Invited Paper

Growth characteristics of red rain microbes at temperatures below 100°C

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ABSTRACT

The red rain microbes, which caused red rain phenomenon in Kerala, India, exhibit many characteristics much different from conventional microorganisms. Previous study indicates that these microbes are possibly of extraterrestrial origin. Their ability to multiply at extreme high temperature of 300 °C and the unusual autofluorescence of their biomolecules are some of their extraordinary properties. Their molecular composition is yet to be identified. In this paper we report the growth pattern of these novel microbes at temperatures below 100 °C as a minimal approach to show their biological nature. Automated turbidity measurement of the cell culture indicate standard microbial growth curve. Increase in the cell population is faster at higher temperatures. Details of this investigation and results are discussed.

Keywords: red rain microbes, hyperthermophiles, extraterrestrial life detection, turbidity measurement, microbial growth curve

1. INTRODUCTION

The major event of red rain phenomenon occurred in Kerala in India during July to September 2001. Rain water appeared colored in random locations and random point of times in Kerala during this period. More than 120 cases of colored rain events were reported from various parts of the State separated by few hundred kilometers. In majority of events the color of the rain water was red. In some of the cases the color was found to be yellow. Red color of the rain water was due to the finely dispersed red particles in the rain water. Under microscope these particles had clear structure of biological cells. An estimated total quantity of at least 50000 kg of these cells has fallen in Kerala through various red rain events. On the basis of geographical and time distribution pattern of the red rains in Kerala which occurred over the period of July to September in 2001, we argued that the red cells which colored the rainwater may have possibly originated from cometary meteor fragments that disintegrated in the upper atmosphere [1]. A huge explosive sound heard by the residents early in the morning before the first occurrence of the red rain was also a possible link to the suspected meteor origin. Thus these red rain cells are considered as possibly of extraterrestrial origin. If cells can land on earth from cometary fragments as red rain events then Kerala red rain can be considered as a case of Cometary Panspermia, this argument was raised by us in another paper [2]. Having considered the fact that the cells can be cultured at extreme high temperatures like 300°C and also considering the fact that the cells show absence of DNA or RNA we have argued that this is a new kind of biology with unknown biomolecules supporting the argument that the cells are extraterrestrial but possibly related to Earth life at a more fundamental level [3]. These ideas have created public interest in the topic and resulted in popular articles [4] and TV documentaries. In an independent study of the historic cases of red rain events McCafferty [5] observed that many cases of historic red rains are related with meteoric activity and Kerala red rain should not be ignored and must be subjected to more investigation. Kerala red rain is not a unique recent event; in August 2002 Vietnam had a red rain event [6] and in July 2008 a colored rain event was reported from Bagado in Colombia [7].

The cultured red rain cells are highly autofluorescent and this autofluorescence is unusual type. For certain wavelength region the emission peak is found to shift with the shift in excitation wavelength. Spectroscopically the emission peak is supposed to remain constant. Having studied this fluorescence behavior we have proposed that the cells contain unconventional biomolecules [8]. This observation further supported the argument that these cells represent a new kind of biology.

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During the following years after 2001 minor cases of colored rain was also reported in Kerala during the months of July to September. This is consistent with the idea that Earth may be passing through a crossing orbit of color rain causing special meteoric bodies during these months. The red colored rain occurred in Vietnam on July 29 in 2002 and the colored rain occurred in Colombia, on 31 July 2008. Yellow rain was also reported in Kerala in the month of July 2008 on days 18th, 25th and 29th. Kerala and Colombia are near to equator but are on diametrically opposite side of the Earth. Meteoric origin is the most likely explanation for the appearance of similar kind of colored rains on subsequent days on either side of the Earth. Our study [9] of the fluorescence properties of the yellow rain from Kerala showed that it has the same fluorescence spectral signature as that of the cultured red rain cells which establish common origin for red and yellow rains.

In another independent study [10] it was reported that IR spectra of red rain cells show a number of infrared features which correlate with the emission spectra of unidentified infrared bands in protoplanetary nebulae thus proposing a possible extraterrestrial origin for the red cells.

Having argued that these red rain cells are extraterrestrial and after reporting that their biology is different with absence of DNA or RNA it remains to be established that they are living organisms and not a chemical artifact. For this it is logical to follow a minimal approach to life detection as applicable to extraterrestrial life detection experiments [11]. As stated by Merek and Oyama, "should the biochemistry of extraterrestrial organisms differ from that of terrestrial organisms, a simple growth experiment could succeed, whereas systems designed to measure specific molecular species might fail because of the potential inapplicability of extrapolating terrestrial biochemistry to the universe as a whole" [12]. Microbial growth and reproduction can be monitored by a variety of optical and electrochemical methods [13-18]. In this paper we report the results of our study of the growth of red rain microbes at temperatures below 100°C by optically monitoring the turbidity of the culture medium. Even though these microbes are capable of growing at much higher temperatures, present experimental setup only permits real time turbidity measurements below 100°C.

2. EXPERIMENTAL TECHNIQUE

2.1 Method

The microbial growth in a liquid culture medium produces increasing turbidity in the medium due to the increasing number of cell count. This is due to the light scattering by the micrometer sized biological cells which are increasing in an active culture medium. If a beam of light is allowed to pass through the culture medium the emerging light is incident light minus the scattered and absorbed light. If scattering increases due to cell multiplication then emerging light intensity will decrease in proportion. This decrease in intensity can be monitored using a phototransistor light sensor arrangement. The output of this light sensor can be continuously recorded to a computer file using a Data logger.

The principle of the present experimental setup is shown in the block diagram (Figure 1). There is provision for two culture bottles of which one can be used for the seeded culture and the other for the unseeded control. White Light Emitting Diodes (LED) which gives an inherently focused beam of white light is used as light source. Current supply to both LEDs can be varied simultaneously for changing the light output. The light after passing through the culture bottle falls on the phototransistors. The output of the phototransistor sensors are suitably scaled and applied to the input of the Data logger (PicoLog model No. 1012). The light sensor output voltage data is available through the recorded computer file. The data logging is done using the manufacture supplied Pico Log software. A separate microprocessor based temperature controller (Selec Model No. TC533) is used for controlling the temperature of the culture bottles. Both bottles are held at the same temperature. Figure 2 shows a photograph of the experimental setup. Two culture bottles can be seen inserted in the heating slot. The data logger is not shown in the photograph. The culture bottles have volume capacity of 15 ml. A typical microbial growth curve has characteristic features like a lag phase, a logarithmic growth phase, a stationary phase and a decline phase. Depending on conditions the whole process can take several hours or even days. Automated recording facility of the present setup can monitor growth activity for such long durations.

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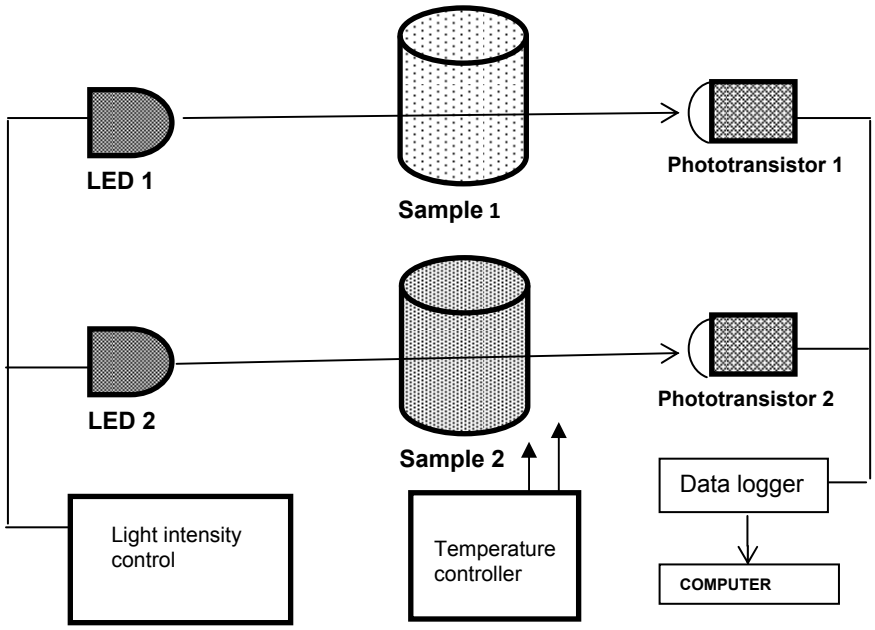

Figure 1. Block diagram of the experimental setup. One of the sample cultures is seeded with the microbe and the other is a control sample culture without seeding.

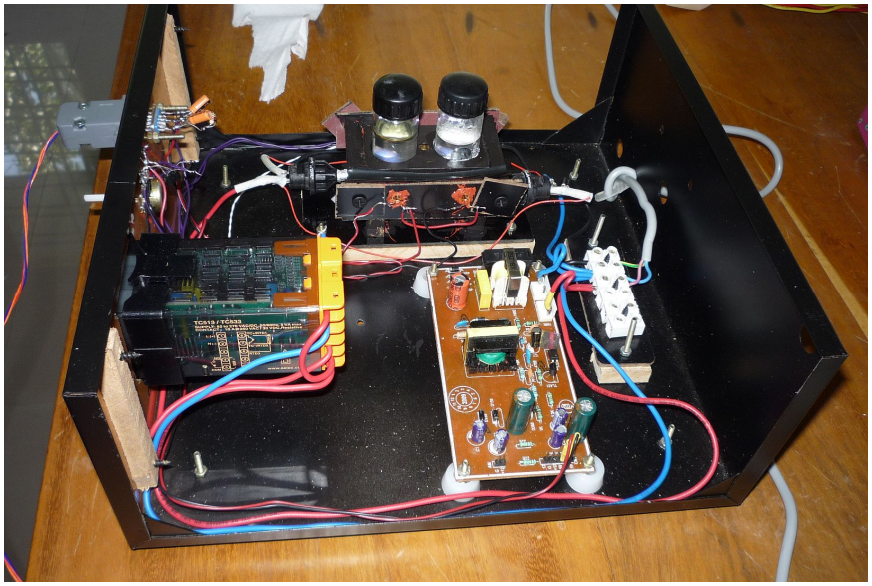

Figure 2. Photograph of the apparatus. Culture bottles are placed in the cavity of a solid brass block and the temperature of the brass block is controlled. Suitable holes are included in the brass block for light passage from LED to phototransistor.

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2.2 Growth experiments

In the present work we have used two types of culture mediums one is a mixture of 1ml of 5% povidone iodine solution (Betadine), 2ml of 100 % ethanol and 10ml of water and the other medium is 10 ml of water with few drops of Cedar wood oil. Active red rain microbes were found to grow in both these chemically different mediums. In iodine-ethanol nutrient medium the growth experiment was done at different temperatures like at 70, 80 and 90°C. In Cedar wood nutrient medium the growth experiment was done at 80°C. The result of the growth at 70°C is shown in Figure 3 and the result of the 90°C growth experiments is shown in Figure 4. Simultaneous measurement of seeded and unseeded control was done at 80°C and the results shown in Figure 5. Result of the culture experiment at 80°C using cedar wood oil nutrient is shown in Figure 6. Clear visual difference in turbidity can be observed in the photograph shown in Figure 7. Photomicrograph of the cultured cells with 100x objective is shown in Figure 8. Scanning electron microscope images of the cultured cells are shown in Figures 9 and 10.

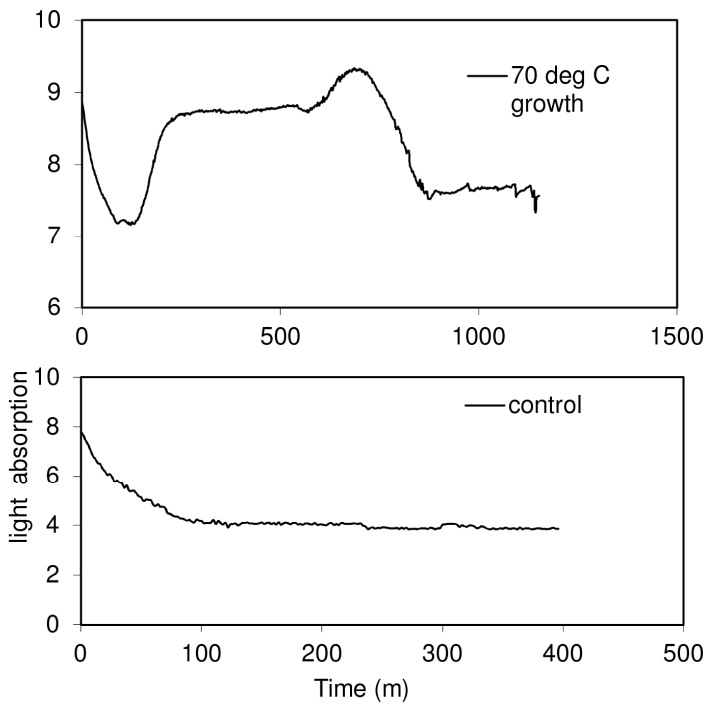

Figure 3. Results of the culture experiment done at 70°C. Curve on top shows the seeded culture and the curve at bottom represents a control experiment without seeding. The nutrient used is Povidone Iodine, ethanol and water mixture. The initial high value of light absorption is due to the brown color of iodine and its discoloration takes place after some time.

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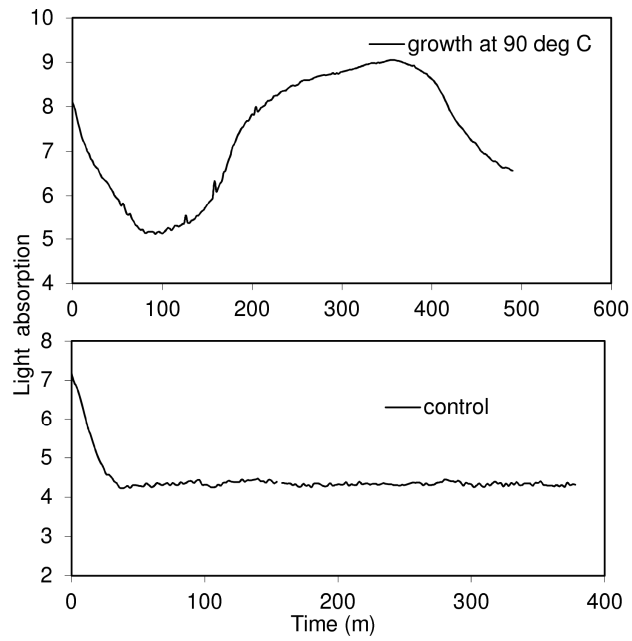

Figure 4. Results of the culture experiment done at 90°C. Curve on top shows the seeded culture and the curve at bottom represents a control experiment without seeding. The nutrient used is Povidone Iodine, ethanol and water mixture. The initial high value of light absorption is due to the brown color of iodine and its discoloration takes place after some time.

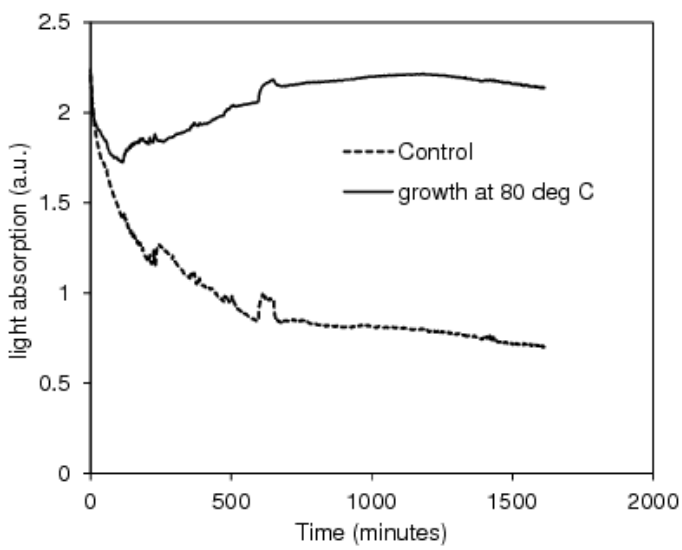

Figure 5. Results of the culture experiment done at 80°C. with present experimental setup using simultaneous monitoring of the turbidity in the seeded and control cultures. The nutrient used is Povidon Iodine, ethanol and water mixture. The initial high value of light absorption is due to the brown color of iodine and its discoloration takes place after some time. Clear variation is observed for seeded and control.

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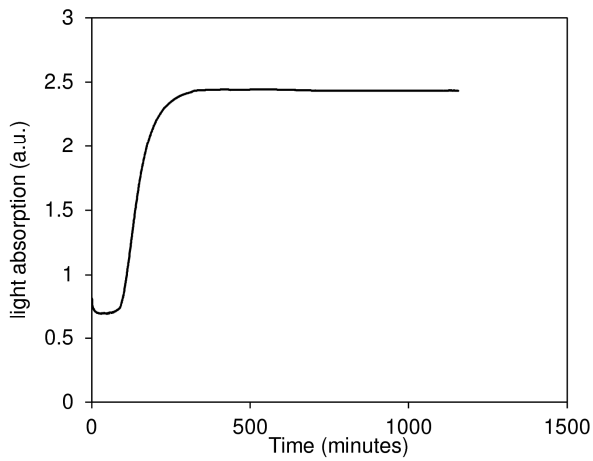

Figure 6. Result of the culture experiment done at 80°C. The nutrient used is cedar wood oil and water. The characteristic lag phase, exponential growth phase and steady phase are recorded over a period of 20 hours.

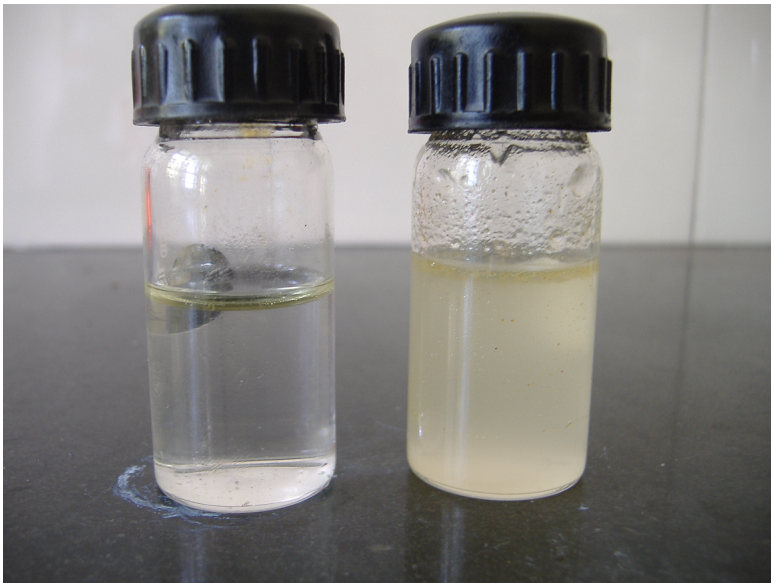

Figure 7. Photograph of the culture bottles after growth experiment using cedar wood oil and water as nutrient. The oil drops remain unchanged and no turbidity is developed in the unseeded bottle on the left. Seeded bottle on the right shows turbidity due to microbial growth.

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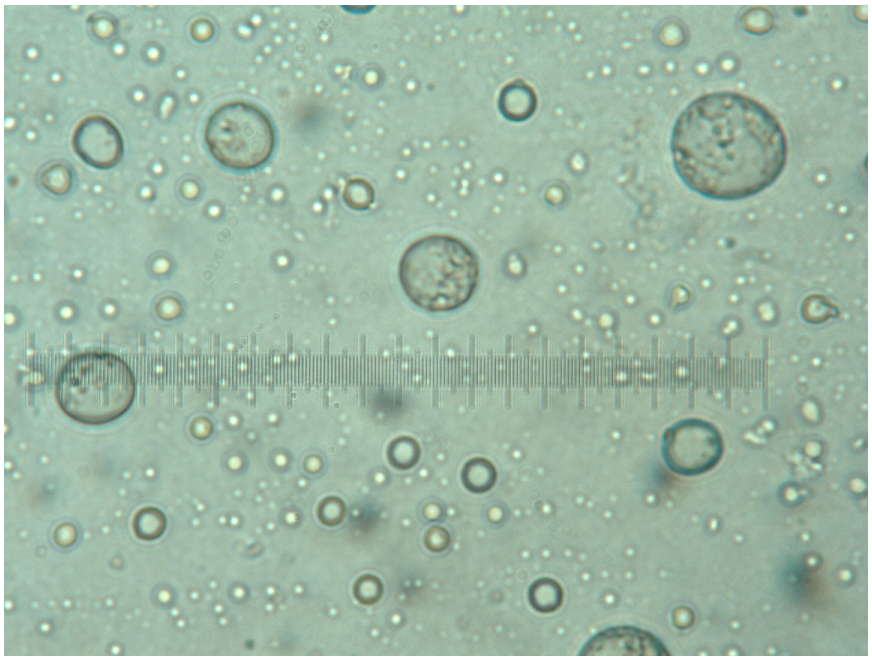

Figure 8. Photomicrograph of the cultured cells with 100x objective. The cells are colorless and spherical in shape and are few micrometers in size. Submicrometer sized small daughter cells are not visible in optical microscopy.

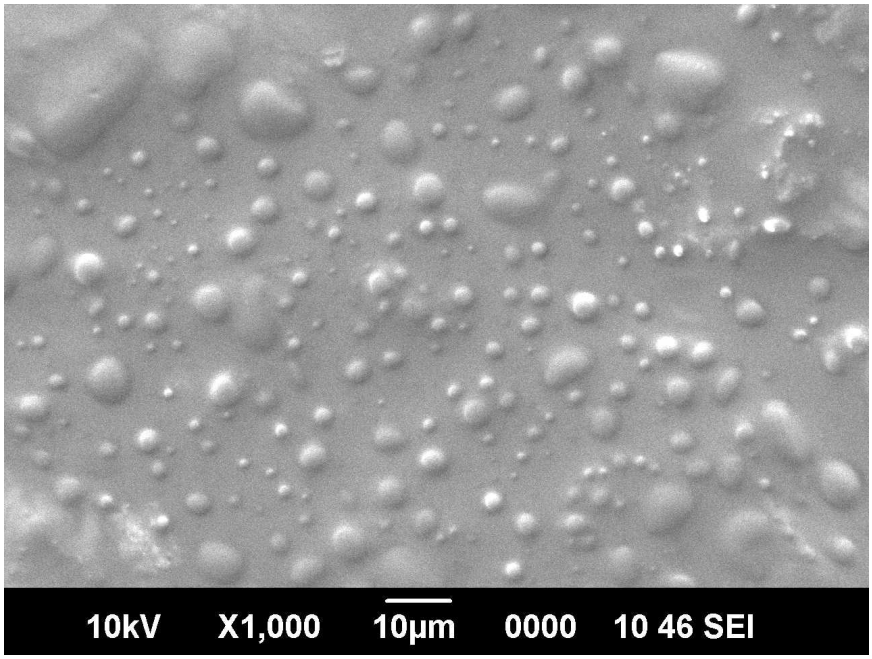

Figure 9. Scanning electron microscope images of the grown cells at 1000x magnification. Cell clusters similar to what is observed in optical image can be seen.

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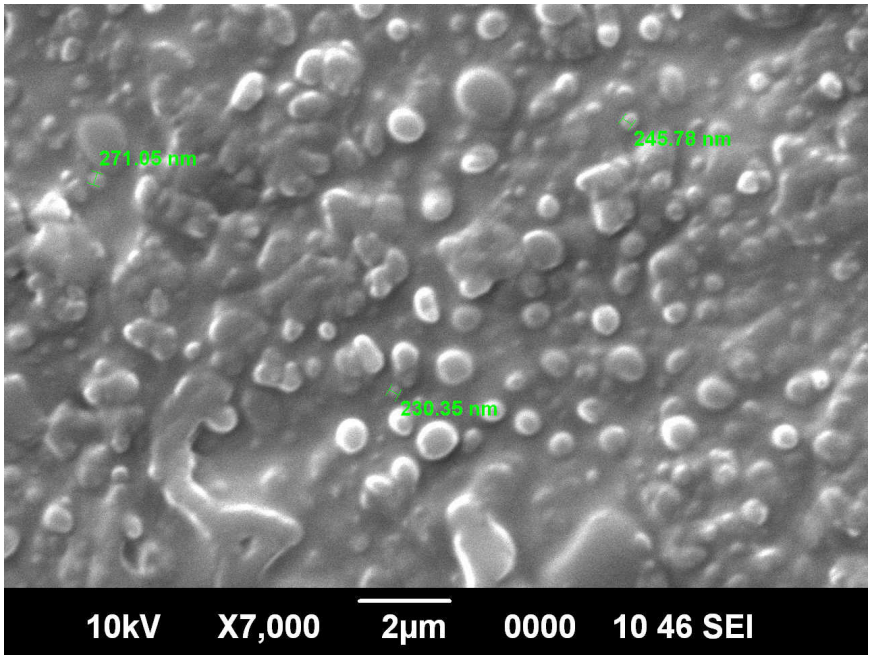

Figure 10. A more magnified scanning electron microscope image of the grown cells at 7000x magnification. Smallest cells are about 0.2 micrometers in size.

3. RESULTS AND DISCUSSION

Growth experiments show that there is a very clear difference between seeded and unseeded cultures. Microbial action in the seeded cultures gives rise to increasing turbidity. Optical and Electron microscopy images show that the cause for the increased turbidity is high count of cells in the culture medium. There appears no reason to consider this increasing turbidity as chemical artifact. Seeding in two different chemicals gives rise to the growth of same kind of cells as expected in biology. The growth curves show standard features of microbial growth. The cells show increased growth rate at higher temperatures as expected from a thermophilic organism. Thus red rain microbes exhibits one fundamental property of life that is their ability to grow and multiply when supplied with food under proper growth temperature. However the contradicting facts are: that DNA and RNA could not be detected in these cells; that the cells grow at temperatures much above the currently known upper temperature limit of Life [19] and at temperatures like 300°C conventional biomolecules cannot possibly survive so life is not expected at such high temperatures. These contradictions will vanish if the red rain microbes have an alternate biochemistry which appears increasingly possible now.

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