UNUSUAL MICROORGANISMS FOR NOVEL INDUSTRIAL APPLICATIONS

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Introduction. The size of the planet prokaryote diversity is increasing every year with the discovery of novel lineages from conventional and extreme environments. Despite such diversity, the prokaryote applications are still limited to the traditional industrial species.
Here we show that non conventional microorganism may be exploited for generating novel products for solving practical problems and to respond to market needs. We describe two case studies where i) novel probiotics for improving the health of the declining honeybees, and ii) sulfate-reducers (SRB) for removing sulfate-based black crusts from precious marble artworks, are exploited.

Methods. Case study i): A variety of molecular microbial ecology, microbiology, entomology and immunology approaches have been applied to screen, study and test the effectiveness of novel symbionts to support bees challenged with by the American Foulbrood Disease (AFB).
Case study ii): With an analogous set of approaches, including marble and crusts geochemical characterizations, a *Desulfovibrio* SRB strain has been used for cleaning a precious statue seriously stained by unaesthetic black crusts.

Results. Case study i): The AFB, an acute disease of honeybee larvae that can only be fought by burning the affected beehive, has been used as a model pest for testing the hypothesis that probiotic bacterial symbionts can help the host in counteracting stressing factors. We have chosen AFB as stress model because it is caused by the cultivable bacterium *Paenibacillus larvae* and can be reproduced in the laboratory. We compared by 16S rRNA gene based PCR-DGGE and pyrosequencing the bacterial diversity associated to the bee larvae. We identified bacterial groups, including acetic acid and lactic acid bacteria and bifidobacteria whose community structure is strongly modified by the invasion of the AFB pathogen determining a huge bacterial dysbiosis in the gut [1].

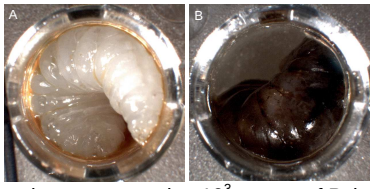

Fig.1 Honeybee larvae exposed to 10^3 spores of P. larvae. A) larva previously treated with a selected probiotic symbiont. B) Untreated larva

As a successive step we have isolated bacteria from the gut of healthy honeybees that have been screened *in-vitro* for their direct antagonism against the AFB pathogen. In an *in-vivo* assay finalized at the selection of those strains capable of decreasing the mortality of larvae exposed to *P. larvae*, we have selected a series of strains that are capable of preventing the infection and the disease (Fig. 1). Such capability, confirmed in the beehive in the field, was shown to be determined by a combination of mechanisms including competitive displacement of the pathogen and elicitation of the host defenses.
Case study ii): the effects of an SRB strain (*Desulfovibrio vulgaris* subsp. *vulgaris* ATCC 29579) coupled with a non-ionic detergent has been studied on a one-century-old artistic marble statue polluted by sulfate-based crusts and grey deposits [2]. The treatment removed the deposits without affecting the sound original marble (Fig. 2).

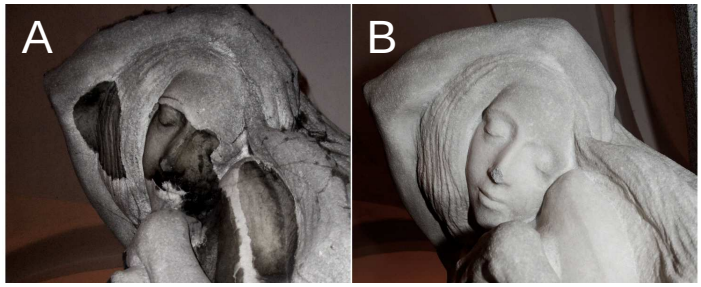

Fig.2 A particular of the statue realized by Lina Arpesani in 1921 in memory of "Neera", the poetess Anna Zuccari, before A) and after the treatment B) with *D. vulgaris*.

The co-treatment with a mild non-ionic detergent enhanced the crust removal with a 70% reduction of the number of biological applications but retaining all the advantages of the biocleaning approach. Geochemical analyses of the crusts and the marble surface showed the removal of the crust mineral without affecting the mineralogical composition of the sound marble.

Conclusions. The two examples proposed here show that the microbial world is plenty of microbes that have the potential to be exploited for safe and sustainable non-polluting novel biotechnological applications.

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