Characterization of microbial communities in bioenzymes made from vegetable and citrus fruit peels

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***Abstract*— Bio-enzyme is a natural, non-toxic, non-flammable liquid fermented from organic wastes that improves engineering quality of soil facilitating higher compaction and stabilization. Bioenzymes are also used as fertilizers, bio-cleaners and for bioremediation in waste water treatment. These organic wastes are transformed into various biologically available nutrients to plant contributing to enhanced plant growth. Since these are natural, they have profound advantage over uasage of chemical fertilizers in agriculture. Microorganisms contain various enzymes involved in various biogeochemical processes which have immense impact upon the environment. This study aimed to characterize the microbial composition of bioenzymes prepared from organic wastes (vegetable and fruit peels). The microbes were grown in specialized media and microbial diversity were studied. These microbes were further examined for enzymatic activities. Altogether, this study provided an insight onto the microbial composition of bioenzymes made from organic wastes and their contribution to microbial degradation.**

***Keywords*— Bio-enzyme**

I. INTRODUCTION

Bioenzymes contains living micro-organism which, when applied to seeds, plant surfaces or soil colonize the rhizosphere or the interior of the plant and promote growth by increasing the supply or availability of primary nutrients to the plant. Fruits and vegetable wastes are produced in large quantities in market and constitutes a source of nuisance in municipal landfills because of their high biodegradability (Virtrurtia *et al*. 1989). The improved fermentation represents a vinegar-like liquid empowered by mineral salts, enzymes and natural proteins which can be used for several purposes (Chelliah, 2015). Various microorganisms possess enzymes that are involved in various biochemical reactions (Palani 2017). In this context, different types of stabilizers ranging from inorganic to organic have been reported to evaluate their suitability as soil stabilizer. Recently bio-enzymes have emerged as a new solution for soil stabilization (Pinang 2012). These organic compounds show their application in various industries to develop edible films, food industries for probiotics and other industries for valuable products. The utilization of these low-cost waste horticultural wastes for producing the value-added product is a novel step in its sustainable utilization (Kumar *et* al., 2020). Since microorganisms are the key players in this scenario, the various enzymatic activities of these microorganisms contribute to the applicability and functionality of these bioenzymes. The present study focused on the characterization of bioenzymes extracted from organic wastes and the microbial composition of the same. The enzymatic activities of the isolated microorganisms were also studied which provided an insight onto the characteristic of the microflora present in the bioenzymes extracted from organic wastes.

II. MATERIALS AND METHODS

Bioenzyme preparation

The bioenzyme solution (10 lt) was prepared with distilled water, vegetable or citrus feels and jaggery powder in the ratioof 10:3:1 (Dhavale *et al*., 2020). The mixture was allowed to ferment for 1 month in a cool, dry place, avoiding direct sunlight, in presence of yeast. During this period of fermentation, the biogas was released at a time interval of every 5 days. The fermented enzyme was then filtered and used for experimental purposes.

Media preparation

Nutrient agar (NA) and Potato Dextrose agar (PDA) media were prepared as per protocol and autoclaved. Media was poured onto sterile petriplates and used or stored for experimental purposes. Nutrient agar plates were used for initial isolation of microbes from both the bioenzyme solutions. 0.5% Carboxymethylcellulose (CMC) was used a supplement in the enriched media used for experiments. Mac Conkey medium was also prepared as per protocol and agar plates were used for the examination of microbial growth.

Microbe characterization and enzymatic activity

Determination of amylase activity of the microbes present in the bio-enzyme solutions

Bio-enzymes samples were streaked onto PDA plates, incubated at 37 ^{o}C for 48hrs and observed for microbial growth. Characteristic colonies were further examined for amylase activity by addition of iodine. 0.5% elemental iodine (Universal Chemical, Lot: RH172) and 1% potassium iodide (EMPARTA, Batch no: DF9D691819) mixed in 10 ml of water was used as the reagent to observe starch hydrolysis.

Detection of lactose fermenters present in the bio-enzyme solutions

MacConkey agar is a selective and differential medium used for the isolation and differentiation of non-fastidious gram-negative bacilli, in particular members of the Enterobacteriaceae family and the genus *Pseudomonas*. Bio-enzymes samples were streaked onto Mac Conkey agar plates and microbial growth were observed by incubating the plates at 37 ^{o}C for 48 hrs. The change in pH of the medium indicated growth of lactose fermenting microorganisms.

Determination of cellulose degradation by microbes

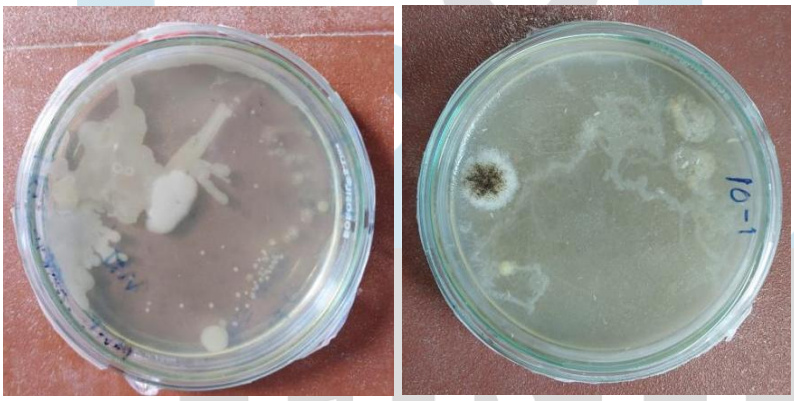
Cellulolytic enzymes play an important role in natural biodegradation process where plant lignocellulosic materials are efficiency degraded by cellulolytic fungi and bacteria. The nutrient agar supplemented with 0.5% Carboxymethylcellulose (CMC) was inoculated with the bio-enzyme samples and incubated at 37°C for 48hrs. Congo red solution (1%) was prepared with 0.5gm Congo red dye dissolved in 50 ml of distilled water and 50 ml of 100% ethanol was added this mixture. The mixture was filtered before usage. After incubation, CMC agar plates were flooded with 1% Congo red solution and allowed to stand for 15 min at room temperature.

III. RESULTS

Characterization of microbes on nutrient agar

Bioenzyme solution from both the vegetable and fruit peels showed bacterial as well as fungal growth on nutrient agar plates. Multiple small white bacterial colonies were observed in the bioenzyme sample isolated from vegetable peels (**Fig. 1A**) whereas most hyphal fungal colonies were observed in the sample from the citrus peels (**Fig 1B**). The colony count in the respective plates are given in the table below:

	No. Of bacterial colonies	No. Of fungal colonies
Bioenzyme from veg peels	57	09
Bioenzyme from citrus peels	69	uncountable

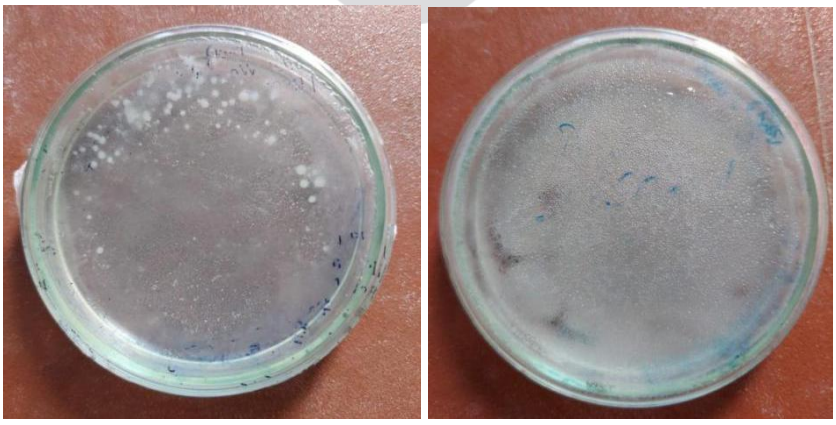


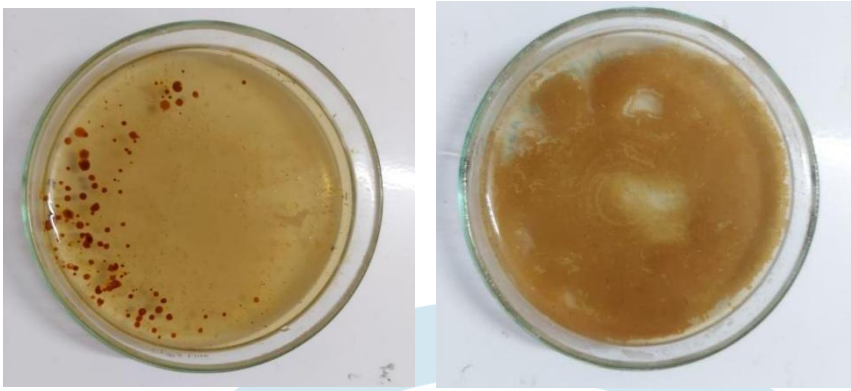
A **B**

Fig. 1: Microbial characterization of bioenzymes prepared from vegetable peels (A) and fruit peels (B) grown on Nutrient Agar media.

Characterization of microbes on PDA

Growth was observed in the PDA plates of both the sample sets from vegetable and citrus peels (**Fig.2**). However, starch hydrolysis was noticed to be more prominent in the plate from the citrus peels sample (**Fig. 2B**) indicating that the fungal colonies profound in the bioenzyme sample extracted from citrus peels were amylase producers.





Lactose fermenters are present in bioenzymes made from citrus peels

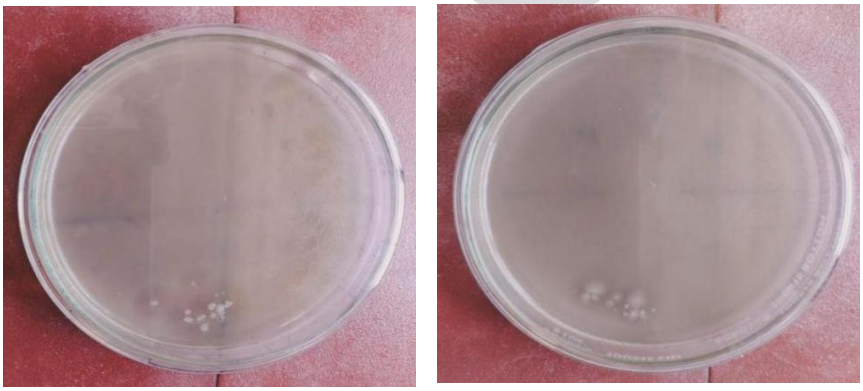
The change in color of the medium indicated acid production by the lactose fermenters present in the sample. Growth on the MacConkey agar plates confirmed presence of gram negative bacteria in the respective samples (**Fig. 3**). Bioenzyme samples extracted from the citrus peels exhibited distinct pink colonies on MacConkey agar plates confirming the presence of lactose fermenters in the sample (**Fig. 3B**) compared to the sample from the vegetable peels, where colourless colonies were observed confirming presence of non-lactose fermenters (**Fig. 3A**). Presence of bright blue colonies on the citrus plates indicated growth of *Klebsiella* or *Pseudomonas* (**Fig. 3B**).

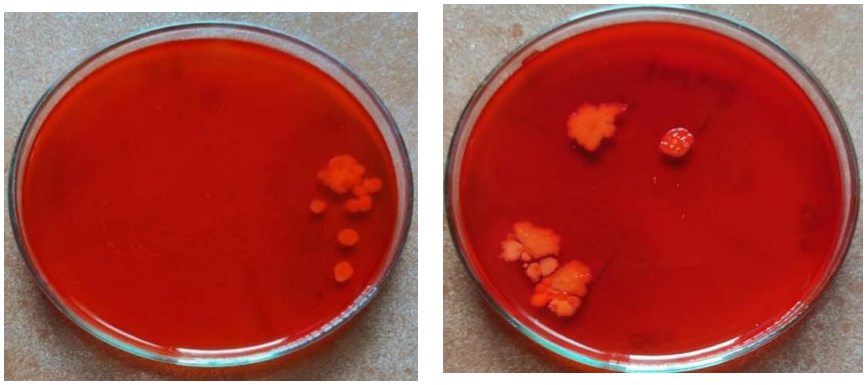
A **B**

Fig. 3: Lactose fermentation of bioenzymes prepared from vegetable peels and fruit peels on MacConkey agar

Microbes present in bioenzymes are capable of cellulose degradation

Both the bioenzyme samples showed microbial growth on carboxymethylcellulose supplemented agar plates indicating the presence of cellulose degrading microorganisms (**Fig. 4**). After addition of congo red, cellulose degradation was more prominent in both the agar plates from bioenzyme samples extracted from both vegetable peels (A) and citrus peels (B).





A **B**

Fig 4: Cellulose degradation by microbes isolated from bioenzyme samples extracted from vegetable (**A**) and citrus peels (**B**)

DISCUSSION

Microorganisms are major producers of enzymes that are of industrial significance. In various sectors, the use of amylases from microorganisms has exceeded synthetic sources (Pandey et al., 2000). They can easily be engineered to produce enzymes of desired attributes and the economic bulk production ability of microbes can be greatly enhanced. Fungi and bacteria which are well-known producers of extracellular proteins have been extensively utilized in producing various enzymes as well as amylases (Kazunari *et al*., 2011). The use of cheap and easily available wastes, such as agro-industrial waste, as an alternate substrate for amylase isolation and production for industrial purpose is a continuous process that helps in solving pollution challenges (Priya *et al*., 2011). The present study demonstrated presence of diverse microorganisms, including both bacteria and fungi, in bioenzymes extracted from both vegetable and citrus peels (**Fig 1**).

Screening of microorganisms with high amylase activities could enhance the discovery of novel amylases needed in commercial processes (Okunwaye *et al*., 2021). In this study, the bioenzyme samples from citrus peels exhibited clear zones of hydrolysis on starch agar plates demonstrating amylase activity (**Fig 2B**).

Bacteria are now being widely explored for cellulases production because of their rapid growth, expression of multi-enzyme complexes, stability at extremes of temperature and pH, lesser feedback inhibition, capacity to colonize a wide variety of environmental niches, and ability to withstand varieties of environmental stress (Rawway *et al*., 2018). Bioenzyme solution extracted from citrus peels exhibited distinct bright pink colonies which confirmed the presence of Gram negative lactose fermenters (**Fig 3B**). Presence of bright blue colonies specified presence of *Pseudomonas* or *Klebsiella*, which needed further confirmation. Presence of colorless colonies on agar plates (**Fig 3A**) confirmed that the bioenzyme extracted from vegetable peels mainly comprised of non-lactose fermenters. Microbial cellulases have many potential industrial and biotechnological applications, and hence are in high demand. In the present study, microorganisms present in the bioenzyme solutions extracted from both vegetable and citrus peels exhibited cellulose degrading ability (**Fig 4**).

Thus, the present study indicated presence of microbes capable of starch hydrolysis due to presence of amylases, cellulose degradation and both lactose and non-lactose fermenters in bioenzymes made from organic wastes (vegetable and fruit peels).

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