XXXV Jornadas Portuguesas de Genética

31 Maio a 2 Junho 2010
Universidade do Minho
Campus de Gualtar
Braga
Molecular identification and differentiation of *Staphylococcus* species of dairy origin

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The *Staphylococcus* genus is widely distributed in nature, with some species inhabiting specific ecological niches. Staphylococci are found living naturally on the skin and mucous membranes of warm-blooded animals and humans; however they are also isolated from a wide range of foodstuffs such as meat, cheese, milk, and from environmental sources such as soil, sand, air, and water. The level and diversity of the *Staphylococcus* spp, occurring in the environment and dairy products of a small manufacturing dairy plant were investigated. Amplified ribosomal DNA restriction analysis (ARDRA) and Multiplex-PCR were used to differentiate among 107 strains of the *Staphylococcus* genus. ARDRA was applied using a universal primer and a fragment of 3.3 Kb was amplified containing the 16S rRNA gene, 16S-23S intergenic spacer region, and about 1436 bp of the 23S rRNA gene. Species-specific restriction patterns were found using the restriction enzymes HindIII, XmnI, SspI, VspI, EcoRV, PvuII, separately. Additionally, classification of staphylococcal enterotoxin (SE) and toxic shock syndrome toxin-1 genes (TSST-1) by Multiplex-PCR was also performed. Nine species were identified in the whole cheese production chain, namely, *S. saprophyticus*, *S. aureus*, *S. epidermidis*, *S. chromogenes*, *S. simulans*, *S. sciuri*, *S. equorum*, *S. haemolyticus* and *S. caprae*. The prevalent strains isolated were: *S. equorum* (32.7%) and *S. saprophyticus* (25.2%) species followed by *S. epidermidis* (14%) and *S. simulans* (10.3%). A low incidence of enterotoxigenic strains was obtained, with only 12 strains (11.1%) being positive for one or more toxin genes. Thus, ARDRA and Multiplex-PCR were proven to be valuable alternative tools for staphylococci identification, allowing a comprehensive insight about their occurrence in a cheese manufacturing plant. Furthermore, it allowed us to conclude that *S. equorum* and *S. saprophyticus* were the prevalent species in this particular cheese plant.

Analysis of molecular and morphological diversity of *Arbutus unedo* in the interior North and Center of Portugal

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In the Mediterranean Basin, the strawberry tree (*Arbutus unedo* L.) has medicinal, ornamental, economical, and environmental importance. During the last decades, several occurrences have caused the decline of the strawberry tree in Portugal being replaced by other species. In this context, the knowledge of genetic variability in the wild populations is essential for a proper conservation. This work aimed to characterize genetically and morphological 38 *A. unedo* genotypes, 36 of which located in Trás-os-Montes e Alto Douro region, and the remaining 2 genotypes in Beira Interior region, to assess their biodiversity. The genetic diversity was assessing by RAPD (Random Amplification of Polymorphic DNA) markers. The dendrogram was obtained from the matrix of pairwise distances through the Nei and Li coefficient using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) grouping method. Morphological characterization was performed through the evaluation of several quantitative traits measured on 40 fresh leaves per plant. The quantitative traits analysed were leave length and width, leave length/width ratio, peduncle length and leave fresh and dry weigh. Distance matrix of morphologic data was assessed using Euclidean distance. A Mantel test was used to analyze correlations between genetic, morphological, and geographical distances. Among a total of 20 arbitrary 10-mer primers, seven produced polymorphic RAPD profile. The highest number of polymorphic loci was exhibited in the Trás-os-Montes samples and the lowest in the Beira Interior samples. The distance UPGMA tree grouped together the genotypes according to their geographical origin, showing that each sample is genetically structured. Morphological differences were also found between genotypes. However, the clustering obtained from the morphological data was different from the obtained in the RAPD analysis. In this work we discuss also the significance of the findings for the genetic variability of *A. unedo*. 