Int.J.Curr.Microbiol.App.Sci (2023) *12*(07): 65-69

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 12 Number 7 (2023)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2023.1207.007

Qualitative Analysis of Candida Species in a Sample of Spoilt Orange

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Keywords

Candida albicans, Orange, citrus fruit, microbiological tests, Pseudomonas

Article Info

Received:
08 June 2023
Accepted:
04 July 2023
Available Online:
10 July 2023

A B S T R A C T

Oranges are a nutritious and delicious source of dozens of nutrients, namely Vitamin C, fiber, Potassium and antioxidants amongst several others. However, much like most other fruits, after 2-3 weeks of plucking, oranges start going bad. They start by going soft and soon after, discoloration starts taking place. Candida is a highly infectious genus of yeasts, known particularly for causing Candidiasis, amongst other skin fungal infections in humans. They are gram positive, ovoid shaped budding yeast fungi. For the purpose of this project, such an orange was taken and a suspension was made of the spoilt fruit with distilled water. This suspension was serial diluted and the subsequently formed solutions were spread on several agar bases, the colonies formed isolated and gram stained to identify the exact species of bacteria, fungi or yeast present in the sample. Further the isolates showing traits characteristic of Candida, a highly infectious type of fungus, were subjected to further biochemical tests to determine the exact subspecies of Candida present in the sample. Today, with the youth being highly immunocompromised, such fungi pose extreme risk if consumed. Such a study could promote more thorough hygiene practices and spread awareness of the harmful microbial particles we consume, if we consume spoiled or dirty fruits.

Introduction

Several strains of Candida including *Candida albicans* are commonly present on the human body at all times, however on overgrowth of this yeast, it causes skin diseases such as Candidiasis. Candida species, however, also grow on several other surfaces such as on spoilt fruits, vegetables amongst others. Orange, a citrus fruit with an average pH of 2.5 to 3.5, is highly acidic which reduces results acquired through microbiological tests. However, to show the presence of rare strains of Candida fungus such as *C. rugosa*, the test was carried out on a sample of spoiled orange obtained from a local fruit seller. The orange used, collected from Dadar Fruit Market, had turned soft and had developed brown patches. Analysis of the fruit showed presence of not only several strains of Candida but also Bacilli and, possibly, Pseudomonas. The Study was conducted between April 24th and May 5th in the R & D Department, Himedia Laboratories Pvt. Ltd, Thane, as a private study as a part of a high school project.

Materials and Methods

Preparation of Required Media for Preliminary Testing

The different bacteria, fungi and yeasts present in the sample must be differentiated for further study. Since it is known that the common pathogens in spoiled fruits include Bacilli, Candida amongst others, appropriate agar bases can be prepared:

Lactobacillus Mrs Agar (M641-Himedia Labs) 67.15 G / 1000ml; Ph- 6.2

Plate Count Agar (M091-Himedia Labs) 23.15 g / 1000mL; pH- 7.2

Sabouraud Dextrose Agar (M063-Himedia Labs) 65g / 1000mL; pH- 6.8

Appropriate quantities of the required agar bases are dissolved in 400mL of distilled water. After they dissolve in the water, their pH values are adjusted to the specific optimum functioning value of the medium.

The Media are then sterilized in an autoclave machine at 121° C, 15-30 PSI, for 1-1.5 hours.

After sterilization, 20mL of each medium is poured into 20 Petri Dishes and left to solidify and dry for 20-25 minutes in a biosafe laminar flow machine.

Formation of Suspension, Serial Dilution, Spreading and Incubation

Formation of Suspension

To conduct the required tests, a suspension of the spoiled fruit in distilled water must be made. To do this, a 10g sample of the spoiled fruit is taken with a sterilized spatula and added to a conical flask containing 90 mL of distilled water. This mixture is then placed on a vortex mixer for 2-3 minutes till the solution becomes turbid.

Serial dilution

Serial dilution of the suspension is done to see the number of colonies formed in different dilutions of the suspension. To do this, 10 test tubes are taken and filled with 9mL of 0.85% Saline Solution. To the first test tube, 1mL of the Suspension is added and the test tube is labeled 10^{-1}. To the next test tube, 1mL of solution from 10^{-1}, is added and this test tube is labeled 10^{-2}. This is repeated for all 10 test tubes and the last test tube is labeled 10^{-10}.

Spreading

100µL from each test tube is added to 2 plates (duplicate) from each type of agar and spread till it becomes a very fine layer. A different spreader is used for each dilution of the suspension to prevent cross-contamination.

Incubation

All MRS Agar Plates are incubated in a Carbon-Dioxide Incubator at 37°.

All Plate Count Agar Plates are incubated at a temperature of 37° under normal pressure.

All Sabouraud Dextrose Agar Plates are incubated in a Biological oxygen demand (BOD) Incubator at 22.5°.

Analysis of preliminary testing data

After Incubation of the different media, the number and types of colonies on the different plates is noted down.

Each different type of colony is then isolated on soy-based agar plates by streaking a sample from each colony onto the plate and incubating at required conditions for 24 hours.

A sample from each type of colony is also Gram-Stained and viewed under a 100x microscope and the results are noted down.

Differential Tests for Candida and Bacilli

Preparation of media bases

Different Bases are prepared to differentiate the types of bacteria in the isolates taken. Since it has been determined that the main types of species are Candida and Bacilli, the media prepared are:

Candida Differential Agar (Hichrome M1297a-Himedia Labs) 42.72g / 1000 Ml; Ph- 6.5

Bacillus Agar Base (Hichrome M1651 - Himedia Labs) 49.22g / 1000ml; Ph- 7.3

Appropriate quantities of the required agar bases are dissolved in 400mL of distilled water. After they dissolve in the water, their pH values are adjusted to the specific optimum functioning value of the medium. The Bacillus Agar Base is then sterilized by placing it in an autoclave machine at 121℃, 15-30 PSI for 1-1.5 Hours. The Candida Differential Agar is sterilized by boiling. After sterilization, 20mL of each medium is poured into 20 petri dishes each and allowed to solidify and dry for 20-25 minutes each.

Streaking of isolates onto differential media

Each isolate is then streaked onto 2 plates of each type of agar using the 4 quadrant method with a sterile loop. The Candida Differential Agar is then incubated in a Biological Oxygen Demand Incubator at 22.5° while the Bacillus Agar Base is incubated at 37°.

Analysis of differential agar results

The technical data sheet of the agar is then used as a reference to infer the results of the differential agar. All isolates that show violet/purple to cream coloured colony growth on the Candida Differential Agar can be confirmed to show Candida. Those isolates which show green/blue, yellow, or white growth in the Bacillus Agar can be confirmed to contain types of bacillus. Swarm-like green growth on this agar is a possible indicator of *Pseudomonas* bacteria.

Biochemical tests for confirmation of *candida* strains

The following medium is prepared as a base for the biochemical tests:

Phenol Red Broth Base (M054-HiMedia Labs) 16.02g / 1000mL; pH- 7.6

After preparation of medium, the liquid is divided into as many test tubes as required and a 1% solution of the following sugars is made:

Cellobiose; Melibiose; Xylose; Dulcitol; Sucrose; Maltose; Lactose; Galactose; Trehalose; Inositol; Raffinose.

A sample from each confirmed candida isolate is added to each of the sugar solutions and the test tubes are allowed to incubate in a biological oxygen demand incubator for 48-72 hours. Post incubation, all sugar solutions that have turned yellow for each isolate are noted and the Candida strain can be determined.

Results and Discussion

Preliminary testing results

Due to acidity of suspension, microbial growth in diluted suspensions was inhibited. Non-diluted plates however showed the following growth:

All 9 types of Colonies were isolated and assigned serial numbers from 1-9 and isolates so obtained were gram-stained. Results were as follows:

ISO 1- Gram Positive Rods
ISO 2- Gram Positive Rods
ISO 3- Gram Positive Rods
ISO 4- Gram Positive Rods
ISO 5- Gram Negative Rods
ISO 6- Gram Positive Oval
ISO 7- Gram Positive Oval
ISO 8- Gram Positive Rods
ISO 9- Gram Positive Rods

Differential Testing Results

On observing the results, it could be seen that only 2 plates show growth of candida and both candida were of the same type. The results from the bacillus differential agar also confirmed the fact that Isolates 6 and 7 were not bacilli.

Candida Identification Via Biochemical Tests Results

After comparing the results of the biochemical tests with the technical data sheets of the test, it was ultimately determined that the species of candida present in the sample was *C. rugosa*.

Table.1

Suspension Plate Serial Number	No. of Colonies on MRS Agar	No. of Colonies on Plate Count Agar	No. of Colonies on Sabouraud Dextrose Agar
Plate Number 1	3	1	0
Plate Number 2	2	3	0

Table.2 Candida Differential Agar

Isolate Number	Description of Growth
1	No Change
2	No Change
3	No Change
4	No Change
5	No Change
6	Pinpoint Violet Colonies
7	Pinpoint Violet Colonies
8	No Change
9	No Change

Table.3 Bacillus Agar Base

Isolate Number	Description of Growth
1	Agar Yellow With Yellow Pinpoint Colonies
2	Agar Yellow With Yellow Pinpoint Colonies
3	Agar Yellow With Yellow Pinpoint Colonies
4	Agar Yellow With Greenish Yellow Mucoid Colonies
5	Agar Green With Swarm Like Growth
6	No Change
7	No Change
8	Agar Yellow With Yellow Mucoid Colonies
9	Agar Yellow With Yellow Mucoid Colonies

Table.4 Following were the results of the reaction of the two isolates with the 10 sugar solutions

Sugar in Solution	Result- ISO 6	Result- ISO 7
Cellobiose	No Change	No Change
Xylose	Solution Turned Yellow	Solution Turned Yellow
Sucrose	No Change	No Change
Maltose	No Change	No Change
Lactose	No Change	No Change
Galactose	Solution Turned Yellow	Solution Turned Yellow
Trehalose	No Change	No Change
Melibiose	No Change	No Change
Dulcitol	No Change	No Change
Inositol	No Change	No Change
Raffinose	No Change	No Change

References

Himedia Labs Lactobacillus MRS Agar Technical Data Sheet

Himedia Labs Plate Count Agar Technical Data Sheet

Himedia Labs Sabouraud Dextrose Agar Technical Data Sheet

Himedia Labs HiChrome Candida Differential Agar Technical Data Sheet

Himedia Labs HiChrome Bacillus Agar Technical Data Sheet

How to cite this article:

Kaiwaan Dalal. 2023. Qualitative Analysis of Candida Species in a Sample of Spoilt Orange. *Int.J.Curr.Microbiol.App.Sci.* 12(07): 65-69. **doi:** https://doi.org/10.20546/ijcmas.2023.1207.007