Rapid detection of contaminating bacteria in the *Rhodospirillum rubrum* bioreactor of the life support system MELiSSA

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For a lunar base or a mission to Mars, a reliable life support system is essential to replenish the food and water supplies and manage the production of gases and wastes. MELiSSA (Micro Ecological Life Support System) is a model of regenerative life support system, targeting complete recycling of gas, liquids, and solid wastes, by using the combined activity of different living organisms $i.e.$ microbial communities in 4 succesive microbial bioreactors (CI, CII, CIII, CIVa), a plant compartment, and a human crew (http://www.estec.esa.nl/ecls/). In order for the MELiSSA system to function properly, the organisms inhabiting the MELiSSA loop need to perform their tasks as optimally as possible.

One important aspect is to control the axenicity of the MELISSA compartments CII, CIII, CIVa, because contaminants constitute a major concern in the proper functioning and maintenance of a closed artificial ecosystem. The first compartment of the MELiSSA loop, wherein the organic waste is liquefied by a bacterial consortium originating from the waste itself, is a likely source of pathogens. Hence, the second compartment, in which *R. rubrum* converts the in CI produced volatile fatty acids into minerals and biomass is probably an axenically vulnerable compartment within the MELiSSA loop, due to its direct link with the first compartment.

Methods to check any loss of axenicity in the compartment of *R. rubrum* are presented and evaluated in the present communication. Flow cytometry in combination with specific fluorescent probes, matrix assisted laser desorption ionisation mass spectrometry, and polymerase chain reaction (PCR) based methods were applied to detect the presence of contaminants. A new method involving peptide nucleic acid (PNA) probes specifically targeted at *R. rubrum*, offered the opportunity to selectively amplify unknown contaminants present in *R. rubrum* cultures. The PNA backbone is not charged, thereby conferring to a much stronger binding between a PNA/DNA dimer than a DNA/DNA dimer. In a PCR reaction a PNA probe can't act as a primer, but will remain annealed with the complementary DNA strand during the amplification step. Due to the tight binding characteristics of a PNA/DNA dimer, amplification of a region

spanning the position, wherein a PNA probe can anneal, is blocked. A PCR reaction using a PNA probe, targeted against a particular organism within the 16SrDNA region, combined with universal primers results in a PCR amplification discriminating against the targeted organism, thereby amplifying the 16SrDNA of any other contaminant present in the sample. The PNA based amplification assay followed with denaturing gradient gel electrophoresis proved to be sensitive enough to detect 1 unknown contaminant out of 10,000 target cells. In addition, this detection method can be further adapted towards identification of the contaminating species by 16S rDNA sequencing of isolated amplicons.

This work has been supported by ESA/ESTEC.

R. Wattiez is Research Associate at the National Fund for Scientific Research.