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The Microbiological Control Problem on Board Manned Spacecraft

V. Guarnieri

Alenia Spazio SpA. Torino (Italy)

As demonstrated by 4 decades of space research [1], in microgravity fungi and bacteria efficiently live and proliferate; man is weaker; synergistic effects among microgravity and other space factors may happen; any material can also be damaged, affecting instrument and system reliability.
In general, hazard related to microbial contamination is directly proportional to the amount of microorganisms, so it is necessary that exceeding of thresholds for air, water and internal surfaces be reported as soon as possible to act suitably.
Detection methods commonly used on ground are unsafe and difficult to enforce in microgravity. Thus, monitoring technologies must be capable of functioning in microgravity, managing multiple sample types, providing analytical data in a short time and must fall within stringent power, weight and volume constraints. For instance, Kilgore et al. identified 28 indirect methods for microbial contamination monitoring [2]. Among these, the most promising were measurement of ATP in bioluminescence, and analysis of DNA by PCR.
Alenia Spazio builds manned spacecraft – hence very concerned about the problem. In particular, to rapidly identify and combat the microbiological risk, our final scope is to provide spacecraft with an *integrated system* for monitoring and control microbiological contamination. A network of "sensors" (automatic miniaturized and working in real time) should form this system, connected to a prediction and control model – when a threshold is reached suitable countermeasures are applied.
Technological experiment T2 (Euromir'95 mission) was the first step of this ambitious project – aimed at the validation of measurement methods *simple*, *rapid*, *significant* and *safe* compatible with long duration manned space missions.
T2 experiment was the result of the fruitful collaboration among Alenia Spazio and: Prof. M. Pizturra, Prof. A. Savino, Dr. C. Pasquarella of the University of Perugia; Prof. G. Dall'Oglio of ESLAB Srl (Milano); Prof. G. Norbiato and Dr. T. Vago of IRCEA & Laboratorio di Endocrinologia dell'Ospedale "L. Sacco" (Milano) - on behalf of the Italian Space Agency - MIRIAM Program.
Innovative sampling and analysis methods of T2 allowed measuring microbial contamination *directly* on-board, by *solid state bioluminescence* & *miniculture* [3]. After flight, part of T2 downloaded dry samples were analyzed by a method based on polymerase chain reaction (*PCR*) [4], with a sensitivity of *one microorganism per sample - with bacteria and fungi detected in the same sample*.
In summary, experiment T2 permitted testing/adjusting the following innovations/developments:

- microbiological contamination *indexes* for standard quantification of the problem
- standard sampling method by *membrane* filter utilization (air, water and surfaces)
- analysis methods on board and on ground:

– *solid state bioluminescence* (threshold analysis method; the multipurpose cuvette was patented in March 2001 by ESLAB Srl, Milan; also other Companies have directly or indirectly used/extrapolated the concept) – see Fig.1

– *miniculture* (analysis method by pinpoint colony counting; some Companies have used/extrapolated the concept) – see Fig.2

- analysis on ground, based on polymerase chain reaction *(PCR),* whose sensitivity (see fig. 3) was obtained thanks to new:

– method to *purify reagents from contaminant DNA*

– method to *simultaneously extract DNA from fungi and bacteria*

In the consequent *MIMOSA study* (Microbial Contamination Monitoring System for ISS Alpha), carried out for ESA, it was demonstrated the feasibility of a microbial contamination detection system – for air, water and surfaces – reasonably compact, almost completely automatic, and able to provide results in few minutes from sampling phase. Such a system could be used aboard spacecraft and have wide terrestrial applications, in both civil and military fields [5]. A preliminary functional diagram of MIMOSA is sketched in Fig.4.

References

[1] Moore D., Bie P., Oser H., 1996. Editors. Biological and Medical Research in Space. An Overview of Life Sciences Research in Microgravity, Springer books.
[2] Kilgore M.V., Zahorchak R.J., Woodward S.S., Pierson D.L., Arendale W.F., 1989. Definition of a near real time microbiological monitor for application in space vehicles. NASA/Johnson Space Center 891541.
[3] V.Guarnieri et al. New Methods for Microbial Contamination Monitoring - An Experiment On-Board Mir Orbital Station.. IAF96.64.04, Acta Astronautica
[4] V.Guarnieri et al. Microbial Contamination Monitoring of Space Stations: PCR Analysis for Downloaded Samples of EUROMIR'95 Experiment T2. SAE paper 981762
[5] MIMOSA - Microbial Contamination Monitoring System for ISS Alpha» - V.Guarnieri et al. IAF/IAA-98-G.3.03

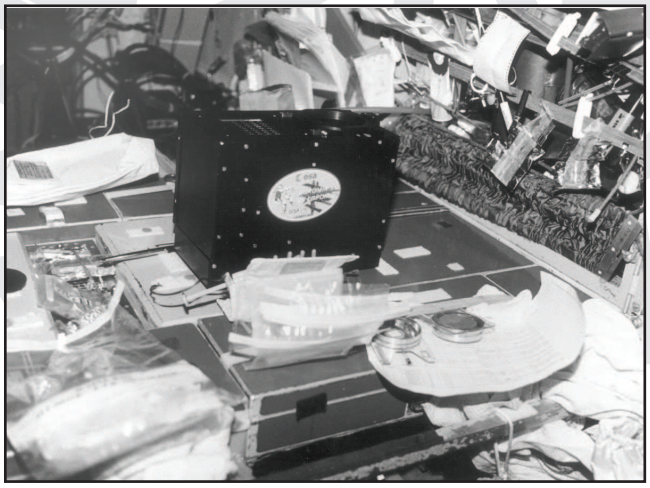

Fig. 1.T2 luminometer (bioluminescence) in flight.

Fig. 2. Minicultures (belonging to "session 1" downloaded samples).

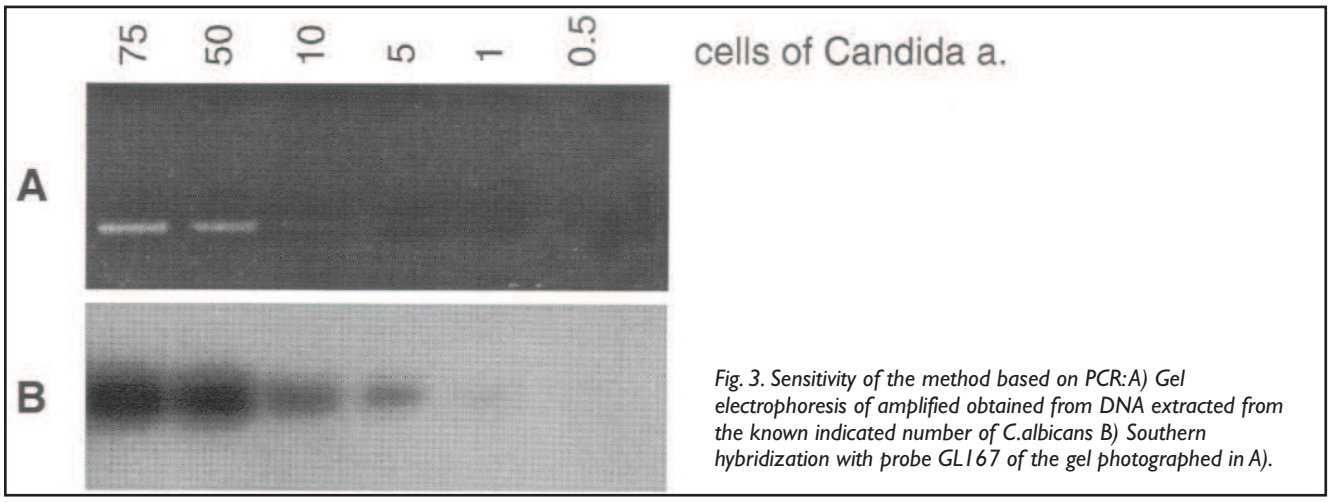

Fig. 3. Sensitivity of the method based on PCR:A) Gel electrophoresis of amplified obtained from DNA extracted from the known indicated number of C.albicans B) Southern hybridization with probe GL167 of the gel photographed in A).

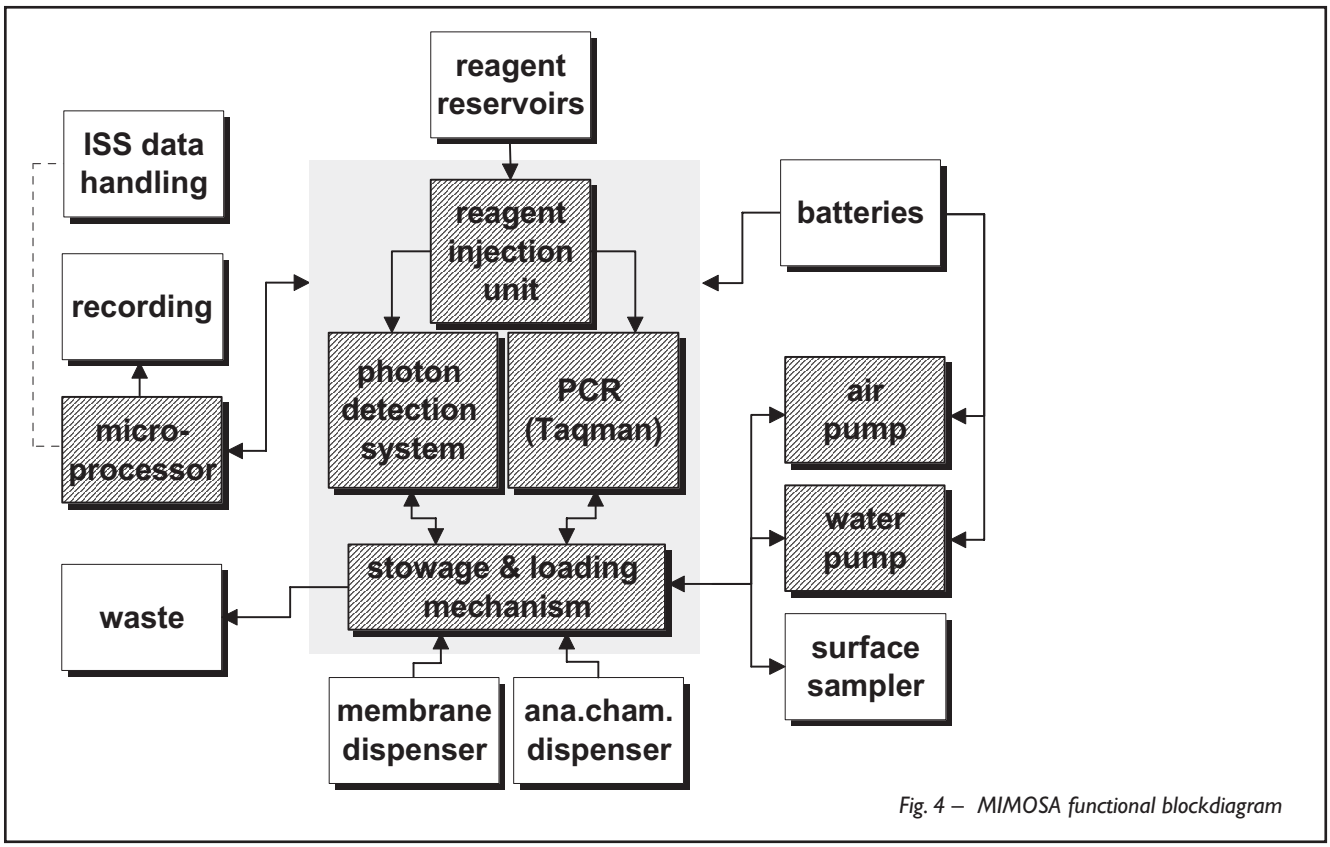

Fig. 4 – MIMOSA functional blockdiagram