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Biosynthesis and Delineate Industrially Prime Exopolysaccharide (MYCtPs) from Marine Yeast *Candida Tropicalis* (MYCt).

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V. Sree Sountharavallee

Department of Biotechnology, Muthayammal College of Arts and Science (Autonomous), Rasipuram - 637408, Namakkal Dt, Tamilnadu. sreesoundarya95@gmail.com.

M. Suresh Kumar

Department of Zoology, Thiruvalluvar Government Arts College, Rasipuram-637401, Tamilnadu, India. chlorosuresh@gmail.com.

K. Kathiresan

Honorary professor, Annamalai University, Center of Advanced Study in Marine Biology, Parangipettai-608502, Chidambaram, Tamil Nadu. kathiresan57@gmail.com.

D. Kavitha

Assistant professor, Department of Biotechnology, Muthayammal College of Arts and Science (Autonomous), Rasipuram-637408, Namakkal Dt, Tamilnadu. ibtkavitha.d@gmail.com.

Correspondence Author: M. Suresh Kumar

Department of Zoology, Thiruvalluvar Government Arts College, Rasipuram-637401, Tamilnadu, India. chlorosuresh@gmail.com.

Keywords

Marine yeast *Candida tropicalis* (MYCt), Polysaccharide (MYCtPs), Biosynthesis, Characterization.

Abstract

The purpose of this research is biosynthesis and characterize the exopolysaccharide extracted from marine yeast isolated from marine soil for manipulate the environmental stress in industrial applications. Biosynthesis of exopolysaccharide (MYCtPs) derived by isolated strain *Candida tropicalis* (MYCt)MCAS01 and delineation of their physiochemical, biochemical, and microbial characteristics and composition. Thermogravimetric analysis confirmed the thermal stability of the MYCtPs along with the reaction kinetics. ¹³C-NMR and GC-MS analysis demonstrated a complex and heterogeneous nature of the MYCtPs. FT-IR, EDAX, and UV spectrum analysis revealed the presence of functional groups such as carboxyl, carbonyl, and ester. The X-ray powder diffraction (XRD) and the zeta potential exhibited the stability of the MYCtPs. The SEM micrograph demonstrated the size of (MYCtPs) is 1264.354 m (d0.5). Thus, study reveals that marine yeast produces novel polysaccharides of potential for applications in propitious bio sectors.

1. Introduction

Marine microbes, including yeasts, lives in the extreme environments, are unique for the synthesis of functional biomolecules (1). The polyphyletic group of ascomycetous and basidiomycetous fungi known as yeasts is distinguished by their ability to grow in a single cell. The survey has taken up some printed words that the marine yeasts are comparatively few and marine mycota is still not well known about yeast are ubiquitous in their distribution. Although terrestrial yeasts have been studied since the dawn of

time, the yeasts associated with marine settings have not proven to be a popular research topic. Yeasts are abundant in marine environments and their occurrence is determined by organic materials and other environmental variables. Marine yeasts can be isolated from seawater, sediments, plants, and digestive tracks of mammals and seabirds (2).

Bioactive substances produced by marine yeasts include glucans, glutathione, toxins, enzymes, phytase, vitamins, and others. (3). Hence, the marine yeasts are beneficial to food, pharmaceutical,

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chemical, and cosmetic sectors, as well as in environmental protection. They are commonly employed in the food sector in the brewing, distillation, and manufacture of carbon dioxide, as well as in baking.

Exopolysaccharides with interesting physicochemical properties and functional qualities can be synthesized by microorganisms from many taxonomic groups of microbes.

High viscosity and good gelling qualities are characteristics of some microbial polysaccharides, and they have a synergistic effect when interacting with other polysaccharides and combine with different salts over many pH and temperature ranges. Individual polysaccharides employed as food additives consists of different structures and properties, which account for their activities such as thickeners, emulsifiers, leavenings, and frothers. Bacterial and fungal compounds such as xanthan, dextran, and scleroglucan are the commercially available biopolymers (4).

Exopolysaccharides can be synthesized by yeasts belonging to genera: *Cryptococcus*, *Hansenula*, *Rhodotorula*, *Lipomyces*, *Bullera*, *Aureobasidium*, and *Sporobolomyces*. Among the polymers, Mannans, glucans, glucomannans, galactomannans, and phosphor mannans are also identified from the yeasts (5). The exopolysaccharides are extracted from culture broth of yeasts than bacteria - produced exopolysaccharides. The mix of the culture media and the growth conditions influence the structural and physical properties of microbial polysaccharides.

The influence of growth-limiting substrates on exopolysaccharide synthesis has been studied extensively, and the results show that the growth medium composition has a significant impact on the specific rate of polymer synthesis (6). The amount of carbon substrate converted to polymer by microbial cells is determined by the composition of the growth media. Polysaccharide synthesis is generally favored by a high carbon-to-limiting nutrient ratio. Although the monosaccharide ratio in the side chains of the polymer can change when ammonium salts are added to the medium, while the carbon and nitrogen sources utilized have no effect on the polysaccharide's qualitative monosaccharide composition (7). The composition of growth medium can also have an

indirect impact on polymer yield (8). With this background, this work was undertaken to investigate the exopolysaccharide-producing capacity of psychrophilic yeast for biosynthetic patterns, dynamic viscosity and relationship with cell physiology and polymer composition.

2. Methodology

Marine yeast isolation and identification

Isolation of marine yeast from soil

The study site is at a mangrove region that has been artificially raised along the Vellar Estuary's banks., Parangipettai, Southeast coast of India (Lat. 110.29'N; Long. 790.46'E). Here, the marine soil was collected and processed. Isolation of marine yeast was carried out by serial dilution and spread plate using the method with slight modifications (9). The yeast isolate was maintained on modified YEPD media (Yeast extract 0.3%, Peptone 0.5 %, (NH₄)₂SO₄ 0.1 %, KH₂PO₄ 0.025 %, Dextrose 3 %) following autoclaving, supplemented with 200 mg/l of chloramphenicol avoiding bacterial growth and incubated at 30°C for 2 days.

Morphological identification of Yeast

A single strain of yeast is identified microscopically by simple staining and morphologically colony color, cell shape and size as well as based on aerobic carbon and nitrogen utilization tests, and carbon fermentation assays.

PCR-Based Identification

According to Ben Ali (2020), the internal transcribed spacer (ITS) region (10) (5.8S rRNA) sequence was used to identify a few marine isolates using the primers ITS4 and ITS5 (11). The PCR master mix for each reaction contained 8 µl of 10 PCR buffer, 1 unit of Taq DNA polymerase, 25 pmol of each forward and reverse primer, 100 µM of each deoxy nucleoside triphosphate, and sufficient distilled water to form a 50 µl total reaction volume. Using a micropipette tip, a small quantity of yeast growth from a culture (24–48 h) was taken, suspended in 50 l of deionized water, and then incubated for 10 min at 95 °C. The warmed yeast suspension was then diluted by 4 l and added to the PCR tubes as a DNA template. The tubes were then placed in a thermocycler, which was set up as

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follows: initial denaturation at 94 °C for 30 s, followed by 35 cycles at 94 °C for 15 seconds, 52 °C for 45 seconds, and 72 °C for 30 seconds, with a final extension step at 72 °C for 10 min. Electrophoresis separation using 1% agarose gel with 4 µl of ethidium bromide in TBE buffer was used to identify the PCR products (0.09 M Tris, 0.09 M boric acid, and 2 mM EDTA, pH 8.3). Prior to sequencing, the MinElute Reaction Cleaning Kit (QIAGEN) was employed for purification. For comparison with already accessible sequences, the acquired sequences were aligned using BLAST analysis (<http://www.ncbi.nlm.nih.gov/BLAST>).

All PCR clones' core sequences were used for the ITS phylogenetic comparisons, and any lengthy sequences were shortened to ensure that all bases were included in the multiple sequence alignments. We did not include any too-short sequences. Megalign, DNA Star, and ClustalW embedded in Megalign were used to create the alignments and create the cladograms of the phylogenetic relationships. One thousand produced trees were used in the bootstrap analysis.

Synthesis of Exopolysaccharides (MYCtPs) from MYCt

The strains generated slimy colonies and were identified as EPS generating yeast after being streaked onto Modified YEPD agar plates and cultured for 48 hours at 30°C. The chosen strains were added to 100 ml of modified YEPD medium, which contained (NH₄)₂SO₄ 0.1%, peptone 0.5%, yeast extract 0.3%, and dextrose 4%. The culture was then incubated for 15 days with continuous agitation of 100 rpm at room temperature (12). The following procedure involved centrifuging the culture at 10,000 rpm for 10 minutes at 4°C, transferring the supernatant to a separate tube, putting twice as much ethanol and isopropyl alcohol in there, and letting EPS precipitate overnight. For decolorization, the pellet was precipitated with ethanol. Next, 1 ml of sterile, distilled water was used to dissolve the pellet (13).

Characterization of Exopolysaccharide (MYCtPs).

FT-IR spectroscopy

By comparing the polymer's molecular structure and group composition with recognised standard polysaccharides, the molecular structure of the polymer was examined using a Perkin Elmer infrared

spectrophotometer. In practise, the process included creating small salt discs that were mechanically compressed from a mixture of 300 mg of pure dry KBr and 1 mg of the tested substance. Following that, the disc was assessed using the specified infrared spectral range of 500 to 4000nm (14).

GC-MS analysis of exopolysaccharide

5 mg of pure MYCtPs were hydrolyzed with 2 mL of 2 M trifluoroacetic acid (TFA) at 120 °C for 2 hours in to determine the monosaccharide composition of MYCtPs. After that, the hydrolysates undergo N-acetylation using acetic anhydride (CH₃CO) O₂ and potassium borohydride (KBH₄) in ammonium hydroxide (NH₄OH) solution. By combining Gas Chromatography and Mass Spectrometry with a Bruker GC remote control MS workstation, the derivative products were used to determine the monosaccharide composition (15).

Using an Agilent auto-sampler and a source temperature of 250 °C, 1 l of samples were injected into the back inlet in a 10:1 ratio. The HP-88 capillary column, which has a 100 m length, 0.250 mm width, and a 0.20 m (88% cyanopropyl) arylpolysiloxane film, was used to conduct the separation. The carrier gas employed was helium, and the flow rate was held constant at 1 mL/min. The temperature was initially set at 50 degrees Celsius with a 1-minute hold period before increasing to 175 degrees Celsius at a 10-minute ramp rate, 210 degrees Celsius at a 5-minute ramp rate, and then 230 degrees Celsius at a 5-minute ramp rate. For the purpose of identifying sugar, a comparison was done with standard glucose and starch.

¹³C NMR study

After drying under pressure for a whole night at 40 °C, the exopolysaccharides were identified using a ¹³C-NMR study in chloroform at a concentration of 15 mg. mL⁻¹. Bruker Avance III HD Nanobay 400 MHz FT-NMR SPECTROMETER with 5 mm multinuclear probes for fluid studies broad band frequency range ¹³C was used to record the experiment (16).

SEM analysis with EDX

To study the morphology and elemental makeup of MYCtPs, the SEM and EDS were conducted using

JEOL JSM Thermo Scientific 7500 and 5600-LV with a voltage increased from 10 to 15 kV. The materials had previously been lyophilized, homogenised into a fine powder, and then dispersed under vacuum to attach to a tiny aluminium sample holder. With the TESCAN OXFORD programme VEGA 3 INCA, carbon was used as a conducting element (17).

TGA & DTA analysis

Analysis of Thermal Stability In a nitrogen-free atmosphere, thermogravimetric analysis (TGA) is performed, and the weight is measured as a function of rising temperature. The device also keeps track of the temperature variation between the specimen and one or more reference pans (differential thermal analysis or DTA).

On NETZSCH-NJA - STA 2500 Regulus - Proteus Software, TGA and DTA were carried out. To assess the thermal stability of the EPS and CPS in comparison to the control bacterial polysaccharides, thermograms for TGA and DSC were generated in the range of 30-700 and 50-550°C, respectively (dextran and guar gum).

UV-Spectrum Analysis

The phenol- sulfuric acid technique was used to determine the amount of total carbohydrates in the MYCtPs (18). In summary, a 1.0 mL aliquot of the MYCtPs was combined to with 1.0 mL of 2.5% phenol and 5.0 mL of conc. H₂SO₄, and the mixture was immediately vortexed. A UV-Vis spectrophotometer was used to measure the absorbance at 480 nm against a blank reagent after the mixture had been incubated at room temperature for 20 minutes (JENWAY 6800 Japan).

XRD analysis

By collecting X-ray diffraction patterns on a Malveran panaltical Advance diffractometer with Cu Ka radiation produced at 45 kV and 40 mA, the crystallinity of MYCtPs was ascertained. The MYCtPs were compressed into flat pieces and put on a quartz sampler holder after being lyophilized. The reflection mode was used to produce data. The generated data was acquired at room temperature in the 2 range of 20°- 80° with a scan rate of 1.0° min⁻¹ (19).

Zeta potential analysis

The particle electrophoresis equipment "Malvern instrument" was used to measure the electrical charge (-potential) of MYCtPs (Nanosizer Instruments, USA). The direction and speed of the particle motion in the applied electric field were measured to calculate the potential. The experiment was carried out at 25 °C with a biopolymer concentration diluted to around 104 g. L⁻¹.

The diluted solution was well mixed before being injected into the particle electrophoresis device's measuring chamber. The average and standard deviation of measurements made on three recently manufactured samples were used to calculate the - potential values using the Smoluchowski equation in an aqueous medium with moderate electrolyte content (20)

$$\xi = \eta \mu / \epsilon \quad \xi = \eta \mu / \epsilon$$

Whereas,

μ : Electrophoretic mobility

η : Viscosity

ϵ : Dielectric constant of the solvent.

3. Result:

Isolation of marine yeast

The marine yeast strains isolated from sediments of the Vellar estuary, Parangipettai, (Figure.1) Southeast coast of India and the isolates were maintained on modified YEPD media. According to a review of the literature, there have been relatively few studies on marine yeasts, and knowledge of marine mycota is still limited. Marine yeasts have a special potential and can survive in harsh environments.



Figure 1- Map of study area: Vellar estuary, Parangipettai, Southeast coast of India

Morphological identification of *MYCt*

Using a trinocular microscope, isolated yeast was morphologically recognised before being stained (Figure.2). Initially, yeast isolate *MYCt* was screened for EPS (*MYCtPs*) production in modified YEPD media and the kind of ubiquinone (coenzyme Q) present in different yeasts.



Figure 2 (i): Morphology of yeast (*MYCt*) on Plate.
 (ii): Microscopic view of Yeast isolate *MYCt*

Phylogenetic analysis of the isolate

The phylogenetic analysis of 18S rRNA demonstrated that the yeast isolate *MYCt* belonged to the genus of the *Candida tropicalis* (Figure 3). The nucleotide sequence of the marine Yeast isolate *MYCt* has been submitted to the NCBI Database under accession number MZ723324.1 in the name of *Candida tropicalis* (*MYCt*) isolate MCAS01.

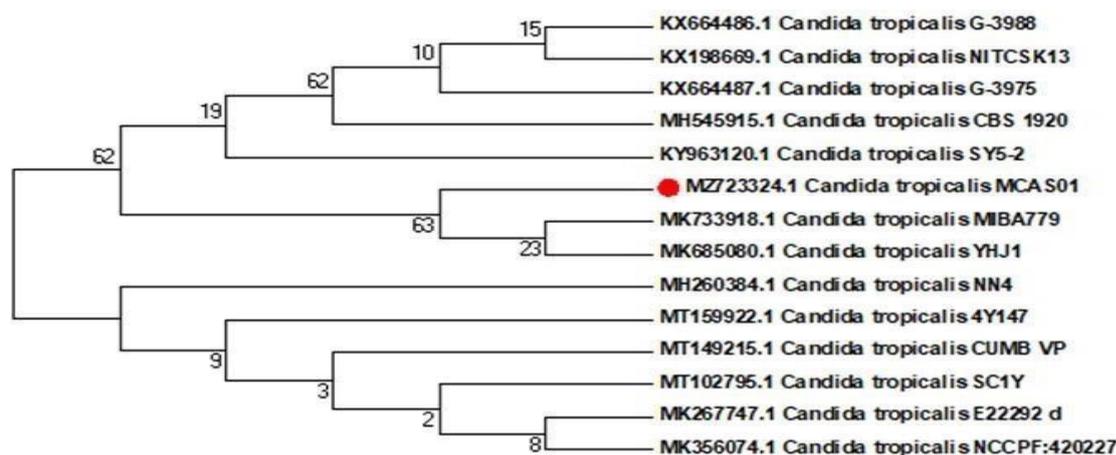


Figure 3. The Phylogenetic position of Yeast isolate *MYCt* obtained with neighbor-joining method. The optimal tree with the sum of branch length = 0.37221527 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The tree was constructed by a neighbor-joining method using the Mega 6 tools and the sequences were aligned by clustal W and are in the units of the number of base substitutions per site. Evolutionary analyses were conducted in MEGA 6.

Accession number MZ723324.1 and the name of *Candida tropicalis* isolate (*MYCt*) MCAS01.

SEM analysis with EDX

The lyophilized *MYCtPs* SEM micrograph shows associations of some crystals primarily related to minerals as well as irregular blocks aggregates and compressed layers-like structures with smooth surfaces. Particles of extracellular polymeric material range in size from 49.492 (d0.1) to 1634.192 (d0.9) μm , on average, and have 0.0651344 m^2g^{-1} specific surface areas (Figure 7).

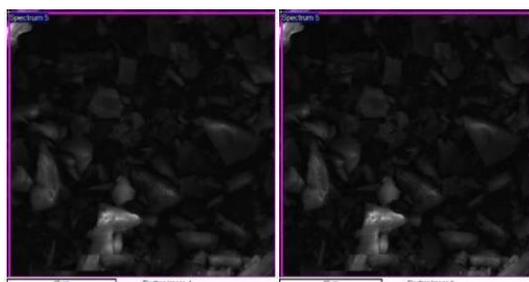


Figure 7. SEM Analysis of extracted *MYCtPs*

Energy dispersive X-ray spectroscopy is one of the X-ray fluorescence spectroscopy versions used to examine *MYCtPs* elements (EDS or EDX). By measuring the X-rays that a sample emits in response to being bombarded by charged particles, electromagnetic radiation and matter interactions are the focus of EDX analysis. A polymer may be crystalline or amorphous, and phase identification of *MYCtPs* using X-ray powder diffraction (XRD) is a common quick analytical approach.

The structure, quantity, and composition of *MYCtPs* all affect their rheological and textural characteristics. SEM was used to examine the EPS made from *Candida tropicalis* (*MYCtPs*) (Figure 7). The produced EPS is structurally extremely compact, as can be seen from the microstructure of *MYCtPs* surface view.

Energy dispersive X-ray spectroscopy, also known as EDS or EDX, is a type of X-ray fluorescence spectroscopy that is used to analyze a sample's elements. By examining the X-rays that a sample emits in reaction to being struck by charged particles, EDX examines how electromagnetic radiation interacts with the material in a sample. The weight and atomic percentage of the elements present in *MYCtPs* were determined by quantitative elemental analysis performed by EDX (Table 1).

Table 1. Energy dispersive X-ray spectroscopy (EDX)

Element	Weight%	Atomic%
C K	57.25	65.09
O K	39.09	33.36
Na K	0.50	0.30
P K	0.23	0.10
S K	1.52	0.65
Cl K	0.43	0.16
K K	0.32	0.11
Ca K	0.66	0.23
Totals	100.00	

TGA & DTA analysis

Exopolysaccharides temperature and rheological properties heavily influence how useful they are. A practical analytical method called thermogravimetric analysis (TGA) evaluates the weight loss of a substance as a function of temperature. An amorphous solid will become less viscous as temperature rises, and at a specific temperature, known as the crystallisation temperature, the molecules have adequate freedom of motion to spontaneously arrange themselves into a crystalline state. A considerable thermal transition of *MYCtPs* was revealed by differential scanning calorimetric analysis, which is an exothermic process that occurs when an amorphous solid transforms into a crystalline solid (Figure 8).

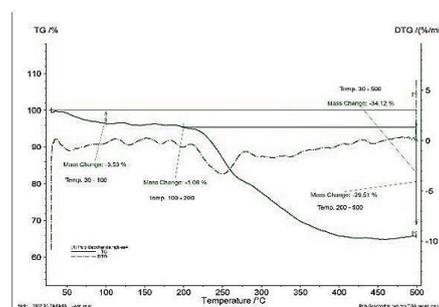


Figure 8. TGA and DTA analysis

The DTA curve of MYCtPs shows one exothermic peak at 444.7 0C, while the TGA of MYCtPs revealed a mass loss of 7.81% between 33 and 240°C, another loss of 63.53% between 240 and 400°C, and finally a loss of 28.38% between 400 and 660°C. The DTA curve displayed two exothermic peaks at 325.54 and 542.10 degrees Celsius, respectively, whereas the Dextran TGA exhibited a mass loss of 12.73 percent between 33 and 2600°C, a further loss of 63.78 percent between 260 and 4000 degrees Celsius, and lastly a loss of 23.1 percent between 400 and 6300 degrees Celsius. The MYCtPs show a mass loss between 30 and 2300 C of 11.127% and a loss between 230 and 7000 C of 64.873% when compared to the dextran and guar gum employed as controls. The CPS shows a mass loss of 9.33% between 33 and 2600 C and another loss of 26.86% between 401.55 and 549.99 C. The DTA curve has three exothermic peaks at 401.55, 502.55, and 549.99 C, respectively (Figure 8). The three polymers displayed a mass loss of around 72–75% after being exposed to increasing temperatures and exothermic peaks, demonstrating their thermal stability and reaction kinetics.

UV-spectrum analysis

MYCtPs' UV spectrum study revealed that there was a sizable amount of absorption in the UV region, with the largest absorption occurring at 190 nm. According to, the 190 ~ 230 nm wavelength area often results from n-σ* and/or π-π* transitions, which is found in many functional groups such as carboxyl, carbonyl, and ester.

XRD analysis

The most frequently used quick analytical method for determining a crystalline material's phase is called X-ray powder diffraction (XRD). The XRD profile of MYCtPs obtained from *Candida tropicalis* isolate MYCt (Figure 9) exhibited the characteristic

diffraction peaks at, 11.9022°, 14.9077°, 20.9836°, 29.8299°, 2.99527°, 31.9652° and 49.1517° with interplanar spacing (d-spacing) 7.43578, 5.94274, 4.23371, 2.79989 and 1.85367Å, respectively.

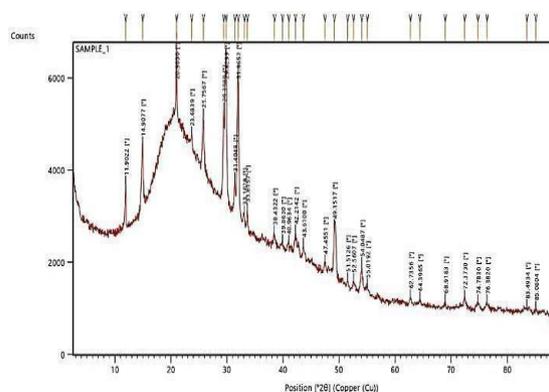


Figure 9. XRD Analysis of the Extracted MYCtPs

ZETA potential analysis

For particles, zeta potentials between - 30 mV and + 30 mV are regarded as stable (26). For particles, zeta potentials between - 30 mV and + 30 mV are regarded as stable (26). Silver nanoparticles' zeta potential values of - 20.87 mV, - 23.65 mV, and -16.41 mV, respectively, in Figure 10 made it very evident that the particles were tables.

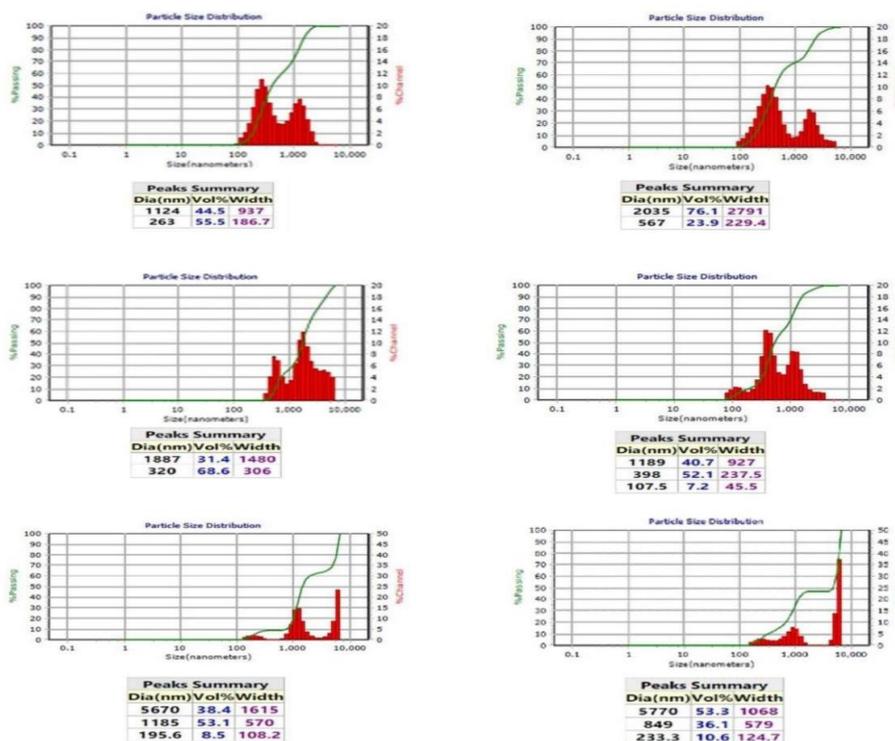


Figure 10. Zeta potential analysis of MYCtPs

Figure 10. Zeta-potential analysis of MyCtPs.

4. Discussion

According to a review of the literature, there have been relatively few studies on marine yeasts, and knowledge of marine mycota is still limited. The Similar isolation done by kutty *et al.* (21), is Major genera isolated in this study were *Candida*, *Cryptococcus*, *Debaryomyces* and *Rhodotorula*. The distribution of the species, their numbers, and their metabolic traits were discovered. Delineation of polysaccharide were done, Initially, yeast isolate MYCt was screened for EPS (MYCtPs) production in modified YEPD media and the kind of ubiquinone (coenzyme Q) present in different yeasts the described by to Balluo *et al.* (22). Extracellular-polysaccharide EPS was extracted from *Saccharomyces cerevisiae* growth medium after 10 days from incubation at 25°C and pH 7 on liquid Saubroud's medium (23) comparatively in this work yield of polysaccharide increased in just 48 hours 30°C in the normal Yeast extract medium.

Mishra., (24) describes polymer is in crystalline or amorphous give good phase of identification. MYCtPs polymer seen crystalline or amorphous in the nature. The FTIR analysis confirmed the functional groups of

the EPS (25) similarly MYCtPs FT-IR and NMR shows well peaks indicates presence of functional groups. Physio-chemical analysis using UV-Vis estimated the carbohydrate contents (14) MYCtPs polymers also measured in UV-Vis estimation. For particles, Zeta potentials between - 30 mV and + 30 mV are regarded as stable (26). MYCtPs polymers also got good potential range as it was showed in zeta potential figures. In conclusion a good application in industrially prime Products in pharmaceutical, cosmetics and textile industries.

5. Conclusion:

Yeast was isolated from the soil of banks along the Vellar estuary, Parangipettai, Southeast coast of India and named MYCt. By using a trinocular light microscope and simple staining, isolated yeast was morphologically identified. The phylogenetic analysis of 18S rRNA demonstrated that the Yeast isolate MYCt belonged to the genus of the *Candida tropicalis* sp. Marine yeasts are widely distributed in nature and have a wide range of uses. The fresh water and energy crises can both be resolved with the help of marine yeasts.

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FT-IR study revealed the presence of alkenes, ketones, alkyl hydrogen and aliphatic groups, alcohols, ethers, esters carboxylic acids and phenols groups. When the glycosyl composition was analysed using GC/MS, it was discovered that the main components were acetyl glucosamine, mannose, and glucuronic acid, with very modest amounts of glucose and rhamnose and moderate levels of galactose and fructose. The structure of *MYCtPs* was investigated by ¹³C-NMR which demonstrated a complex and heterogeneous nature of the *MYCtPs*. The SEM micrograph demonstrated the size between 1264.354 m (d0.5) and 0.0651344 m²g⁻¹ specific surface areas. The reaction kinetics and thermal stability of the polymer are both confirmed by thermogravimetric analysis (TGA). UV spectrum analysis of *MYCtPs* revealed that the presence of functional groups namely carboxyl, carbonyl, and ester. X-ray powder diffraction (XRD) and the zeta potential exhibited the stability of the exopolysaccharide. In conclusion *MYCt* exopolysaccharide *MYCtPs* can be used in various fields of cosmetics, Pharmaceuticals & food industries due to stability and chemical nature.

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