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ABSTRACTS**

46°

**Congreso de la
Sociedad Española
de Bioquímica y
Biología Molecular**

A Coruña

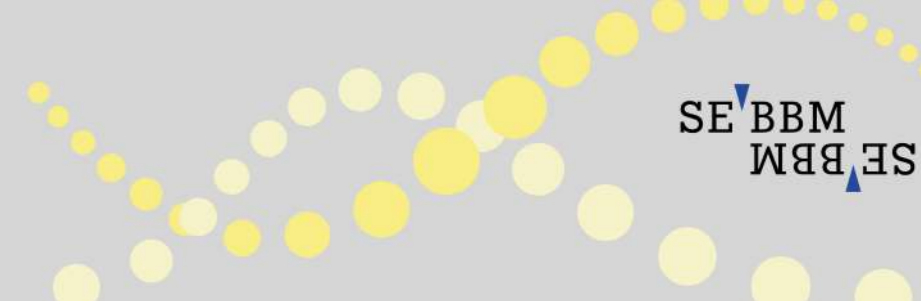
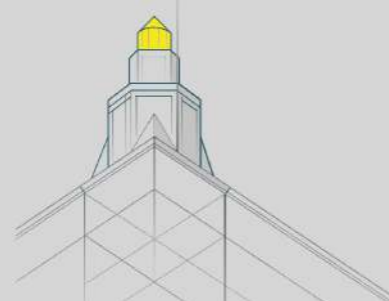
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2024**

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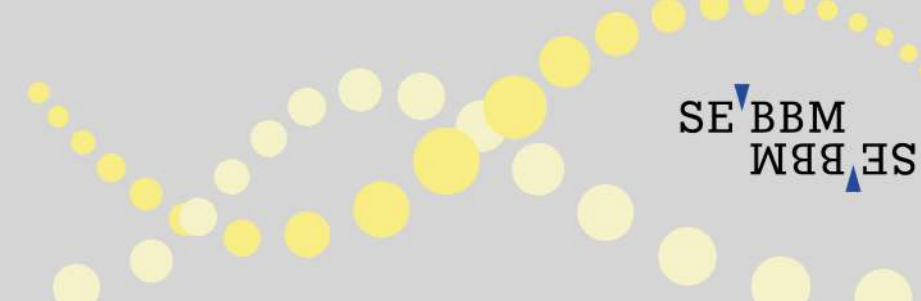
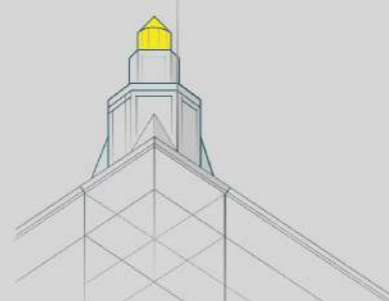
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Surface Display (YSD) to identify high-affinity HLA-peptide complexes, thereby facilitating the identification of novel neoantigens.

References:

(1) Torre, L. A. et al. *CA. Cancer J. Clin.* 68, 284–296 (2018) 10.3322/caac.21456

(2) Bobisse, S. et al. *Nat. Commun.* 9, 1092 (2018) 10.1038/s41467-018-03301-0

Keywords: Epithelial Ovarian Cancer (EOC), Tumor-Infiltrating Lymphocytes (TILs), Single-Cell Sequencing, T-Cell Receptor Sequencing

G03 - 190 - P

Serum Proteome Responses in Sea Bass (*Dicentrarchus labrax*) Following Immunization

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Finding correlates of protection after fish vaccination is highly dependent on factors such as genetics, immunological history, type of vaccine...

As immunization is the main preventive strategy used in aquaculture, defining the traits that provide protection could be a stepping stone towards the rational design of vaccines, as well as the monitoring of fish immune status, which would lead onto the improvement of vaccine efficiency.

In the present study, the serum protein response after immunization with a *Photobacterium damsela* subsp. *piscicida* vaccine was analyzed in individually marked sea bass. Fish were finally challenged against the pathogen. Fish sera of immunized and control groups were classified depending on fish survival. Groups were compared by qualitative and semi-quantitative (label-free liquid chromatography mass spectrometry) proteomic analysis.

A great variation in serum proteins was observed. This correlates with heterogeneity of fish populations.

Protein groups specific of fish survival in immunized fish were identified. Besides, protein groups present on survivor fish, regardless of being vaccinated, were also identified as traits of fitness. These proteins clustered into groups showing different responses to vaccination, with identification of protein groups belonging to classical immune system, acute phase molecules, other proteins, (many related to immune system) and some uncharacterized proteins (which shows the status of the lack of information contained in fish protein

databases).

Overall, this study provides new knowledge of fish serum proteome improving our understanding of sea bass immunization and providing prospective paths of study for correlates of protection identification, as well as for vaccine development.

Keywords: Proteomics, Serum, Seabass, Photobacterium, Vaccine

G03 - 225 - P

Identification of immunogenic proteins in the pathogen *Aeromonas salmonicida* for inclusion in a subunit vaccine.

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Furunculosis is one of most common fish diseases on aquaculture, and it is caused by Gram-Negative bacterium *Aeromonas salmonicida*. *A. salmonicida* causes septicemia and wounds in the musculature, called furuncles, increasing the probability of colonization by opportunistic pathogens, worsening the infection. Although furunculosis affects different fish species, in this study we focused on its occurrence in turbot, since currently, the number of proteins studied related to the adhesion of *A. salmonicida* to this host is scarce. Expanding the number of known proteins found within the infectious pathway of this bacterium would help to develop a subunit vaccine, which roughly speaking, is a vaccine designed from virus or bacterial components, usually proteins. In this study, a search for outer membrane proteins of *A. salmonicida* was carried out for subsequent incorporation into a subunit vaccine effective against different isolates of this pathogen in turbot. For this purpose, we approached a strategy based on bioinformatics, a second based on proteomic analysis, and finally, a third based on a literature search, including in our list of antigens several *A. salmonicida* proteins whose expression on the outer surface of the bacterium is well established. Thus, by combining these three strategies, we tried to maximize the accuracy in the search for these membrane antigens.

Keywords: Shaving, Immunoinformatics, Vaccines, *Aeromonas*, Turbot

G03 - 249 - P

Evaluation of DNA-metabarcoding as a new approach for honey's botanical authentication

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Honey is one of the foods most prone to be adulterated, with botanical origin mislabelling being one of the most frequent frauds. Honey's botanical origin is traditionally determined by microscopic analysis of pollen grains, a laborious method requiring expertise and often providing only family-level identification. Pollen identification by DNA-metabarcoding would allow for a faster, simpler, and more accurate determination. However, the reliability of using the number of sequences reads to estimate pollen percentages in honey remains unclear.

To address this issue, the number of pollen grains per mg in 13 pollen samples was determined using a Neubauer chamber. Then, four pollen mock mixtures were created, two containing 5 species and two 13 species. In each case, one of the mock mixtures was prepared with an equal mass of each pollen species (corresponding to varying amounts of grains), and the other was prepared using a similar percentage of each pollen species. Each mock mixture was also added to agave syrup (naturally pollen-free) to simulate the honey matrix. The pollen and agave mock mixtures were subjected to DNA extraction, PCR and ITS2-metabarcoding, and parallelly to pollen microscopic analysis.

DNA-metabarcoding results aligned well between the pollen mixtures and the agave syrup mixtures. In both cases, a few species were overrepresented and others underrepresented but, in general, the quantitative profile was according to the expected. In contrast, microscopy results closely matched the expected composition of the pollen mock mixtures, but significant discrepancies were observed in the agave samples.

Acknowledgment: to project "Mel I.D" financed by PNASA 2023-2027, PRIMA project "MEDIBEES" and FCT PhD scholarships (2021.08119.BD, 2020.05155.BD)

Keywords: DNA-Metabarcoding, Honey, Authentication

G03 - 250 - P

Comparison of different sequencing approaches to uncover botanical adulteration in herbal products

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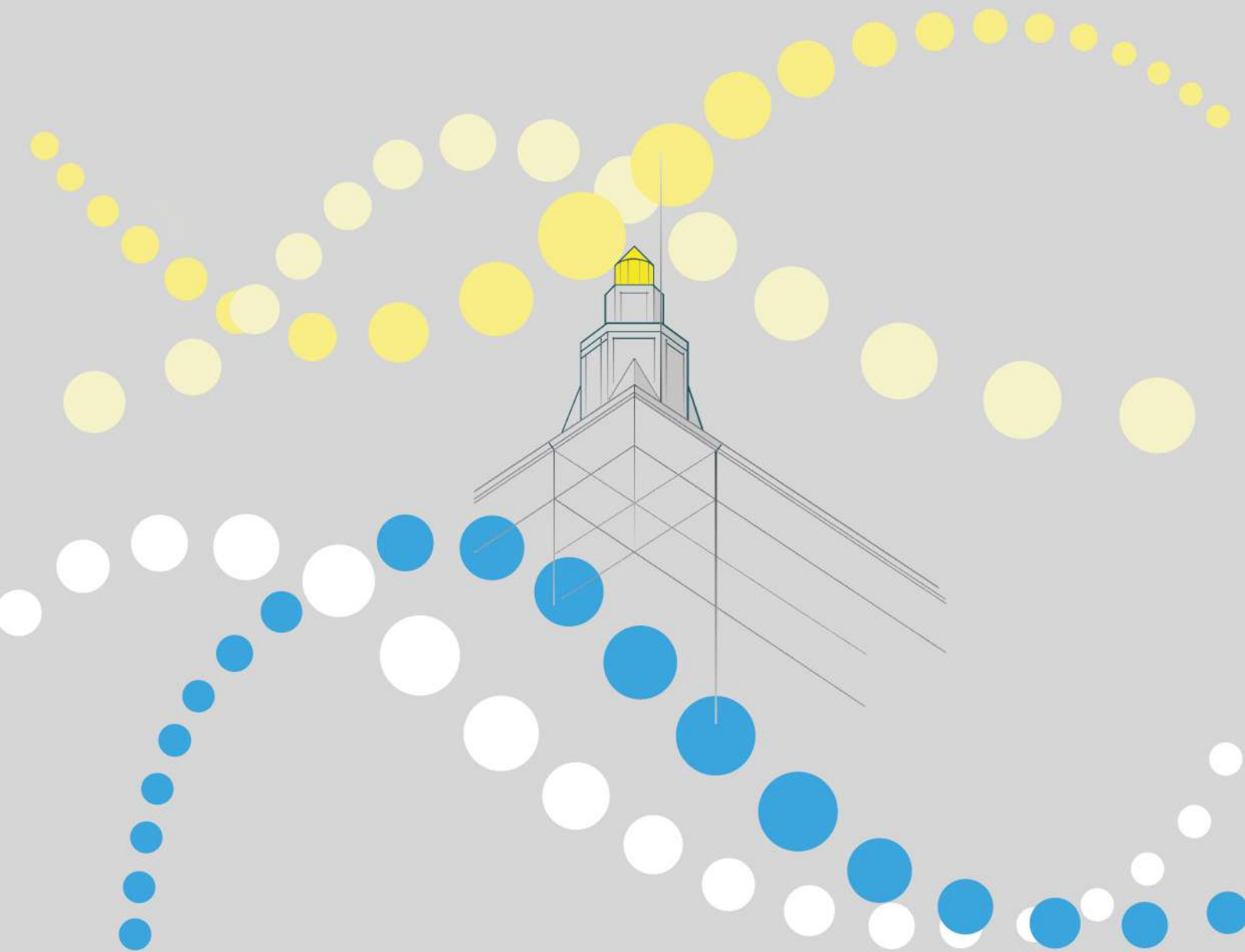
Due to the increasing demand for popular medicinal plants, herbal products are prone to botanical adulteration. Aiming for fraud detection, DNA-barcoding emerges as one of the most suitable approaches for species identification in medicinal plants.

In this study, 58 commercial herbal products labelled with plant species traditionally used for cognitive, mood, or sleep improvement were submitted to DNA extraction and PCR targeting three barcodes. *MatK* and *rbclA* amplicons were sequenced by Sanger, while *ITS2* amplicons were sequenced by NGS for plant species identification. The Sanger sequences were identified by BLAST using a custom script. The NGS sequences were identified using a bioinformatic pipeline that included three *ITS2* custom databases: one containing sequences of plants traditionally used for improving brain health, one containing medicinal plant sequences, and the third was a curated global database containing 307,977 sequences representing 111,382 species of vascular plants. NGS results were analysed by a script that sequentially went through these databases to ensure that a maximum number of sequences were identified as accurately as possible. Of the evaluated samples, *MatK* was able to correctly identify 62% labelled samples, *rbclA* 76%, and *ITS2* 68%, with only 28% of the samples being correctly identified by the three barcodes simultaneously. NGS revealed that 29% of samples with a correct identification by Sanger sequencing were in fact mixtures of species, with the labelled one being the most representative. Overall, only 34% of samples conformed to the label, while 56% were mixtures containing the labelled species.

Acknowledgments: FCT project POIROT (PTDC/SAU-PUB/3803/2021) and PhD scholarships (2021.08119.BD, 2020.05155.BD)

Keywords: DNA Barcoding, Botanical Adulteration





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