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Research Article

STUDY OF GROWTH PATTERN OF ACTINOMYCETES SPECIES IN VARIOUS NUTRIENT MEDIUM

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ABSTRACT

Dental diseases are presently seemed to be very painful and critical to diagnose. The microbial flora of oral cavity is characteristic and changes in its composition may result in to development of dental diseases. An important microbial species found in oral cavity is Actinomycetes. Actinomycetes are not only responsible for causing actinomycosis but also responsible for tooth decaying and ultimately periodontal diseases. The first objective of this study is to study the growth pattern and habit of Actinomycetes species by using various nutrient medium. The present study revealed that the growth of Actinomycetes species shows declination at neutral pH (pH-7.0) and at both extreme pH ranges it showed increase in growth. Further by adding sucrose in the medium the growth was found to be more as compared to addition of sorbitol in the medium. This shows that pH of medium and presence of sugar is playing an important role in growth of this species.

Keywords: Oral microbial flora, Actinomycetes species, Nutrient media, pH dependant growth.

INTRODUCTION

The Teeth are the accessory structures of the digestive system located in sockets of alveolar process. The teeth surfaces provide shelter to lots of microorganisms which are harmful and which causes many of the oral problems like Dental Caries, Periodontal Diseases. Getting rid of all these organisms is a part of maintenance of Oral Hygiene.

The different Microorganism which are responsible for the Oral Problems

1. Dental Caries/ Tooth decay caused by Streptococcus mutans in association with Actinomycetes species

2. Periodontal disease caused by Streptococcus mutans, Streptococcus salivaris, Actinomycetes israeli.

3. Actinomycosis caused by Actinomycetes species.

One of the main causative agent in all dental problems is Actinomycetes Israeli, which is an a gram positive, filamentous, branching, nonsporing, anaerobic or microaerophilic, non-acid fast organism and exists as an obligatory commensals in oral cavity[1].

WHAT ARE DENTAL DISEASES

Dental plaque

Dental plaque is the soft, sticky layer of bacteria on the surface of gums and teeth, particularly along the gum-line. Dental carries or Tooth decay involves a gradual demineralization of enamel and dentin. Dental carries begin when bacteria acting on sugars give off acids that demineralise the enamel. Dextran a sticky polysaccharide produced from sucrose causes bacteria to stick to teeth. The major portion of Plaque constituents are Bacteria and remainder being carbohydrate and other substances of saliva.

Calculus

Calculus is a hard, pale, brittle substance formed on the tooth by precipitation of calcium phosphate from saliva onto plaque. Calculus above the gum margin (supra-gingival calculus) is very common and not normally a problem. The rarer form, sub-gingival calculus, harbors plaque under the gum margin, preventing it from being brushed off. This can allow periodontitis to set in.

Gum disease

If environmental conditions promote the overgrowth of bacterial plaque (e.g. poor oral hygiene), changes in the quantity and quality of the plaque occur. This causes gingivitis, an inflammation of the gingivae adjacent to the plaque layer, which is observed as a swelling and reddening of the gum tissue. Gingivitis is an early response of the body to a build-up of plaque bacteria, and the effect of the toxins which the bacteria produce[1, 2].

Bad breath

Bacterial metabolism can produce organic thiols (sulfur-containing compounds), which give rise to bad breath or halitosis. Thorough brushing with a dentifrice or rinsing with a mouthwash which has anti-caries and anti-plaque properties is an effective way of reducing bad breath. Additionally, some formulations contain active ingredients which reduce bad breath by reacting with thiols. Advanced caries lesions can also contribute to bad breath, particularly when an abscess is formed[1].

Stained teeth

The acquisition of colored substances from our diet (tea, coffee, red wine etc.) onto the tooth pellicle make teeth to lose their natural white color. Other habits as smoking are also responsible for stained teeth. The stained pellicle cannot be removed by normal brushing. To remove the stains a tooth paste must contain an abrasive which gently abrades away the stained pellicle. Abrasives are carefully chosen not to damage the underlying enamel (or dentine if exposed) [1,3].

Periodontal disease

Periodontal disease is a collective for a variety of conditions characterized by inflammation and degeneration of the gingivae, alveolar bone, periodontal ligament and cementum. Periodontal diseases frequently caused by poor oral hygiene, by local irritants, such as bacteria, impaired food and cigarette smoke or by a poor bite [1,2,3,4].

Actinomycosis

A chronic granulomatous infection caused in human beings. It is characterized by indurate swellings, mainly in connective tissue suppuration and the discharge of Sulphur granules. Actinomycosis in human beings is an endogenous infection. Actinomycosis is more common in rural people and in agricultural workers. Young male persons (10-30years old) are most commonly affected. About 60% of cases are cervicofacial and 20% are abdominal[1.2.3,4].

The aim of present work revolves around three objectives. First to study the microorganisms present in oral cavity of human being, second to study the effect of different environmental conditions of medium on the growth pattern of Actinomycetes and third and of

crucial importance is to study the effect of sugar and sorbitol on the growth of Actinomycetes.

The selection of Sorbitol and Sucrose are carrying intentional purpose. The Sorbitol is D- glucitol by chemical nature. It is a hexahydric alcohol related to mannose and is isomeric with mannitol. Sorbitol is claimed to be not fermented by oral microorganisms and has little effect on dental plaque pH, hence generally considered to non-cariogenic. Thus we want to confirm the Sorbitol really nonfermentable by Actinomycetes too. If we will get confirmation then use of Sorbitol in Oral hygiene products can be recommended[5].

Sucrose is α-D- glucopyranosyl-β-D-fructofuranoside by chemical nature. Sucrose is considered to be more cariogenic than other carbohydrates since it is more easily converted to dental plaque. For this reason its use in oral formulations is declining. This made us to re-evaluate the cariogenic effect and relevant growth of Actinomycetes species.

MATERIALS AND METHOD

Digital Colorimeter EQ-650-A is used for present work to determine the turbidity (microorganism growth) manufactured by EQUIP-TRONICS. Sorbitol, Agar, Peptone are purchased from LOBA Chemie, Mumbai and Dextrose, Meat extract from Research-Lab, Mumbai and Sodium Hydroxide, Hydrochloric Acid, Sodium Chloride from Qualingens Fine Chemicals. For the purpose of sterisation the LAB-HOSP is used. To check the growth of microorganism species the laboratory purpose incubator manufactured by KUMAR MFGERS is used.

Preparation of medias

In the present research work the two medias are used namely Potato Dextrose Agar medium for maintaining in house culture of Actinomycetes species and Nutrient medium for growing Actinomycetes in different nutrient medias. For the preparation of Potato Dextrose Agar medium, the potatoes are scrubbed but didn't peeled and cut into 12mm cubes. Boiled 200g in 1litre of water for 60 min. Squeezed as much of the pulp as possible through a fine sieve. Added 20 g of Agar and boiled until it gets dissolved. Added 20g Dextrose and made up to 1litre. Autoclave at 115°C for 30 min. Cooled to 50°C and poured approximately 20ml amounts into Petri dishes. For the preparation of Nutrient medium, Meat Extract 1.5 g and Peptone 5 g dissolved in 500ml water. The pH of medium is adjusted to 7.4. For the purpose of pH adjustments the 0.1N NaOH and 0.5N HCl are used. All prepared medias are sterilised in autoclave at 115°C for 30 min. The Actinomycetes species sample are gifted by Modern College of Biotechnology, Pune. The all glass accessories including pipettes and beakers are sterilised in the oven for 30 minutes at 150°C.

Preparation of distilled water at different pH ranges 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0.

10ml of distilled water taken in each of 7 sterilised test tubes and pH adjusted to 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0. The two control samples are kept, one of plain distilled water (Control 1) and other is distilled water with inoculation (Control 2). After adjusting the pH the culture of Actinomycetes species is inoculated directly with Nichrome wire loop to the test tubes containing medium in all the 8 test tubes and then kept for incubation at 37°C for 48 hrs in the incubator[6,7,8].

Preparation of Nutrient Broth Medias containing 5%w/v Sucrose solution at pH ranges 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0.

The 10ml of sterilised Nutrient broth medium added in each of 7 test tubes and pH adjusted to 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0 after addition of 5ml of 5% w/v Sucrose solution. Remaining two test tubes mainly contain the Distilled water (Control 1) and other is simply 15ml of 5% w/v Sucrose solution (Control 2). All contents are sterilised and then the Actinomycetes species is inoculated directly with Nichrome wire loop in all test tubes[9].

Preparation of nutrient broth medias containing 5%v/v Sorbitol solution at pH ranges 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0.

The 10ml of sterilised Nutrient broth medium added in each of 7 test tubes and pH adjusted to 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0 after addition of 5ml of 5% v/v Sorbitol solution. Remaining two test tubes mainly contain the Distilled water (Control 1) and other is simply 15ml of 5% v/v Sorbitol solution (Control 2). All contents are sterilised and then the Actinomycetes species is inoculated directly with Nichrome wire loop in all test tubes[9,10].

RESULTS AND DISCUSSION

Study of absorbance pattern of actinomycetes species with only distilled water after inoculation

The sample culture is inoculated with help of nichrome wire loop in sterile environment. This study revealed us the probable growth of Actinomycetes species in simple Distilled water. The pH adjustments done at same pH ranges to confirm and evaluate any variations present in growth[11].

Table 1: Findings of growth of actinomycetes by using distilled water

Solutions	Observations	Optical density
Distilled Water	No Turbidity	0.00
Control	Turbidity	0.01
Test tube 1 pH 5.0	Turbidity	0.02
Test tube 2 pH 5.5	Turbidity	0.02
Test tube 3 pH6.0	Turbidity	0.02
Test tube 4 pH 6.5	Turbidity	0.01
Test tube 5 pH 7.0	Turbidity	0.01
Test tube 6 pH 7.5	Turbidity	0.01
Test tube 7 pH 8.0	Turbidity	0.02

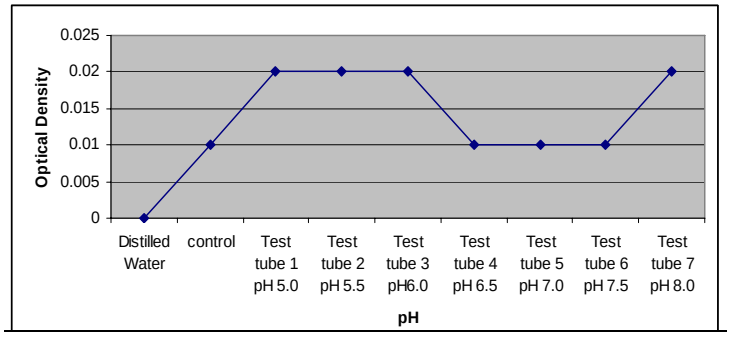

Fig. 1: Graphical presentation of growth of actinomycetes in distilled water

The graph focuses on two concepts, first growth in Distilled water is minimal compared to specific media and second that the growth still decreased at pH 7.0.

Study of Absorbance pattern of Actinomycetes species with Nutrient medium and 5% w/v Sucrose solution after inoculation

This study revealed the effect of Sucrose on growth of Actinomycetes species and also combines effect of use of Sucrose and particular pH ranges.

The optical density values showed the persistent growth in all sample pH ranges. The surprising result enlightened here that the growth declination at neutral pH.

Study of Absorbance pattern of Actinomycetes species with Nutrient medium & 5% v/v Sorbitol after inoculation

The Sorbitol also shows the increase in growth of Actinomycetes species. The growth declination at neutral pH still continued with Sorbitol.

Table 2: Findings of growth of actinomycetes by using nutrient medium and 5% w/v Sucrose solution

Solutions	Observations	Optical density
Distilled Water	No Turbidity	0.00
Control	Turbidity	0.29
Test tube 1 pH 5.0	Turbidity	0.36
Test tube 2 pH 5.5	Turbidity	0.42
Test tube 3 pH6.0	Turbidity	0.46
Test tube 4 pH 6.5	Turbidity	0.41
Test tube 5 pH 7.0	Turbidity	0.46
Test tube 6 pH 7.5	Turbidity	0.45
Test tube 7 pH 8.0	Turbidity	0.49

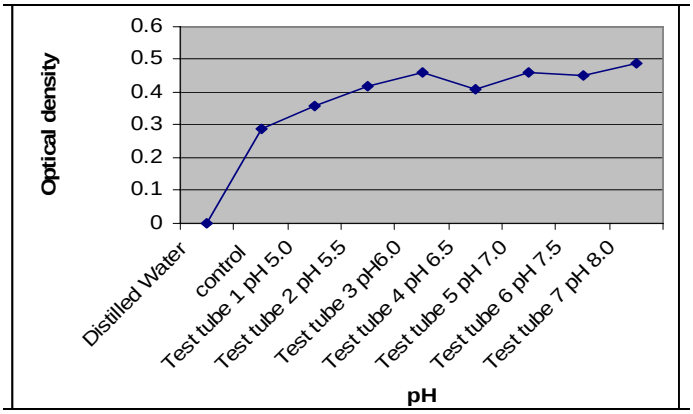

Fig. 5: Graphical presentation of growth of Actinomycetes in Nutrient medium and 5% w/v Sucrose.

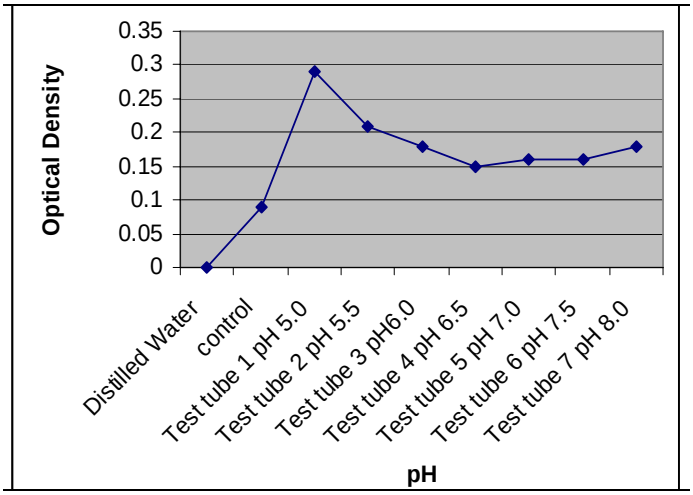

Fig. 6: Graphical presentation of growth of actinomycetes in nutrient medium + 5% v/v Sorbitol.

Comparison between the results

The comparison between the results of inoculation of Actinomycetes species with only Distilled water, with Nutrient broth medium and 5% w/v Sucrose solution, with Nutrient broth medium and Sorbitol 5% v/v shows confined growth in respective pH ranges.

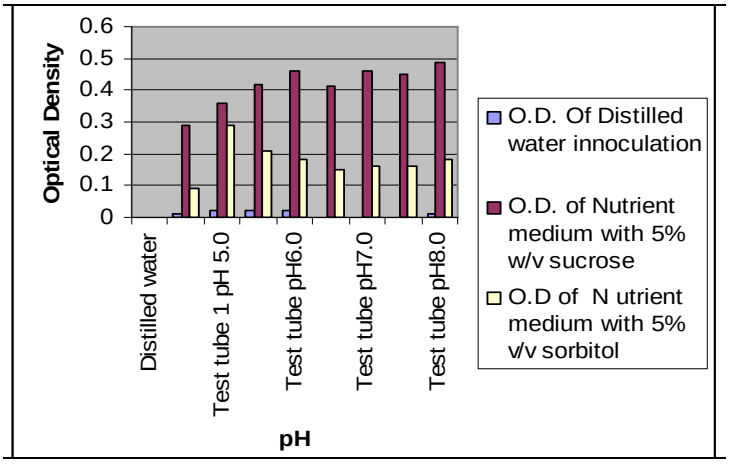

Fig. 7: Comparative graphical presentations

The growth of Actinomycetes species in nutrient medium, nutrient medium with 5% w/v Sucrose and nutrient medium with Sorbitol 5% v/v at pH ranges 5.0, 6.0, 7.0, 8.0 showed declination at neutral pH i.e. pH 7.0 and growth increased at both extreme pH ranges.

In the next section when growth compared with control i.e. Distilled water, nutrient medium with 5% w/v sucrose, nutrient medium with 5% v/v sorbitol at pH ranges 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 showed declination at neutral pH i.e. pH 7.0 and growth increased at both extreme pH ranges.

In future the studies can be explored for all other microorganisms found in oral cavity and are involved in periodontal diseases.

This study can be used as a base for study of one single isolated strain amongst Actinomycetes species. In future the metabolic pathway of growth of act can be studied in detail and can be used in formulation of oral hygiene product. This research may help in formulation of and oral hygiene product which is aimed at antibacterial action.

Table 3: Findings of growth of Actinomycetes by using Nutrient medium + 5% v/v Sorbitol

Solutions	Observations	Optical density
Distilled Water	No Turbidity	0.00
control	Turbidity	0.09
Test tube 1 pH 5.0	Turbidity	0.29
Test tube 2 pH 5.5	Turbidity	0.21
Test tube 3 pH6.0	Turbidity	0.18
Test tube 4 pH 6.5	Turbidity	0.15
Test tube 5 pH 7.0	Turbidity	0.16
Test tube 6 pH 7.5	Turbidity	0.16
Test tube 7 pH 8.0	Turbidity	0.18

Table 4: Comparison of results

Solutions	O.D. Of distilled water inoculation	O.D. of nutrient medium with 5% w/v sucrose	O.D of nutrient medium with 5% v/v sorbitol
Distilled water	0.00	0.00	0.00
Control	0.01	0.29	0.09
Test tube 1 pH 5.0	0.02	0.36	0.29
Test tube 2 pH5.5	0.02	0.42	0.21
Test tube 3 pH6.0	0.02	0.46	0.18
Test tube 4 pH6.5	0.00	0.41	0.15
Test tube 5 pH7.0	0.00	0.46	0.16
Test tube 6 pH7.5	0.00	0.45	0.16
Test tube 7 pH8.0	0.01	0.49	0.18

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