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Isolation and Characterization of *Pityrosporum ovale* – the causative agent of dandruff.

Abstract

Pityrosporum ovale commonly known as *Malassezia furfur* is a lipophilic, dimorphic, yeast like fungus, occurring in human skin as an opportunistic pathogen, causes diseases such as dandruff, seborrheic dermatitis etc. Specialized media for culturing the collected sample were standardized. A modified medium especially SDA (Sabourad's Dextrose Agar) for culturing *Pityrosporum ovale* has been proposed. Microscopic observations along with different biochemical analysis has been done against the isolated fungus and the results are discussed.

Key Words: *Pityrosporum ovale*, Dimorphic, Opportunistic pathogen, SDA, Biochemical analysis

Introduction

Dandruff is a minor infection of the scalp caused by an overabundance of microorganism called *Pityrosporum ovale* commonly known as *Malassezia furfur*, a yeast like fungus that is part of the normal skin flora. In other words dandruff is a condition, which causes small white flakes of skin to separate and fall from the scalp. People who suffer from dandruff have an overactive sebaceous gland which makes their scalp oily. In the past, dandruff and seborrheic dermatitis were seen as two different conditions. Seborrheic dermatitis was treated with corticosteroids and dandruff was viewed as more of a cosmetic

concern, and treated with shampoos supplied by the cosmetic industry. But later in 1960s and 70s, dermatologists began to use a number of new techniques to study skin structure and functions, including the growth of epidermal cells, which showed that epidermal cell turnover (the production and shedding of the uppermost layer of the skin), was increased in patients with dandruff. At that time dermatologists assumed that it was the increased epidermal cell turnover, or "hyperproliferation" that was causing dandruff and that the *Pityrosporum ovale* colonization was secondary. It is now evident that the opposite is true i.e. *Pityrosporum ovale* overgrowth occurs first and increased epidermal turnover is secondary.

Materials and Methods

Reagents and chemicals

Crystal violet, Gram's iodine and Saffranin were obtained from HiMedia Laboratories Pvt.Ltd, Mumbai, India. Deionized double distilled water were used for the preparation of SDA (Sabouraud's Dextrose Agar) media. The SDA media used in the experiment were purchased from HiMEDIA Chemicals, India. For isolation and growth experiment, required amount of fatty substance like butter or olive oil (2ml) was added singly to sterile medium before inoculation. Materials required for biochemical analysis were obtained from Merck, Mumbai, India.

Selection of Samples

Two samples were selected from two different individuals of coal belt zone having variable ages. One of them suffering from mild dandruff (Fig 1) without hair loss and the other suffering from severe dandruff (Fig2), itchy scalp and hair loss resulting in baldness. Questionnaires and physical observations as shown in Table 1 and Table 2 were used to obtain informations such as previous history, seasonal occurrence, dandruff type and demographic data such as family status, age, sex and nationality from the respondents.

Sample Collection

The collection of samples were carried out using a sterile scalpel blade. Dandruff in the form of white flakes and infected hairs were scrapped from the scalp usually from the frontal midline from where the hair growth begins. The collected sample was taken in a sterile petriplate for further growth in a required media.

Isolation of the collected samples

Sabouraud's Dextrose Agar (SDA) were used for culturing the above collected samples i.e. dandruff in the form of white flakes and infected hairs. Both the samples were used for culturing. 6.5 gms of SDA was dissolved in 100ml of water and was autoclaved at 121°C at 15lb/inch^2 for 15 minutes. The sterilized SDA combined with 2ml of butter was then poured on to sterile petriplates under aseptic condition and were kept for solidification inside Laminar Airflow System.

Then with the help of sterile cotton swab dipped in saline citrate solution, the scrapped sample (White flakes of dandruff) was streaked on the SDA plate. The plates were then incubated at 25⁰C for 6 days.

Morphological Characteristics

Gram staining was done with the isolated culture and examined under high power objective of the microscope, and the characters were recorded. Budding Yeasts were observed.

Biochemical Tests

The culture was biochemically analysed by using gelatin hydrolysis test, litmus milk reaction, carbohydrates viz., dextrose, xylose, rhamnose, raffinose and mannitol fermentation tests also performed and the results were recorded.

Results and Discussion

In SDA, the isolate was developed as cream coloured colonies along with smooth pasty yeast like appearance over the medium as shown in figure 2. Microscopically, the cells are bottle shaped with purple colour appearance indicating the gram+ve nature of the cells.

The biochemical tests proved that dextrose and xylose produced acid but there was no gas production. Maltose, lactose, rhamnose, raffinose and

mannitol were not fermented by the isolate .Acidification of litmus milk along with curdling occurred after 6 days.

Conclusion

Thus from the present investigation, it can be concluded that the isolate so cultured is *Pityrosporum ovale*, a yeast like fungus. Commonly Sabouraud's Dextrose Agar is used for the culturing of all dermatophytes. Emmon's in 1970 modified this medium by adding corn oil for the culturing of *M. furfur* . But, the present study clearly established that the growth of *P.ovale* was more favoured in the presence of butter than corn oil. Hence, it is suggested that Emmon's modified medium can be further modified by supplementing Sabouraud's dextrose agar medium with butter in place of corn oil.

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