Microbiological Analysis of Portugal Northern Coastal Beach Sands

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Introduction

The Blue Flag award as a symbol of environmental quality conferred to beaches, has been the sole criterion used to validate the quality of a seaside beach. However, the microbiological analysis of the sand, which can represent a potential risk to human health, is not being included as a quality parameter.

The main objective of this work was to evaluate the microbiological flora, namely the yeast flora of clinical interest, in order to contribute for a more accurate evaluation of the Portuguese beaches. Microbiological surveys were carried out in two northern public bathing beaches of Portugal, Labruje and Salgueiros, both in Winter and Summer. The 58 total samples were collected from five different sites, at three depths (0, 0.5, 1 m), along the sea coast of each beach. Only the Salgueiros beach exhibited the Blue Flag award.

Methods

Sample collection: Samples of 30g of sand from each site and depth were collected in sterile Falcon tubes and kept at 4°C until microbiological analysis. Sample analysis: 0.4g of sand and 10ml of a sand suspension (fell of sterilized water and 3g of sand) were separately plated onto YEPA, YM, YEPA 10% NaCl and YEPA 0.01% Chloramphenicol. The number of colony forming units was calculated from decimal dilutions of each sand suspension, spread on YEPA plates.

Results and discussion

The present results concern only the winter of 2003.

Sample analysis: The different groups of microorganisms were analyzed and yeasts were isolated regarding further characterization and identification.

Bacteria analysis: After submitting the bacteria to Gram staining we observed a dominance of Gram bacteria in almost all sand samples (Fig. 2).

Yeasts characterization: In order to search for the existence of urease enzyme, the traditional physiological and biochemical tests of urea hydrolysis was performed. Urease positive yeasts turned the colour of the medium into bright pink due to urea hydrolysis. The percentage of urease positive and negative yeasts was calculated (Fig. 3).

Yeasts identification: The 302 isolates were screened and grouped according to the results of the PCR-RFLP analysis (Fig. 4). The molecular weights of the amplified and digested products were determined using the Lab Image program and compared with available databases for species identification.

The present results concern only the winter of 2003.

Final Remarks

In what concerns to the results obtained up to now, we may point out:

- During winter, in both studied beaches, the Gram bacteria were prevalent, even when each depth was considered.
- In spite of the geographic neighboring of both beaches, they presented a distinctive and unique yeast flora.
- The size of the PCR products and the restriction analyses with the three restriction endonucleases (CfrI, HaeIII, HindIII) yielded a specific pattern for each species, allowing isolates screening and grouping.

- A total of nine yeast species belonging to seven different genera was identified.
- Only one yeast species (Cryptococcus laurentii) was common to the two beaches.
- In what concerns to yeast flora of medical interest, only the genera Cryptococcus was present.
- The data obtained could be a reference point for further studies.

Table 1 - Spatial distribution of the yeast flora present in each studied beach in winter 2003

<table>
<thead>
<tr>
<th>Season</th>
<th>Depth (meters)</th>
<th>Labruje</th>
<th>Salgueiros</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dry zone</td>
<td>Wet zone</td>
<td>Dry zone</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>Winter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>Cryptococcus laurentii</td>
<td>*</td>
<td>Cryptococcus laurentii</td>
</tr>
<tr>
<td>0.5</td>
<td>*</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>2</td>
<td>Cryptococcus sp.</td>
<td>*</td>
<td></td>
</tr>
</tbody>
</table>

* yeast groups for which identification is in progress.

Acknowledgments

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