



**SOCIETÀ ITALIANA DI
PROTISTOLOGIA (SIP) *onlus***

**XXXI Congresso della Società Italiana di Protistologia *onlus*
Pineto (TE) 14-16 settembre 2016
Villa Filiani e Torre del Cerrano**

Programma e riassunti dei contributi

Organizzazione:

Graziano Di Giuseppe (Presidente SIP *onlus*), Università di Pisa

Adriana Vallesi (Direttivo SIP *onlus*), Università di Camerino

Marina De Ascentiis, Responsabile Scientifico Museo del Mare, Area
Marina Protetta Torre del Cerrano

Con il patrocinio di:



UNIVERSITÀ
DI CAMERINO



14 settembre

Villa Filiani

Via D'Annunzio, 90 – Pineto (TE)

12:00 Apertura segreteria e Registrazione partecipanti

13:00 Buffet di benvenuto

15:00-15:30 Saluti: Graziano Di Giuseppe (Presidente SIP), Leone Cantarini (Presidente Area Marina Protetta Torre del Cerrano)

15:30-16:00 Seminario d'apertura FEDERICO BUONANNO: **Behavioral and physiological interactions among ciliated protists and/or metazoans.**

I SESSIONE: INTERAZIONI TRA PROTISTI E ALTRI ORGANISMI

Moderatori: Santovito e Miceli

16:00-16:15 ILEANA FEDERIGI, ANNALaura CARDUCCI, GRAZIANO DI GIUSEPPE, MARCO VERANI: **The protective role for Human Adenovirus against water chemical disinfection of *Acanthamoeba polyphaga*.**

16:15-16:35 (premio Nobili-Franceschi) OLIVIA LANZONI, MICHELE CASTELLI, GIULIO PETRONI: **Bacterial endosymbionts in ciliates: from environmental samples to massive genomic extraction.**

16:35-17:00 Coffee Break

17:00-17:20 (premio Nobili-Franceschi) CRISTIANA SIGONA, MASAHIRO FUJISHIMA, GIULIO PETRONI, MARTIN HAHN, CLAUDIA VANNINI: **An investigation on the role of betaproteobacterial endosymbionts in *Euplotes* (Ciliophora, Spirotrichea) by trans-infection experiments.**

17:20-17:35 KESAVA PRIYAN RAMASAMY, SANDRA PUCCIARELLI, PATRIZIA BALLARINI, MATTEO MOZZICAFREDDO, ANDREA TELATIN, MARTINA SCHRALLHAMMER, VALENTINA LOGGI, MARCO ZANOTTI, RITA GIOVANNETTI, CRISTINA MICELI: **Characterisation of the microbial consortium associated with the psychrophilic Antarctic ciliate *Euplotes focardii*.**

17:35-17:55 (premio Nobili-Franceschi) CHIARA PASQUALETTI, FRANZISKA SZOKOLI, GIULIO PETRONI, MARTINA SCHRALLHAMMER: **Transmission of bacterial *Paramecium* endosymbionts influenced by salinity stress.**

17:55-18:10 OLIVIA LANZONI, NATALIA LEBEDEVA, ALEXEY POTEKHIN, GIULIO PETRONI: **Artificial ecosystems as source of potentially pathogenic bacteria associated in symbiosis with ciliates.**

20:00 Cena presso Hotel Centrale, Pineto

15 settembre

Villa Filiani

Via D'Annunzio, 90 – Pineto (TE)

II SESSIONE: I PROTISTI NEL BIOMONITORAGGIO E NELLE BIOTECNOLOGIE

Moderatori: Petroni e Ortenzi

9:00-9:20 SANDRA PUCCIARELLI: **MSCA-RISE-MetaBLE project: searching for novel biocatalysts from extremophiles with biotechnological value.**

9:20-9:40 (premio Nobili-Franceschi) CÉLINE FUSI: **Tossicità acuta e bio-accumulo di metalli pesanti in protisti ciliati dulcacquicoli del genere *Euplotes*.**

9:40-10:10 Coffee break

10:10-10:25 A. PIERSANTI, K. JUGANSON, W. WEI, J. ZHANG, Z. ZHAO, S. PUCCIARELLI, M. MOZZICAFREDDO, C. MICELI, W. MIAO: **RNA-seq analysis of stress response to silver nanoparticles in *Tetrahymena thermophila*.**

10:25-10:40 CÉLINE FUSI, FRANCESCO PAOLO FRONTINI, GRAZIANO DI GIUSEPPE: **Acidophilic microbes in a tuscan acidic mine drainage and potential application of *Euglena mutabilis* in bioremediation.**

10:40-10:55 MARIA CRISTINA ANGELICI, RICCARDO SCARPELLI: **Water-borne protozoan infections in Italy in the globalization scenario.**

11:00 Partenza per Torre del Cerrano

Torre del Cerrano

Strada Statale Adriatica Km431 – Pineto-Silvi (TE)

www.torredelcerrano.it

INCONTRO PUBBLICO

**11:30 INAUGURAZIONE SALA PROTISTI DEL MUSEO DEL MARE,
SALA ESPOSITIVA ALLESTITA DALLA SIP *onlus*
IN COLLABORAZIONE CON L'AREA MARINA PROTETTA TORRE DEL CERRANO**

Introduzione:

FABIANO ARETUSI (Vice-Presidente Area Marina Protetta Torre del Cerrano),
MANOLA DI PASQUALE (Presidente Istituto Zooprofilattico Abruzzo e Molise).

Illustrazione e Visita:

FABIO VALLAROLA (Direttore Area Marina Protetta Torre del Cerrano),
GRAZIANO DI GIUSEPPE (Presidente SIP *onlus*),
MARINA DE ASCENTIIS (Responsabile Scientifico Museo del Mare dell'AMP Torre del Cerrano).

ANNULLO FILATELICO DEDICATO AL CONGRESSO

Apertura ufficio postale distaccato
dalle 9,00 alle 13,00
con ANNULLO FILATELICO

Mostra filatelica dedicata al tema marino

Realizzate 4 cartoline ricordo illustrate



12:30 Buffet

Rientro previsto ore 14:00

15 settembre **Sala Polifunzionale** Via Trieste, 7 – Pineto (TE)

WORKSHOP APERTO ALLA CITTADINANZA

MICROBI EUCARIOTI E NON SOLO: IL LORO UTILIZZO PER L'AMBIENTE E LA SALUTE DELL'UOMO

15:00 **Saluti di apertura:**

ROBERT VERROCCHIO, Sindaco di Pineto

15:30 **Introduzione:**

GRAZIANO DI GIUSEPPE, Presidente SIP *onlus*
SILVIO BROCCO, Liofilchem.

15:30-16:00 ANGELO FONTANA (CNR – Istituto di Chimica Biomolecolare, Napoli): **Diatomee: tra ruolo in Natura e uso biotecnologico.**

16:00-16:30 GUIDO FAVIA (Scuola di Bioscienze e Medicina Veterinaria, Università di Camerino): **Il triangolo della malaria è un quadrato?**

16:30-17:00 coffee break

17:00-17:30 TELATIN ANDREA (BMR Genomics, Padova): **Un primo sguardo al microbioma intestinale degli Italiani.**

17:30-18:00 CARDUCCI ANNALAURA (Dipartimento di Biologia, Università di Pisa): **Microrganismi nelle acque e problemi sanitari.**

18:00- 19:45 Assemblea dei Soci (votazioni) e Tavola rotonda - La Protistologia in Italia: passato, presente e futuro.

20:15 partenza per la cena sociale

16 settembre

Villa Filiani

Via D'Annunzio, 90 – Pineto (TE)

III SESSIONE: BIODIVERSITÀ, GENOMICA E EVOLUZIONE

Moderatori: Verni, Angelici

9:30-9:50 CRISTINA MICELI: **Ciliate genomics: on-line available resources and news about potential new model organisms.**

9:50-10:05 MICHELE CASTELLI, B. FRANZ LANG, GERTRAUD BURGER, GIULIO PETRONI: **Genome analysis of an obligate bacterial endosymbiont of *Paramecium*.**

10:05-10:20 FEDERICO BUONANNO, ANDREA ANESI, GRAZIANO DI GIUSEPPE, GRAZIANO GUELLA, CLAUDIO ORTENZI: **Erythrolactones, the chemical defense weapons of the ciliated protist, *Pseudokeronopsis erythrina*.**

10:20-10:40 (premio Nobili-Franceschi) GLORIA SILVERI: **Characterization and localization of dicer-like proteins during the nuclear development of *Paramecium tetraurelia*.**

10:40-11:10 Coffee break

11:10-11:25 DIANA FERRO, RIGERS BAKIU, FRANCESCA CORRÀ, FRANCO CATTALINI, LAURA GUIDOLIN, PAOLA IRATO, FRANCESCO BOLDRIN, ESTER PICCINNI, GIANFRANCO SANTOVITO: **New insights into reactive oxygen species homeostasis in ciliates: molecular characterization of the catalase gene in *Tetrahymena thermophila*.**

11:25-11:35 FRANCESCA RICCI, FEDERICO LAURO, GIANFRANCO SANTOVITO, PIERANGELO LUPORINI, ADRIANA VALLESI: **Anti-oxidant defense of the bipolar ciliate, *Euplotes nobilii*: characterization of the methionine-sulfoxide reductase-B gene family.**

11:35-11:50 CLAUDIO ALIMENTI, ADRIANA VALLESI, BILL PEDRINI, PIERANGELO LUPORINI: **Insights into the evolution of *Euplotes* pheromone and pheromone-gene structures.**

11:50-12:10 (premio Nobili-Franceschi) RACHELE CESARONI: **Development of a system to detect protein-protein interaction in *Paramecium tetraurelia*.**

12:10-12:25 TJOTA NATALIE, FERRO DIANA: **Intra- and intergenerational effects of photoperiodic variation on *Paramecium* growth.**

13:00 Pranzo presso Hotel Centrale e proclamazione vincitore premio Nobili-Franceschi.

SEMINARI

Behavioral and physiological interactions among ciliated protists and/or metazoans. F. BUONANNO. Laboratory of Protistology and Biology Education, University of Macerata, 62100 Macerata, Italy.

Earth life is represented by multicellular and single-celled organisms, the latter comprising procaryotes, and the large eukaryotic group of protists that appeared relatively early in evolution, about 1.8 billion years ago, soon after bacteria. During this very extensive time period, most species of protists have developed a number of behavioral, morphological, and physiological strategies intended to mediate interactions among protists and/or metazoans.

Interactions involving free living ciliated protists, and those involving protists and multicellular organisms, are mostly mediated by protein and non protein molecules. Among proteins, a paradigmatic case is represented by the ciliate pheromones, diffusible molecules to date structurally characterized in *Blepharisma* and *Euplotes* triggering autocrine (growth-promoting) or paracrine (mating-inducing) cell responses.

With regard the non-protein molecules, different classes of secondary metabolites are, until now functionally and structurally characterized in ciliates, many of which belonging to the chemical families of polyketides and terpenoids, and essentially used in predator/prey interactions with other protists or metazoans. Among these, we can mention blepharismins from *Blepharisma japonicum*, stentorins from *Stentor coeruleus*, climacostol from *Climacostomum virens*, spirostomins from *Spirostomum teres*, hydroquinone from *Spirostomum ambiguum*, euplotins from *Euplotes* species, maristentorin from *Maristentor dinoferus*, amethystin from *Stentor amethystinus*, the keronopsamides and keronopsins from *Pseudokeronopsys riccii* and *Pseudokeronopsys rubra*, respectively.

In addition, these kind of interactions could be mediated also by mechanical processes such as those involving trichocysts (subpellicular non-toxic extrusive organelles) used for defense by some ciliate species such as *Paramecium*, *Frontonia* or *Pseudomicrotorax*.

Diatoms between natural relevance and biotech potential. ANGELO FONTANA. Bio-Organic Chemistry Unit, CNR- Istituto di Chimica Biomolecolare, Via Campi Flegrei 34, 80078 Pozzuoli, Napoli, Italy.

Diatoms are unicellular photosynthetic microorganisms that play a major role in global primary production and aquatic biogeochemical cycling. Although these organisms give origin to massive blooms that sustain life in marine and freshwater environments, their metabolism and biotechnological potential has received little consideration in comparison to other plant-like lineages. In the last years we have been carried out an extensive survey on the lipid metabolism of these organisms and their use of for commercial and industrial applications as the carbon neutral synthesis of fuels, pharmaceuticals, health foods and materials relevant to nanotechnology. Here I report a brief summary of these studies underlying the importance of basic studies in the development of applied products.

I SESSIONE
INTERAZIONI TRA PROTISTI E ALTRI ORGANISMI

The protective role for Human Adenovirus against water chemical disinfection of *Acanthamoeba polyphaga*. ILEANA FEDERIGI¹, ANNALaura CARDUCCI¹, GRAZIANO DI GIUSEPPE², MARCO VERANI¹. ¹Laboratory of Hygiene and Environmental Virology, Department of Biology, University of Pisa, Pisa, Italy; ²Protistology-Zoology Unit, Department of Biology, University of Pisa, Pisa, Italy.

Adenoviruses are important human pathogens that are responsible for both enteric illnesses and respiratory and eye infections. Recently, these viruses have been found to be prevalent in any kind of aquatic environment, such as rivers, coastal waters, swimming pool waters, and drinking water supplies worldwide. *Acanthamoeba* is the most common free-living environmental amoeba, it may serve as an important vehicle for various microorganisms living in the same environment, such as viruses. This study aimed to investigate the interaction between Human Adenovirus type 5 (HAdV 5) and *Acanthamoeba polyphaga* in water environment in order to highlight the role of protection from chemical disinfection of internalized viruses.

In the first part of the study, *Acanthamoeba polyphaga* at mean concentration of 105 cells/ml was co-cultured in 24-well plates with Human Adenovirus (3.16 x 10⁴ DCP50/ml) in water for 1 day at 25°C and the viral uptake was assessed by direct immunofluorescence. In a second series of experiments, the efficacy of sodium hypochlorite disinfection against *Acanthamoeba* and HAdV either singly, by cultural methods, or when co-cultured, as above method, was assessed. In particular, 3 different concentrations in water were tested: 5; 2.5 and 1 mg/L for 24 h contact time.

The data obtained by the co-culture trials demonstrated that HAdV was incorporated into the host amoeba in water. In singly disinfection tests, the results revealed that amoebae still remained alive with 5 mg/L sodium hypochlorite while the viruses loss the infectivity with 2.5 mg/L. In co-cultured trials, at this disinfectant concentration, we found HAdV in *Acanthamoeba polyphaga* cytoplasm. The results of the study confirm and underline the possible role of protection of *Acanthamoeba polyphaga* for Human Adenovirus against chemical disinfection in water environment. More deeply studies in co-cultured experiments can clarify if HAdV infectivity is still present in amoebae after disinfection, revealing a new system of viral resistance in water environment.

Bacterial endosymbionts in ciliates: from environmental samples to massive genomic extraction. OLIVIA LANZONI¹, MICHELE CASTELLI^{1,2}, GIULIO PETRONI¹. ¹Department of Biology, University of Pisa; ²Department of Veterinary Medicine, University of Milan.

Intracellular bacteria, traditionally called endosymbionts, are very common in all living organisms, and ciliated protists are considered one of the most suitable model organisms for the study of endosymbionts because of their small size and simple cultivation techniques.

The aim of this Thesis was to perform a “complete study” approach on bacterial endosymbionts of ciliates. The study was split into two distinct but related parts, due to time constraints.

The first part consisted in the characterization of symbionts from newly isolated hosts present in environmental samples. It was essential to isolate ciliates from their natural environment and establish cell cultures. The subsequent step consisted in performing the screening of ciliates cultures to detect the presence of possible bacterial endosymbionts, with fluorescence *in situ* hybridization (FISH) using a universal 16S rRNA gene-directed probe. Subsequently the endosymbiont retrieved in a selected culture was molecularly characterized.

The second part consisted in setting up the conditions to perform massive total DNA isolation on the selected bacterial endosymbiont *Holospira caryophila*, preliminary to whole genome sequencing. A particular strain of *Paramecium octaurelia* called GFg infected with *H. caryophila* was selected for our purpose. This intracellular bacterium is uncultivable outside host cells, furthermore in literature it has been reported that rapid growing lines of *Paramecium* could lose the endosymbiont. Therefore, to obtain the highest amounts of bacterial cells, a growth experiment was designed establishing three different host feeding conditions. For each of them the host cells were enumerated and FISH experiments were performed with a *H. caryophila*-specific probe to check the infection level. After having found the best feeding condition, massive culture was established in order to isolate the symbiont cells from lysed hosts and subsequently extract their genomic DNA. Different kinds of approaches, previously used in literature for the isolation of other ciliate intracellular bacteria, were adapted and performed. Finally, to evaluate how the isolation procedures worked out, two distinct approaches were set up and utilized: FISH and real-time PCR.

An investigation on the role of betaproteobacterial endosymbionts in *Euplotes* (Ciliophora, Spirotrichea) by trans-infection experiments. CRISTIANA SIGONA¹, MASAHIRO FUJISHIMA², GIULIO PETRONI¹, MARTIN HAHN³, CLAUDIA VANNINI¹. Dipartimento di Biologia, University of Pisa, Pisa, Italy; ²Department of Environmental Sciences and Engineering, Yamaguchi University, Yamaguchi, Japan; ³Research Institute for Limnology, University of Innsbruck, Innsbruck, Austria.

In the obligate symbiosis between a monophyletic group of *Euplotes* species and betaproteobacterial cytoplasmic endosymbionts, the bacteria are essential for the host reproduction and survival. It was suggested that these bacteria carry out a function lost by the common ancestor of host species. Symbionts belong to the species *Polynucleobacter necessarius*, or to the species "*Candidatus* Protistobacter haeckmanni". Other studies showed the existence of free-living strains of the species *P. necessarius*. Object of this study was to investigate the level of functional specificity of these kinds of bacteria with respect to the different potential ciliate hosts. The host species *E. harpa* FSP1.4 and *E. woodruffi* POH1 were studied. They hosts, respectively, the symbionts *P. necessarius* and "*Candidatus* P. heckmanni". Removal of the symbionts was done by antibiotic treatment. Two kinds of re-infection experiments were performed: acquisition of the bacteria by phagocytosis and microinjection of the bacteria directly in the cytoplasm of the hosts. The ability of bacteria to colonize the cytoplasm and to restore ciliates division were checked. Phagocytosis experiments showed that the bacteria were acquired: the FISH detected their presence within the vacuoles, and only in a case also in the cytoplasm after two days; but they were never retained in the cytoplasm in the following days. Microinjection experiments showed the re-establishment of the symbiosis only for the *P. necessarius* symbiont with its original host. It was inferred that: 1) the free-living *P. necessarius* is neither able to replace the symbiotic one from a functional point of view, nor able to colonize the cytoplasm of *Euplotes*; 2) the microinjection technique can be applied to *P. necessarius*, but not to "*Candidatus* Protistobacter"; 3) there is a certain degree of specificity for the host: the symbiotic *P. necessarius* was not able to establish the symbiosis with the host of "*Candidatus* P. heckmanni". Therefore, the level of specificity of these symbiosis is high.

Characterisation of the microbial consortium associated with the psychrophilic Antarctic ciliate *Euplotes focardii*. KESAVA PRIYAN RAMASAMY¹, SANDRA PUCCIARELLI¹, PATRIZIA BALLARINI¹, MATTEO MOZZICAFREDDO¹, ANDREA TELATIN², MARTINA SCHRALLHAMMER³, VALENTINA LOGGI⁴, MARCO ZANOTTI⁴, RITA GIOVANNETTI⁴, CRISTINA MICELI¹. ¹ School of Bioscience and Veterinary Medicine, University of Camerino, Italy; ²BMR Genomics, Padova, Italy; ³Institute of Biology, University of Freiburg, Germany; ⁴School of Science and Technology, University of Camerino, Italy.

The ocean comprises about 70% earth surface and represents a rich source of microbial species. Microorganisms are highly diverse and abundant group of organisms that constitute about 60% of the Earth's biomass. Ciliates (Phylum- Ciliophora) are heterotrophic protozoans and are colonised by different types of bacteria, in particular many unrelated genera of proteobacteria. They can be present as endosymbionts, or localized in the outer surface as ectosymbionts. The psychrophilic marine ciliate *Euplotes focardii* (Class Spirotrichea, Subclass Hypotrichia) colonized the harsh oligotrophic Antarctic environment. It has been isolated from sandy sediments of the Ross Sea near Terra Nova Bay located in Antarctica. In this study, we focus on the characterization of members of the bacterial consortium associated to this Antarctic ciliate. By using culture enrichment technique with diesel oil, methanol, cadmium and copper chloride, we isolated five different bacterial strains: *Marinomonas* sp, *Rhodococcus* sp, *Bacillus* sp, *Pseudomonas*, and *Brevundimonas* respectively. In situ hybridization experiments were performed to analyse the cellular localization of the isolated bacterial strains using designed 16SrRNA probes. The whole genomes of these strains have been sequenced with Next Generation Sequencing (NGS) using a whole Genome Shotgun (WGS) approach. PROKKA pipeline has been used to annotate the WGS data. Preliminary C.O.D. (Chemical Oxygen Demand) experiments showed that *Marinomonas* sp and *Pseudomonas* sp are capable of utilizing diesel as food, suggesting that they may be used in bioremediation. Furthermore, the characterization of the bacterial consortium in *E. focardii* contributes to understand how different organisms cooperate for environmental adaptation.

Transmission of bacterial *Paramecium* endosymbionts influenced by salinity stress. CHIARA PASQUALETTI^{1,2}, FRANZISKA SZOKOLI^{1,3}, GIULIO PETRONI¹, MARTINA SCHRALLHAMMER².

¹Dipartimento di Biologia, Università di Pisa, Italy; ²Mikrobiologie, Institut für Biologie II, Albert-Ludwigs-Universität Freiburg, Germany; ³Institut für Hydrobiologie, Technische Universität Dresden, Germany

Paramecium can be found in freshwater and brackish environments. For the latter, changes in salinity concentrations represent an important environmental stress which influences the ecology and physiology of these microorganisms. It was proposed that protists, including *Paramecium*, living in brackish environments are more frequently infected with bacterial endosymbionts. This might imply an evolutionary advantage for symbiont-bearing microorganisms in habitats which are exposed to salinity stress.

In this study four salinity stress levels (no, weak, strong, and lethal stress) were defined by growth analyses of several strains at different salinity concentrations. Using this data, it was possible to calculate the relative reduction of the total cell number in relation to the maximal cell number reached in freshwater (0‰). Out of this, we could define stress levels as: I) no osmotic stress 0‰, II) weak osmotic stress 1.8‰, III) strong osmotic stress 4.5‰, IV) lethal osmotic stress 7‰ (not considered in following experiment). Analyzed species showed differences regarding to their salinity tolerance.

Furthermore, the possibility of horizontal endosymbiont transmission from strains infected by ‘*Candidatus Megaira polyxenophila*’ (donor strains) to symbiont-free strains (receiver) was investigated by co-cultivation under different salinity stress conditions. Additionally, the ability of paramecia to maintain bacterial endosymbionts under different osmotic stress levels was verified. Population and infection dynamics were observed by fluorescence *in situ* hybridization (FISH) using probes specific for the symbiont species and/or the *Paramecium aurelia* species complex. A first set of experiments showed that at control conditions (no salinity stress) no symbiont transmission was observed. On the contrary, at weak salinity stress, symbiont transmission was observed. At high salinity stress condition, donor strains lost their endosymbionts. To confirm these results, a second round of experiments was performed using genetically identical infected (donor) and symbiont-free (receiver/aposymbiotic) cell lines that had been produced using tetracycline on infected cells. In this case, transmission at weak salinity stress was not observed, whereas endosymbionts loss under high stress conditions was confirmed.

Finally, the effect of endosymbionts on the growth rate of genetically identical infected and symbiont-free cell-lines exposed to different salinity stress was determined. The presence of ‘*Candidatus Megaira polyxenophila*’, turned out to be beneficial for *P. biaurelia* strain YE9 since infected lines grew better than uninfected lines under osmotic stress condition (exception at 2‰). In the case of *P. primaurelia* strain LgJac2III, a negative effect of the bacterium on host growth was observed.

Obtained results suggest that: 1) the symbiosis between *Paramecium* and ‘*Candidatus Megaira polyxenophila*’ can be either parasitic or mutualistic, according to involved hosts species and strains; 2) weak salinity stress levels could favor symbiont transmission; 3) high salinity stress favors the loss of the symbiont.

Artificial ecosystems as source of potentially pathogenic bacteria associated in symbiosis with ciliates
OLIVIA LANZONI¹, NATALIA LEBEDEVA², ALEXEY POTEKHIN³, GIULIO PETRONI¹. ¹ Department of Biology, University of Pisa, Pisa, Italy; ² Centre of Core Facilities “Culture Collections of Microorganisms”, Research Park, Saint Petersburg State University, Saint Petersburg, Russia; ³ Department of Microbiology, Saint Petersburg State University, Saint Petersburg, Russia.

Ciliates (Ciliophora) play a double role in artificial ecosystems (e.g. activated sludge plants and fish-farms), indeed they control the abundance of bacteria, which are highly present in such factories, and may become reservoirs of potentially pathogenic bacteria. As matter of fact, ciliates as phagotrophic predators are able to engulf bacteria present in water and establish symbiotic associations offering many cell compartments and protection from adverse conditions. In this manner, bacteria escape from disinfection systems and spread in the natural environment.

In this study, ciliates isolated from various artificial ecosystems have been screened in order to detect the possible presence of intracellular bacteria. Endosymbionts have been molecularly characterized following the full-cycle rRNA approach and in some cases preliminary phylogenetic analysis and ultrastructural investigations have been performed. Bacteria have been found frequently associated with *Paramecium*, but also with other genera such as *Euplotes* and *Colpidium*. The host cell usually presented symbionts in the cytoplasm or in the nuclear apparatus. However, in some cases the presence of multiple symbiotic associations has been recorded within the same host cell.

Several new species of endosymbionts have been characterized and some of them appeared to be related to pathogenic bacteria (e.g. *Rickettsiaceae*). Numerous novel strains belonging to the recently described “*Candidatus Megaira*” genus proved that “*Ca. Megaira*” might be the most widespread bacterium in protist world.

**II SESSIONE
I PROTISTI NEL BIOMONITORAGGIO E NELLE
BIOTECNOLOGIE**

MSCA-RISE-MetaBLE project: searching for novel biocatalysts from extremophiles with biotechnological value. SANDRA PUCCIARELLI, School of Biosciences and Veterinary Medicine, University of Camerino.

The aim of the MSCA-RISE-MetaBLE project is to acquire new insights about the mechanisms of environmental adaptation, particularly for cold-adapted organisms, and to discover novel metabolic pathways and enzymes with potential industrial value. We intend to accomplish this goal by establishing a collaboration and a knowledge-exchange network between industrial and academic partners aiming at developing tools for genomic analysis and directed evolution. The partners present high complementarity: the University of UNICAM (IT) holds genomic sequences from psychrophilic and mesophilic ciliates that need to be annotated and analysed. The University of CAMBRIDGE (UK) provides the know-how for the discovery of new metabolic pathways valuable for understanding environmental adaptation. BMR Genomics (IT) and the University of COMSATS (PK) possess all the facilities for the sequencing and analysis of genomes from extremophiles. Epigenetiks Inc. has specialized expertise in comparative genome analysis in a pathway related context to identify differentially activated and deactivated pathways upon environmental adaptation. The University of Milano-Bicocca owns the facilities for the heterologous expression of gene sequences in bacteria and yeasts and technologies to assess and implement the properties of novel enzymes towards industrial application. We are confident that the establishment of the international research network proposed will significantly contribute to push all the involved laboratories to the top level in the field of production of active biomolecules and in the discovery of mechanisms of molecular adaptation under extreme environmental conditions. These discoveries may help the manufacturing of a new generation of ordinary facilities, as cleaning agents, biofuels, replacing chemicals in industry as a green technology applications.

RNA-seq analysis of stress response to silver nanoparticles in *Tetrahymena thermophila*. A. PIERSANTI¹, K. JUGANSON², W. WEI³, J. ZHANG³, Z. ZHAO³, S. PUCCIARELLI¹, M. MOZZICAFREDDO¹, C. MICELI¹, W. MIAO³. ¹School of Biosciences and Veterinary Medicine, University of Camerino, Italy; ²National Institute of Chemical Physics and Biophysics, Tallinn, Estonia; ³Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China.

Silver nanoparticles are increasingly used as biocides in various consumer products and if released in the environment they can affect non-target organisms. Nanoparticles specific surface area increases in reversal proportion to the particle size; thus, the smaller the particle, the greater the proportion of atoms that lay close to or at the surface resulting in higher reactivity of the particle. These properties, which make nanomaterials more efficient in industrial applications, might make them also more harmful to living organisms since they could be able to penetrate physiological barriers and interact with subcellular structures. Therefore, understanding the toxicity mechanisms is crucial for both the design of more efficient nano-antimicrobials and for the design of nanomaterials that are biologically and environmentally benign throughout their life-cycle. *Tetrahymena thermophila* has been largely investigated and functional genomic databases are available. Therefore, it is an optimal model system for studying molecular bases of environmental responses, since molecular data obtained in different environmental conditions can be easily compared. We recently used *T. thermophila* to elucidate the environmental effects of silver nanoparticles by analysing the gene expression profile by RNA-seq after exposure to silver nanoparticles (AgNPs) and comparing with the effect of the soluble silver salt, AgNO. We tested two different toxicant concentrations at two time points, for 2 and 24 hours. By several bioinformatic tools the experimental sequences are compared with the control to evaluate quantitatively the inhibition or increase of gene expression due to nanoparticles or silver ions. Some biological processes are targets of both toxicants. In addition to many similarities in affected genes, some effects were different for soluble silver ions with respect to AgNPs. This research provides evidence that silver nanoparticles might be toxic due to combined effects of soluble silver ions released from the particles and the particles themselves.

Acidophilic microbes in a tuscan acidic mine drainage and potential application of *Euglena mutabilis* in bioremediation. CÉLINE FUSI, FRANCESCO PAOLO FRONTINI, GRAZIANO DI GIUSEPPE.

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Many studies have illustrated the potential role of eukaryotes in bioremediation of Acidic Mine Drainage (AMD), especially algae and fungi that inhabit this environment. It is largely reported that algae can generate alkalinity and reduce level of heavy metals, directly and indirectly. In this study, two abandoned mines were analysed: Pollone Mine and Monte Arsiccio Mine (Lucca, Italy). Here, pyrite dissolution that generates acidic drainage is accelerated by the activity of prokaryotes. With PCR amplification (16S rDNA), these bacteria were detected: *Acidithiobacillus ferrooxidans*, *Acidithiobacillus ferrivorans*, *Sulfobacillus* sp., *Leptospirillum ferrooxidans* and *Leptospirillum* sp. Moreover, inside and outside mines, some forms of protists were found. With molecular method (PCR amplification of the 18S rDNA) the ciliated protozoan *Oxytricha acidotolerans* was identified and with morphological approach an alga was identified as *Euglena mutabilis*. This last protist is a typical inhabitant of acidic environment and it can accumulate metals such as Fe and As; furthermore, a specific detoxification pathway for As was found. A biological and passive method of remediation is proposed: exploiting the endemic alga *E. mutabilis*, it's possible to promote the alkalinity of effluents and reduce the concentration of some heavy metals.

Water-borne protozoan infections in Italy in the globalization scenario. MC ANGELICI, R.

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The so-called waterborne diseases are all the infections acquired through the contact with both drinking and recreational water. Some parasite protozoa show an aquatic stage of their biological cycle because cysts and oocysts are able to survive long-time in water. *Giardia duodenalis*, *Cryptosporidium parvum*, *Cyclospora cayetanensis*, *Toxoplasma gondii*, have been isolated frequently by piped and surface water and have been described as cause of several widespread outbreaks. These protozoa infections are maintained by a zoonotic cycle and transmitted by an oral-fecal route. Some countries, like USA, Australia and New Zealand, have organized well-structured surveillance systems, to identify cause-effect relationship in this environmental transmission. European countries have not a surveillance plane, except of Great Britain, Germany, Austria and Ireland nevertheless several risk factors exist. Considering the globalization scenario and the migratory flow to which the continent is submitted, this knowledge lack can no longer be justified for its public health involvement. A monitoring and epidemiological survey have been promoted to study the effective risk degree for the waterborne protozoan infections in Italy. Results indicate a certain number of human cases for these infections and correlation with environmental monitoring data almost for all the protozoan species. Particularly some species are less frequent than in other Countries (*Cryptosporidium*) whereas some others are more frequent and emergent (*Blastocystis*, *Dientamoeba*) both in feces and water samples analysis. A surveillance-standardized network for these protozoan infections is desirable in Europe and efforts have to be employed on this field.

**III SESSIONE
BIODIVERSITA', GENOMICA E EVOLUZIONE**

Genome analysis of an obligate bacterial endosymbiont of *Paramecium*. MICHELE CASTELLI^{1,2}, B. FRANZ LANG³, GERTRAUD BURGER³, GIULIO PETRONI². ¹Dipartimento di Medicina Veterinaria, University of Milano, Milano, Italy; ²Dipartimento di Biologia, University of Pisa, Pisa, Italy; ³Departement de Biochimie and Robert-Cedergren Centre for Bionformatics and Genomics, Universite de Montreal, Montreal, Canada.

Ciliates are well known for harbouring a variety of bacterial endosymbionts, however, the molecular mechanisms of interaction and the effect on the protist are poorly understood. A peculiar case is represented by bacteria of the genus *Holospora* (*Alphaproteobacteria*), which are endosymbionts of *Paramecium* spp. *Holospora* displays a distinctive life cycle, involving two shapes: the reproductive form (RF) and the infectious form (IF). The RF is a Gram-negative rod, which resides and multiplies in the host nuclear apparatus. Under certain conditions, which can be triggered by host starvation, the RF differentiates into the elongated IF, which is unable to divide and is released into the external medium by vesicles. When the IF is phagocytosised by another *Paramecium* cell, the bacterium is activated, escapes digestion, and enters the host target nucleus, where it eventually produces another cycle of RFs. Our study is focusing on the genome sequence of *Holospora caryophila*, a macronuclear endosymbiont of *Paramecium octaurelia*. We have produced a draft assembly (111 scaffolds: 987,513 bp; N50: 105,107 bp; GC%: 33.6), which represents the almost complete genome of *H. caryophila*; high-copy number repetitive regions hamper further joining of scaffolds. The metabolic capacity of the endosymbiont was inferred from the annotated gene set, and compared with selected obligate intracellular and free-living *Alphaproteobacteria*. *H. caryophila* reveals to be a highly specialized obligate endosymbiont, lacking several pathways typical for free-living bacteria, therefore heavily relying on the host even for ATP production. We will present hypotheses on *H. caryophila* evolutionary history, including the possibility of horizontally transferred genes, as well as on the interaction with *Paramecium* – to be confirmed by future analytical and experimental studies.

Erythrolactones, the chemical defense weapons of the ciliated protist, *Pseudokeronopsis erythrina*. F. BUONANNO¹, A. ANESI², G. DI GIUSEPPE³, G. GUELLA^{2,4}, C. ORTENZI¹. ¹Laboratory of Protistology and Biology Education, University of Macerata, 62100 Macerata, Italy; ²Bioorganic Chemistry Laboratory, Department of Physics, University of Trento, 38050 Povo, Trento, Italy; ³Department of Biology, University of Pisa, Via Alessandro Volta 4, 56126, Pisa, Italy; ⁴Biophysical Institute, CNR, Via alla Cascata 56/C, 38123 Povo (Trento), Italy.

Pseudokeronopsis erythrina produces three new pigmented secondary metabolites, erythrolactones A2, B2 and C2, and their respective sulfate esters (A1, B1, C1), whose structures have been recently elucidated on the basis of NMR spectroscopic data coupled to high resolution mass measurements (HR-MALDI-TOF). We investigated if the erythrolactones stored in pigment granules of *P. erythrina* are involved in chemical defense against predators, by evaluating the toxic activity of these secondary metabolites on a panel of free-living ciliates and micro-invertebrates, and the activity of each purified erythrolactone on three ciliate species. This allowed us to recognize the structural trait that increases the biological activity of one of the three protozoan molecules.

We also performed some observations on the prey/predator interaction of *P. erythrina* with unicellular and multicellular predators which highlight that erythrolactones can exert their defensive function by means of a complex and differentiated set of effects that can simply deter or discourage predation, or eventually lead to the predator's death.

New insights into reactive oxygen species homeostasis in ciliates: molecular characterization of the catalase gene in *Tetrahymena thermophila*. DIANA FERRO¹, RIGERS BAKIU², FRANCESCA CORRÀ³, FRANCO CATTALINI³, LAURA GUIDOLINI³, PAOLA IRATO³, FRANCESCO BOLDRIN³, ESTER PICCINNI³, GIANFRANCO SANTOVITO³. ¹Institute for Evolution and Biodiversity, Westfälische Wilhelms-Universität, Münster, Germany; ²Department of Aquaculture and Fishing, Agricultural University of Tirana, Tirana, Albania; ³Department of Biology, University of Padova, Padova, Italy.

Peroxisomes are very metabolically active organelles and are a very important source of reactive oxygen species (ROS), mainly hydrogen peroxide (H₂O₂). Peroxisomes have also the capacity to can also protect cells from oxidative stress expressing catalase (CAT), a metalloenzyme that catalyze the dismutation of H₂O₂ to water and molecular oxygen. With the aim to study the molecular and physiological features of CAT in the ciliated protozoan *Tetrahymena thermophila*, we characterized the gene codifying this protein. In the GenBank database only one CAT-like sequence from *T. thermophila* is present; we studied this gene, demonstrating its effective transcription after cloning and sequencing analyses. The deduced protein, named TthermCAT, show a good level of sequence conservation with orthologues of some protozoon and animal species, especially regarding residues that are important for the catalytic activity. Preliminary phylogenetic analyses indicate that the *T. thermophila* CAT is well correlated with orthologues from other Alveolata species (*Ichthyophthirius multifiliis* in particular) and clearly separated from CATs of metazoa. We used copper, a metal with pro-oxidant proprieties, in order to stimulate gene activation. Copper-dependent regulation of CAT expression was investigated by measuring both mRNA accumulation and enzyme activity in response to acute exposure to subtoxic doses of the metal (500 µM). RT-qPCR analyses show that the time-course of mRNA accumulation is higher in Cu-treated cells but CAT activity did not vary if compared to control cells. These results suggest that the activation of the CAT gene is finalized to a preventive mRNA accumulation, and that the immediate risk of oxidative stress is counteracted by other enzymes that are able to eliminate the H₂O₂, as GPXs, whose increase of enzymatic activity has already been demonstrated in the same experimental conditions. (Grant by M.I.U.R.)

The anti-oxidant defense of the bipolar ciliate, *Euplotes nobilii*: characterization of the methionine-sulfoxide reductase-B gene family. FRANCESCA RICCI¹, FEDERICO LAURO², GIANFRANCO SANTOVITO³, PIERANGELO LUPORINI¹, ADRIANA VALLESI¹. ¹School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy; ²Singapore Centre on Environmental Life Sciences Engineering, Nanyang Technological University, Singapore, Singapore; ³Department of Biology, University of Padova, Padova, Italy.

In polar coastal seawaters, life must face high oxygen concentrations and enhanced UV radiations. These abiotic stresses cause the intracellular accumulation of reactive oxygen species that are particularly noxious to the activity of biological macromolecules. Polar organisms have thus evolved particularly effective mechanisms to cope with the oxidative damage. We studied these mechanisms in *Euplotes nobilii*, a species distributed in both Antarctic and Arctic coastal waters, in comparison with its phylogenetically closest relative, *E. raikovi*, dwelling in temperate mid-latitude sea waters. Doses and times of exposure to hydrogen peroxide that were lethal to *E. raikovi*, did not affect at all the viability of *E. nobilii*. To shed light on the genetic basis underlying this powerful response of *E. nobilii* to the oxidative stress, we focused particular attention on the genes encoding Met-sulfoxide reductases of type B (MsrB), that repair proteins carrying Met-sulfoxides with the sulfur atom in the stereoisomeric R-form. Following a previous characterization of one of these *msrB* genes via molecular cloning, three new other *msrB* genes have now been characterized via an in-silico search on a draft assembly of the transcriptionally active genome of the *E. nobilii* somatic macronucleus. They are constitutively expressed and encode four structurally distinct MsrB isoforms. Each takes its own specific localization inside the cell, cytoplasm, nucleus, mitochondria and endoplasmic reticulum, thus ensuring an integrated protection of various cellular compartments of *E. nobilii* against the oxidative damage.

Insights into the evolution of *Euplotes* pheromone and pheromone-gene structures. CLAUDIO ALIMENTI¹, ADRIANA VALLESI¹, BILL PEDRINI², PIERANGELO LUPORINI¹. ¹School of Biosciences and Veterinary Medicine, University of Camerino, Italy; ²Paul Scherr Institute, Villigen PSI, Switzerland.

Cell-cell communication via diffusible signalling protein pheromones is a pervasive phenomenon among *Euplotes* species. The panel of species, namely *E. raikovi*, *E. nobilii*, *E. octocarinatus* and *E. crassus*, that have mostly contributed to the knowledge of the structures of these pheromones and of their coding genes (which are expressed in the cell somatic macronucleus), has recently been expanded to *E. petzi* and *E. focardii* that occupy quite distant positions in the *Euplotes* phylogenetic tree. The former, together with *E. sinicus*, represents the earliest divergent clade of the tree, while the latter clusters close to *E. crassus* into a late branching clade. The study of this wider panel of species is providing new information on the evolution of the pheromone and pheromone-gene structures, that can be synthesized with the aphorism: the earlier, the shorter and simpler. The *E. petzi* pheromones have in absolute the shortest amino acid sequences of only 32 residues and the simplest molecular structures characterized by only two short alpha-helices and an irregular 4-residue helical turn. Analogously, the *E. petzi* pheromone genes have the shortest nucleotide sequences of only 700 bp, with no apparent insertion of intron-sequence. On the other hand, in *E. focardii*, the pheromone sequences extend for 85 residues and the pheromone genes have sequences of 1900 bp, three-fourths of which belong to the 5' leader un-transcribed region.

Intra- and intergenerational effects of photoperiodic variation on *Paramecium* growth. TJOTA NATALIE, FERRO DIANA, Institute for Evolution and Biodiversity, Westfälische Wilhelms-Universität, Münster, Germany

In the last century, a large number of studies highlighted the presence of strong relationships between metabolic states (e.g. reactive oxygen species homeostasis) and environmental-induced fluctuations of the circadian rhythm. The circadian programming pathways, involved in the regulation of the internal metabolism, show high levels of gene conservation in flies, mammals and fish. In humans, internal clock factors are essential for maintaining the energetic balance and allow for plastic adaptations, not only in case of environmental changes, but also in presence of diseases like thyroid malign cancers. Ciliates, like *Paramecium tetraurelia* and *Tetrahymena termophila*, are unicellular eukaryotic models, highly important for modern evolutionary molecular biology. In these alveolata species only little information is available about the circadian rhythm. Moreover, the effect of fluctuations in photoperiod on genome and proteins evolution remains poorly investigated. In this work, different light / dark conditions were tested with physiologically synchronized cell lines of *P. tetraurelia* in order to identify the possible presence of relationships between the metabolic state and photoperiod fluctuations. We monitored the cell division and the mortality rate over five sexual generations. Our preliminary results demonstrate not only the presence of a strong light-related effect on cell division, within and across experimental generations. These observations highlight that ciliates can be used as model organism to study the relationship between endogenous clock regulators and stress resistance in an evolutionary context.