Environmental factors as possible causes of DNA fragmentation in human sperms

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Literature data demonstrated that some environmental factors could have a key role in the remarkable and continuous decline of sperm quality observed in the last fifty years. Specifically, in the Taranto area, data about the detrimental effects of environmental pollution are alarming because of the high level of poisons released in the atmosphere. Pollution coming from the plant causes health and fertility risks, mainly due to the exposure to the dioxin. Our study analyzed sperm samples from three patients groups: i) workers of local steel factories; ii) Taranto residents; iii) Controls. Results demonstrate that patients from the "factory workers" group, constantly exposed to environmental pollutants for professional reasons, show a mean percentage of DNA fragmentation above 30%. In contrast, patients from group "Taranto residents" and patients coming from Palermo considered as "Controls" group show mean percentages of 25 and 16.8%, respectively. We observed an increase of spermatic DNA fragmentation (DFI) in the "factory workers" and "Taranto residents" groups, compared to "Controls". These ones are patients of an *in vitro* fertilization clinic, with supposed fertility issues. It is known that a spermatic DFI less than 15% is physiologic, while above 30% is related to fertility issues. It is also known that interrupting the sperms damaging source might bring back the DFI level to normal values. So, moving away from the sperms damaging source, patients from "factory workers" and "Taranto residents" groups could restore spermatogenesis. The research methods employed in this study were found to be specific and valid for these analysis.

Omics approaches to elucidate the molecular physiology of lantibiotc NAI-107 production in *Microbispora* ATCC-PTA-5024

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The filamentous actinomycete *Microbispora* ATCC-PTA-5024 produces the lantibiotic NAI-107 [1], which is an antibiotic lanthipeptide effective against multidrug-resistant Gram-positive and some Gram-negative bacteria [2]. In actinomycetes, antibiotic production is often associated with a physiological differentiation program controlled by a complex regulatory and metabolic network that may be elucidated by the integration of genomic, proteomic and bioinformatic tools [3]. Accordingly, an extensive evaluation of the proteomic changes associated with NAI-107 production onset and maintenance during growth was performed on *Microbispora* ATCC-PTA-5024 by combining two-dimensional difference in gel electrophoresis, mass spectrometry and gene ontology approaches. Biomass samples were collected during growth at five time-points corresponding to different profiles of biomass and NAI-107 accumulation to perform differential proteome analyses. A total of 303 gene products, participating into 241 molecular/metabolic functions (the 14.5% of the total ones predicted from genome) were identified having a NAI-107-dependent accumulation profile. In particular, during NAI-107 production nutritional signals, regulatory cascades and primary

metabolism shift-down trigger the accumulation of protein components involved in nitrogen metabolism, cell wall biosynthesis/maturation, lipid metabolism, osmotic stress response, multi-drug resistance, and NAI-107 transport. An interesting finding was the increasing abundance of a TetR-like regulatory protein during growth progression. The over-expression of this TetR-family regulator exerted a stimulatory effect on morphological and physiological differentiation. This work, reporting the first omic-based study of *Microbispora* ATCC-PTA-5024, provides a net contribution to the elucidation of the molecular, metabolic and regulatory pathways controlling physiological differentiation and eventually leading to NAI-107 production. In addition, this study further supports the relevance and the powerfulness of proteomics in revealing novel players of antibiotic biosynthesis regulation in actinomycetes.

[1] Maffioli SI. et al. (2014) *J Nat Prod.* **77(1)**:79-84.
[2] Jabès D. et al. (2011) *Antimicrob Agents Chemother.* **55(4)**:1671-6
[3] Gallo G. et al. (2010) *Microb Cell Fact.* **26(9)**:95.

Touch DNA a quarter of a century after the fact

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A broken shotgun forestock was collected at a homicide scene in 1988. The object was repeatedly scrutinized by experts in different fields in the following years, until DNA analysis was ordered in 2013. The genetic profile of a suspect and those of eight experts that had came in contact with the object were made available for comparison. Single-point swabbing methodology was used to probe the entire surface of the object; in total, 40 spots were sampled. Genotyping was performed by an ABI PRISM 3500 Genetic Analyzer with the software Gene Mapper IDX v1.3 using the PowerPlex® ESX-17 kit and AmpFLSTR NGM SElect™ Kit. In the end, 78 independent amplifications/detections of 16 autosomal STR markers were obtained. Most sample profiles were complex mixtures; however, a single major contributor was inferred in two spots; one of the two (named E1) remained unknown, the other (A18) turned out to be first- or second degree relative of the suspect with probability > 99%. Analysis of allele sharing between each of the eleven available single-person profiles (the eight experts, E1, A18, and the suspect) and all the 78 sample profiles (analytical threshold = 50 RFU) showed that, namely, two experts, A18, and the suspect, ranked highest. Suspect's profile was fully compatible with a single spot (composite method), and the likelihood ratio computed by the Forensim package for four different scenarios was 105 – 5 x 105; the corresponding value for the consensus method (four dropouts) was 103 – 3 x103. The value of the RMNE (random man not excluded) statistics for the same spot was < 10-6. For a second spot, suspect's profile showed two dropouts (composite method), and the LR for the same four scenarios was 400 – 4000. The report to the jury asserted that the data provided very strong support to the hypothesis that suspect's DNA was present on the object.

Cigarette smoke alters the DAB2IP expression in epithelial cells from COPD patients: a risk factor for lung cancer

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Cigarette smoking, one of risk factor of Chronic Obstructive Pulmonary Disease (COPD), activates epithelial cells. The alteration of the methyltransferase EZH2 has a role on the expression of the oncosuppressor DAB2IP and therefore, the cancer progression; it is often overexpressed and it promotes cell proliferation and invasion, inhibits apoptosis and enhances angiogenesis in lots of tumor. We evaluated the effect of