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Research Article

Growth Pattern of Economically Important Fungi in Fruits Peel Waste Media

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Abstract

Fruits peels, the unused outer covering part, are considered as fruits peel waste which can be simply used in different microbiological media preparing in various industries. This study was aimed to formulate growth media for some economically important fungi by using fruits peel waste materials such as Pineapple, Papaya, Green Banana, Yellow Banana, Orange and Pomegranate. Fresh Fruits were purchased from Local market of Dharan and obtained fruits peels were air dried and grinded separately into fine particles using mechanical blender and then sieved with 1 mm sieve size. Fungi isolates (*Aspergillus niger*, *Rhizopus stolonifer* and *Saccharomyces cerevisiae*) were inoculated separately into the medium prepared in conical flask by using fruits peel powder (4 g in 100 mL distilled water) and incubated at room temperature (25°C±1) for 5 days. Growth of *Aspergillus niger* was recorded in the medium containing Pineapple, Orange, Green Banana, Yellow Banana and Pomegranate except Papaya while *Rhizopus stolonifer* growth was observed in the medium containing Pineapple, Green Banana, Yellow Banana and Pomegranate except Papaya and Orange. However, the growth of *Saccharomyces cerevisiae* was recorded in the medium containing Pineapple, Green Banana, Orange and Papaya except Yellow banana and Pomegranate. This study showed that some economically important fungi can be successfully grown in some fruits peel waste media.

Keywords: Fruits peel waste; economically important fungi; *Aspergillus niger; Rhizopus stolonifer; Saccharomyces cerevisiae*

Introduction

Cultural medium is defined as any material in which microorganism find nourishment for their growth and development (Pelczar *et al*., 1993). Generally, growth media for fungi contain carbon and nitrogen sources, and most fungi require several specific elements for growth and reproduction (Gao *et al*., 2007). Because of Modern efficient agricultural practices, there are huge productions of fruits and vegetables throughout the world. The most widely acceptable fruits are banana, pineapple, mango and papaya (Jamal *et al*., 2012).

Unused portions such as peels, pulp and seeds of fruits considered as wastes that constitute about 40 % of the total mass. Improper disposal of these fruits wastes create huge environmental disorders (Lim *et al*., 2010). Economically important fungi are those fungi which play an important role in medicine yielding antibiotics, in agriculture by maintaining the fertility of the soil and causing crop and fruit diseases, forming basis of many industries and as important means of food. Some of the fungi are important research tools in the study of fundamental biological processes (Pelczar *et al*., 1993; Saranraj and Anbu, 2017).

Fungi are generally grown on very expensive media found in market such as Potato dextrose agar (PDA), Sabouraud's dextrose agar (SDA), Rose bengal agar (RBA) and Corn meal agar (CMA) (Saranraj and Anbu, 2017).

As commercially available media are very expensive, routine practical require large investment in the form of

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media on regular basis for streak plate, pour plate and spread plate experiments. The search for alternative, cheap media providing rich in nutrients for use in laboratory agents for routine microbiological experiments is going on (Ravimannan *et al*., 2014).

The cultivation of microbial cells (bacteria, yeast, and fungi) that converts fruit wastes into value added products through fermentation such as biomass that can serve as animal feed supplement is a unique approach. Simple and complex sugars present in the fruits peel wastes are metabolized (Saheed *et al*., 2013) and the byproducts such as bio-ethanol, biogas and animal feed are produced (Tijani *et al*., 2012).

Production of more valuable products by culturing of fungi utilizing agricultural wastes as a substrate has been reported. Cellulase can be produced by some fungi culturing on pineapple waste. Carotenoids and cellulose production can be carried out on agricultural waste using *Blakeslea trispora* and *Aspergillus niger* respectively (Papaioannou and Liakopoulou Kyriakides, 2012). Sugarcane bagasse has been also reported as an energy source for the production of lipase by *Aspergillus fumigatus* (Naqvi *et al*., 2013)

This study was designed to formulate a cost effective and efficient medium for economically important fungi such as *Aspergillus niger*, *Rhizopus stolonifer* and *Saccharomyces cerevisiae* using fruits peel wastes as raw material.

Materials and Methods

Fruit Collection and Peel Waste Preparation

Fresh fruits were collected from house garden and some of the fruits were brought from the local market of Dharan. The fruits were then thoroughly washed and peeled out. Fruits peel were air dried at room temperature for 10 days to remove the moisture. The dried peels were then grinded separately into fine particles by mechanical grinder and sieved with 1 mm sieve size to give powdery form. The powder was stored in polythene bag at room temperature.

Isolation and Identification of Fungi

Aspergillus niger was isolated from air by open plate technique while *Rhizopus stolonifer* was isolated from serial dilution techniques. On the other hand, *Saccharomyces cerevisiae* was isolated from marcha sample.

The potato dextrose agar (PDA) plates were prepared and were plated aseptically. Some plates were then opened in the laboratory and outside for around 15 minutes. The plates were incubated at room temperature for 3-5 days at 28° C. Fungi were also isolated from soil samples collected from college premises by spread plate technique followed by serial dilution up to 10^{-4}. The plates were incubated for 3-5 days at 28°C.Similarly, *Saccharomyces cerevisiae* was directly isolated from marcha sample by spread plate technique on MYGP agar plate followed by crushing marcha into fine powder and performing serial dilution of 1 g of it up to 10^{-2}. The plates were incubated at 28° C for 3-5 days.

Isolated fungi were identified by Lactophenol Cotton blue (LPCB) staining technique under compound microscope (40X).

Maintenance of Fungal Isolates

The pure fungal colonies were sub-cultured in freshly prepared PDA and MYGP agar plates and stored at 4° C for further use.

Inoculum Preparation

The suspension of 4 days old cultures of fungi (*Aspergillus niger*, *Rhizopus stolonifer* and *Saccharomyces cerevisiae*) were used to study the qualitative and quantitative growth analysis. They were prepared in saline solution (0.85% sodium chloride) separately. The fungal cultures were inoculated into conical flask containing 50 ml of saline solution and incubated at room temperature for 5 hours.

Preparation of Fruit Peel Waste Media

About 4.0 grams of dried fruit peels powder were added into the 100 ml of distilled water and sterilized by autoclaving at 121° C for 15 minutes. The fruit peels broths were cooled and then 1 ml of prepared fungal inoculums was transfer in to it.

Qualitative Analysis of Fungal Growth

The inoculums added broths were incubated at room temperature for 5-7 days. The presence/absence of fungal growth in fruit peels broth media was visually observed.

Results

Isolation and Identification of Economically Important Fungi

Economically important Fungi were isolated from air, soil and marcha samples. Different types of fungal colonies were isolated in Potato Dextrose Agar and MYGP agar plates initially. The well grown fungal colonies were identified by lactophenol cotton blue staining technique and colonial characteristics (Table 1). Among them, economically important fungi were chosen after identification of fungal isolates and subcultured on respective agar plates i.e. *Aspergillus niger* and *Rhizopus stolonifer* in PDA and *Saccharomyces cerevisiae* in MYGP agar plates.

Qualitative Growth Analysis of Economically Important Fungi in Fruit Peel Waste Media

The effect of six different fruit peel media *viz*., Pineapple, Papaya, Orange, Yellow Banana, Green Banana and Pomegranate on the qualitative growth of *Aspergillus niger*, *Rhizopus stolonifer* and *Saccharomyces cerevisiae* were studied and the results were given in Table 2.

It was observed that the *Aspergillus niger* growth was recorded in the medium which contains Pineapple, Pomegranate, Orange, Yellow Banana and Green Banana but not in the medium containing Papaya. *Rhizopus stolonifer* grew in the medium that contains Pineapple, Green Banana, Yellow Banana and Pomegranate. The *Rhizopus stolonifer* growth was not recorded in the medium containing Papaya and Orange did not allow growing *Rhizopus stolonifer*. However, the *Saccharomyces cerevisiae* growth was noticed in the medium which contains Papaya, Pineapple, Orange and Green Banana whereas the *Saccharomyces cerevisiae* growth was not recorded in the medium containing Yellow Banana and Pomegranate.

Table 1: Colonial characteristics of fungal isolates on PDA and MYGP agar plates

S.N	Media used	Colony morphology	Probable identity
1	PDA	Velvety, black, creamy	*Aspergillus niger*
2	PDA	Colonies grow rapidly, resemble cotton candy. Turned into blackish colony due to ageing.	*Rhizopus stolonifer*
3	MYGP	Flat, smooth, moist, glistening and cream in color.	*Saccharomyces cerevisiae*

Table 2: Qualitative growth analysis of fungal in fruit peel wastes

S.N.	Fruit peel waste	*Aspergillus niger*	*Rhizopus stolonifer*	*Sccharomyces cerevisiae*
1.	Pineapple	+	+	+
2.	Papaya	-	-	+
3.	Pomegranate	+	+	-
4.	Yellow banana	+	+	-
5.	Green banana	+	+	+
6.	Orange	+	-	+

[Note: (+) = Positive growth; Nil Growth = (-)]

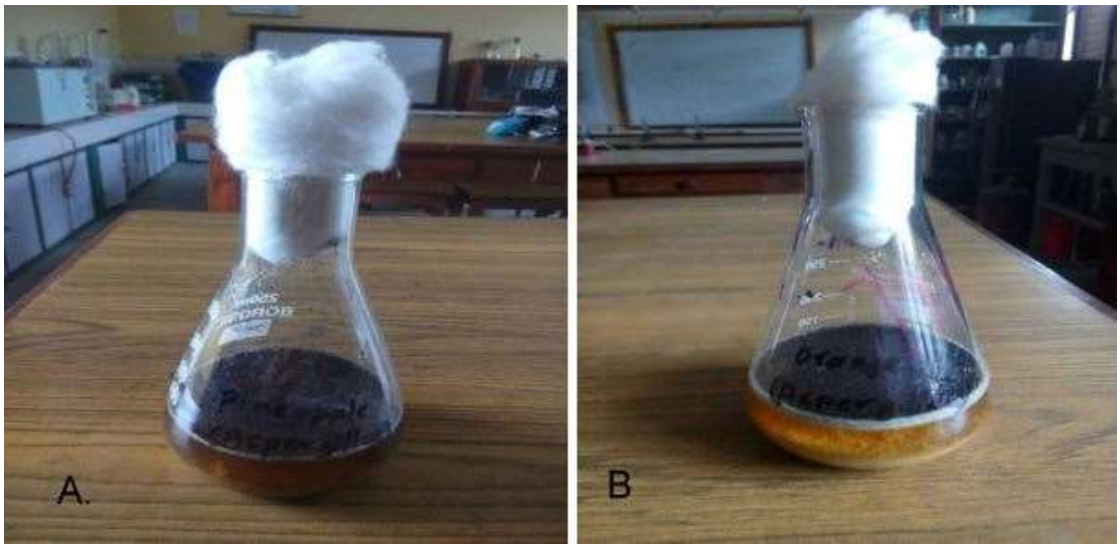

Fig.1: Growth of *Aspergillus niger* on fruit peel media (A. Pineapple peel media; B. Orange peel media)

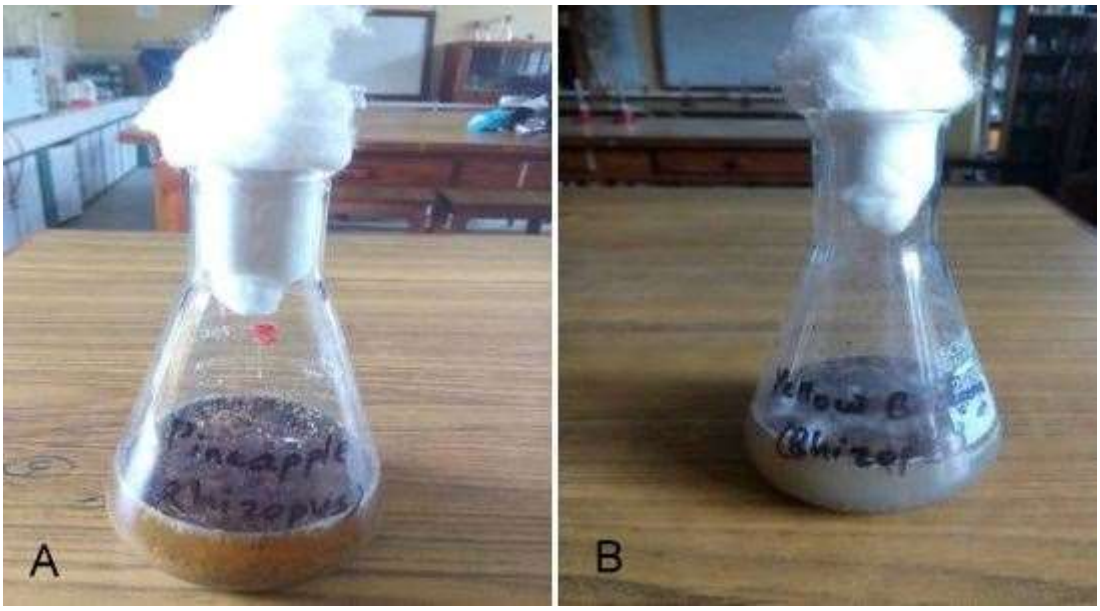

Fig. 2: Growth of *Rhizopus stolonifer* on fruit peel media (A. Pineapple peel media; B. Yellow Banana peel media)

Fig. 3: Growth of *Saccharomyces cerevisiae* on fruit peel media (A**.** Pineapple peel media; B. Papaya peel media)

Discussion

Fungi Isolation Backup

For this study, fresh fruits were brought from local market of Dharan. Then, they were subsequently washed peeled, and the peels were dried under shade.

According to (Gyawali 2013) immediate drying prevents microbial fermentation and degradation of metabolites. In addition, protection from direct sunlight is essential to minimize chemical reactions induced by ultra violet rays. Fruit peels might get discolored and even lose its important minerals and vitamins during direct sun drying, so best dried under shade.

Each fruit peel waste was grinded to powdery form using mechanical blender. Economically important Fungi were isolated from air, soil and marcha samples. *Aspergillus niger* and *Rhizopus stolonifer* were isolated from air by open plate technique and from soil by serial dilution technique in PDA media plates while *Saccharomyces cerevisiae* was isolated from marcha sample using MYGP media.

A loopful of sub cultured fungal colony was incubated in 0.85% of saline solution at room temperature. Lastly, the saline solution containing fungi was poured into the flask containing autoclaved and sterile respective fruits peel solution (peel powder in distilled water) at room temperature for analysis of qualitative growth of respective fungi. Saline solution balances osmotic pressure of fungi (Saranraj and Sanbu, 2017).

The agricultural based industries generate significant quantities of organic wastes including peels from cassava, plantain, banana, oranges and straw from cereals

(Nwabueze and Otowa, 2006). Banana peel, pineapple peel, mango peel and papaya peel (Pp) are major wastes generated by fruit processing and agro-allied industries (Jayabalan *et al*., 2010**).** Tropical and subtropical fruits processing have considerably higher ratios of by-products than the temperate fruits **(**Schieber *et al*., 2001).

Agricultural waste materials support the good growth of fungi (Domsch and Anderson 1980). The nutrients in the fruits wastes include protein, carbohydrate and minerals. Protein constitutes a significant portion of microbial cells and thus is necessary for the growth of microorganisms (Prescott and Harley, 2002). These wastes contain simple and complex sugars that are metabolizable by microorganisms through secretion of extracellular products **(**Saheed *et al*., 2013). The nutrients in the wastes included protein, carbohydrate and minerals. Protein constitutes a significant portion of microbial cells and thus is necessary for the growth of microorganisms (Prescott and Harley, 2002). Fruit wastes rich in carbohydrate content and other basic nutrients could support microbial growth. Apple, turnip, papaya and banana peels were used for alcohol fermentation and biomass production by Kandari and Gupta (2012). The protein content of the formulated media must have ensured a good supply of nitrogen while the carbohydrate content served as additional carbon source both of which are essential for good fungal growth. The mineral content of the wastes in the formulated media was probably useful for some aspects of the fungi's metabolism (Pelczar *et al*., 1993).

A fruit peel waste may meet needs nutritional requirements of fungi for their growth and development. Fruits peel media can add a benefit of minimal contamination in the cultures because it does not meet the needs of every microbe (Ravimannan *et al*., 2014) [10]. The protein content of the formulated media must have ensured a good supply of nitrogen while the carbohydrate content served as additional carbon source both of which are essential for good fungal growth. The mineral content of the wastes in the formulated media was probably useful for some aspects of the fungi's metabolism (Pelczar *et al.*, 1993).

These fruits waste materials can be utilized for further industrial purposes to convert them into useful products by fermentation, extraction of bioactive components, extraction of functional ingredients etc. It is now realized that these waste can be utilized as cheap raw materials for some industries or as cheap substrates for microbiological processes (Nwabueze and Otowa, 2006).

Cucumber and orange peels were evaluated for the production of single cell protein culturing *Saccharomyces cerevisiae* by submerged fermentation (Mondal *et al.*, 2012).

Pineapple cannery waste materials have been used as substrate for the microbial production of vanillic acid and vanillin by the use of some fungi species (Ong *et al.,* 2014).

Fruit peels, which constitute a huge part of the waste streams, provide anchorage for filamentous fungi during bioconversion process (Essien *et al*., 2005). Bioconversion of single fruit waste is a common practice in valorization of fruit peels. Pineapple waste, palm tree waste and cassava waste have received attention for their conversion to bio-ethanol, biogas and animal feed (Dhanasekaran *et al*., 2011; Tijani *et al*., 2012). Apple, orange, banana and other fruits locally available and thus serve as readily available raw materials for the separation of ethanol yeasts (Eghafona, 1999).

The disposal of agricultural wastes on land and into water bodies is common and has been of serious ecological hazards (Smith *et al*., 1987).Fruit waste dumping sites provide favorable environment for vectors, pathogenic bacteria and yeast to multiply and spread diseases. A popular approach to mitigating poor handling of fruit wastes in landfill and incineration cause an acute air pollution problem by generating massive leachates that contaminate ground water and destroy aquatic lives (Ali *et al.*, 2014).

Thus, environmentally polluting by-products such as fruits peel waste could be converted into products with a higher economic value than the main product (Saranraj and Sanbu, 2017) because they contain many reusable substances of high value.

Routine laboratory experiments for streak plate pour plate and spread plate techniques require large amount of media. As the commercially available media have very high costs, the search for alternative and cheap media for use in laboratory agents for routine microbiological experiments is going on. (Ravimannan *et al.*, 2014)

The need to develop alternative media has become imperative as the conventional media are either not readily available or expensive in most developing countries (Weststeijn and Okafor, 1971).

The Growth of the fungi on the formulated media implies that the fruits peel wastes which were used in formulating the media contained the required nutrients for fungal growth (Ruth *et al*. 2012). Amadi and Moneke (2012) also reported higher mycelia growth rate in Purple sweet potato dextrose agar than in Yam dextrose agar.

According to Meletiadis *et al*. (2010), optimal nutrient medium should provide not simply adequate growth but the best possible growth in order to allow molds and yeast grow without restriction and express all phenotypes.

A wide range of media are used for isolation of different groups of fungi. These media influence vegetative growth, and colony, morphology, pigmentation and sporulation

depending on their composition, pH, temperature, light, water availability and surrounding atmospheric gas mixture (Kuhn and Ghonnoum, 2003).

Conclusion

This study showed that fungi such as *Aspergillus niger*, *Rhizopus stolonifer* and *Saccharomyces cerevisiae* can be successfully grown in various fruits peel waste media. The fruits peel waste media can fulfil nutritional requirements of industrially important fungi because they are rich in minerals and nutrients. Thus, they can be utilized as alternative materials in the formulation of culture media for the *in vitro* cultivation of fungi for industrial and research purposes. Available fruits (pineapple, pomegranate, orange, green banana, yellow banana and papaya) peel waste can be taken as the base for formulating cost effective and useful fungal media. It is necessary to study fungal physiology by modern tools and methods for using nutrients of respective fruits peel for their growth. This study has tried to solve the problem of the shortage of culture media.

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