Hexavalent chromium tolerant fungal species identified from urban vegetable farm and effluent waste in Addis Ababa & Rift valley, Ethiopia

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Abstract

Hexavalent chromium is resistant to degradation and sever toxic substance to environment and community health. Physico chemical treatment methods are demanding high cost, used large quantities of chemicals & energy, release large amount of secondary toxic degradants. Mycoremediation is an eco-friendly alternative treatment method. The main objective of this research is to isolate and characterize chrome (VI) tolerant yeast from farm soil & industry effluent for mycoremedation role. The screening and isolation of yeast was carried out on potato dextrose agar media. Solid agar and broth assay test for yeast tolerance on hexavalent chromium at different concentration, temperature and pH was evaluated. Yeast species was identified biochemically using Biolog Microstation depending on carbon utilization and chemical sensitivity test. The result revealed that 11 yeast species was identified from effluent waste and farm soil based on their probability ≥75% and similarity index ≥0.5 as well as their hexavalent chromium tolerance ability up to 2500ppm. These are *Yarrowia lipolytica* (100%,0.7) *Cryptococcus luteolus(*100%,0.64), *Rhodotorula aurantiaca* A (100%,0.62), *Ustilago maydis* (100%,0.58) *Trichosporon beigelii B* (100%,0.51),*Cryptococcus terreus* A (100%,0.62), *Zygosaccharomyces bailii* (98%,0.65), *Nadsoniafulvenscens* (90%,0.62), *Schizoblastosporon starkeyi henricii* (*89%,0.56), Endomycopsis vivi* (84%,0.62*), Rhodotorula pustula* (Sim 0.59). Two yeast species *Yarrowia lipolytica* and *Nadsonia fulvenscens* show the highest mean Optical density (OD) measure (0.953±0.43), (0.96±0.400) respectively at pH4 &25OC. Cr (VI)-tolerance ability of these yeast strains used after further HPLC analysis& molecular characterization in the development of chromium-bioremediation technologies provide an alternative method for chromium sequestration.

Introduction

Industrial expansion & environmental pollution are uncompromised issue for many decades. Hexavalent chromium is one of sever toxic heavy metal released from industrial and anthropogenic activities which pollute terrestrial and aquatic ecosystem. Many industries like textile, tannery, electroplating, dye, metallurgy are the source of chromium waste (Nakkeeran et al., 2018; Lian et al., 2019). Those industries built near the river side in Addis Ababa and different part of Ethiopia drain unmanaged & untreated effluent waste water in the rivers. This waste water in turn serve for direct irrigating of the urban vegetable farming activities in Addis Ababa and neighboring area. CSA (2017) reported that chemical contamination in water bodies are very high where about 13% are estimated to be water free from chemical contamination. Cr^{+6} is water soluble and highly mobile which is bio-available in all environment therefore farmlands, underground and surface water, vegetables are highly contaminated by these potentially toxic heavy metal (Elahi, 2020). The accumulation of potentially toxic elements(PTEs) in the environment and on the edible parts of vegetables with high concentrations could have a direct impact on the community health (Aschale et al., 2019).The primary health hazard caused by chromium are skin rashes, nausea, vomiting, skin irritation, skin allergies, upset stomach and ulcer, cancer, respiratory problem, weakened immune system, kidney and liver damage, alteration of genetic material, reproductive and developmental problems and death (Chatterjee,2015). According to the U.S. Environmental Protection Agency (USEPA) regarded the Cr(VI) as one of the supper environmental contaminants and categorized it into group “A” human carcinogen (Dhal et al. 2013).

When the concentration of Cr(VI) reach 5 to 100mg kg^{-1}in the soil, it is very toxic to most plants, as the result of Cr^{6+} oxidizing potential cause biological systems to be distorted resulting mutagenic and carcinogenic effect (Garg et al., 2012). Different study carried out in Ethiopia where the level of heavy metal in farmland, water body and vegetables are beyond the threshold level. In Addis Ababa particularly Kera, Bulbula, Meknisa, Goffa German, Pickok, Akaki urban vegetable farming area the level of chromium and other heavy metal are very high (Ewers 1991; Kabata-Pendias and Pendias 1992; Weldegebriel et al., 2012). Other studies reaffirmed that Akaki river was highly contaminated because of the discharge of the partial treated or untreated and appropriately not managed industrial effluents into the river

(Aregawi, 2014, Amare, 2019). In another study the physicochemical analysis of effluents from Sabata river and industries around Addis Ababa showed that beyond the safe limits of World Health Organization (Gemeda et al.,2020). Physicochemical treatment method has some limitation because chromium waste treatment are high cost and energy demanding, large quantities of chemical utilization for treatment, it releases other secondary degraded compound waste and need high technology. Therefore chromium pollution in all environments can be treated using alternative eco-friendly mycoremedial approach for pollution mitigation. There for this study aim at screening and identifying hexavalent chromium tolerant fungi for mycoremediation role.

Material And Methods

Sampling area description

Soil and effluent sample were collected from Addis Ababa urban farming site particularly from Pickok, German Goffa, Akaki kality, Mekanisa, Lafto-saris, kolfe-Liquanda and rift valley area. The vegetable farm was irrigated from polluted river using pumping motor directly. However the river was connected with different industries effluent outlet. AddisAbaaba is located at geographic coordinate, latitude: 9°01'29" N, longitude: 38°44'48" E, elevation above sea level 2355 meters above sea level. Some of the soil sample was collected from part of Rift valley at geographic coordinate 6° 2' 0" N, 37° 33' 0" E at an elevation of 1285 meters above sea level.

Sample size and collection method

10g of soil from vegetable farm and 10mL wastewater samples from effluent waste was collected and kept in sterilized falcon tube (45mL) from Addis Ababa urban farming site, polluted rivers, effluent outlets and rift valley area. Totally 150 sample were collected. Sampling point were clustered depending on vegetable types and polluted rivers, these were, Sald, Cabbage, Carrot, Potato, Garlic, Cauli flower, Swiss chard farm and forest . The collected samples were transported into Addis Ababa University Mycology laboratory.

Soil enriching method

150 soil and effluent samples were pooled into 15 composite samples depending on similar property from each site and 10gram of soil from each farm and effluent were taken. A total of 150gram soil were kept in 500mL Erlenmeyer flask 300mL distil water and 100mL potassium dichromate($K_2Cr_2O_7$) solution (500-2500ppm) were added within 3 days interval subsequently by increasing the concentration into the flask where potassium dichromate used as a source of hexavalent chromium and kept at rotary shaker at 150rpm for 15days.

Fungi Isolation

After 15days from enriched soil sample & effluent, 10mL was transferred into 90 mL distil water and 1 mL subsequently transferred into 9mL distils water containing test tube. Serial dilution was prepared from 10^{-1} to 10^{-6}mL. From each test tube using micropipette 0.1mL diluted sample were spread on potato dextrose agar (PDA) aseptically & incubated for 24-48 hrs at 26°C. Fungi will be picked and sub cultured twice on PDA until pure colony appeared. Fungi isolates cultures will be maintained in PDA slant agar and kept at 4^{o}C until further analysis employed.

Experimental design

The interactions of three independent variables: temperature (25^{o}C, 30^{o}C, and 35^{o}C), pH (values 4, 7, and 9) and hexavalent chromium concentration (500 ppm, 1000 ppm and 1500 ppm, 2000ppm, 2500ppm) were investigated using a response surface design.

In vitro screening of Cr (VI) tolerant yeast

PDA media was amended with different concentration of $K_2Cr_2O_7$ ranging from 500-2500 ppm and evaluated at different pH (4, 7 and 9) and Temperature range (15^0C, 25^0C, 35^0C) A loop full of fresh yeast isolates was transferred to this growth mediaand incubated at 26^0C for 48–72 hours. The plates were observed visually for any yeast colony growth after incubation. The yeast tolerance to Cr (VI) and colony appearance in the presence of the highest concentration was chosen for further studies.

Screening of Cr (VI) tolerant yeast at different pH, Temperature & Concentration

100mL Potato dextrose broth (PDB) in 250mL Erlenmeyer flask was amended by 500,1000,1500,2000, 2500ppm $K_2Cr_20_7$ and antibiotic. Each PDB concentration was adjusted at pH (4, 7 and 9) and Temperature (15^OC, 25^0C, &35^oC). 1ml of yeast culture $1.5x10^8$CFU/mL was inoculated each flask and incubated on incubator shaker at 200 rpm. Potato dextrose broth was also used for control without inoculation of fungi. Jenway 6305 UV/Visible model spectrophotometer was used to measure the optical density(OD) at 600nm within 24 hour interval for 3 days. 3mL sterile fresh PB broth in cuvette was used to blank the Spectrophotometer and the cell suspension cuvette was placed into the cuvette slot and the cuvette chamber was closed. The absorbance of the cell suspension was recorded and the process will be repeated at 24 hour interval for 72h. All experiment carry out tri-replicate set up.

Macro morphology characterization

Fungi colony was characterized like colony size, opacity, color, margin, texture, pigment and elevation using morphologic key of pictorial atlas of soil and seed fungi (Watanabe, 2010). Data and photograph will be taken.

Biochemical characterization of fungi for different carbon utilization

YT Micro Plate consists of 96 phenotypic tests (94 biochemical tests pluses 2 control). Culture suspensions are tested with a panel of pre-selected assays, then incubated for 24-96h. At the incubation period, fungi were utilizing compound in the well that reduce the tetrazolium dye forming a formazon purple color or initiate growth leading to an increase in turbidity by producing a characteristic pattern or metabolic fingerprint from discrete test reactions. Each metabolic pattern was read by a Micro Station (Biolog Inc) at a single wavelength of 590 nm and compared to Biolog databases as well as interpreted by micro log3 software ver. 4.20.05 (Biolog, Hayward, CA). Colorimetric or turbidity change in each well was referenced against negative control wells. Micro plate wells are recorded as negative, (**-**), as positive <**+**>, borderline (****) for its carbon assimilation and oxidation.

Yeast identification using biolog microstation

Pure yeast colony cultured on biolog universal agar (BUYA) at 26 °C for 24-48h. 10mL sterile distilled water was used for preparation of yeast inoculums suspension. Using sterilized cotton swab pure yeast cell transferred into distil water containing test tube and adjusted to 47T$\pm$2 by using biolog YT turbidiameter. 100 µ L of inoculums was added to each wells of the biolog YT Micro Plate using digital pipettes and incubated at 26°C for 24-72h. The YT micro plate measures both metabolic reactions as well as turbidity growth to produce identifications. YT micro plate was read by the micro station reader within 24 h time intervals up to 72 h at a single wavelength of 590 nm. The biolog software micro log3 ver. 4.20.05 compared the results obtained with the test strain to the database and provided identification based on distance value of match and separation score. The recommended and acceptable species identification result must have similarity index value $\geq$ 0.5and probability $\geq$ 75% (Biolog, 1993).

Statistical Analysis

The data analysis carried out using descriptive & inferential statistics such as mean, standard deviation, percentages frequency, correlation. SPSS Ver 23 statistical software package and design expert ver13 was employed to analyze obtained data.

Result And Discussion

Fungi isolation & percentage frequency

Totally 533 fungi isolates were cultured on PDA media and screened. All isolates were clustered by using hand lens and microscope based on their colonial morphology and genera. The percentage frequency of fungi on PDA media revealed that 181 (34%) was the yeast isolates and 352(66%) filamentous fungi in this study.

Hexavalent chromium tolerant yeast

Yeast isolates were screened depending on only their colony growth on PDA solid agar amend with $K_2Cr_20_7$ at different concentration (500, 1000, 1500, 2000, 2500ppm), Temperature (15, 25,35^oC), pH (4, 7, 9). The result revealed that totally

fourteen yeast species were selected visually having good growth and tolerance from 500 to 2500ppm Cr(VI). Two yeast species *Yarrowia lipolytica* and *Nadsonia fulvenscens* show the highest tolerance at 2500 ppm at pH4 &25^{0}C, Four yeast species are tolerant at 2000ppm of Cr(VI), *these are Cryptococcus terreus A, Trichosporon beigelii B , Endomycopsis vivi, Ustilago maydis* and Five yeast species tolerant to 1500ppm these are *Rhodotorula pustula, Zygosaccharomyces bailii, Rhodotorula aurantica A, Schizoblastosporon starkeyi-henricii, Cryptococcus luteolus.* Three yeast tolerant to 500ppm (Figure.1,2,3).

Fourteen yeast species were tested for their ability to grow and tolerate on liquid assay amended with 500-2500ppm $K_2Cr_2O_7$ concentration at different temperature(15,25,35^{0}C) and pH ranges(4,7,9). The optical density reading at 24 hour interval up to 72h indicated that *Yarrowia lipolytica and Nadsonia fulvenscens* showed the highest mean Optical density (OD) measure (0.953$\pm$0.43) ,(0.96$\pm$0.400) respectively at pH4 & 25^{O}C (Figure.4).).Six yeast grow best at pH4 &25^{0}C, six yeast grow at pH7 and 25^{0}C. One yeast grows best at pH9 & 25^{o}C. The interactions of three independent variables: temperature, pH and concentration also analyzed using surface response design. The significant interaction were seen in Chrome concentration and Temperature in pareto chart (Figure 1-7).

Macro morphology of yeast

Species	Shape	Color	Size	Optical property	Elevation	Texture
Cryptococcus luteolus	Round	White	Medium	Translucent	Raised	Dry
Schizoblastosporon starkey henricii	Irregular	Dull white	Large	Translucent	Flat	Dry
Rhodotorula aurantica A	Round	White	Medium	Translucent	Raised	Dry
Yarrowia lipolytica	Ovoid to Spherical	Tanish white	Medium	Glistering to Dull	Raised	Dry
Zygosaccharomyces bailii	Round	White pink	Medium	Translucent	raised	Dry
Ustilago maydis	Round	White	Medium	Translucent	Raised	Dry
Nadsonia fulvenscens	Round	White	Medium	Translucent	Raised	Mucoid
Endomycopsis vivi	Round	Pink	Small	Glistering to Dull	Raised	Dry
Trichosporon beigeliiB	Round	White	Medium	Translucent	Raised	Dry
Cryptococcus terreus A	Round	Cream	Medium	Translucent	Raised	Mucoid
Rhodotorula pustula	Round	White	Medium	Translucent	Raised	Shiny dry

Table.1. Macro morphology of yeast characterization

Characterization of yeast species for carbon utilization on YT-micro plate

Yeast were characterized biochemically for their carbon utilization on YT-microplate, when incubated at different temperature the yeast reduce the tetrazolium dye tagged on YT-Microplate forming a formazon purple color or initiate growth leading to an increase in turbidity. Colorimetric or turbidity difference in each well was compared against negative control wells. The result revealed that five yeast isolates commonly utilize, D-Cellobiose, Sucrose, D-Trehalose, D-Xylose, D-Gluconic Acid. Four yeast isolates utilized Tween 80, Maltotriose, L-Glutamic Acid, D-Sorbitol. Three yeast isolates utilized D-Melibiose, D- Glucuronic Acid plus D-Xylose, Dextrin plus D-Xylose, D-Galactose plus D-Xylose, m-Inositol plus D-Xylose, Palatinose, Salicin, D-Arabitol, D-Mannitol, Quinic Acid. Each species substrate utilization are summarized in Table2.

Yeast species	Assimilated carbon source by yeast species
Cryptococcus luteolus	D-Cellobiose, D-Melibiose, Sucrose, D-Trehalose,Succinic Acid Mono-Methyl Ester plus D-Xylose, N-Acetyl-L-Glutamic Acid plus D-Xylose, D- Glucuronic Acid plus D-Xylose, Dextrin plus D-Xylose, α -D-Lactose plus D-Xylose, D-Melibiose plus D-Xylose,D-Galactose plus D-Xylose, m-Inositol plus D-Xylose, 1,2- Propanediol plus D-Xylose
Schizoblastosporon starkeyi-henricii	D-Gluconic Acid, D-Xylose, D-Galactose plus D-Xylose
Rhodotorula aurantica A	Tween 80, 2 -Keto-D- GluconicAcid, D-Gluconic Acid, D-Cellobiose, Maltotriose, Palatinose, Sucrose, D-Trehalose, Amygdalin, Arbutin, Salicin, D-Arabitol, L-Arabinose, D-Xylose, N-Acetyl-L- Glutamic Acid plus D-Xylose, Quinic Acid plus D-Xylose, D- Glucuronic Acid plus D-Xylose, Dextrin plus D-Xylose, D-Melibiose plus D-Xylose, m-Inositol plus D-Xylose
Yarrowia lipolytica	L-Aspartic Acid, L-Glutamic Acid, L-Proline, D-Gluconic Acid, N-Acetyl-D-Glucosamine, Glycerol, Tween 80, Fumaric Acid, L-MalicAcid, N-Acetyl-D–Glucosamine.
Zygosaccharomyces bailii	D-Trehalose
Ustilago maydis	Bromosuccinic Acid, D-Gluconic Acid, D-Cellobiose, Maltose, Maltotriose, Palatinose, Sucrose, D-Trehalose, N-Acetyl-D-Glucosamine, D-Glucosamine, Salicin, D-Mannitol, Tween 80, D-Ribose, D-Xylose, N-Acetyl-L-Glutamic Acid plus D-Xylose, Quinic Acid plus D-Xylose, D- Glucuronic Acid plus D-Xylose, D-Melibiose plus D-Xylose, m-Inositol plus D-Xylose, 1,2-Propanediol plus D-Xylose
Nadsonia fulvescens	L-Glutamic, Acid, D-Sorbitol
Endomycopsis vivi	Sucrose
Trichosporon beigelii B	D-Raffinose,Fumaric Acid, L-Malic Acid, Succinic Acid Mono-Methyl Ester, Bromosuccinic Acid, L-Glutamic Acid, D-Cellobiose, Gentiobiose, Maltose, Maltotriose, D-Melezitose, D-Melibiose, Palatinose, Stachyose, Sucrose, D-Trehalose, N-Acetyl-D –Glucosamine, D-Glucosamine, α -D-Glucose, D-Galactose, D-Psicose, L-Rhamnose, L-Sorbose, a-Methyl-D-Glucoside, ß-Methyl-D-Glucoside, Amygdalin, Arbutin, Salicin, D-Mannitol, D-Sorbitol, Adonitol, D-Arabitol, Xylitol, i-Erythritol, Glycerol, Tween 80, L-Arabinose, D-Arabinose, D-Ribose, D-Xylose, N-Acetyl-L-Glutamic Acid plus D-Xylose, Quinic Acid plus D-Xylose, D-Glucuronic Acid plus D-Xylose, Dextrin plus D-Xylose, D-Melibiose plus D-Xylose, D-Galactose plus D-Xylose, m-Inositol plus D-Xylose, 1,2- Propanediol plus D-Xylose, Acetoin plus D-Xylose.
Cryptococcus terreus A	Dextrin,D-Cellobiose, D-Sorbitol,D-Arabitol,D-Gluconic Acid, Maltotriose, D-Xylose
Rhodotorula pustula	L-Glutamic Acid, L-Proline, Gentiobiose, α-D-Mannitol, D-Sorbitol, Tween 80

Table.2. Assimilated carbon source by yeast species

Hexavalent chromium tolerant yeast from1000-2500ppm.

Fungi species Biolog identification result

Biolog micro-station consists of automated and semi automated identification system as a result of yeast cellular respiration for a metabolic fingerprint.YT-micro-plat consists of different carbon source and Redox tetrazolium dye in 96 wells. As the result of color change of tetrazolium into purple formazon due to respiration and turbidity. A similarity index was calculated based on the reaction profiles of dehydrated carbon sources. At 24 h an acceptable species identification must have similarity index value 0.75 or above, and subsequent reading 48 to 72 h having similarity index value of 0.5 or above is needed. By comparing with the yeast database (Micro Log TM System Release 4.2 User Guide 2001, Biolog), the result revealed that eleven yeast species were read by Micro-station. 6 yeast species have above 0.5

similarity index value and probability value above 75% for species identity and 3 yeast species do not have probability result.(Table.3)

Yeast species	Code	Probability	Similarity	Distance	Specific area identified	Sample Type
Yarrowia lipolytica	AA13	100%	0.7	4.46	Kera	Cabbage rhizospher soil
Cryptococcus luteolus	AA7	100%	0.64	4.16	Kera	Cauliflower rhizospher soil
RhodotorulaaurantiacaA	AA12	100%	0.62	5.80	Saris	Sald rhizospher soil
Ustilago maydis	AA21	100%	0.586	6.31	Saris	Sald rhizospher soil
RhodotorulaaurantiacaA	AA20	100%	0.510	4.37	Kera	Sald rhizospher soil
TrichosporonbeigeliiB	AMF1	100%	0.514	7.68	Riftvalley	Farm soil
RhodotorulaaurantiacaA	AMF2	100%	0.585	6.47	Rift valley	Farm soil
Cryptococcus terreus A	NSF1	100%	0.629	5.72	Rift valley	Forest soil
Zygosaccharomyces bailii	AA16	98%	0.650	5.12	Kera	Swiss chard rhizospher soil
Nadsonia fulvenscens	AA1	90%	0.627	4.61	Kera	Swiss chard farm
Schizoblastosporonstarkeyi-henricii	AA11	89%	0.56	7.83	Saris	Cabbage rhizospher soil
Endomycopsis vivi	AA2	84%	0.619	4.03	Picock	Polluted River
Rhodotorula pustula	HLW	-	0.591	4.88	Rift valley	Lake
Cryptococcus luteolus	AA3	-	0.716	4.27	Kera	Cabbage rhizospher soil
Cryptococcus curvatus A	22A	-	0.401	9.71	Saris	Cabbage rhizospher soil
Rhodotrula acheniorum	AA22	-	0.265	13.15	Kera	Sald rhizospher soil
Rhodosporidium sphaerocarpum	AA6	-	0.337	7.73	Kolfe	Carrot rhizospher soil

Table.3. Yeast species Biolog identification result

Discussion

Chromium pollution are the most serious environmental problems specially in farm land, groundwater, surface water and community health due to urbanization, industrialization and anthropogenic activities (Anirudhan and Sreekumari 2011;Netzahuatl Munoz et al., 2015). Exposure to this heavy metal cause serious human diseases such as respiratory

problems, kidney pathology, neurological disorders, cancers, anemia, skin lesions and respiratory problems (Mohammadi et al., 2020).Chromium generally considered as major pollutant due to its highly toxic, teratogenic, mutagenic and carcinogenic nature and the U.S. Environmental Protection Agency (USEPA) also categorized it into group “A” human carcinogen.(Elahi *et al*.,2020). This heavy metals are generally toxic, even at very trace amount and non-biodegradable and often withstand to physicochemical treatment (Bai&Abraham, 2003). Conventional treatment methods are not effective due to consuming large amount of chemical, uneconomical, release secondary degraded metabolite and energy demanding (Elahi *et al*.,2020). Mycoremediation is an alternative strategy for chromium sequestration and removal as well as ecofriendly. Fungi are capable enough in tolerating and detoxifying heavy metals by various mechanisms and enzyme versatility are known as potential biosorbents for heavy metal (Igiri et al., 2018).

In the present study fungi were screened from soil and effluent waste from Addis Ababa urban farming area and rift valley site where high industry activities and heavy metal contamination seen. The result revealed that a total of 552 fungal isolate were screened on PDA growth media. The percentage frequency of fungi on PDA media accounted 181 (34%) was the yeast isolates and 352 (66%) filamentous fungi from these contaminated soil and effluent waste. For identification of the yeast species Biolog micro station machine were used. The YT MicroPlate™ test panel provide a standardized method using 94 biochemical tests having different tagged carbon source to identify and characterize a wide range of yeasts. The result revealed that 11 yeast species were identified with full Identification result from urban farming area and effluent waste from Addis Ababa & Rift valley with full species identification having ≥ 75% probability and ≥ 0.5 similarity Index, these are, *Yarrowia lipolytica* (100%,0.7), *Cryptococcus luteolus* (100%,0.64), *Rhodotorula aurantiaca* A *(*100%,0.62), *Ustilago maydis* (100%,0.586), *Zygosaccharomyces bailii* (*98%,0.65), Schizoblastosporon starkeyi-henricii (89%,0.56), Nadsonia fulvenscens* (90%,0.63), *Endomycopsis vivi* (84%,0.61), *Trichosporon beigelii* B(100%,0.514), *Cryptococcus terreus A* (100%, 0.63), *Rhodotorula pustula* (0.591)(Table.3).

Yeast species are ubiquitous in divers habitats throughout all biomes and do have complex role in the ecosystems. Gizaw et al.,(2016) reported that Yeast identified from coffee waste are *Hanseiaspora valbyensis, Hyphopichia burtonii A Rhodotorula hylophila, Rhodotrula aurantiaca A and Pichia amenthionina var. menthionina.* The genera *Barnettozyma, Candida, Hanseniapora, Lachancea, Lodderomyces, Metschnikowia, Pichia, Rhodotorula, Saccharomyces, Saturnispora, Trichosporon, Wickerhamomyces* and *Yarrowia* Strain were isolated from the fruit (Barriga et al.,2011).

Many taxa exist in soil also live above the soil surface (e.g., in decaying fruits, flower, on leaves, and in association with trees and are incorporated into soil by a variety of mechanisms. *Schizoblastosporion, Schwanniomyces spp. Cryptococcus, Lipomyces, and Rhodotorula spp*. survive in soil. Phylloplane region consists of yeast species includes, *Bullera, Bensingtonia, Cystofilobasidium, Cryptococcus, Leucosporidium, Pseudozyma, Rhodotorula, and Sporobolomyce*s (Maksimova and Chernov., 2004). Rhodotorula species isolated from deep igneous rock aquifers, 200–400 m below the surface in the Baltic Sea and also from deep ice cores of Greenland glaciers (Ekendahl et al. 2003). Some species, including *Debaryomyces* (*Schwanniomyces*) *occidentalis*, *Lipomyces* spp., *Schizoblastosporion starkeyihenricii*, *Cryptococcus* and certain *Rhodotorula* and *Sporobolomyces* species, are isolated exclusively from soils (Hagler & Ahearn 1987). Slavikova and Vadkertiova (2000) showed that *Cryptococcus laurentii, Cystofilobasidium capitatum, Leucosporidium scottii, Rhodotorula aurantiaca*, and *Trichosporon cutaneum* were the most frequently isolated species in forest soil.

When this study compared with the other researcher work, Mafakher et al.(2010) reported *Yarrowia lipolytica* species isolated from agro-industrial waste water. Erer,et al.,(2000) reported *Trichosporon beigelii* is a ubiquitous yeast inhabiting the soil which is similar to this research finding. Aljohani et al.,(2018) reported yeast identified from agriculture soil in cameroon which is different to this research work, these are *Cryptococcus laurentii, Candida boidinii, Candida pseudolambica, Candida tropicalis, Cyberlindnera saturnus, Cyberlindnera subsufciens, Debaryomyces*

nepalensis, Saccharomyces cerevisiae, Torulaspora delbrueckii, and Torulaspora globosa. Corresponding with this research Boguslawska, &Dabrowski (2001) also reported that some species from *Rhodotorula, Candida, Trichosporon and Pichia* genera survive in polluted waters where they are degrading various pollutants including aromatic compound and heavy metals, they are good weapon for pollution. Dynowska (1997) reported that *Trichosporon sp., Rhodotorula sp., Candida sp. and Cryptococcus sp* can be used as indicator fungi for water pollution. The genus Cryptococcus and Trichosporon have also been reported as capable of biotransformation of a wide range of substrates as well as xenobiotics substances (Johnson, 2013) .

In this study fungi were evaluated for Cr(VI) tolerant ability at different concentration, 500ppm,1000ppm,2000ppm and 2500ppm where eleven yeast were obtained maximum tolerant from 1000- 2500ppm Cr(VI). *Yarrowia lipolytica* and *Nadsonia fulvenscens* show the highest tolerance at 2500PPm whereas three unidentified yeast tolerant at 500ppm (Fig. 1 &Table,3). When this research is compared with the work of other scholars reported by Liaquat et al.,(2020) showed the highest Cr tolerance was seen in *Komagataella phaii* resistance at 4000 ppm. Elabed et al.,(2020) also reported *Hansenula fabianii* strain able to survival on Cr (VI) toxicity up to 1400 (mg/L). Ono and Weng (1982) many strains of *Saccharomyces cerevisiae* do not grow on YEPD agar containing 750 µgm $^{-1}$ Cr_2O_3 but their mutants could grow up to 1000 µgmL^{-1} Cr_2O_3.

Kamarudheen et al.,(2020) investigation showed that Ustilage spp was found to act on the Cr (VI) contaminated soil and observed there is a remarkable reduction of Cr (VI) amount. Serrano-Gómez,& Olguín (2015) reported the adsorption potential of *Ustilago maydis* for Cr (VI). Corresponding with our study *Ustilago maydis* identified from contaminated urban farming area and tolerant up to 2000ppm Cr(VI). The yeast isolates of this research compared with the chromium tolerance ability with filamentous fungi, the work of Fukuda et al., (2008); Sen, (2012) indicated that *Aspergillus lentulus, Penicillium sp.*, and *Fusarium solani* isolated from contaminated sites have been reported to tolerate 1000 ppm Cr(VI)

whereas, *A. terreus* can tolerate Cr(VI) upto 1200 mg L-1 (Mishra. and Malik, 2014). Our research result showed that yeast isolates have good tolerance and growth performance against Cr(VI), this is may be due to sequestration capacity and fast growth ability and also have great industrial and biotechnological application for mycoremediation role.

Conclusion

Eleven Yeast species isolated from effluent waste, urban farming and rift valley area soil important for bioremediation service. High Cr(VI) tolerance growth ability of tested yeast strains will be used in the development of chromium-bioremediation technologies providing a potential alternative to physicochemical methods for chromium decontamination. The identified yeast species also have great industrial and biotechnological role. They are important for bioinnoculant formulation in chrome waste treatment after further HPLC analysis and molecular characterization. Understanding what fungal community exists in wastewater also helps to design mycoremediation technology.

Declarations

Conflict of Interests

The authors have not declared any conflict of interests.

Acknowledgment

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References

1. Anirudhan TS, Sreekumari SS (2011) Adsorptive removal of heavy metal ions from industrial effluents using activated carbon derived from waste coconut buttons. J Environ Sci 23(12):1989–1998
2. Aljohani R, Samarasinghe H, Ashu T, Xu J (2018) Diversity and relationships among strains of culturable yeasts in agricultural soils in Cameroon. Sci Rep 8(1):1–11
3. Amare A (2019) Corporate environmental responsibility in Ethiopia: a case study of the Akaki River Basin. Ecosyst Health Sustain 5(1):57–66
4. Aregawi T (2014) Peculiar Health Problems Due to Industrial Wastes in Addis Ababa City: The Case of Akaki Kality Industrial Zone. Unpublished master's thesis, Addis Ababa University, Addis Ababa Ethiopia
5. Aschale M, Sileshi Y, Kelly-Quinn M (2019) Health risk assessment of potentially toxic elements via consumption of vegetables irrigated with polluted river water in Addis Ababa, Ethiopia. Environ Syst Res 8(1):1–13
6. Bai RS, Abraham TE (2003) Studies on chromium (VI) adsorption–desorption using immobilized fungal biomass. Bioresour Technol 87(1):17–26
7. Bankar AV, Kumar AR, Zinjarde SS (2009) Environmental and industrial applications of *Yarrowia lipolytica*. Appl Microbiol Biotechnol 84(5):847–865
8. Barriga EJC, Libkind D, Briones AI, Iranzo J, Portero P, Roberts I, Rosa CA(2011) Yeasts biodiversity and its significance: case studies in natural and human-related environments, ex situ preservation, applications and challenges.Changing Diversity in Changing Environment,55–86
9. Biolog.1993. YT Microplate: Instruction for use.Biolog, Inc. https://www.biolog.com
10. Bogusławska-Wąs E, Dąbrowski W (2001) The seasonal variability of yeasts and yeast-like organisms in water and bottom sediment of the Szczecin Lagoon. Int J Hyg Environ Health 203(5–6):451–458
11. Chatterjee S (2015) Chromium toxicity and its health hazards. Int J Adv Res 3(7):167–172
12. Central statistical agency (2017) Agricultural sample survey 2016/2017 (2009 E.C).volume I report on area and production of major crops (private peasant holdings, Meher season, Addis Ababa
13. Dhal B, Thatoi HN, Das NN, Pandey BD (2013) Chemical and microbial remediation of hexavalent chromium from contaminated soil and mining/metallurgical solid waste: a review. J Hazard Mater 250:272–291
14. Dynowska M1997.Yeast-like fungi possessing bio-indicator properties isolated from the Łyna river.Acta Mycologica, 32(2),279–286
15. Elahi A, Rehman A (2018) Oxidative stress, chromium-resistance and uptake by fungi: isolated from industrial wastewater. Brazilian Archives of Biology and Technology, p 60
16. Elabed A, Ezziat L, Ibnsouda S, Elabed S, Erable B (2020) Bioelectrochemical Characterization of Heavy Metals Resistant yeast: *Hansenula fabianii* Isolated from Tannery Wastewater. Int J Electrochem Sci 16:140128
17. Erer B, Galimberti M, Lucarelli G, Giardini C, Polchi P, Baronciani D, Izzi G (2000) *Trichosporon beigelii*: a life-threatening pathogen in immune-compromised hosts. Bone Marrow Transplant 25(7):745–749
18. Ewers U (1991) Standards, Guidelines and Legislative Regulations Concerning Metals and their Compounds. In: Maria E (ed) Metal and their Compounds in the Environment: Occurrence, Analysis and Biological Relevance. VHC, Wenheim, pp 458–468

19. Elahi A, Arooj I, Bukhari DA, Rehman A (2020) Successive use of microorganisms to remove chromium from wastewater. Appl Microbiol Biotechnol 104(9):3729–3743
20. Ekendahl S, O'neill AH, Thomsson E, Pedersen K (2003) Characterisation of yeasts isolated from deep igneous rock aquifers of the Fennoscandian Shield. Microb Ecol 46(4):416–428
21. Fukuda T, Ishino Y, Ogawa A, Tsutsumi K, Morita H (2008) Cr(VI) reduction from contaminated soils by Aspergillus sp. N2 and Penicillium sp. N3 isolatedfrom chromium deposits. J Gen Appl Microbiol 54:295–303
22. Garg SK, Tripathi M, Srinath T (2012) Strategies for chromium bioremediation of tannery effluent. Reviews of Environmental Contamination and Toxicology Volume 217:75–140
23. Garavaglia E, Audrey S, Annalisa I, Stefano F, Iacopo T, Laura C, Massimo C (2015) Adenomyosis and its impact on women fertility. Iran J reproductive Med 13(6):327
24. Gemeda FT, Guta DD, Wakjira FS, Gebresenbet G(2020) Physicochemical characterization of effluents from industries in Sabata town of Ethiopia.Heliyon, 6(8), e04624
25. Gizaw B, Tsegaye Z, Tefera G (2016) Isolation, identification and characterization of yeast species from coffee waste collected from Sidama and Gedio zone. J Yeast Fungal Res 7(6):47–53
26. Gonzalez-Lopez CI, Szabo R, Roland SB, Gaillardin C (2002) Genetic control of extracellular protease synthesis in the yeast *Yarrowia lipolytica*. Genetics 160:417–427
27. Hagler AN, Ahearn DG (1987) Ecology of aquatic yeasts. In: Harrison JS (ed) The yeasts. AH Rose, 2nd edn. Academic Press, London, UK, pp 181–205
28. Ito H, Inouche M, Tohoyama H, Joho M (2007) Characteristics of copper tolerance in *Yarrowia lipolytica*. Biometals 20:773–780
29. Kamarudheen N, Chacko SP, George CA, Somachandran C, Rao KB (2020) An ex-situ and in vitro approach towards the bioremediation of carcinogenic hexavalent chromium. Prep Biochem Biotechnol 50(8):842–848
30. Johnson EA (2013) Biotechnology of Non-Saccharomyces Yeasts. The Basidiomycetes. Appl Microbiol Biotechnol 97:7563–7577
31. Igiri BE, Okoduwa SI, Idoko GO, Akabuogu EP, Adeyi AO, Ejiogu IK(2018) Toxicity and bioremediation of heavy metals contaminated ecosystem from tannery wastewater: a review. Journal of toxicology, 2018
32. Kabata-Pendias A, Pendias H(1992) Trace elements in soils and plants–CRC Press. Boca Raton, FL, 356
33. Liaquat F, Munis MFH, Haroon U, Arif S, Saqib S, Zaman W, Liu Q (2020) Evaluation of Metal Tolerance of Fungal Strains Isolated from Contaminated Mining Soil of Nanjing, China. Biology 9(12):469
34. Netzahuatl-Muñoz AR, Cristiani-Urbina MDC, Cristiani-Urbina E(2015) Chromium biosorption from Cr (VI) aqueous solutions by *Cupressus lusitanica* bark: Kinetics, equilibrium and thermodynamic studies.PLoS One, 10(9), e0137086
35. Maksimova IA, Chernov IY (2004) Community structure of yeast fungi in forest biogeocenoses. Microbiology 73(4):474–481
36. Mafakher L, Mirbagheri M, Darvishi F, Nahvi I, Zarkesh-Esfahani H, Emtiazi G (2010) Isolation of lipase and citric acid producing yeasts from agro-industrial wastewater. New Biotechnol 27(4):337–340
37. Mishra A, Malik A (2014) Novel fungal consortium for bioremediation of metals and dyes from mixed waste stream. Bioresour Technol 171:217–226
38. Mohamadi M, Goodarzi A, Aryannejad A, Fattahi N, Alizadeh-Khoei M, Miri S, Bodaghabadi M (2020) Geriatric challenges in the new coronavirus disease-19 (COVID-19) pandemic. A systematic review. Med J Islamic Repub Iran 34:123
39. Ono BI, Weng M (1982) Chromium resistant mutants of the yeast *Saccharomyces cerevisiae*. Curr Genet 6:7l–77

40. Pepi M, Baldi F. Modulation of chromium (VI) toxicity by organic and inorganic sulfur species in yeast from industrial wastes.BioMetals5,179–185
41. Weldegebriel Y, Chandravanshi BS, Wondimu T (2012) Concentration levels of metals in vegetables grown in soils irrigated with river water in Addis Ababa, Ethiopia. Ecotoxicol Environ Saf 77:57–63
42. Lian G, Wang B, Lee X, Li L, Liu T, Lyu W (2019) Enhanced removal of hexavalent chromium by engineered biochar composite fabricated from phosphogypsum and distillers' grains. Sci Total Environ 697:134119
43. Nakkeeran E, Patra C, Shahnaz T, Rangabhashiyam S, Selvaraju N (2018) Continuous biosorption assessment for the removal of hexavalent chromium from aqueous solutions using *Strychnos nux vomica* fruit shell. Bioresour Technol Rep 3:256–260
44. Slavikova E, Vadkertiova R (2000) The occurrence of yeasts in the forest soils. J Basic Microbiol 40:207–212
45. Serrano-Gómez J, Olguín MT (2015) Separation of Cr (VI) from aqueous solutions by adsorption on the microfungus *Ustilago maydis*. Int J Environ Sci Technol 12(8):2559–2566
46. Sen M (2012) A comparative study on biosorption of Cr(VI) by *Fusarium solani* under different growth conditions. J Appl Sci 2:146–152
47. Solieri L, Landi S, De Vero L, Giudici P (2006) Molecular assessment of indigenous yeast population from traditional balsamic vinegar. J Appl Microbiol 101(1):63–71
48. Teoh AL, Heard G, Cox J (2004) Yeast ecology of Kombucha fermentation. Int J Food Microbiol 95(2):119–126
49. Watanabe T(2010) **Pictorial Atlas of Soil and Seed Fungi**. **Morphologies of Cultured Fungi and Key to Species**, **Third Edition**.**pp**.**1-426**

Figures

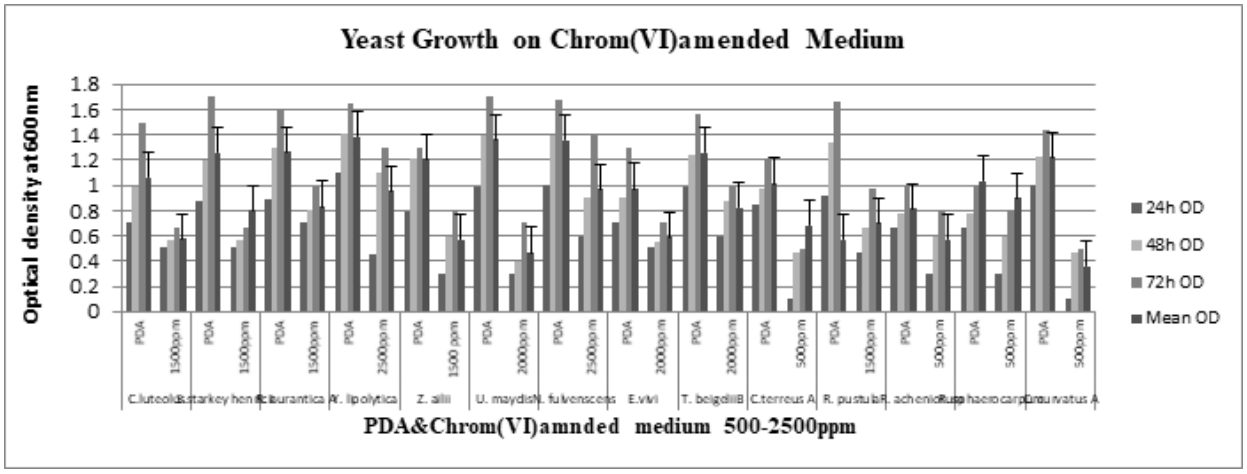

Figure 1

Yeast optical densities M±SE (OD600nm)

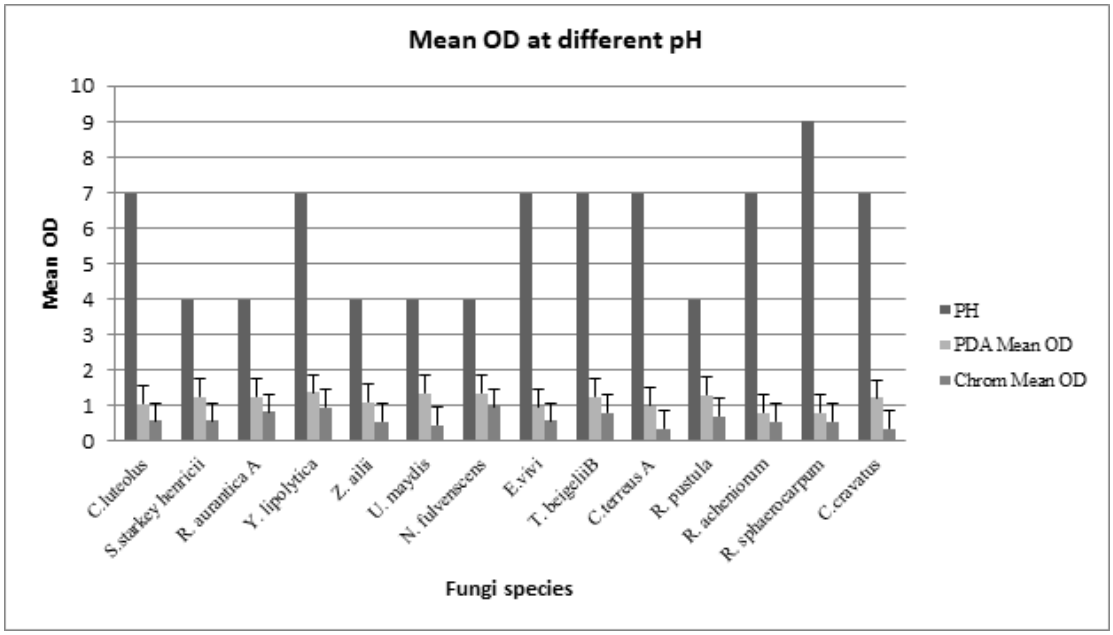

Figure 2

Optical density at different pH

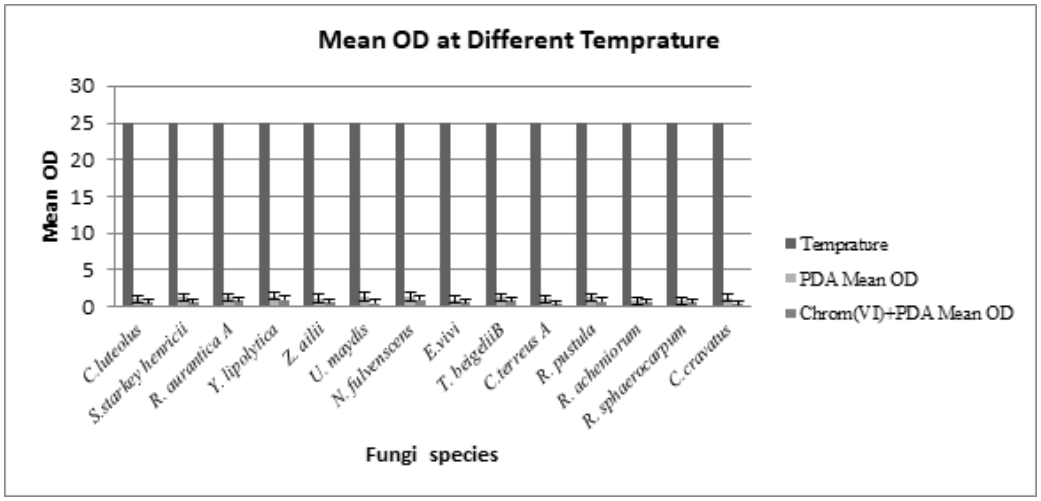

Figure 3

Optical density at different Temperature

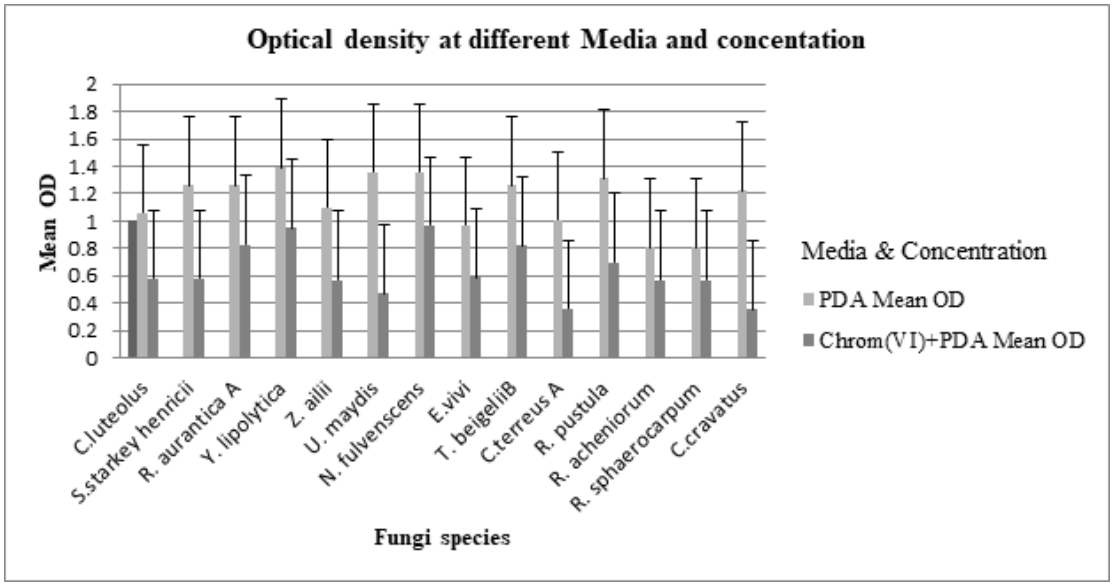

Figure 4

Optical density at different Media &Concentration

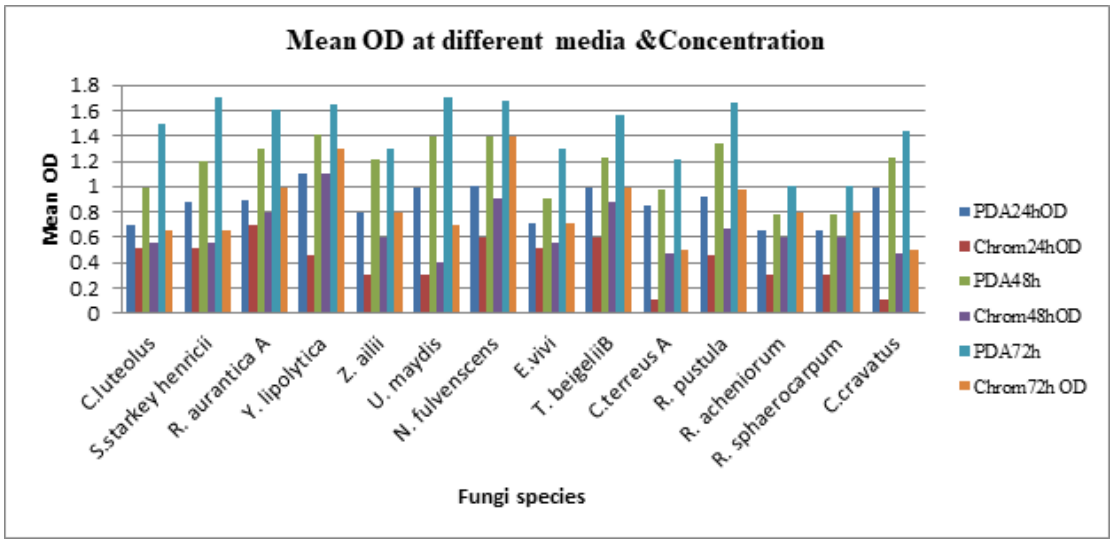

Figure 5

Optical density at different Media &Concentration

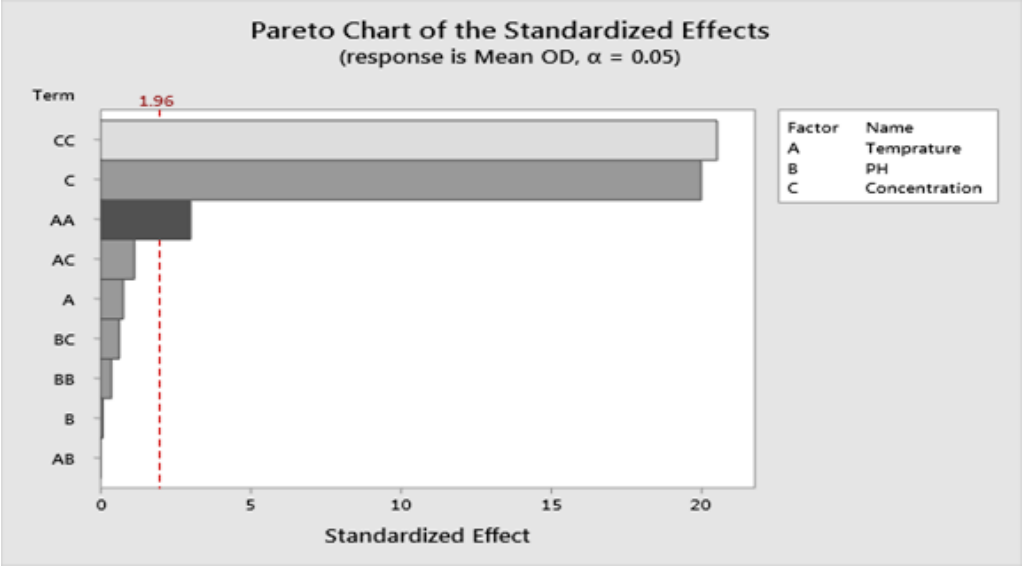

Figure 6

Interaction of factors

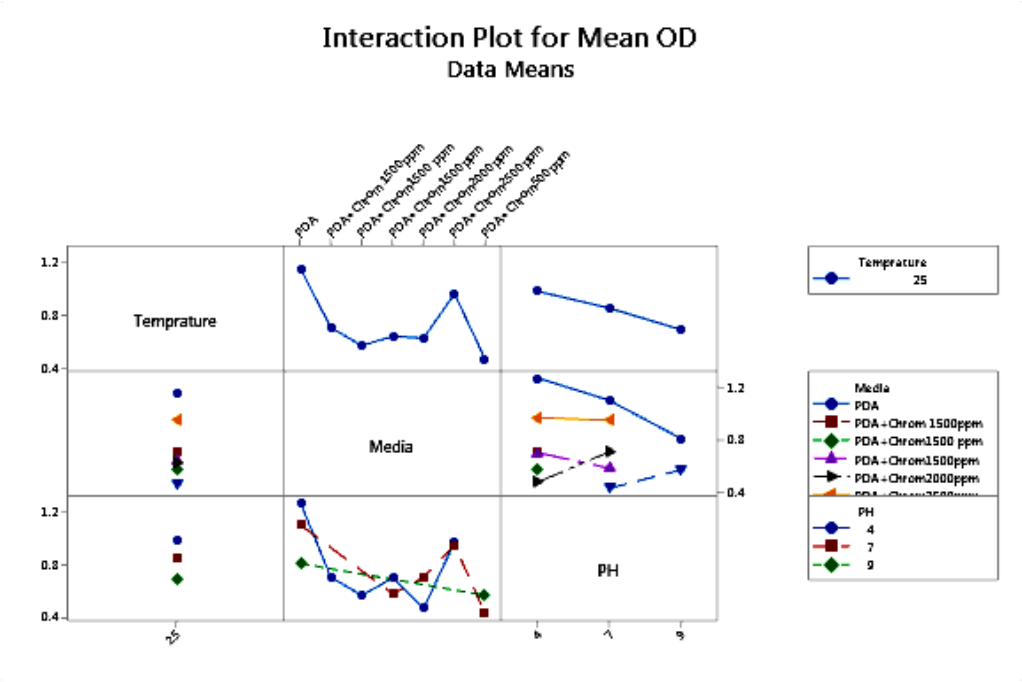

Figure 7

Interaction effects of treatment factors

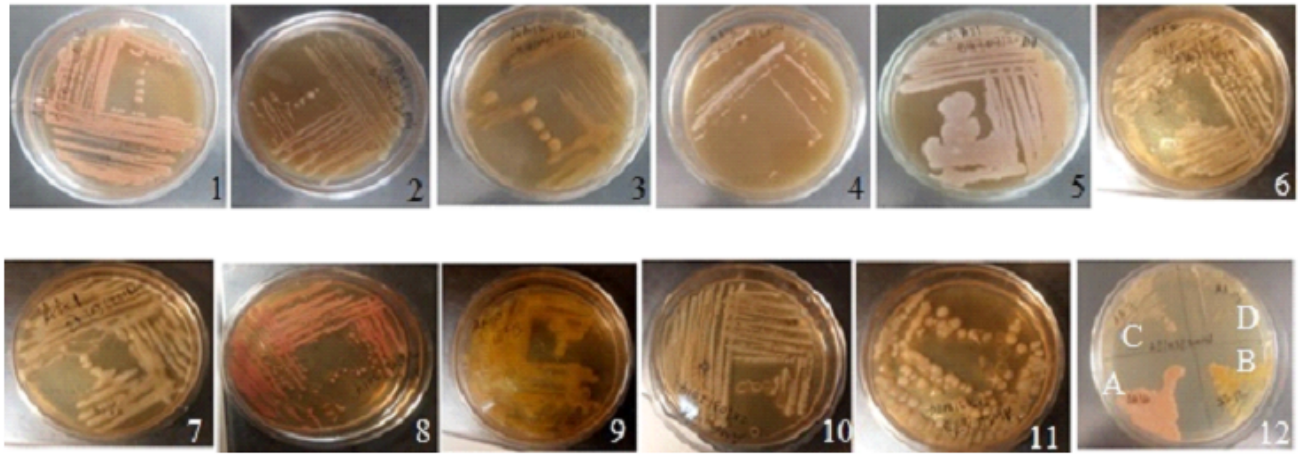

Figure 8

Zygosaccharomyces bailii, 2. Ustilago maydis,3. Rhodotorula aurantiacaA,4. Cryptococcus luteolus.,5., Schizoblastosporonstarkeyi-henricii,6, Cryptococcus terreus A 7. Nadsonia fulvenscens.,8 Endomycopsis vivi.,9, Trichosporon beigelii B 10, Rhodotorula aurantiaca A 11, Rhodotorula pustula, 12 C, Cryptococcus luteolus, 12D,Yarrowia lipolytica.