Harnessing Aquatic Yeasts For Developing A New Generation of Sunscreens

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ABSTRACT:

Around 100 yeast genera and 800 species have been isolated and characterized. Nagahama (2006) has reviewed yeast biodiversity in freshwater, marine and deep-Sea Environments. Our work aims at systematic biodiversity assessment of freshwater aquatic yeasts from Goa with major focus on carotenogenic basidiomycetes yeasts which on exposure to potentially damaging levels of solar ultraviolet radiation (UVR) accumulate photoprotective compounds (PPC) such as mycosporine-glutaminol-glucoside (myc-glu-glu, peak absorption at 309-310nm) that server as sunscreens and /or antioxidant. These yeasts are a potential source of a particularly diverse family of such substances, collectively referred to as mycosporine- like amino-acids (MAAs). This paper presents the potential of exploiting MAAs to develop a new generation of ecofriendly sunscreens.

INTRODUCTION:

Yeasts are eukaryotic microorganisms that dominate fungal diversity in the oceans. Basidiomycetous yeast have been reported from freshwater lakes, streams, brackish water to fully saline sea water. Many aquatic organisms are exposed to damaging levels of solar ultraviolet radiation (UVR) and accumulate photoprotective compounds (PPC). The accumulation of UV-screening compounds clearly represents an adaptive benefit against the harmful effects of UV radiation, which, especially the highly energetic UV-B waveband (280–315 nm), affects organisms in several ways. Among the adverse effects are DNA- damage by the formation of thymine dimers, DNA strand breaks and lipid peroxidation, and impairment of motility and orientation [1]. The major group of PPC's are melanins, carotenoids, mycosporine- like amino acids (MAA), Mycosporine is a general term for a group of about 10 to 12 compounds. Mycosporines are water-soluble compounds of low molecular weight, composed of either an aminocyclohexenone or an aminocyclohexenimine ring, bearing nitrogen or aminoalcohol substituents. The mycosporine found in yeast has been identified as mycosporine- glutaminol- glucoside (myc-glu-glu), a compound originally found in terrestrial fungi [2], palythinol,palythene, porphyra-334, mycosporine- glycine:valine, shinorine and MAA 357. MAA have been considered interesting candidates as sunscreen compounds because of their outstanding UV-absorption, coupled with a high photostability. It is considered as safe alternative to synthetic UV-sunscreens (e.g., Helioguard 365TM, HelionoriTM). Ecofriendly natural sunscreens have a huge market. Novel sources of MAAs therefore need to be identified and screened. However, relatively poor attention has been paid to systematic screening programmes to identify promising aquatic yeast strains as potential sources of MAAs. The aim of the present work was set out in the direction of filling this gap. It was thought that by utilizing the local biodiversity of aquatic yeasts a promising strain could be identified with potential applications in development of a new generation of MAA based sunscreens[3].

AIM:

In the ongoing study of biodiversity and bioprospecting of aquatic yeast in our department several interesting cultures of carotenogenic basidiomycetous yeast cultures were recovered. A pinkish strain of carotenogenic basidiomycetous yeast identified as *Sporobolomyces* sp. and designated as GFCC 11001 was screened to detect presence of MAA.

MATERIALS AND METHODS:

Collection of water sample:

The water samples were collected near a sacred grove, Western Ghats forest, Sanguem, Goa, India. Standard techniques [4] were used to isolate the yeast. Identification was done using standard keys. Pure cultures were designated and maintained on MEA and also in 10 % sterile glycerol in cryovials.

Extraction and analysis of pigments: Pure culture of *Sporobolomyces* sp. GFCC 11001 on MEA (3 days old) >

Submerged culture in PDA broth (100 ml in 250 ml Ehlrenmeyer flasks) (1:4 , V/V) (200 rpm)
↓
Routine observations to record pigment and viscosity
↓
Aseptic harvesting of the medium
↓
Centrifugation, 5000 rpm, 20 min.
↓
Pellet and culture filtrate

- Stereomicroscope → Colony morphology
- microscope → cell morphology
- Washed cell pellet (after 21 days)
  - Homogenization (Tissue grinder) (Aksu & Eren, 2007)
  - Ultrasonication (20KHz, 5 min.)

↓
Centrifugation (5000g, 20 mins)
↓
Extraction in Acetone and ethyl acetate (50:50, V/V)
↓
UV- Visible spectrophotometer (Schimadzu) (190nm - 700nm)

RESULTS:

The waxy shiny colonies with smooth margin and plano convex elevation on MEA produced a pinkish orange pigment. Micromorphologically the culture showed typical cylindrical to ellipsoidal cells (2 x 8 µm) with ballistoconidia. The culture was identified as *Sporobolomyces* sp. –a carotenogenic basidiomycetous yeast. Its' teleomorphic stage found in nature is known as *Sporidiobolus* sp-a Urediniomycetes

In submerged culture the pigment was seen on third day and its' intensity reached a peak within 7-10 days and then there was no change. The medium became progressively turbid and viscous. After 21 days a sediment could be observed at the bottom. After biomass harvesting, the culture filtrate appeared pale yellow. The separated cell biomass showed unique and complex pellet morphology with honeycomb and laminate structures with heavy amount of mucilage. Microscopic examinations in wet mount showed heavy yeast cell sediment and thick biofilms composed of yeasts. Refrigerant and guttulate cells were found in every field. The teleomorphic stage was also recovered with the pelletized biomass. Filamentous mycelial fragments were stained reddish and hence found positive for extracellular polysaccharides (EPS) in ammoniacal congo red. The hyphal morphology showed characteristic basidiomycetous nature with, simple dolipore septation, somatogamy, distinct branch initials and anastomosis. The teleomorphic status was confirmed by the formation of mycelial cords, differentiating microstructures such as cystidia and macrocystidia. Hyphae were found to carry intracellular refrigerant lipid like inclusions. It is known that *Sporobolomyces* sp. produce MAAs in only the anamorphic basidiomycetous carotenogenic yeast phase. It is inferred that the source of MAA's are from the anamorphic yeast stage.

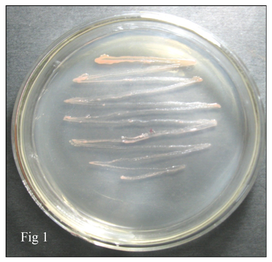


Fig 1

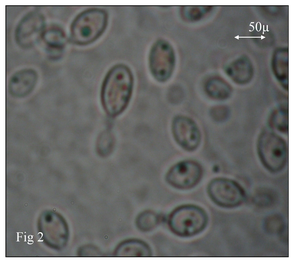
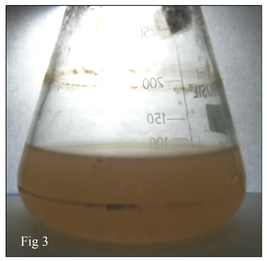


Fig 2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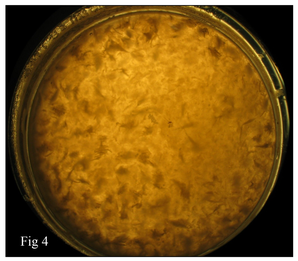
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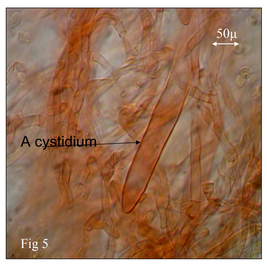

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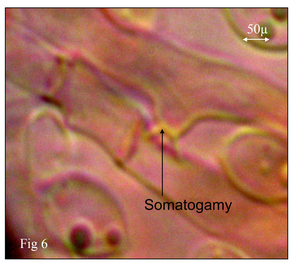


Fig 6

Legend:

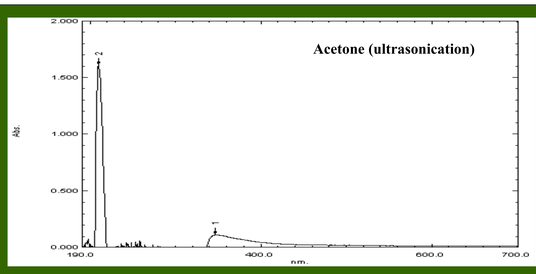
Fig1- Carotenogenic *Sporobolomyces* sp. GFCC 1100 Colony morphology (6 days) on MEA; Fig 2- Typical ballistosporic cells, Wet mount, phase contrast (x1000) Fig 3- submerged culture showing pigment production; Fig 4- Complex pellet morphology; Fig 5- *Sporidiobolus* teleomorph showing cystidium formation Fig 6- Somatogamous hyphae in *Sporidiobolus* teleomorph.

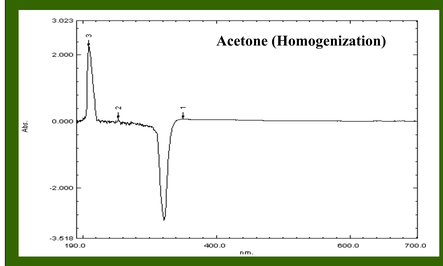
Sporobolomyces sp. is reported to be connected to a natural teliomorph *Sporidiobolus microsporus* sp. nov. [Mycologia 41:687. (1949) 1950] of Class Urediniomycetes, Order Sporidiales , Family Sporidiobolaceae. However we claim that development of *Sporiodiobolomyces* teliomorph in shaken culture is a novel finding confirming for the first time that basidiomycetous carotenogenic yeasts such as *Sporobolomyces* can produce a holomorph in liquid shaken culture under laboratory conditions (results in separate publication). The spectroscopic conformation 292 ,321 ,321.5 ,332.5 ,346 , 349 nm (table 1), of MAA's which absorb within UV-A and UV-B also indicates the potential of the aquatic yeast as an excellent source for formulating a new generation of Zymogenic sunscreens. The detection of absorbance at 416 nm indicates the presence of carotenoid compounds. It has been established that *sporobolomyces* sp. are rarely reported as animal pathogens. Its' teleomorph *Sporidiobolus* sp., a Urediniomycetes is also known to be benign to animals. This makes the carotenogenic yeast strain employed in present work , i.e. *Sporobolomyces* GFCC 11001 a promising potential candidate for MAA production.

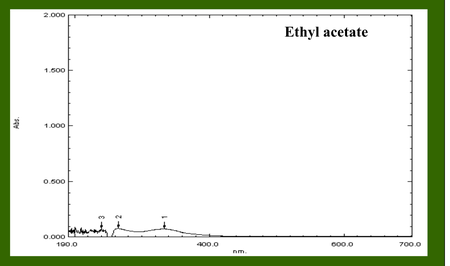
Having obtained positive leads in detection of MAAs, further work is in progress in the Mycology Laboratory to screen other promising strains and standardize the conditions to maximise production of MAAs considering their potential in development of a new generation of ecofriendly sunscreens. ***It is claimed that the present work has reported for the first time in the world , the identification of Sporobolomyces holomorph Sporobolomyces (anamorphic yeast stage) and Sporidiobolus (teleomorphic mycelial, state) in submerged culture under laboratory conditions***. Previous reports had indicated its' natural occurrence (Mycologia 41:687.(1949) 1950) . We have also identified prolonged aeration as a positive trigger for induction of teleomorphic stage. (Both these results would be published separately). Besides we aim to focus on the physiological, morphological and genetic aspects of the interesting anamorph-teliomorph connection in ***Sporobolomyces –Sporidiobolus*** which is currently under investigation. It is possible to study this system as a model in eukaryotic cell differentiation and regulation of gene expression. It has also not escaped our notice that simultaneous detection of carotenoid pigments in *Sporobolomyces* also affords a possibility to utilize the strain as a good source of carotenoids.

Table1:UV-Visible spectral detection of MAAs

Solvents	Method	λmax (nm)
acetone	Ultrasonication	321
		349
	Homogenization	321.5
		346
Ethyl acetate		332.5
		292







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