Edited by Antonio Mendez-Vilas

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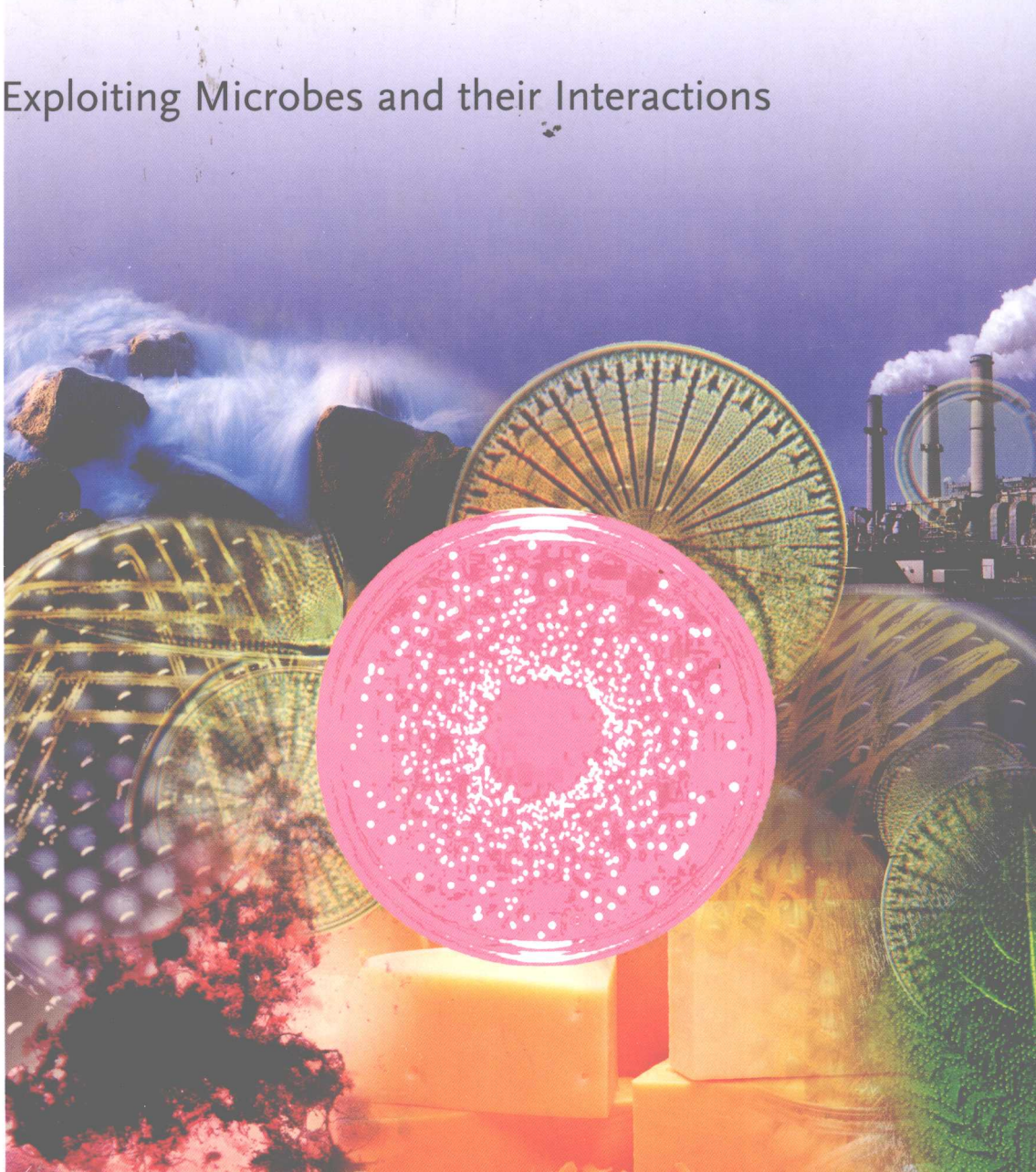
Modern Multidisciplinary Applied Microbiology

Exploiting Microbes and their Interactions



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Preface

This book contains selected papers related to contributions that were presented in short during the 1st International Conference on Environmental, Industrial and Applied Microbiology (BioMicroWorld-2005), held on March 15–18th, 2005 in Badajoz (Spain). http://www.formatex.org/biomicroworld2005, and it is intended to give an overview on the current state-of-the-art of the field.

Focus of the Conference. While Microbiology is about the study of microorganisms (bacteria, viruses, algae, fungi, and protozoa) and of related topics such as microbes interactions, the immune response and molecular genetics, Applied Microbiology is quite interdisciplinary, overlapping aspects of several other academic branches, some of them traditionally near, such as cell biology, molecular and cell biophysics, physiology, parasitology, biochemistry, genetics, medicine, pharmacology, and medical technology, and other not so near as physics, physical (bio)chemistry, materials science, nanotechnology, computer science, information technology, instrumentation, but collaboration with which is resulting in extraordinary advances in this post-genomic world. Thus, cross-disciplinary cooperation in Microbiology has made possible that microbiologists can not only study traditional microorganisms, but also aspects of molecular genetics, biosensors, cancer, aging, immunodeficiency diseases, animal and plant cell cultures, and microscopy, among others. Modern microbiology includes a broad variety of scholarly approaches which lead to a better understanding of all living things at the micrometer-scale/cellular and nanometer-scale/molecular level, and which produce beneficial applications in medicine, agriculture, industry, and ecology.

In this context, the Conference called for papers reporting interdisciplinary resear-chers, relating Microbiology with other Sciences as Physico/Chemistry, Environ-mental Science, Genetics, Pharmacology, Nanoscience, Microscopy/Imaging Science, etc. In other words, we are specially (but not exclusively) interested in reports applying the techniques, the training, and the culture of Microbiology to research areas usually associated with other scientific and engineering disciplines. Over 750 participants from over 60 countries attended the Conference, 15% of which participated with a grant from the conference organization. Over 1100 works were presented during the different oral and posters sessions. Good examples of modern interdisciplinary applied microbiology were the works represented by the three Plenary Speakers:

David C. White, Director of the Center for Biomarker Analysis, University of Tennessee, USA
Lecture: *Biomarkers to define Interactions in the Environment and Health*

Alexander Steinbüchel, Institut für Molekulare Mikrobiologie und Biotechnologie, Münster, GERMANY
Lecture: *Unspecific Microbial Enzymes for Template-independent Biosynthesis of Polyoxoesters, Polythioesters and Polyamides*

Timo Lövgren, Department of Biochemistry and Food Chemistry/Biotechnology, University of Turku, FINDLAND
Lecture: *Novel time-resolved Fluorescence based Immunoassays and Real-time PCR assays in Microbiological Applications*

I am are very grateful to all members of the Organizing Committee for the hard work done in the Conference preparation (which began over a year before the conference) and for the good job that made the Conference so successful that there are already several candidates to host next edition of BioMicroWorld. We would also like to thank the members of the International Scientific Advisory Committee, as well as the reviewers, for their advice, which has certainly helped to improve the quality, accuracy and relevance of this conference Program and publications.

Finally, we would like to thank *BIOMEDAL S.L.-Advances for the Postgenomic Era (http://www.biomedal.es)* for sponsoring the Conference.

Hoping that this edition will stimulate further meetings focusing on the interdisciplinary nature of current relevant applied research, we hope that readers will find the content fruitful and interesting.

Antonio Mendez-Vilas
Editor

FORMATEX, Badajoz, Spain
June, 2006

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A new potential indicator of virological contamination of surfaces

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1 Introduction

TT virus is a widespread infectious agent of humans identified in 1998 (1). The studies carried out till now have evidenced a large diffusion of the virus in the world population with a persistent viremia in infected people characterized by a low pathogenic potential. TTV is present in blood, serum, faeces, pharyngeal and nasal swabs and other biological fluids, it has a high environmental resistance and several ways of elimination (2,3). All this characteristics, linked to the technical problems and great limits that present the direct research of pathogenic viruses in environment, suggest the utilization of TT virus as a new possible indicator of presence of haematic and entero-oral transmission viruses (4). TTV could be researched in particular settings like hospitals, clinical laboratories, etc. in witch the presence, at high concentration, of these pathogenic viruses represent a real risk for health-care workers (5).

2 Materials and methods

The study was carried out in two stages:

2.1 Estimating the sensitivity limits of the technique for TTV detection on surfaces artificially contaminated.

2.2 Environmental monitoring utilising the moist sensible technique.

2.1.1 Preparation of artificial samples

The serum from a TTV virus-infected subject with viral title of 46×10^6 copies/ml has been diluted from 10^{-1} to 10^{-4} in bovine fetal serum negative for TTV-DNA presence. 10 microliters of the whole serum and the dilutions, in addition to a negative control were spread on a sterilized stainless steel plate. The plate was then dried for about 10 minutes.

2.1.2 Estimation of sensitivity limits of sample purification and DNA extraction methods

Two analytical protocols have been compared on artificial samples:

a) Two eluents were tested contemporary: beef extract (BE) 3% at pH 9 and bovine serum albumin (BSA) 1% with NaCI 0.85%. For elution trials, the surface was repeatedly wiped with a cotton swab impregnated with 1 ml of eluent. The swab was then dipped in a test tube containing 1 ml of the same eluent, kept in a refrigerator for 2 hours at 4°C during which time it was shaken every 15 minutes, and finally mixed with vortex. The eluates were then recovered and extracted using a commercial kit "QIAamp DNA Mini Kit" (Qiagen). The recovery test was repeated three times.
b) Application of a commercial kit (DNA IQ™ SYSTEM), generally used in forensic field for the detection and purification of DNA present in several biological samples, on the body and on objects for personal use; it was modified in order to achieve the present work using the Lysis Buffer of the kit as eluent to wash the artificially contaminated surface (250 µl) and cutting the swab of 1 cm long to allow its introduction in a spin basket put in a centrifuge test-tube containing 50 µl of the same eluent. Subsequently the swab was treated as indicated by the protocol of the commercial kit. Also in this case the recovery tests were repeated four more times.

2.1.3 Qualitative detection and quantitative determination of the TTV genome by PCR

The qualitative detection of the TTV genome was carried out by "nested PCR", which uses specific primers drawn by the UTR region of the TTV genome (6). For every reaction negative and positive controls were inserted. In order to quantify the TTV genome the TaqMan-Applied Biosystems Prism 7700 (Poster City, California) system has been used, drawing the primers and probe from the UTR region (7).

2.2 Application of the selected method for the environmental monitoring

The most sensible technique is actually used for an environmental monitoring in different sites of an hospital associated with research of an other biological indicator: haemoglobin (Table 1). A total of 74 selecting points were sampled, chosen mainly for the high probability of becoming contaminated, such as work benches, centrifuges, biosafety cabinet, and other instrumentation.
For TTV and haemoglobin detection, cotton swabs soaked in the relative eluent were wiped repeatedly on area of 36 cm squares. For the detection of haemoglobin a kit used for the blood detection hidden in faeces (Kit OC-Hemocard-Alfabiotech®-Wasserman) was modified and applied to the purpose (8).

Table 1 Environmental monitoring

ENVIRONMENT	ANALYZED SAMPLES
Clinical Lab.	12
Surgery	31
Cardiac Unit Intensive Terapy	16
Rianimation	9
Surgery passage	5
TOT	74