biodiversity

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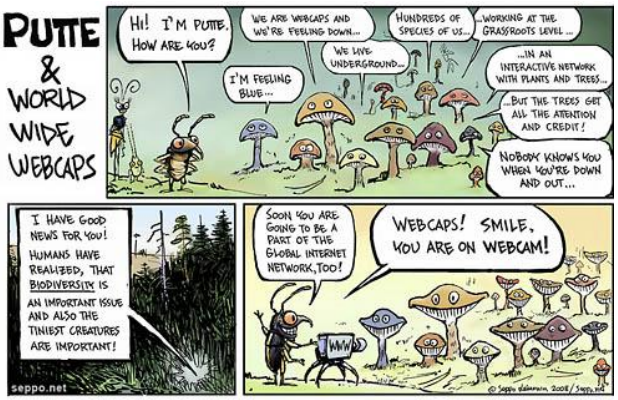

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Editorial

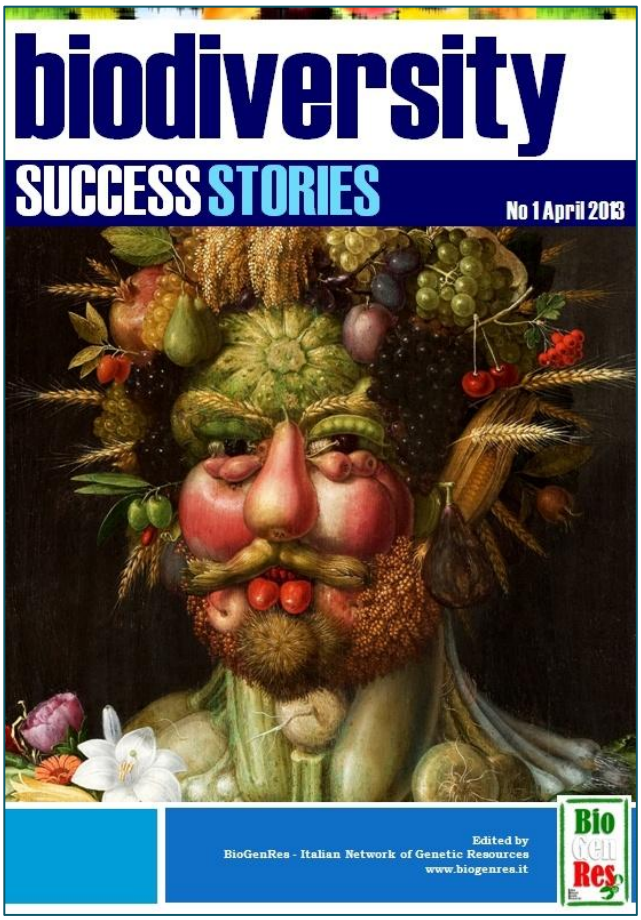
Biodiversity Success Stories, here we are

Trovandoci nella situazione di annunciare la nascita di un nuovo magazine sulla biodiversità, viene spontanea la necessità di "giustificarlo" agli occhi di un mondo che, addetti ai lavori o no, sente ormai parlare di questo concetto in modo caotico e quasi invadente.

Sembra passare l'idea che la tutela della biodiversità, da esigenza imprescindibile di un pianeta che si distrugge inesorabilmente con le stesse mani che lo hanno arricchito, sia diventata quasi uno spot inflazionato o l' hobby di cercatori di fondi e venditori di "verde fumo ecologista".

Biodiversity success stories nasce, nel nostro intento, come un racconto, o meglio un puzzle di racconti, strettamente legato alla trasferibilità di risorse genetiche disponibili nel BioGenRes Network. Non si ambisce a creare tediose sovrapposizioni con chi, prima, meglio e più di noi, ha operato e opera nella conservazione e valorizzazione del patrimonio naturale globale. Si sposta, semmai, l' obiettivo verso storie e protagonisti molto più vicini a noi, al rapporto complesso ed affascinante tra i problemi e le esigenze sollevate dal mondo produttivo, tecnologico, medico o alimentare, e le risposte che prova a dare la scienza. Facendo leva proprio sulla conoscenza via via meno pionieristica e più specializzata, di quel bacino inesauribile rappresentato dalla biodiversità dei viventi.

Tra le pagine di quello che speriamo riuscirà ad imporsi con la consuetudine di un appuntamento piacevole ed atteso troverete allora brevi flash e attraenti richiami sul lavoro dei tanti ricercatori e operatori italiani che operano nelle svariate applicazioni pratiche di un concetto, la biodiversità, che lasciato cadere nel linguaggio comune, senza



chiarirne le connessioni concrete, finisce per risultare astratto e fine a se stesso.

L' augurio è che il nostro magazine contribuisca a far luce su quanto di bello si sta facendo e quanto ancora si può fare in questo ambito.

Con la consapevolezza che, come afferma il Prof. Edward Osborne Wilson, unanimemente riconosciuto come il padre della Biodiversità, "*We should preserve every scrap of biodiversity as priceless while we learn to use it and come to understand what it means to humanity*". Buona Lettura.

The Editorial Team

Table olives
the microorganism's point of view

Dr. Giancarlo Bleve, Institute of Sciences of Food Production, CNR-ISPA Italy

The Problem

Because of their worldwide economic impact, table olives are the most important fermented vegetables. The European Union (EU), with 1.4 million tons, is the main producing area in the world. Greece and Italy have a long tradition in table olive production: 15.2 and 9.6 % of the table olives marketed in the EU, respectively. Following the natural process, generally olives are washed, put into containers and then filled with freshly prepared brine. Natural olives are incubated in the brine until they lose totally or partially their natural bitterness and they undergo the fermentation process whose characteristics depend on the cultivar and on the applied conditions. At the end of the process the olives acquire typical characteristics of final products.

The time necessary for this process might change depending on several factors, such as the variety and olive size, the salt concentration and temperature. Nowadays there are neither physicochemical nor

microbiological well established fermentation descriptors to control and evaluate the good performance of the ongoing fermentation process as well the end of the fermentation process.

The Solution

In olive fruits the epiphytic microbial population consists of yeast, fungi, and both Gram positive and Gram negative bacteria but throughout the fermentation process, Enterobacteriaceae, lactic acid bacteria (LAB) and yeasts are the most relevant microorganisms. Although it has been generally established that LAB are responsible for the fermentation of treated olives, LAB and yeasts compete for the fermentation of untreated olives, and in some cases yeasts can be exclusively responsible for fermentation on untreated olives.

The study of the microbial dynamics during fermentations of different olive cultivars will allow to know the microbial species (moulds, yeasts and LAB) associated to a fermentation processes and to determine the influence of parameters such as salt, temperature, anaerobiosis, necessary for successful fermentations.

A new protocol for selection of yeast and LAB suitable to drive and control olive fermentation was set up. The microbial isolate selection was carried out on Model Brine (MB) during the first step of the proposed procedure. Then, the microbial isolates were tested in order to evaluate two key strain-specific technological properties: the presence of beta-glucosidase activity (requested for oleuropeina degradation) and the inability to produce biogenic amines and to produce protease activity. Then, the yeast and LAB isolates characterized by the above described enzymatic activities were identified at the molecular level. The fermentation process was described by analysis of sugars, organic acids, alcohols profiles, mono and polyphenols profiles, sensory analysis of olives and brines. Selected yeast and LAB has been used as starters to inoculate pilot-scale fermentation of olives.

'Farro': a new history for an ancient crop

Dr. Domenico Catalano, Dr. Gaetano Laghetti, Institute of Plant Genetics, CNR-IGV Italy

The Problem

In Italy 'farro' is a term that is used to indicate all the three cultivated species of hulled wheat: emmer (*Triticum dicoccon* Schrank), spelt (*T. spelta* L.) and einkorn (*Triticum monococcum* L.). In time these ancient crops, well known by old Romans, have been gradually replaced by the more productive naked cultivars of durum wheat (*Triticum durum* Desf.) and common wheat (*T. aestivum* L.). However, in Italy and elsewhere, they are becoming promising niche crops. Their success is mainly due to their sensorial, nourishing and wholesome qualities in line with some medical data. From a nutritional point of view, farro is characterized by higher protein, ash, Se, Li, Mg, P and Zn content compared to common wheat.

Agronomically, farro is a very hardy low input crop and may increase the use of marginal and hilly agricultural zones. In many cases it is a valid economic alternative to durum wheat, mainly for its lower production costs. Both einkorn and emmer cultivated in Italy show an high resistance to stem rust, leaf rust and powdery mildew. Indeed, this ancient cereal is grown in several farms following organic or biodynamic agro-technique. In Italy, a new flourishing market for products based on farro has emerged. Many guests of farm holidays want to taste farro recipes.

The Solution

Some years ago the Istituto di Genetica Vegetale of CNR patented the following four pure lines of farro "Farvento", "Triventina", "Lucanica" and "Forenza", selected from genotypically heterogeneous accessions (Laghetti *et al.*, 1993, Perrino *et al.*, 1996). These four patented varieties were selected after six years of agronomic trials in Central-South Italy and according to their nourishing traits. Nowadays these are the first and unique farro patented varieties in Italy that guarantee the quality and origin of the product. This material has been widely used by Italian and foreign farmers' organizations and agribusinesses that today still pay the relative royalties to the CNR.

According to our opinion and experience farro market in central southern Italy is still economically profitable, particularly following initiatives tending to quality or élite markets, or by means of a bettering the organization of agricultural production, trading and transformation. The evidence of this conviction are the many recent requests of expressions of interest to stipulate agreements with IGV by foreign agricultural firms intending to use in their countries (e.g. Austria, Australia, Brazil) the patented varieties selected by IGV.

Autochthonous fermentation starters to valorize regional wines properties

Dr. Francesco Grieco, Institute of Sciences of Food Production, CNR-ISPA Italy

The Problem

Wine productions are typical because they are based on selected varieties of grapes and a peculiar fermentation process that determines the quality of the wine. The grape must fermentation is a complex process conducted by a number of yeasts and bacteria naturally present on the grapes or added to the must. The alcoholic fermentation is the biological event of greater importance in the production of wine in fact it is delegated "imprinting" of quality and characteristic of each fermentation. A stabilization and control of the production of wine passes through the use of starter, consisting of selected yeasts. The selected yeast strains available on the market today are limited in number, with provenance often unknown and very similar enological properties. Their use in a systematic way of musts from vines native to areas or regions of interest can become a cause of flattening of the quality of the wines. It is therefore very important to value and preserve the characteristics of a wine as a typical product as belonging to a particular territory.

The Solution

In these last year the research activity of CNR-ISPA has been directed to the exploitation of autochthonous microbiota to enhance the quality of regional wines. The research activity has generated a virtuous circle, thus allowing the standardization of protocols for wine yeast oenological selection and biomass production, the constitution of a Yeast Collection but, most of all, the transfer of technology to a large number of Small and Medium Enterprises belonging of the wine production chain.

Natural fermentations of Negroamaro, Primitivo and Susumaniello musts have been performed and the *Saccharomyces cerevisiae* population has been analyzed and characterized, allowing the identification of four indigenous *S. cerevisiae* strains candidate as autochthonous fermentation starters.

A novel protocol for the selection of *Saccharomyces cerevisiae* starter cultures and for the preparation of their biomasses has been optimized and validated by industrial-scale production of Negroamaro wine in two different local wineries. The procedure consisted of the following steps: i) selection of no H_2S-producer isolates; ii) yeast identification at species level; iii) yeast typing at strain level; iv) strain evaluation by fermentation test; v) chemical analysis of fermented must; vi) estimation of strain-specific enzymatic properties; vii) statistical analysis of obtained data; viii) starter biomass production and its use as inoculum for industrial-scale must fermentations.

The enological properties of the above strains have been evaluated during the vintages 2006-2012, by performing more than 40 large scale vinification trials in 21 different industrial cellars in Apulia. The selected strains demonstrated that they were always able to dominate the fermentation process and to produce a final product characterized by excellent oenological and organoleptic features.

Bioremediation of creosote treated wood by *Pleurotus ostreatus*

Dr. Emanuela Galli, Institute of Agro-environmental and Forest Biology, CNR-IBAF Italy

The Problem

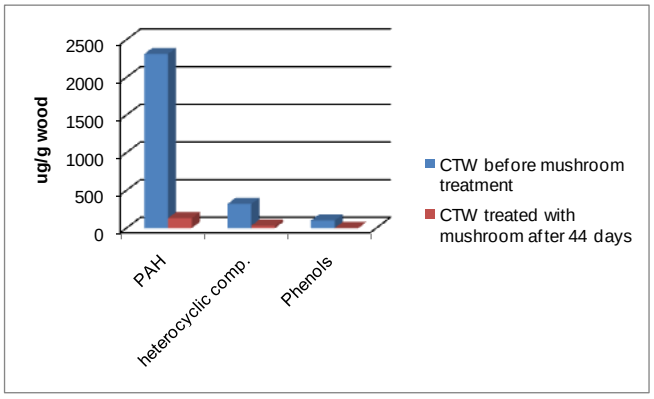
Creosote treated wood (CTW) has been classified as dangerous waste on the basis of the existing law dispositions (EU Directive 2001/90/CE).

Creosote oil is an industrial material obtained by distillation of coal tar, used as a wood preservative owing to its bactericide, fungicide and insecticide properties. It is rich in polycyclic aromatic hydrocarbons (PAH) and phenols and has been already recognized as very harmful to humans and environment and potentially cancerogenous.

The Solution

A mycoremediation system for creosote treated wood was performed, based on the detoxifying capability of *Pleurotus ostreatus*, a ligninolytic fungus. Non-sterilized chipped contaminated wood was mixed at various ratios with wheat straw on which *Pleurotus* mycelium was grown.

At 1:2 initial ratio contaminated wood:wheat straw, chemical analyses demonstrated an almost complete degradation of creosote oil components after 44 days, also confirmed by a significant reduction of ecotoxicity. The best creosote degradation was obtained by a stepped up wood addition.

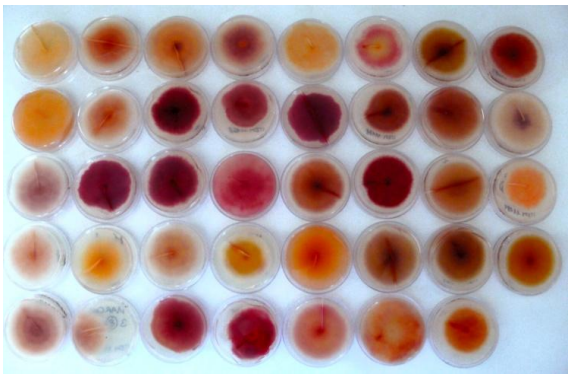


Wheat and maize safety and early detection of toxigenic *Fusarium*

Dr. Antonio Moretti, Institute of Sciences of Food Production, CNR-ISPA Italy

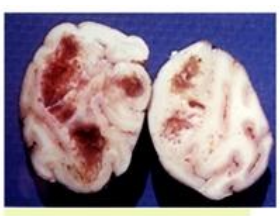
The Problem

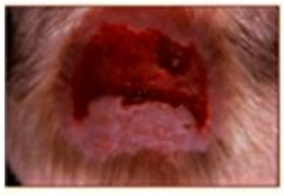
Fusarium head blight (FHB) of wheat and *Fusarium* ear rot (FER) of maize are worldwide severe fungal diseases that causes significant yield and economic losses. Several *Fusarium* species are involved in these diseases and produce a wide range of mycotoxins, in particular trichothecenes and fumonisins, related to a wide range of animal and human health problems. Each *Fusarium* species has its own mycotoxin profile. Moreover, these species are morphologically very similar and then difficult to distinguish each other.



Colonies of *Fusarium* species grown on Petri dishes

Therefore, a reliable and early identification of *Fusarium* species that contaminate wheat and maize kernels are important tools for a correct evaluation of the risk associated to cereal contamination and setting up appropriate prevention strategies to reduce the mycotoxin accumulation in cereal grains and by-products.





Leukoencephalomalacia in horses caused by fumonisins (left) and skin irritation caused by trichothecenes (right)

The Solution

For an early and accurate identification of main toxigenic *Fusarium* species that are involved in *Fusarium* ear rot of maize and *Fusarium* head blight of wheat, a set of modern molecular tools based on the DNA analyses have been developed.

The study of DNA variability within some housekeeping gene regions, such as calmodulin and elongation factor 1-α, of a wide range of *Fusarium* strains belonging to the species involved in FHB and FER has allowed to develop rapid PCR methods for the identification of these pathogens from wheat and maize kernels.

Fusarium head blight of wheat (left) and ear rot of maize (right)

These methods contemplate the use of species-specific primers that are fast and reliable genetic markers for detecting *Fusarium* species both in pure fungal cultures and from plant materials, and thus have a positive impact on plant, animal and human health.

Use of probiotics to formulate new functional juices

Dr. Filomena Nazzaro, Institute of Food Sciences, CNR-ISA Italy

The Problem

"Consumer convenience" and "health" symbolize, without doubt, the 2 most important trends in the food industry. Since recent decades, probiotics have been found or increasingly incorporated into commercial dairy products, as a response to the consumer demand for healthy food alternatives capable to meliorate overall health. However, the demand for alternatives to dairy products is growing due to problems with intolerance and allergy to milk proteins or lactose, as well as to try to meet to vegetarian and vegan alternatives desire.

The Solution

Fruit or vegetal juices may represent a good medium for functional ingredients like probiotics. They can be certainly positioned as a healthy food product, due to their strong antioxidant activity and a noticeable amount of healthy components such as polyphenols, carotenoids, glucosynolates, anthocyanins, vitamins, thus resulting capable to exert numerous positive benefits on the human health.

Researchers of the CNR-ISA, and of CNR-IBP, used fruit and vegetal juices as appropriate media for the growth of different lactic acid bacteria, such as *Lactobacillus acidophilus*, *Lactobacillus plantarum*, and *Lactobacillus rhamnosus*. A fruit juice particularly rich of anthocyanins, such as the berry juice, represented an excellent basis for the growth of *Lactobacillus acidophilus*; indeed, such microorganism was also capable to absorb a noticeable amount of anthocyanins, which content, in two grams of dried microbial cells, was similar to that present in a liter of red wine.

Because not all probiotics are capable to grow into fruit and vegetal juice and to survive during gastro-intestinal transit, researchers identified microencapsulation in food grade polymers (e.g. alginate) as an excellent way both to allow for the growth of more sensitive microorganisms, such as *Lactobacillus plantarum* and the probiotic yeast *Saccharomyces cerevisiae*, which were capable to grow and ferment berry juice, and to express a greater resistance against the attack of gastric and pancreatic juices. Indeed, microencapsulated cells showed a prolonged shelf life (8 weeks) compared to free cells (1 weeks) during their storage at 4°C.

The sensory evaluation of the presence of probiotics in fruit juice systems has vital commercial importance. It will be need a deeper knowledge about the sensory impact that probiotic cultures might have on nondairy systems, and to determine how probiotic fortification influences the consumer acceptance and preference for fruit juice, related to its appearance, aroma, texture or taste, with the purpose to convey the focus to an optimal development and formulation of these products.

Fungal plant pathogens as sources of novel natural herbicides

Dr. Maurizio Vurro, Institute of Sciences of Food Production, CNR-ISPA Italy

The Problem

Controlling weeds is one of the major problems in agriculture, currently still strongly dependent on the use of synthetic chemical products.

A large number of herbicides have already been withdrawn for regulatory reasons because of their hazardous effects or because they have become ineffective, due to the increasing weed resistance. These compounds are not being replaced, causing serious difficulties to farmers for managing those biotic constraints. The withdrawal of a number of herbicides has increased the need for novel, effective and environmentally compatible alternatives, renewing the interest in the development of biological alternatives to synthetic agrochemicals.

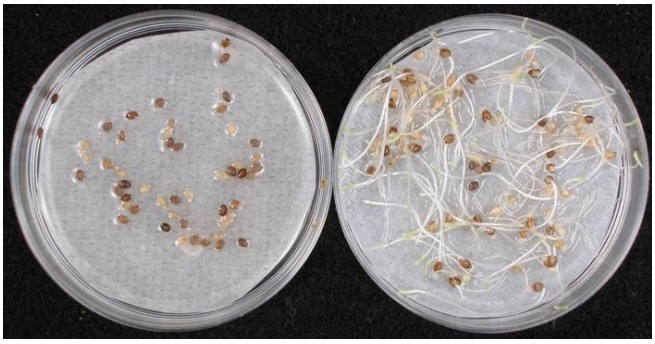
The Solution

Fungi play a major role in bioregulatory systems in natural ecosystems and utilize a large range of biologically active secondary metabolites having biological activities of great ecological relevance. These compounds are widely expected to represent the source of new biologically active compounds, chemical structures, mechanisms and site of actions, to be used in crop protection.

These compounds are the result of co-evolution of the producing organism and its biotic environment, and can have higher target selectivity, with reduced risks for humans and non-target organisms. Moreover, they can have a shorter environmental half-life than synthetic compounds, thus reducing potential environmental impact. Isolating and identifying these compounds was an arduous task in the past, but modem high throughput instrumentation has simplified and reduced the cost of this process.

In the case of searching for potential natural herbicides, fungal pathogens of plants could represent an extraordinary source of new compounds. Thus, selecting pathogens which cause those kind of symptoms can increase the probability of choosing interesting and novel toxin producers, and then to find toxins with herbicidal properties.

By using this research approach for years, researchers of the Institute of Sciences of Food Productions, were able to produce, purify, and chemically and biologically characterize a very large number of novel metabolites obtained by growing fungal pathogens belonging to several genera, namely, among others: ascaulitoxin, phomentrioloxin, ophiobolins, cytochalasins, phyllostictine, ascosonchin, agropyrenol. Those metabolites proved to have very unusual structural features and promising biological characteristics, and have been or are under study for their full biological characterization and test for their practical application.



Inhibitory effect of phyllostictine A on germination of Setaria viridis seeds (control on the right)

Thus, microorganisms could be the new frontier of discovery of novel metabolites to be used as safe and environmentally friendly agrochemicals which will pave the road towards sustainable pest control in the 3rd millennium.

Microbes and microbial by-products for plant health

Dr. Claudio Altomare, Institute of Sciences of Food Production, CNR-ISPA Italy

The Problem

Crop plants are subjected to a number of biotic constraints, particularly microbial diseases and insect pests, which cause big losses of both yield and quality of agricultural products. Every year, farmers spend large amounts of money for pesticides in order to prevent or limit the outbreak of plant diseases and pests. Over time, the continuous and massive use of chemicals for pest management has led to environmental pollution, onset of resistance in several key pests and loss of efficacy of active principles, and concerns for consumers health.

For these reasons, a number of pesticides are being withdrawn from the market every year and there is urgent need for low-environmental impact and safer alternatives to chemicals.

The Solution

Fungi and their by-products may offer valuable environmental-friendly alternatives for reduction of the use of conventional pesticides for plant pest and disease control. Biological pesticides relieve the selective pressure on pests caused by heavy and continuous use of synthetic agrochemicals and thus hamper the onset of resistant populations of plant pathogens and insects.

We have isolated and selected the soil fungus *Trichoderma harzianum* strain ITEM 908, which is an effective antagonist of plant pathogenic fungi that cause root diseases of vegetables. This fungus colonizes the root system and the soil portion around the roots and thus prevents the infection by root-rotting fungi.

The plants whose root system is protected by ITEM 908 grow healthier and bigger. The strain ITEM 908 is being developed in cooperation with a private company as a commercial biopesticide which has already obtained the inclusion in Annex I to Council Directive 91/414/EEC concerning the placing of plant protection products on the market. Products based on the strain ITEM 908 are a useful tool for pest management in organic or integrated farming and they may give a contribution to the diffusion of low environmental impact agriculture systems.

From another species of the genus *Trichoderma*, namely *T. citrinoviride*, we isolated some saturated or unsaturated long-chain alcohols which exhibit a strong fagodeterrent activity towards major aphid pests of fruit and cereal crops.

Aphids restrain from feeding on plants treated with the fagodeterrent compounds and both the direct damage caused by subtraction of sap and the indirect damage due to virus transmission are significantly reduced. For all of the active compounds an international patent (PTC IB2012/052383) has been filed.

Cultures of *Trichoderma* spp. are a valuable source of fungal biomass and by-products with a technological use for plant protection against diseases and insect pests.

Epididimal sperm: a tool for improving gene banking

Dr. Flavia Pizzi, Institute of Agricultural Biology and Biotechnology, CNR-IBBA Italy

The Problem

The farm animal term refers to animals domesticated for producing commodities for man such as food, fiber and draught. Farm animal biodiversity is integral to our culture, history, environment, and economy. Efficient ex situ and in situ conservation strategies are obligatory tools in order to implement an appropriate action for the conservation of farm animal biodiversity.

Brianzola sheep breed

Cryopreservation for ex-situ management of genetic diversity sometimes is not routinely feasible, due to the lack of facilities (Artificial Insemination centres, laboratories) and expertises near the local breeds farming area. Moreover in local breeds few males are available for semen collection on farm and semen collection training is more complicated due to the fact that animals are not accustomed to contact with farmers. Access to new technologies is important for gene banks to develop their collections.

The Solution

When semen collection through standard procedures is problematic, the post mortem recovery of epididymal sperm can be the only possibility to preserve male gametes from animals of high value or from endangered species. After developing the knowledge concerning post mortem testicles conservation and epididymal sperm recovery (Turri *et al.*, 2012.) we have implemented these procedure for setting the genetic reserve of two Italian local breeds: the Brianzola sheep breed and the Nero Siciliano pig breed. In these local breeds, farmed in extensive breeding system in rural and marginal area, no trained males were available to be collect semen directly on farm. In Brianzola breed testicles were collected at local slaughterhouse, after that semen samples were extracted and frozen in our laboratories in Lodi. Whereas in Nero Siciliano testicles were collected on farm in Sicily, after surgical castration, then were transported by air at 5°C and processed within 24 hours in our laboratories. Epididymal sperm were collected by using the retrograde flushing technique.

Nero Siciliano pig breed

Semen volume, total number of sperm and sperm motility were assessed. Overall the quality of the sperm recovered was good allowing the production of viable semen doses. These experience suggests the possibility to extract viable epididymal sperm from testicles transported and processed within 24 hours increasing the opportunities to create semen storages for local genetic resources farmed in marginal areas.

The BioGenRes Network

BioGenRes, the Italian Network of Genetic Resources, has been set up by the the CNR Department of Bio-Agrifood Sciences (DiSBA) to harmonize all genetic resources available in the CNR system and other institutions, making them available for the entire scientific and business national and international community.

The goal and work of BioGenRes will be preparatory to identify and propose supporting and coordinated actions aimed to raise funds for the maintenance of collections, especially for a continuous and organic functional and genetic characterization. The availability of genetic resources aims to be useful for understanding the mechanisms that shape biodiversity, both for their effective exploitation or as base for genetic improvement programs for a sustainable food production.

More detailed information about the BioGenRes resources and activities is easily available on the online database www.biogenres.it designed to create a web-based, permanent information system to serve as documentation platform for a possible national genetic resources inventories.

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