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, mainly 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, Salgueiros and Labruje, both in Winter and Summer. The 58 sand 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 100µl of a sand suspension (9ml of sterilized water and 3g of sand) were separately plated onto YEPDA, YMA, YEPDA 10% NaCl and YEPDA 0,05% chloramphenicol. The number of colony forming units was calculated from decimal dilutions of each sand suspension, spread on YEPDA plates. Gram staining allowed an estimate of the relative percentage of Gram$^+$ and Gram$^-$ bacteria.
Only yeasts were isolated regarding further studies.
Yeasts characterization and identification: Yeast characterization included morphological, physiological and biochemical tests. Molecular identification was performed by PCR-RFLP analysis (endonucleases *Cfo*I, *Hae*III and *Hinf*I) of the intergenic transcribed spacers (ITS) of the rDNA and the results were compared with available databases. Whenever PCR-RFLP did not allow yeast identification to species level, it was determined by sequencing the D1/D2 domain of the large-subunit (26S) rDNA using primers F63 (5'-GCATATCAATAAGCGGAGGAAAAG) and LR3 (5'- GGTCCGTGTTTCAAGACGG).

Results and discussion

The present results concern only the winter of 2003

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

Fig. 1 – Direct sand sampled on YEPDA

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

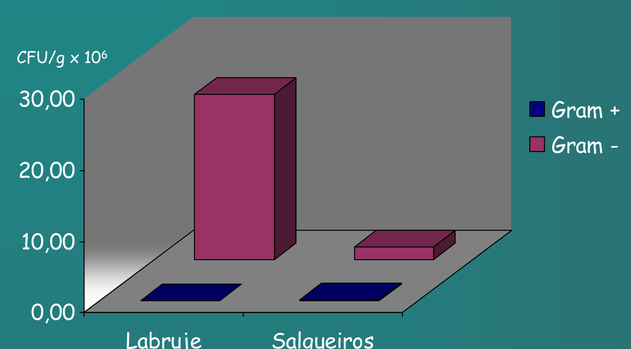


Fig. 2 – Total number of bacteria CFU/g sand present in the two beaches

Yeasts characterization: In order to search for the existence of urease enzymes, the traditional physiological and biochemical test 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).

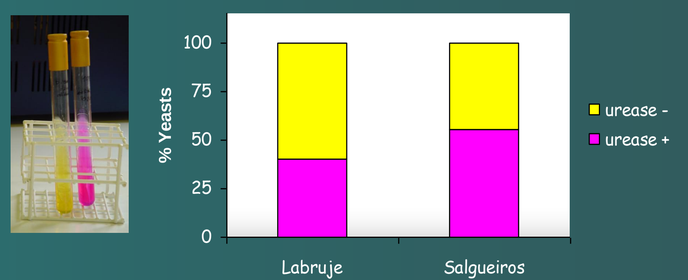
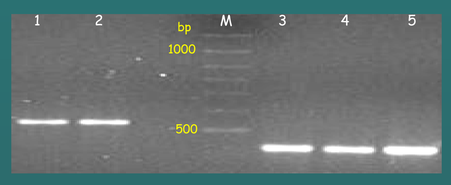


Fig.3 – Percentage of urease positive and negative yeasts present in the sand of the two beaches

Yeasts identification: The 102 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.

PCR amplification products



Restriction analysis

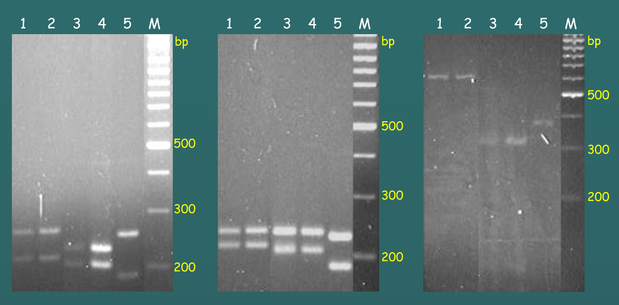


Fig. 4 - Agarose gel electrophoresis of the PCR amplification products, using primers ITS1 and ITS4, and of the fragments produced by *Cfo*I, *Hae*III and *Hinf*I digestion.

M - DNA Molecular weight marker (100 bp ladder);
1, 2 - *Cryptococcus laurentii;* 3, 4 – *Metschnikowia zobellii;* 5 - *Candida sorbophila.*

Yeasts identification: Yeasts identification was based on morphological, physiological, biochemical tests and PCR-RFLP analysis. Whenever identification to species level was not possible once some data did not match any described profile, D1/D2 domain of the large-subunit (26S) rDNA sequencing was performed. Table 1 summarises the results obtained by the combination of the different methods.

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

		Beach									
		Labruje					Salgueiros				
Season	Depth (meters)	Dry zone			Wet zone		Dry zone			Wet zone	
		E1	E3	E5	E2	E4	E1	E2	E4	E3	E5
Winter	0		*Cryptococcus laurentii*		*Metschnikowia zobellii* *	*Cryptococcus laurentii*	*Rhodotorula graminis* *Rhodotorula sloofiae* *		*Cryptococcus laurentii*	*	*Debaryomyces hansenii*
	0.5					*Metschnikowia zobellii* *Candida sorbophila*		*Torulaspora delbrueckii*		*Trichosporon domesticum*	
	1	*Cryptococcus sp.*				*			*Debaryomyces hansenii*	*	

* yeast groups for which identification is in progress

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 evaluated.
- In spite of the geographic neighbouring 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 (*Cfo*I, *Hae*III, *Hinf*I) 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.

ACKNOWLEDGMENTS
We thank to A. Correia for her useful contributions and suggestions