

Determining the Sensitivity of PCR to Detect Methanogens in a Martian Soil Simulant

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Introduction

Methanogens are a group of anaerobic micro-organisms that produce methane as a waste product from hydrogen gas and carbon dioxide. They will usually not tolerate even brief exposure to oxygen. These organisms live in a range of anaerobic environments, such as marine and fresh-water sediments, intestinal tracts of animals, ocean vents, and hot springs. Methanogens belong to the Archaea domain, one of the three domains life (Figure 1). Their ability to inhabit extreme environments is of great interest to astrobiologists who search for the possibility of life on other planets. Mars has become the main area of focus for the search of life in space due to its unique environment which closely resembles many of Earth's extreme habitats. Recently, a trio of research teams independently probing the Martian atmosphere for signs of methane have confirmed the presence of the gas. The source of the methane can either be from volcanism or, what many scientists hope, from subterranean microbes.

PCR, as a molecular diagnostics test, has proven to be an effective tool for detecting methanogens present in a Martian soil simulant through prior research (Lewter, 2003). The ability to detect specific methanogens in a soil sample by using PCR is a great advantage for researchers, because the organisms do not have to be alive for the technique to work.

Objectives

- To get ESEM (Environmental Scanning Electron Microscope) images of all five methanogen strains.
- To determine the sensitivity of the PCR technique by quantifying the number of microbes in a soil sample necessary for detection.
- Test this technique on other soil types, such as sand and potting soil, to determine its versatility and to establish a range of varying simulants that support methanogen growth.

Methods and Materials

Culturing samples:

Five strains of methanogens were obtained from stock cultures. These five strains are *Methanosarcina barkeri*, *Methanobacterium formicum*, *Methanogenium frigidum*, *Methanococcus maripaludis*, and *Methanothermobacter wolfeii*. Microscopic views of four of the five methanogens used in the experiment are shown to the lower right of this text. The organisms were grown in specific media. For *M. wolfeii*, "MM" and "MS" media were used. MS media requires 0.4 grams of yeast extract, 0.2 grams of Trypticase peptones, and 0.2 grams of Mercaptoethanesulfonic acid in addition to the ingredients needed to make MM media. Seven tubes of varying concentrations were accomplished by the serial dilution method. This gave a range of dilutions from undilute to 10⁻⁶. 2 mLs of each dilution containing the organisms were then added to 5 grams of soil simulant.

DNA extraction and PCR:

The Puregene DNA Isolation Kit for extraction was used in this experiment (Gentra, Minneapolis, MN). One gram of soil simulant was used for the extraction. Figure 2 shows all the primers that were used for PCR (polymerase chain reaction). The ME 1 and ME 2 primers are the universal methanogen primers used to distinguish them from all other organisms. Mfrig, Mwolf and Mform are the reverse diagnostic primers specific for each strain. It required 1 µL of extracted DNA to run the PCR. The PCR program goes through 40 complete cycles, with each cycle doubling the amount of DNA synthesized in the previous cycle. The first three cycles of the process are depicted in Figure 3.

Primer	Sequence
ME1	5'GCMATGCARATHGGWATGTC
ME2	5'TCATKGCRTAGTTDGGRTAGT
Diagnostic primer	Sequence
Mfrig-R	5'GCTGCGGGGTCCGGGTT
Mwolf-R	5'TGTTCAAGTCCGTAGAAC
Mform-R	5'TGTTGCCATACATACACCA

Figure 2: The five primers used to amplify specific base pairs of the MCR α-subunit gene of all strains except *M. barkeri*.

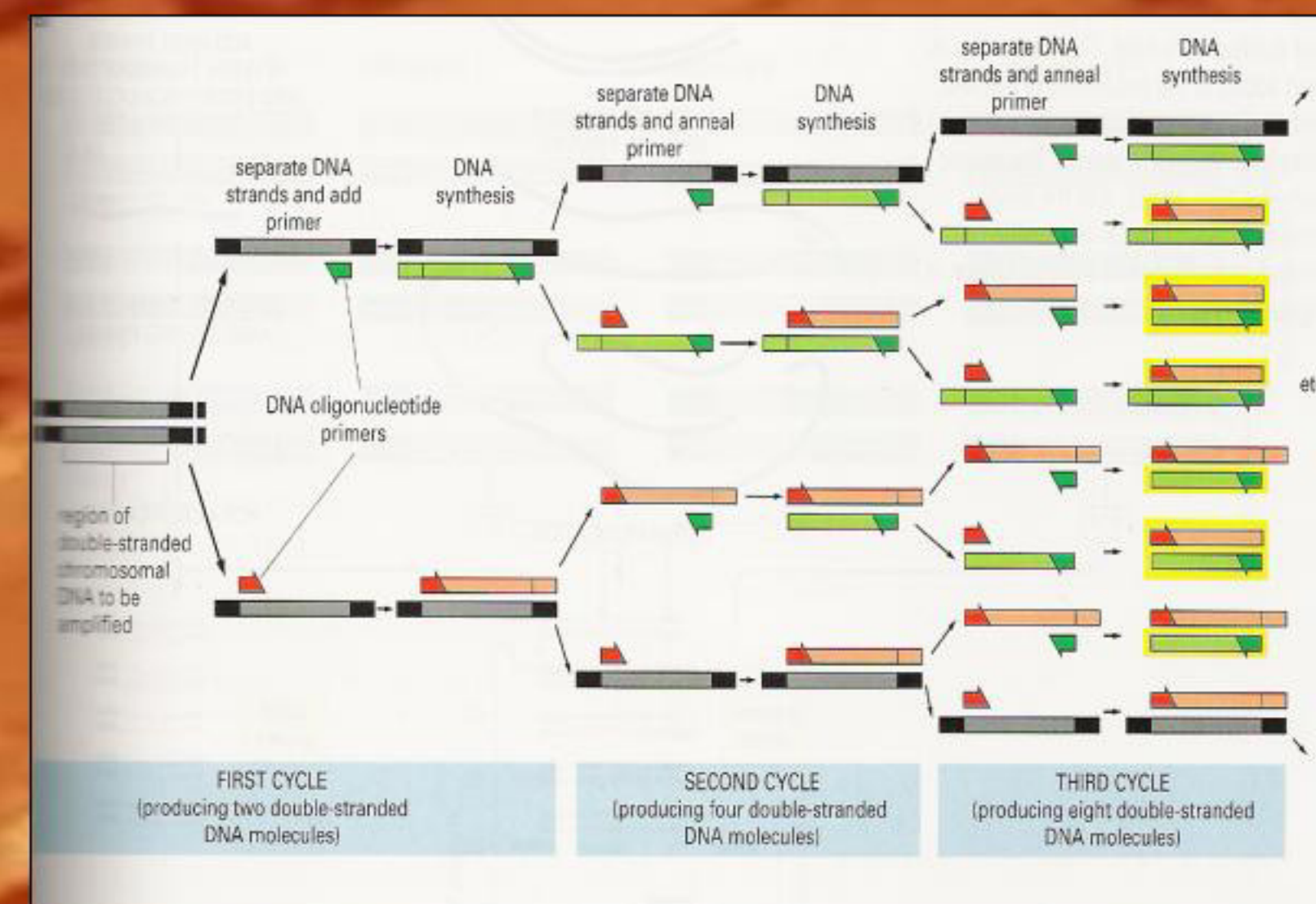
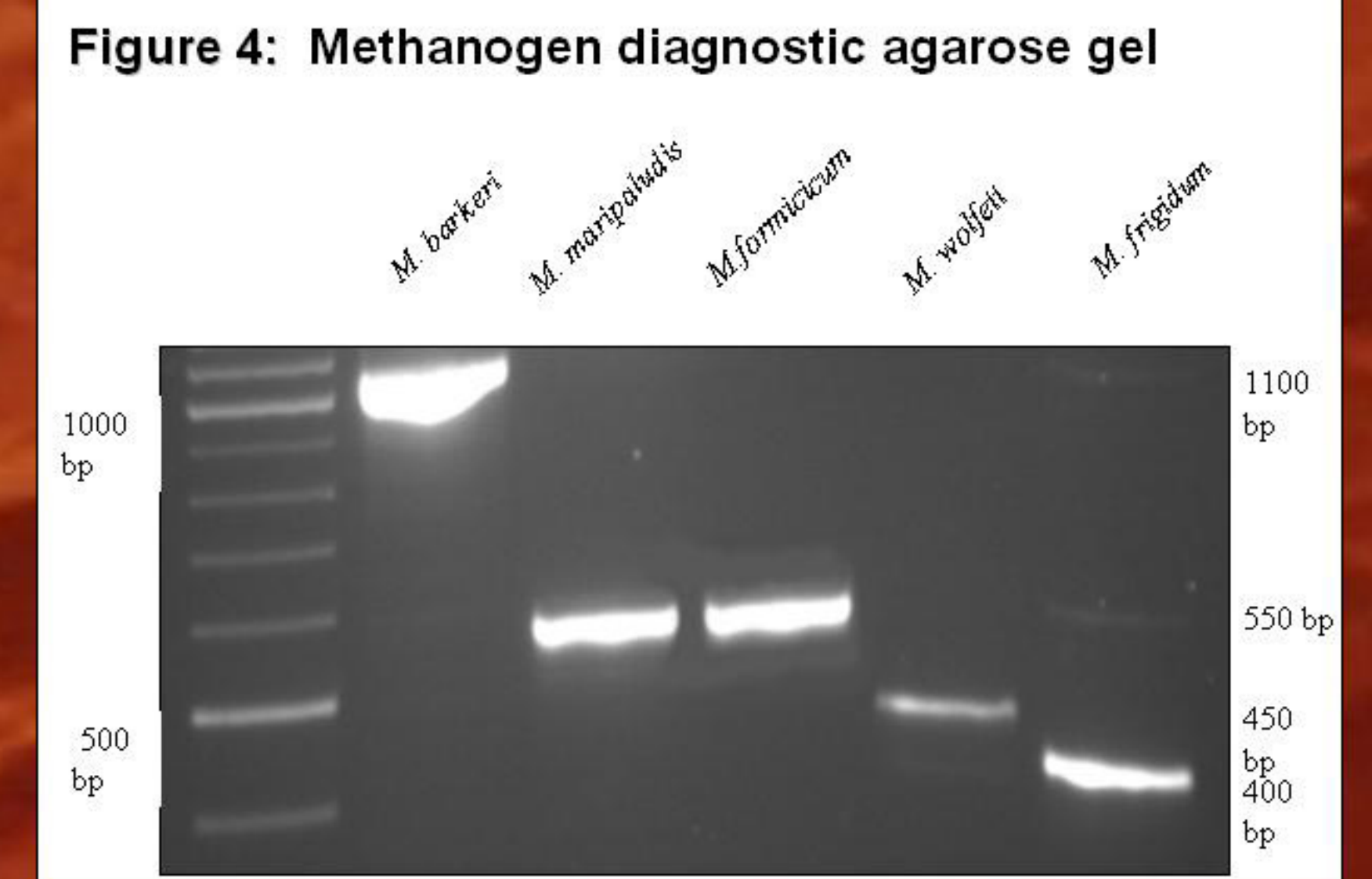


Figure 3: This demonstrates three cycles of the PCR procedure. Note how each cycle doubles the amount of DNA from the previous one. By the time 40 cycles are complete, an amplification factor of around one billion can be obtained.

Expected Results

Below, in Figure 4, are results from prior research of a methanogen diagnostic agarose gel. If the PCR technique is able to detect the methanogens at each dilution level in the Mars soil simulant, then the bands will show up at the approximate base pair mark. This procedure is also expected to produce similar results with the other soil types. ESEM images will also be taken to confirm the microscopic appearance of the methanogens.



Chad Elliott and Travis Altheide using the ESEM in the physics lab at the University of Arkansas.

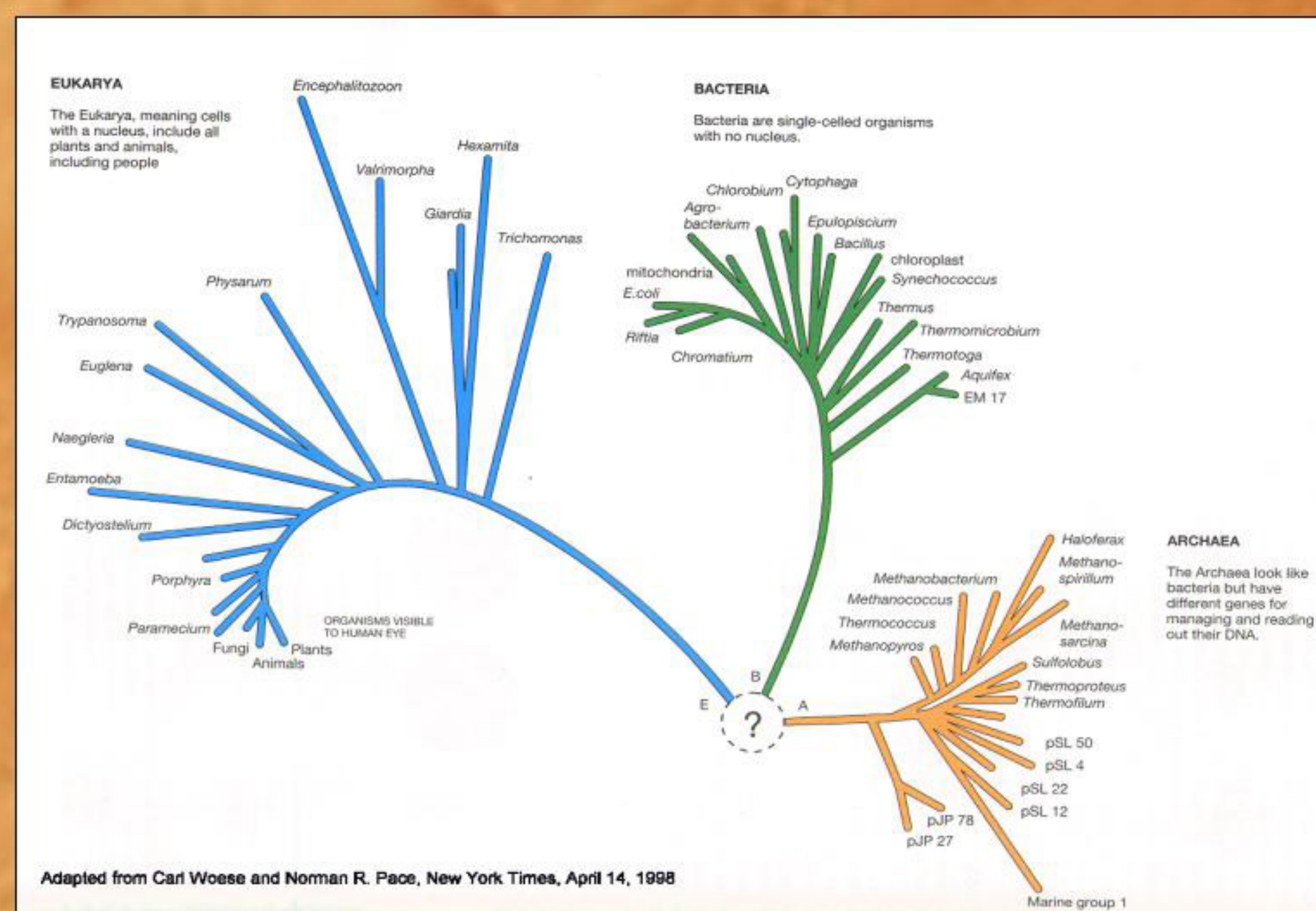
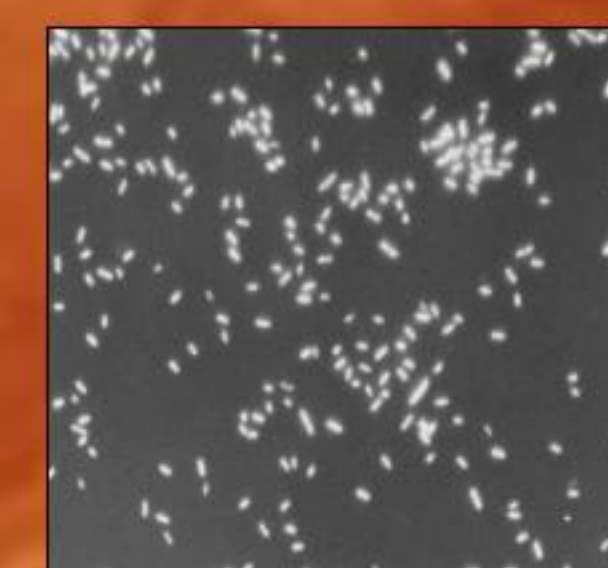
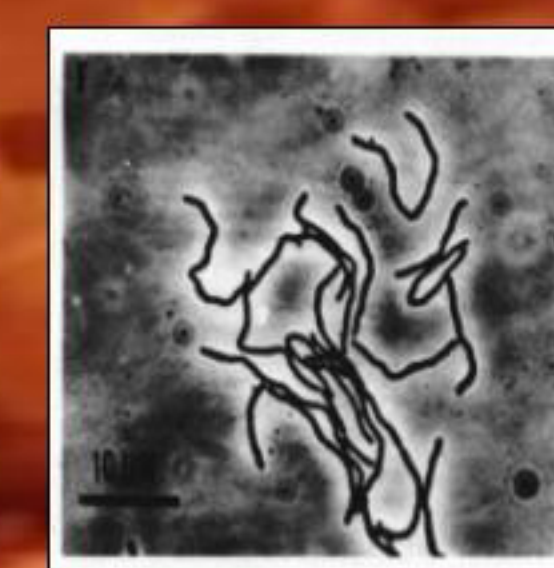


Figure 1: The three domains of life of which all organisms belong. The methanogens belong to the Archaea domain.



M. formicum



M. wolfeii



M. maripaludis



M. barkeri

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