, media opinion, particularly in the UK, has suggested that the Kerala red rain is composed of dust, fat globules or red blood corpuscles. However, the studies by Louis and Kumar (2006) clearly show that the redness of the Kerala rain is due to the presence of cells, a fact that is confirmed by the scanning electron microscope image show in Fig. 3a; the suggestion that these cells are erythrocytes (some have suggested that they originate from bats) is probably based on the fact that they often show an annular appearance when viewed under the environmental SEM (Fig.4a). Such blood-like cells are an artefact produced by the vacuum of the SEM. However, as Fig.3a shows, some cells are sufficiently rigid to avoid this effect and appear as ovoid spheres, approximately 4 µm in diameter. The annular appearance is caused by the due to the fact that the Indian red rain has a very thick wall. The UK red rain, is 20 µm in diameter (Fig. 3b) and in contrast, to the Indian red rain, has thin walls; the artefacts produced during SEM examination are completely different in structure, appearing more like punctured balls than red blood cells (Fig. 4b). Both types of rain were found to be insoluble in carbon tetrachloride, and are clearly not fat globules. The Indian red rain cells are morphologically similar to red algae (notably species of *Porphyridium*) but do not contain the stellate chloroplasts that are typical of this alga. The morphology of the cells, taken together with other biological characteristics, clearly show that neither type of red rain is made up of dust particles, a fact confirmed when EDAX analysis of the cells indicated the presence of carbon, but no mineral elements, including silicon; this last result contradicts the findings of Louis and Kumar (2006) that Indian red rain cells contain silicon.

Indian red rain cells proved very resistant to the cellular disruptive effects of sonication, a fact that presents a problem since adequate cell-disruption is essential if the cellular components of red rain, including DNA, are to be sufficiently exposed to allow for them to be detected; cells of the UK red rain, in contrast, was easily disrupted by sonication.

After exposure to a high degree of sonication, disrupted Indian red rain cells were treated with a variety of fluorescent stains to determine if, as suggested by Louis and Kumar (2006), they lack DNA (these tests were not performed on UK red rain). Fig. 5 shows that the cells fluoresced when exposed to SYBR Green II fluorescent stain, a stain that detects both DNA and RNA. Images taken using white light and UV, show that the fluorescence exactly corresponded to the location of the cells. Similar fluorescent staining of the red rain cells was achieved using the DNA sensitive stains DAPI (Vectashield, Mounting Medium with DAPI, H-1200), Live/Dead Stain *Bac*light (Molecular Probes) and ethidium bromide. No evidence was found to suggest that these results were due to auto fluorescence by red rain cells. These results show that, contrary to the findings of Louis and Kumar (2006), red rain does in fact contain DNA.

The above results tell us nothing about the origin of red rain. The fact that the Indian red rain cells contain DNA obviously suggests the possibility that they originated from Earth. It is important however, to note that the theory of panspermia does not necessarily suggest that microorganisms arriving to Earth from space will be novel to the extent of being devoid of DNA. Louis and Kumar (2006) point out that the appearance of red rain in Kerala was associated with a meteorite-associated air burst; further claims by them that red rain exhibits unusual biological properties (including growth at 300^{0}C) and has a panspermic origin. No similar meteorite burst was associated with the fall of the UK red rain. Should

the claims of Louis and Kumar relating to Indian red rain be verified by further work, then the Indian red rain cells are indeed unusual. There is as yet no evidence to suggest that the UK red rain is anything but algal and of Earth origin. However, even if both types of red rain do originate from Earth we are left with having to explain how they were picked up from the oceans or lakes and deposited in large amounts in the rain over both India and the UK. With this in mind, there have been suggestions that the red rain of Kerala is an alga of the genus *Trentepohlia* (Sampath *et al.,* 2001; Kumar *et al.,* 2002); However, if this is the case, it remains difficult to explain how this alga could have reached the atmosphere in such large quantities.

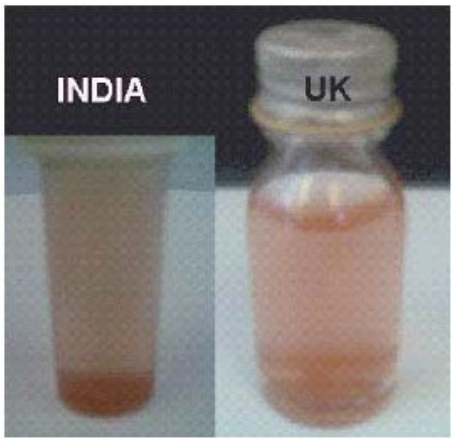

Fig.1: Samples of the two types of red rain

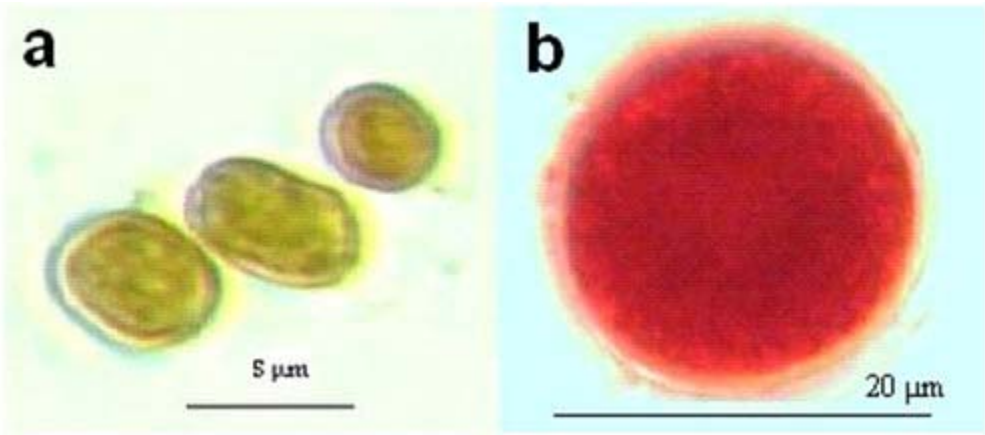

Fig. 2: Red rain as seen under the light microscope (a, Indian b,UK)

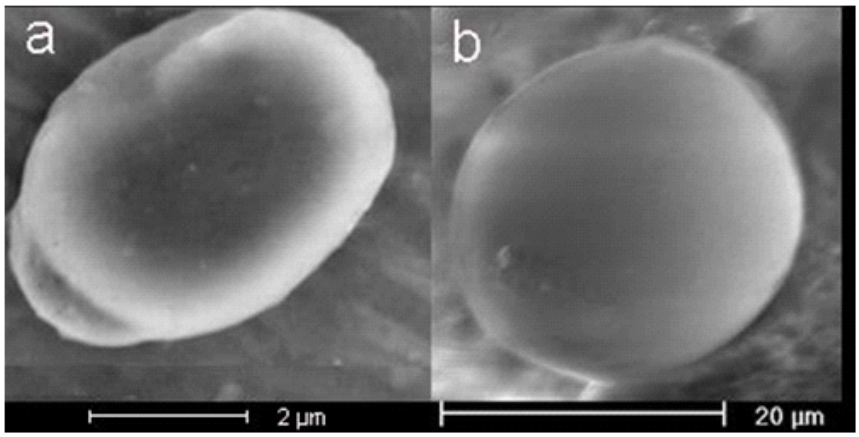

Fig. 3: Red rain under the scanning electron microscope (a, Indian b,UK)

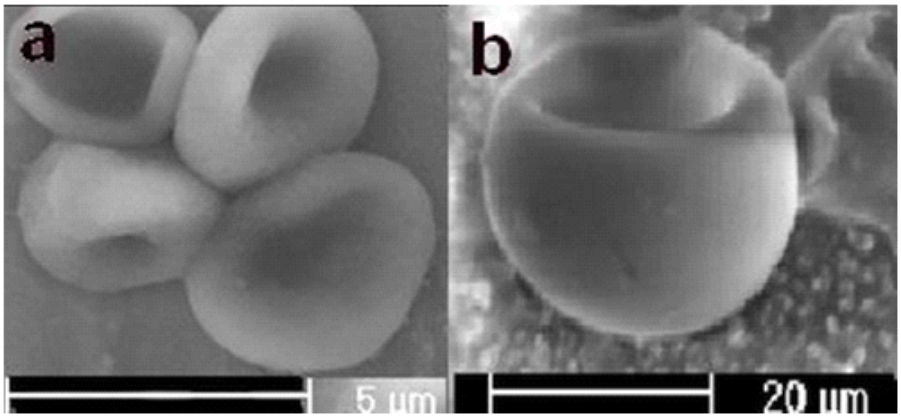

Fig. 4: Collapsed red rain, artefacts caused by scanning electron microscope (a,Indian; b,UK)

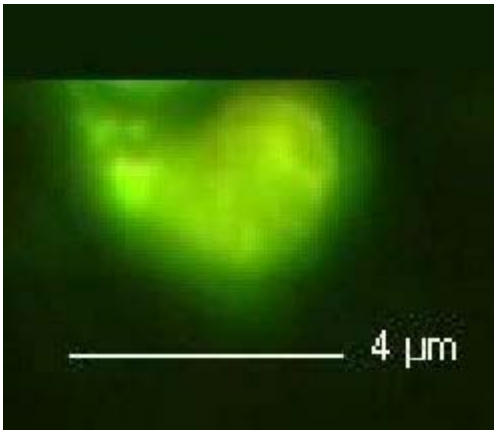

Fig. 5: SBYBR Green II stained Indian red rain, fluorescence indicates DNA

In conclusion the cells of the two types of red rains studied here differ in their morphologies and clearly represent two different organisms which may have differing origins, although this does not imply that either has necessarily an extraterrestrial origin.

A number of historical accounts of the red rain phenomenon have been reported, most of which have been attributed to desert dusts, although in the early 1800s, Ehrenberg commented on a biological nature of some rains. The Kerala red rain and the UK event appears therefore not to have been the first time that large quantities of red rain have fallen, the results from the UK show that it is certainly not the last and that different types of red rain exist; the deposition of large amounts of biological material to Earth in rain water may therefore be a relatively common event.

ACKNOWLEDGMENTS

This study is supported by College of Science Research Center No. (Bot/2009/45). Also I would like to thank Professor Dr M. Wainwright, (The University of Sheffield, UK), for making the facilities of his laboratory available to me.

REFERENCES

1. Louis,G. and Kumar, A.S., The red rain phenomenon of Kerala and its possible extraterrestrial origin, *Astrophysics and Space Science*, **302**:175-187 (2006).
2. Sampath, S., Abraham, T.K., Sasi Kumar, V and Mohanan, C.N., Coloured rain: A report of the phenomenon (of red rain), CESS-PR-114-2001,Center for Earth Science Studies and Tropical Botanical Garden and Research Institute, Palode, India (2001).
3. Kumar, V.S., Sampath, S., Mohanan, C.N., & Abraham, T.K., Colored Rain Falls in Kerala, India, Eos, Transactions, *American Geophysical Union,* **8**(31): 335 (2002).
4. Hoyle, F. & Wickramasinghe, N. C., Comets - A Vehicle for Panspermia, *Astrophys. Space Sci.* **268**: 333-341 (1999).
5. Nature. News in brief: Red rain leaves Indian scientists battling demons: *Nature.* **412**: 670-671 (2001).
6. Sampath, S., Abraham,T. K., Sasikumar,V., and Mohanan, C. N., Colored Rain: A Report on the Phenomenon, *CESS-PR-114-2001*, Centre for Earth Science Studies, Thiruvananthapuram (unpublished) (2001).
7. Satyanarayana, M., Veerabuthiran, S., Ramakrishna Rao, D. and Presennakumar, B., Colored Rain on the West Coastal Region of India: Was it Due to a Dust Storm? *Aerosol Science and Technology* **38**: 24-26 (2004).
8. Surendran, P. K., Mystery of the scarlet rains and other tales. *The Times of India* (2001).
9. Veerabuthiran, S. & Satyanarayana, M., Lidar observations on atmospheric dust transported from south-west Asia to Indian west coast region: A case study of colour rain event occurred during July 2001, *Indian Journal of Radio & Space Physics* **32**: 158-165 (2003).