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Effect of Sodium bicarbonate on *Candida albicans*

Varsha Shegokar[1] and *Sneha Khadse[2]

[1]Kamla Nehru Mahavidyalaya, Nagpur, MS, India
[2]Project Assistant at CSIR –NEERI, Nagpur, MS, India
*Corresponding author Email id: khadsesneha43@gmail.com

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ABSTRACT

The purpose of this study was to evaluate the effect of 1% and 10% sodium bicarbonate on the *Candida albicans.* 5ml of solution from 1% (A) was taken and added to 100ml of Czapek Dox broth and the subcultures were made after every 24 hrs.5ml of solution from 10% (B) was taken and added to 100ml of Czapek Dox broth and subculture were made after every 24 hrs.No sodium bicarbonate was added to 100ml of Czapek Dox broth and subcultures were made after every 24 hrs. The Czapek Dox broth with inoculum (Control flask), the Czapek Dox broth with inoculum with 5ml of solution from 1% (A) and the Czapek Dox broth with inoculum with 5ml of solution from 10% (B) was incubated at 27-30^{0}C for 24hrs. After 24 hrs, 0.1ml broth from control flask was added in control plate and was spread over the PDA plate and kept for incubation for 24 hrs at 27-30^{0}C. Similarly, 0.1ml of inoculum broth from (A) and 0.1 ml of solution from (B) was added in their respective PDA plates and kept for incubation for 24 hrs at 27-30^{0}C. This method was repeated till 168 hrs. By performing the spread plate method, it was found that the growth of *candida albicans* was inhibited after 168 hrs (after 7 days)

Keywords: *Candida albicans*, Sodium bicarbonate, Saliva, PDA, *Candida hiveg*, Czapek Dox Broth.

INTRODUCTION

Candida albicans is diploid fungus that grows both as yeast and filamentous cell. *Candida albicans* have emerged as important cause of mortality in humans immune compromise patient. Candida albicans may form biofilm on the surface. *Candida albicans* lives in 80% of human population without causing harmful effect. *Candida albicans* is a constituent of normal flora comprising microorganisms that lives in human mouth and genital tract. Overlook of fungus results in candidiasis. A common form of candidiasis restricted to the mucosal membrane in mouth or vagina is thrush which is easily cured in people who are not immune compromise. *Candida albicans* is known to be able to escape the intestine and travel around the body in the bloodstream. Oregano oil and cinnamon can kill the yeast in the blood stream.

About 25 per cent of type-2 Diabetics was shown to improve when given cinnamon. This is because the fungus sits on the outer membranes of cells, preventing crucial receptor sites from working properly - in the case of diabetes, those receptor sites are for insulin. However, *candida* also produces a sugar-like waste product. People with higher blood sugar levels develop more cancer; and survive less. But there is a second potential problem. Yeasts like *candid* are anaerobes - this means they generate their energy in the absence of oxygen. Once in the blood stream they can colonise certain local areas of the body and greatly reduce the oxygen levels in that area. The result is that the local cells do not die, but switch their own energy production from an oxygen-based system to one that doesn´t use oxygen. This is the system employed by cancer cells, which also do not use oxygen to generate their energy from food molecules. In fact, Otto Warburg won a Nobel Prize in 1931 for telling the world this property of cancer cells and that oxygen was their enemy. Is it, then, any surprise then that women who had taken antibiotics more than 25 times during their lifetime had twice the risk of breast cancer, according to research covered in Cancer actives research service Cancer Watch? Their friendly bacteria were reduced allowing candida more chance to survive in the gut and move to the blood stream. Their immune systems were weak, indicated by the illnesses that required the antibiotics. And so on. Candida organisms are common saprophytes which reside on the skin and mucous membranes of the gastrointestinal tract in healthy persons, and in the vagina of pregnant and diabetic women. This fungus is ordinarily a weak pathogen which produces mild superficial infection, but occasionally it may cause a systemic disease with involvement of the lung, kidney, endocardium, brain, or reticuloendothelial system. In almost all instances of acute disseminated candidiasis the defence mechanisms of the host have been reduced by predisposing factors such as prior antibiotic therapy (Seelig,1966), systemic adrenocortical isteroids (Louria *et al*.,1962), indwelling intravascular catheters (Louria *et al*.,1962), diabetes mellitus, and other serious chronic diseases. In addition, various neoplastic diseases such as lymphoma and Hodgkin's disease (Casazza, and Carbone, 1966) favour the development of Candida infection and dissemination, but even in these diseases the development of systemic candidiasis was a rare event before modern therapy with bone marrow suppressants, antibiotics, steroids, and roentgen irradiation (Friedman, 1965).

MATERIAL AND METHOD

Media:-Potato Dextrose Agar (PDA)**,** Candida Hi-veg**,**Czapek Dox Broth
Reagents:-Lactophenol cotton blue

Method:-Collection of sample
Oral thrush (Saliva) of human being (Before brushing) was collected. This sample was used in Microbiology Laboratory for the isolation and identification of *candida albicans*.

Isolation of pure culture
The technique used for the isolation of *candida albicans* include:
1) Streak plate method:
Streak plate method is a rapid qualitative isolation method which is essentially a dilution technique that involves spreading a loopful of culture over the surface of an agar plate, dividing the plate into the 4 quadrants and streaking serially.

Procedure:
Place a loopful of sample over the surface of first quadrant of Candida Hi-veg plate Spread it rapidly several times across the surface. Reflame and cool the loop and then turn the plate at 90^0 then touch the loop to the culture of the first quadrant. Continue the same method till the fourth quadrant. The technique used for the inhibition of candida albicans include:

2) Spread plate Method:
Spread plate method requires a previously diluted mixture of micro-organisms that is to be used.
Procedure: During inoculation, the inoculums were spreaded over the complete surface of Potato Dextrose Agar media plate uniformly with a sterile L-shaped bent rod. This procedure was repeated daily for 24 hrs, 48 hrs, 72 hrs, 96 hrs, and so on till the growth of candida albicans is inhibited.

Identification of *candida albicans*.
For identification of candida albicans KOH (Pottassium hydroxide) test was used.
Method:-The isolated colonies are placed directly onto a microscope slide and are covered with 10% or 20% potassium hydroxide. The slide is left to stand until clear, normally between 5 and 15 mins, in order to dissolve the smear. To enhance clearing dimethyl sulphoxide can be added to the slide. To make the fungi easier to see lactophenol cotton blue. The slide is

gently heated to speed up the action of KOH. Place a slide under a microscope and observe.

Preparation of Czapek Dox broth:
In 100ml distilled water adds 3.5gm Czapek Dox broth, if necessary slightly heat to dissolve the medium and sterilize it in autoclave. Innoculate a loopful of culture in the broth. Add 1% sodium bicarbonate in one conical flask and 10% sodium bicarbonate in another conical flask and no sodium bicarbonate in the control flask.

Preparation of 1% sodium bicarbonate:-In 100ml distilled water add 1gm sodium bicarbonate.
Preparation of 10% sodium bicarbonate:-In 100ml distilled water add 10gm sodium bicarbonate.
Procedure:-5ml of solution from 1% (A) was taken and added to 100ml of Czapek Dox broth and the subcultures were made after every 24 hrs.5ml of solution from 10% (B) was taken and added to 100ml of Czapek Dox broth and subculture were made after every 24 hrs.No sodium bicarbonate was added to 100ml of Czapek Dox broth and subcultures were made after every 24 hrs. The Czapek Dox broth with inoculum (Control flask), the Czapek Dox broth with inoculum with 5ml of solution from 1% (A) and the Czapek Dox broth with inoculum with 5ml of solution from 10% (B) was incubated at 27-30^{0}C for 24hrs. After 24 hrs, 0.1ml broth from control flask was added in control plate and was spread over the PDA plate and kept for incubation for 24 hrs at 27-30^{0}C. Similarly, 0.1ml of inoculum broth from (A) and 0.1 ml of solution from (B) was added in their respective PDA plates and kept for incubation for 24 hrs at 27-30^{0}C. This method was repeated till 168 hrs.

RESULT AND DISCUSSION:

In the present study, oral thrush (Saliva) of human being (before brushing) was used as a sample.
1) Isolated colony obtained on potato dextrose agar media plate:

Characteristics:
White, round, soft, and colonies were observed.

2) KOH test was perfomed for the identification of *candida albicans*:
The KOH Test for *Candida albicans*, also known as a potassium hydroxide preparation or KOH prep, is a quick, inexpensive fungal test to differentiate dermatophytes and *Candida albicans* symptoms from other skin disorders like psoriasis and eczema.

Evaluation:
Dermatophytes are easily recognized under the microscope by their long branch-like tubular structures called hyphae. Fungi causing ringworm infections produce septate (segmented) hyphae. Some show the presence of spores formed directly from the hyphae (arthroconidia). Yeast cells appear round or oval and budding forms may be seen. A normal, or negative, KOH test shows no fungi (no dermatophytes or yeast). Dermatophytes or yeast seen on a KOH test indicate the person has a fungal infection.

Plate 1: Isolated *candida albican* Microscopic view of *Candida Albican*

Effect of sodium bicarbonate on *candida albicans:*

Flask 1: Control **A** **B**

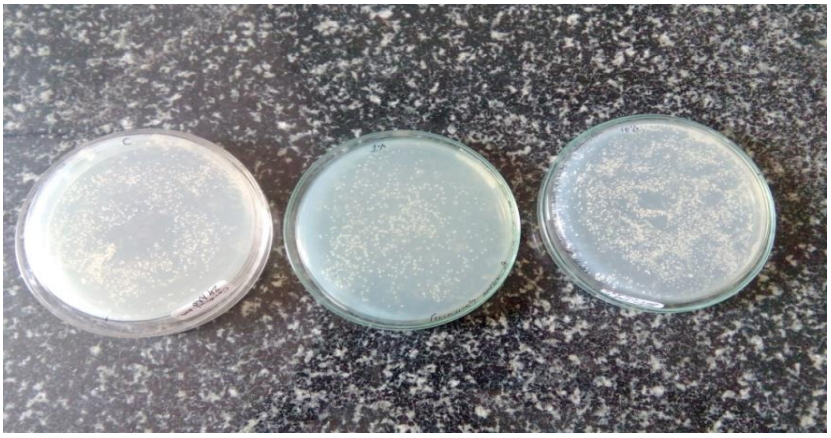


Plate 2: The growth of *candida albicans* was observed after 24 hours

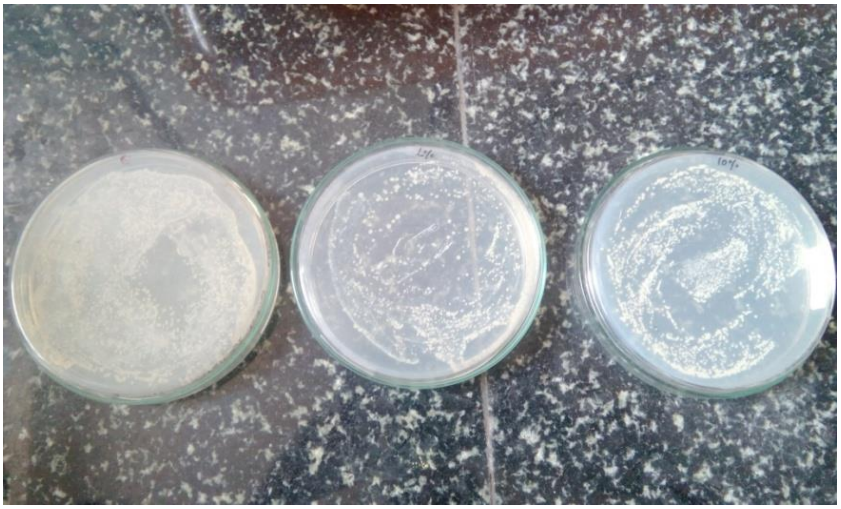


Plate 3: The growth of *candida albicans* was observed after 48 hrs

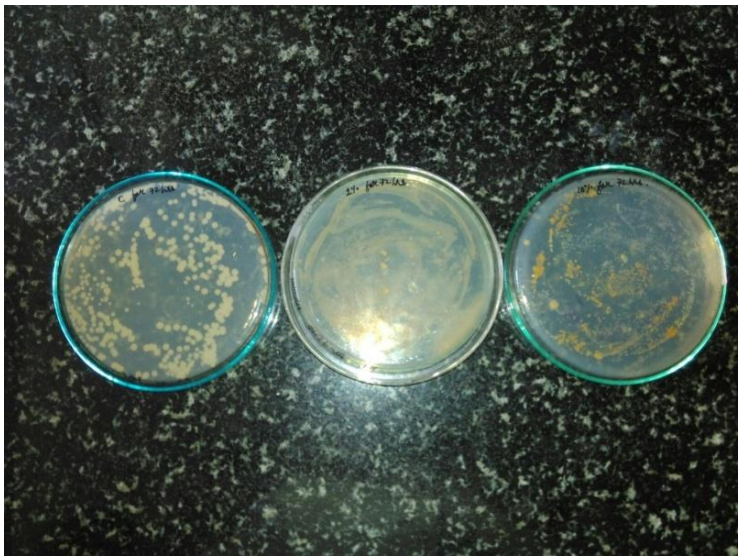


Plate 4: The growth of *candida albicans* was observed after 72 hrs.

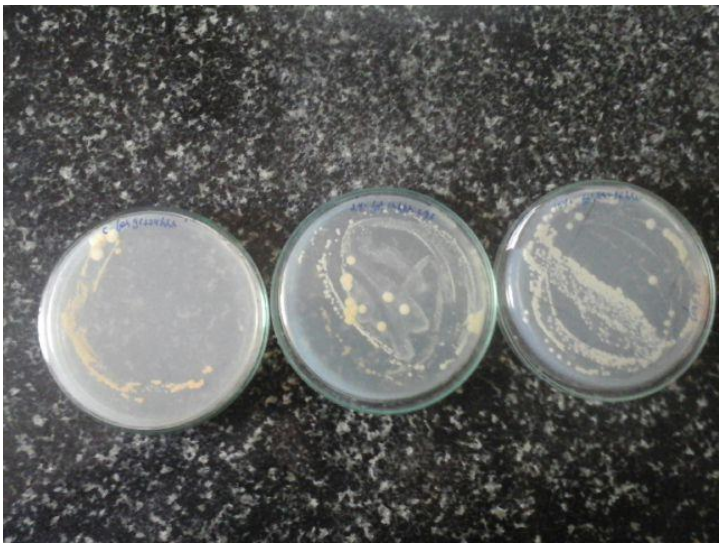


Plate 5: Less growth of *candida albicans* was observed after 120hrs as compared to previous plates

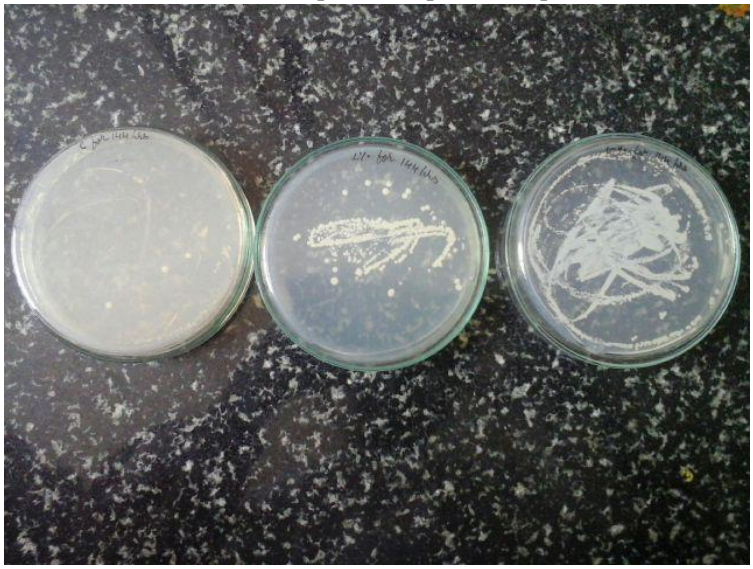


Plate 6: Less growth of *candida albicans* was observed after 144 hrs.

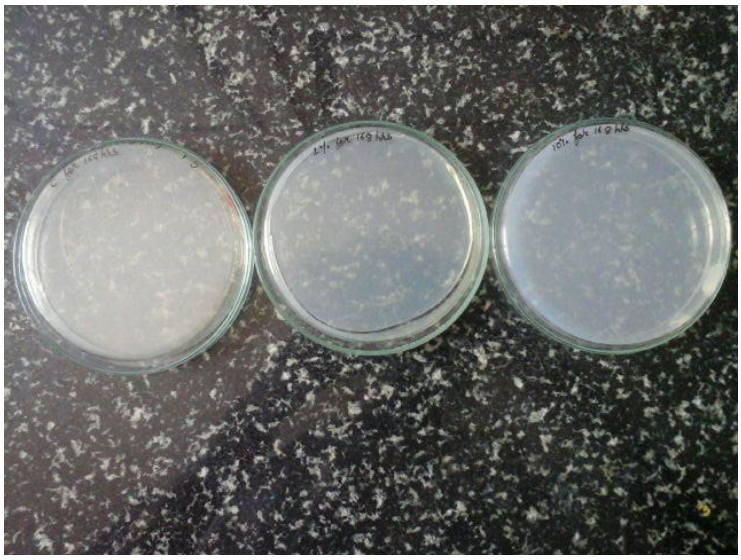
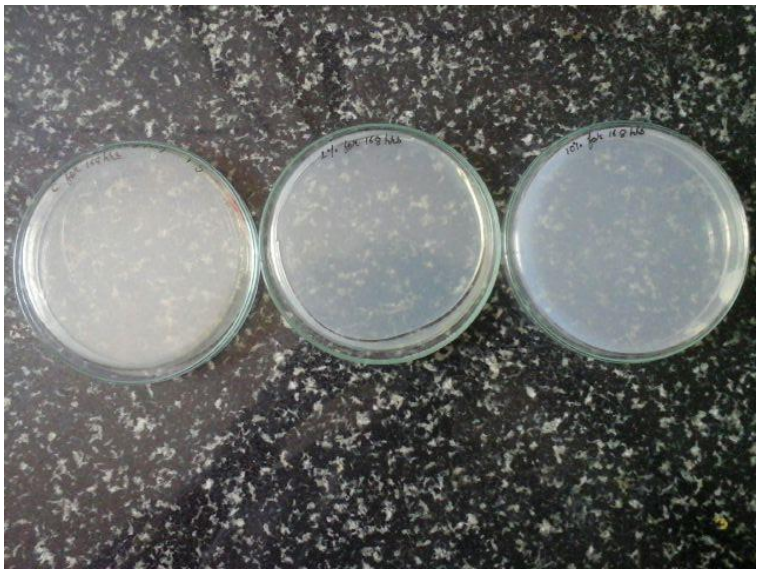


Plate 7: No growth of *candida albicans* was observed after 168hrs
That is the growth of *candida albicans* was inhibited after 168 hrs.

Effect of sodium bicarbonate on *candida albicans*:
By performing the spread plate method, it was found that the growth of *candida albicans* was inhibited after 168 hrs (after 7 days)



The growth of *candida albicans* was inhibited after 168 hrs

CONCLUSION

Oral thrush (Saliva) of human being (before brushing) was used as sample. The organism was isolated from the sample by using PDA and the identification test was performed for the *Candida albicans*. The isolated culture was used for further test of inhibition of *Candida albicans* using sodium bicarbonate From the observation it is concluded that, By using the concentration of 5ml of solution from 1% and 5ml of solution from 10%, the growth of *candida albicans* was observed till 148 hrs. But after the incubation period of 168hrs it was observed that the growth was inhibited by using the concentration of 5ml solution

from 1% and 5ml solution from 10%. By using the concentration of 5ml of solution from 1% and 5ml of solution from 10%,the growth of *candida albicans* was not observed after 168 hrs. It can be concluded from the result that if, the concentration of sodium bicarbonate is increased then the growth *of Candida albicans* may be inhibited in lesser hours.

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Conflict of Interest

The author declares that there is no conflict of interest.

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