IJPSR (2022), Volume 13, Issue 4 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 24 July 2021; received in revised form, 04 October 2021; accepted, 24 March 2022; published 01 April 2022

EFFECT OF SUGARCANE BAGASSE AS A SOURCE OF CARBON FOR *α-AMYLASE ENZYME* AND ANTIBACTERIAL COMPOUND PRODUCING DIFFERENT TYPES OF WILD FILAMENTOUS FUNGI

A. Kalaiyarasan [1], S. Roobamathy [1], B. Vijayaraghavan [1], R. Lakshmi Sundaram [2] and N. Hariram [* 1]

Department of Biotechnology [1], Kalasalingam Academy of Research and Education, Krishnankoil - 626126, Tamil Nadu, India.
Central Research Facility [2], Sri Ramachandra Institute of Higher Education and Research, Porur, Chennai - 600116, Tamil Nadu, India.

Keywords:

Alpha-amylase, Sugarcane bagasse, Antimicrobial, Methanol, SSF (Solid-state fermentation), Thermostability

Correspondence to Author:
N. Hariram

Department of Biotechnology, Kalasalingam Academy of Research and Education, Krishnankoil - 626126, Tamil Nadu, India.

E-mail: n.hariram@klu.ac.in

ABSTRACT: The present investigation screening and characterization of *α-amylase* and antimicrobial compound producing filamentous fungi is used for extensive commercial applications in various sectors and is highly demanded in the Biotech industry. The results reveal that out of the carbon substrates alone, sugarcane bagasse with a combination of Tween-80 was found to yield maximum *α-amylase* (151.76 IU) under solid-state fermentation by different species of fungal species such as KRV_001Chaetomium oryzae, KRV_002 *Penicillium citrinum*, KRV_003 *Aspergillus flavus*, KRV_004 *Penicillium notatum*, KRV_005 *Alternaria longipes* and KRV_006 *Trichoderma polysporum*. Among them, KRV_001 *Chaetomium oryzae*, KRV_002 *Penicillium citrinum*, KRV_005 *Alternaria longipes* were maximum expressed *α-amylase* based on the plate assay by different pH, temperature and different substrate using amylase activity, SDS-PAGE, Antimicrobial activity has been done in Methanol extract exhibited antibacterial activity against *S. aureus* MTCC96 whereas the water extract displayed antibacterial activity. The organism produces volatile and water-soluble antibiotics and shows significant inhibition as the potential value of KRV_*001Chaetomium oryzae* and little less activity of KRV_006 *Trichoderma polysporum* among the isolates was investigated.

INTRODUCTION:

Importance of Microbial Enzyme: In recent years, the uses of microorganisms have become of huge importance to food, textile, baking and detergent industries and sparked a large interest in the exploration of enzyme activity in microorganisms [30].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.13(4).1695-07

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.13(4).1695-07

Amylases are among the foremost important enzymes were hydrolyze starch molecules to offer diverse products, including dextrin and progressively smaller polymers composed of glucose units [18].

These enzymes are among the most important enzymes for biotechnology with great significance, constituting a class of commercial enzymes having approximately 25% of the planet's enzyme market. Amylases are often obtained from several sources, like plants, animals and microorganisms. Today, many microbial amylases are available commercially, and they have almost completely replaced the chemical hydrolysis of starch within

the processing industry. A major advantage of using microorganisms to assemble amylases is the economical bulk production capacity and, therefore, the indisputable fact that they are easily manipulated to get enzymes of desired characteristics [23]. Amylase is often obtained from several fungi, yeast, bacteria, and actinomycetes; however, especially fungi have gained much attention due to fungi's supply and high productivity, which are also amenable to genetic manipulation. Many fungi were good sources of amylolytic enzymes. Many studies indicated that amylases of fungal origin are more stable than those of bacterial origin [31].

Starch is the best substrate for producing yeast cells on a large scale due to its low price and simply available staple in the world. Because most yeasts from environments are safe (GRAS) compared to bacteria, interest in amylolytic yeasts has increased in recent years as their potential value for converting starchy biomass to single-cell protein and ethanol has been recognized. To date, it has been noticed that the terrestrial yeasts which can produce extracellular amylolytic enzymes include *Arxula adeninivorans, Candida japonica, Filobasidium capsuligenum, Lipomyces, Saccharomycopsis, Schwanniomyces* [22].

Amylases can be classified into endoamylases (α-amylase) and exoamylases (glucoamylase). *α-amylase* catalyzes the hydrolysis of α-1,4-glucosidic linkages in the interior of starch molecule in a random manner producing branched and linear oligosaccharides (dextrin, maltose, maltotriose, glucose) of different chain lengths. In contrast, glucoamylases catalyze the hydrolysis of α1,4- and α-1,6-glucosidic linkages in the starch molecule from its nonreducing end-yielding glucose [21]. Genetically modified microorganisms can be used to produce various types of enzymes having different characteristics by the genetic makeup of the interest of our interest. Microbial technology plays an important role in producing industrially important enzymes to make up for their needs, and now a day, they are commercially available. Considering the industrial importance of amylase, in this study, we aimed to isolate and screen amylase-producing fungi and yeasts from different sources and to determine the amylase activity.

Sugarcane is the main agricultural crop cultivated in Brazil. The sugarcane stem is milled to obtain the cane juice, either used for ethanol or sugar production [10]. Each ton of sugarcane processed generates approximately 270-280 kg of bagasse (Rodrigues *et al.* 2003). This bagasse is normally burned in the industries to produce the energy required in the process [10]. However, this agro-industrial by-product may have more valuable uses than direct energy generation through combustion [16]. Sugarcane bagasse has 50% cellulose, 25% hemicellulose, and 25% lignin.

Due to its high availability, it could serve as a substrate for microbial production of value-added products, such as protein-rich animal feed, enzymes, amino acids, organic acids, and compounds of pharmaceutical importance [27] and a source of carbon for the growth filamentous fungi [25]. Filamentous fungi are particularly useful producers of enzymes from the industrial point of view due to the high production level and extracellular secretion of enzymes and the relative ease of cultivation [38]. These fungi produce high levels of polysaccharide-degrading enzymes and are frequently used to produce industrial enzymes [37], such as amylases, cellulases, xylanases, pectinases, and lignin modifying enzymes from lignocellulosic biomass.

These enzymes have several biotechnological applications; for instance, they have been used in the production of textiles, detergents, paper, food for animals and humans for decades [7]. They can also be applied in industrial processes to eliminate the use of high temperatures, organic solvents, and extreme pH while at the same time offering increased reaction specificity, product purity, and reduced environmental impact [9]. In the last few years, research has also been focused on the potential use of these fungal enzymes to degrade lignocellulosic materials, aiming to release fermentable sugars that can be converted to second-generation ethanol by the action of fermentative microorganisms [8]. The breakdown of lignocellulose in the plant cell wall requires the recruitment of glycoside hydrolases with different mechanisms of action in a concerted action with ligninases [18]. Moreover, pretreatment using microbial enzymes for ethanol production is a promising technology due to its advantages, like being an eco-friendly

and economically viable strategy for enhancing enzymatic saccharification rates [33]. Lastly, there has been increasing interest in obtaining new, stable, and more specific enzymes using low-cost carbon sources, such as the sugarcane bagasse, and in searching for new and suitable microbial strains for large-scale cultivation that might be able to enzymes with the appropriate characteristics for biotechnological processes [27]. Therefore, the isolation of new fungal strains from contaminated plant tissue culture labs is the first step for obtaining more efficient and economically accessible enzymes and antibacterial compounds for biotechnological and industrial purposes. Thus, this study aimed to assess the enzymatic activity of some strains of filamentous fungi, using sugarcane bagasse as the sole source of carbon.

The present study screening and characterization of *α-amylase* and antimicrobial compound producing filamentous fungi is used for extensive commercial applications in various sectors and is highly demanded in the Biotech industry. The studies were carried out with different species of contaminated fungi from plant tissue culture lab to produce extracellular α-amylase, and antimicrobial compounds by the effects of sugarcane bagasse as a source of carbon were optimized under the SSF in moisture content, initial pH, incubation period and temperature.

The results reveal that out of the carbon substrates alone, sugarcane bagasse with a combination of Tween-80 was found to yield maximum *α-amylase* (151.76 IU) under solid-state fermentation by different species of fungal species such as KRV_001Chaetomium oryzae, KRV_002 *Penicillium citrinum*, KRV_003 *Aspergillus flavus*, KRV_004 *Penicillium notatum*, KRV_005 *Alternaria longipes*, and KRV_006 *Trichoderma polysporum*. Among them, KRV_001 *Chaetomium oryzae*, KRV_002 *Penicillium citrinum*, KRV_005 *Alternaria longipes* were maximum expressed *α-amylase* based on the plate assay by different pH, temperature, and different substrate using amylase activity, SDS-PAGE, Antimicrobial activity has been done in Methanol extract exhibited antibacterial activity against *S. aureus* MTCC96 whereas the water extract displayed antibacterial activity. The organism produces volatile and water-soluble antibiotics and significantly inhibits KRV_001Chaetomium oryzae alone. The thermostability and the characteristics of the enzyme suggest that this is a promising isolate that produces good quality enzymes and merits further investigations for potential applications in various biotechnological applications.

MATERIALS AND METHODS:

Production of Alpha Amylases by Solid-State Fermentation Using Cheap Substrates Used:

Sugarcane Bagasse: Production of amylase was carried out by SSF using the substrates of zero cost, namely Sugarcane bagasse. For SSF 10gm of powdered wheat bran and rice husk were taken in 250ml flasks and moistened with nearly 50ml of MSM containing the following in gm/l (0.8 g NaCl, 0.8 g KCl, 0.1 g CaCl2, 2.0 g Na_2HPO_4, 0.2g MgSO4, 0.1 g FeSO4, 8.0 g Glucose, 2.0 g NH4Cl pH 6.2). Flasks were autoclaved, cooled to room temperature, inoculated with 1ml of 48 h old grown broth culture showing maximum hydrolysis during screening, and incubated at 28°C for 5 days. Vegetable waste and peels of banana to be used as substrate were washed several times with distilled water, dried under the shed, rinsed with 0.1% H_2SO_4, cut into small pieces, and ground with the help of sterile mortar and pestle. 20 gm of the resultant pastes of vegetable waste and banana peels were transferred into a 250ml flask and moistened with MSM. Both the flasks were autoclaved, cooled to room temperature, inoculated with 1ml of 48 h old grown broth culture showing maximum hydrolysis during screening, and incubated at 28°C for 5 days **Fig. 3.**

Preparation of Inoculum and Cultivation of the contaminated plant tissue culture lab Strain: The fungus was grown in 1% PDA plates at 28-30°C for 48 h. The inocula were prepared by making hyphal discs (0.5 cm diameter). Each disc was used to inoculate 10 ml of medium [20]. The strain was cultivated in SSF (Solid State Fermentation) in 100 mL Erlenmeyer flasks, each containing 5g of Sugarcane bagasse, 20 mL Basal Medium (BM) composed of (g L-1): peptone 0.9; (NH 4)2 HPO4 0.4; KCl 0.1; MgSO4.7H 2O 0.1 and starch 0.25. (pH: 8.0). Fungal colonies were isolated contaminated plant tissue culture lab samples enriched for amylase producing microorganisms by serial dilution method wherein PDA (potato dextrose agar) media was prepared,

autoclaved, and poured in sterile Petri plates. 50µl of soil samples diluted up to 10-5 dilutions were spread on respective solidified PDA plates. The inoculated Petri plates were incubated at 28°C for 48 h. Four different fungal isolates based on physical characteristics obtained after incubation were named KRV_001Chaetomium oryzae, KRV_002 *Penicillium citrinum*, KRV_003 *Aspergillus flavus*, KRV_004 *Penicillium s*p, KRV_005 *Alternaria longipes* and KRV_006 *Trichoderma polysporum*. The isolates were further inoculated on sterile PDA plates by point inoculation and incubated at 28°C for 48 hours to obtain pure fungal plates.

Identification of Fungi: Identification of A Pictorial Guide for the Identification of Mold Fungi on Sorghum Grain (S S Navi *et al* 1999):

Indicator Staining Method: For light microscopy, Labomet 400 binocular microscope was used. Light green and indicator stain Bromocresol purple was obtained from Sigma, USA. Root samples were stained indicator staining method [17]. A drop of indicator stain (Lacto phenol containing 0.1% W/V light green & 0.05 % W/V Bromocresol purple) directly added fungal mass and observed under the microscope. Photography of stained fungal mass were mounted in PVLP iodine mounting medium (0.002 % V/v Phenol; 35% V/V Lactic acid; 0.01% W/V Iodine 60% V/v Glycerol & 0.3% V/V Ethanol) and photographed using Olympus microphotographic kit (Labmet 400 microscope, iPhone8S).

Screening of Fungal Isolates for Amylase Production: All the four fungal isolates were screened for amylase production efficiency in starch agar media comprising the following in gm L-1yeast extract 1.5, peptone 0.5, sodium chloride 1.5, starch 10, agar 15, pH 5.6. All the four isolates were streaked centrally on sterile solidified starch agar plates, a blank without inoculation was also maintained for comparison. Plates were incubated at 28°C for 48 hours; after that, all the plates along with blank were flooded with iodine and observed for a zone of hydrolysis.

Screening of Starch Hydrolysis: The isolate showing the maximum hydrolysis zone was identified based on its physical and staining / microscopic (Lactophenol cotton blue) characteristics. Study of Growth Parameters of the Isolate showing Maximum Starch Hydrolysis during Screening Growth parameters of the isolateKRV_001Chaetomium oryzae, KRV_002 *Penicillium citrinum*, KRV_003 *Aspergillus flavus*, KRV_004 *Penicillium sp*, KRV_005 *Alternaria longipes* and KRV_006 *Trichoderma polysporum* were studied in terms of growth kinetics, the effect of pH, the effect of temperature on growth.

Growth Kinetics: The growth curve of the isolated six isolates was studied to get an idea about phases of growth. For studying the growth curve 100ml PDB was prepared, divided into two flasks containing 80ml and 20ml, respectively, and carbon sources of sugarcane bagasse autoclaved. After cooling the flask containing 80 ml PDB was inoculated with a loop full of fungal isolate showing maximum hydrolysis. The inoculated flask was incubated at 22 °C at 100rmp and the uninoculated flask was stored as blank, the growth of the isolate was tracked for 7 days by reading the absorbance at 600nm against a blank. After that, a curve was plotted between days on the X-axis and OD at 600nm on Y-axis.

Optimization of Temperature: To get optimum production of Amylases by showing maximum hydrolysis during screening the temperature optimization experiment was carried out so that the same can be used during the fermentation procedure. For optimizing the temperature for the best growth of the isolate showing maximum hydrolysis during screening, 60 ml of autoclaved PDA media was poured in four sterile Petri plates and after solidification, all four plates were inoculated with the fungal isolate showing maximum hydrolysis during screening by point inoculation. Plates were incubated at 18°C, 37°C, and 55°C for 48 h and observed for the growth of the isolate.

Optimization of pH: To get optimum production of α amylases by isolate showing maximum hydrolysis during screening, the pH optimization experiment was carried out so that the same can be used during fermentation procedure wherein four flasks containing 20 ml PDB maintained at pH 5, 7.0, and 9.0 respectively were prepared and autoclaved. Before inoculation, 3ml of PDB from

each flask was transferred to sterile and labeled Eppendorf tubes and stored as blank. After that, each flask was inoculated with 10µl of 48 h grown broth, showing maximum hydrolysis during screening. The inoculated flasks were incubated at 28°C at 100rmp for 48 h. Growth in the flasks was studied by reading the absorbance at 600nm against a blank.

Extraction of Crude Enzyme: The crude enzyme was extracted from fermented media by adding 100ml of 100mM Tris buffer pH 8.0, agitating the flask in a shaker at 180rpm for 1 h; the mixture was filtered through a cheese cloth and centrifuged at 12,000 rpm at 4ºC for 15 min. The supernatant was collected and treated as a crude enzyme.

Protein Estimation in Crude Enzyme: The concentration of protein in the crude enzyme (extracted from four flasks containing different substrates) was determined by [24] of protein estimation during which enzyme was reacted with the Lowry's reagents and therefore, the absorbance obtained was compared with a typical graph plotted by reacting a typical protein with known concentrations with the Lowry's reagents and plotting a graph between the concentration of standard protein (BSA) on X-axis and absorbance at 660nm on Y-axis.

Enzyme assay in Crude Enzyme: The enzyme assay was carried out by DNS method of [12] in which 0.5ml enzyme was reacted with 0.5ml of the substrate (1% starch in 100mM Tris buffer) under standard reaction conditions, and the reaction was stopped by adding DNS reagent, amount of maltose released decided by comparing the absorbance reading of the test enzyme at 540 nm with the quality graph plotted by reacting the known concentration of maltose starting from 0.05mg/ml to 0.5mg/ml. One unit amylase activity was defined as the amount of enzyme that releases 1 micromole of maltose per minute under standard reaction conditions.

Antibacterial Activity: All the fungal methanolic extract was placed with the 5mm diameter paper disc was placed on opposite sides of the PDA plates and incubated in the dark at 25 °C. Control was set up by placing the cork bore inoculums of target bacterial strain *S. aureus* MTCC 96 only on the PDA plate **Fig. 13**. Measurements were made by marking the growth of targeting the clinical samples every day, in the presence and absence of test fungus up to 5 days. % of inhibition on growth was calculated in the following manner:

Total area of inhibition = Antibacterial activity X 100 / Total area of disc

RESULTS AND DISCUSSION:

Selection of Fungi from Contaminated Plant Tissue Culture Tubes: Six different fungal isolates were studied colony morphology and named tentatively KRV_001Chaetomium oryzae KRV_002 *Penicillium citrinum*, KRV_003 *Aspergillus flavus*, KRV_004 *Penicillium sp*, KRV_005 *Alternaria longipes,* and KRV_006 *Trichoderma polysporum* **Fig. 1.** All four isolates were subcultures by point inoculation and used for further studies.

FIG. 1: SHOWING DIFFERENT TYPES OF FUNGAL ISOLATES FROM TISSUE CULTURE LAB

Screening of Fungal Isolates for *α-Amylase* Production: All the six fungal isolates were screened by iodine solution and observed for the hydrolysis zone.

Sugarcane Bagasse: Bagasse is typically used to produce heat and electricity in sugar mills (cogeneration), but can also be used for paper making, as cattle feed, and for manufacturing disposable food containers. Currently, bagasse is mainly used as a fuel in the sugarcane industry to satisfy its energy requirements. Sugarcane bagasse is the fraction of biomass resulting from the cleaning, preparation, and extraction of sugarcane juice. Several factors influence sugarcane bagasse composition: 1. the use of fire or another method for straw removal before cutting; 2. harvesting and loading methods resulting in greater or lesser dragging of dirt *vs.* sand and vegetable residue, *i.e.*, manual, mechanical cutting, chopped cane, cutting to include the tip, *etc*.; 3. the type of soil where sugarcane was planted (latosols, sandy soils and other types of soils); and 4. Different procedures used for cleaning sugarcane. Bagasse is heterogeneous in size and particle format and regarding the three predominant components, the polymers: cellulose, hemicellulose, and lignin. **Table 1** shows bagasse potential as a source of reducing sugars from the cellulose and hemicellulose present in the bagasse **Fig. 2** and **3.**

TABLE 1: SHOWN IN COMPOSITION OF PERCENTAGE OF DRY BASE OF SUGARCANE BAGASSE

Composition (%) Dry Base	Bagasse
Glucose	19.50
Xylose	10.50
Arabinose	1.50
Galactose	0.55
Lignin	9.91
Organo solubles	2.70
Reducing sugars	1.85
Uronic acids	1.91
Ash	1.60
Moisture	50.00
Total hexoses	20.04
Total pentoses	12.00

With permission: Industrial waste recovery

***Sarita cândida* Rabelo, *Carlos eduardo* Vaz Rossel, in Sugarcane 2015:**

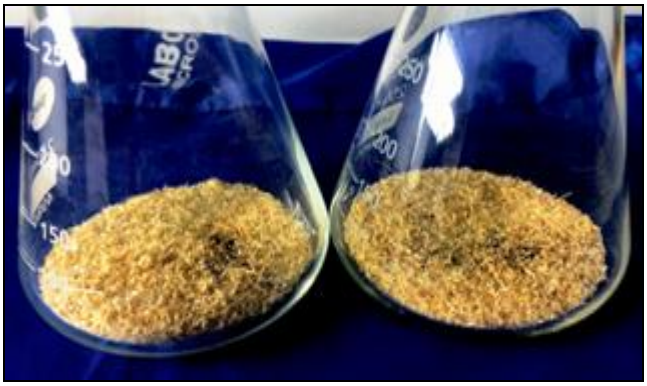

FIG. 2: SHOWING SUGARCANE BAGASSE SAMPLES IN CONICAL FLASK

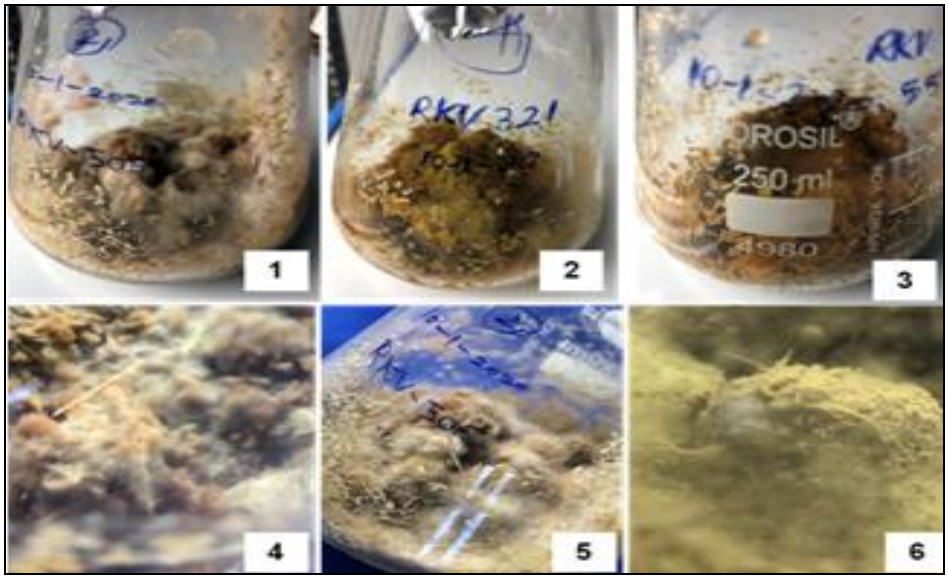

FIG. 3: SHOWING SUGARCANE BAGASSE ON FUNGAL MASS DEVELOPED IN CONICAL FLASK

Chaetomium oryzae: Colony on contaminated tissue culture flask white with the density of mycelium varying from light to dense in agar. The perithecia are found on the seed surface beneath the aerial white mycelium **Fig. 4.** Morphology Perithecia are spherical or elongate, with a pore

opening and a dark, membranous, cellular wall which is covered with conspicuous hairs of various types **Fig. 4**. Chaetomium is distributed worldwide. It has no significance in crop production. However, it is a common saprophyte and secondary invader. Seeds of low germinating capacity are sometimes found to be heavily contaminated with Chaetomium [33].

FIG. 4: SHOWN IN KRV_001 CHAETOMIUM ORYZAE (A-PLATE MORPHOLOGY AND B-E MICROSCOPIC VIEW) PENICILLIUM CITRINUM THORN

The fungus is readily recognized by its penicillin, which consists of 3-5 divergent and usually vesiculate metulae, bearing long, well-defined columns of conidia. Colonies are often dominated by copious, clear to yellow or brown exudate at the centers **Fig. 5**. Like several other *Penicillium metabolites*, citrinin produced by *P. citrinum* is known to be a potentially hazardous mycotoxin. *Citrinin causes* watery diarrhea, increased food consumption, and reduced weight gain due to kidney degeneration in chickens, ducklings, and turkeys. The effect of citrinin on humans is not documented. However, kidney damage appears to be a likely result of prolonged ingestion. *Penicillium citrinum* may well be one of the most common eukaryotic life forms on earth. It is ubiquitous in soil, decaying vegetation, and the air. It is also a powerful biodeteriogen, commonly causing decay and losses in foods, textiles, paints, and plastics [28].

FIG. 5: SHOWN IN KRV_002 PENICILLIUM CITRINUM (A-PLATE MORPHOLOGY AND B-E MICROSCOPIC VIEW)

***Aspergillus flavus*:** The incidence of *A. flavus* infection increases in the presence of insects and any type of stress on the host in the field as a result of damage. Stresses include stalk rot, drought, severe leaf damage, and/or less than ideal storage conditions. Generally, excessive moisture conditions and high temperatures of stored grains and legumes increase the occurrence of *A. flavus* aflatoxin production 1 and 2. In mammals, the pathogen can cause liver cancer through consumption of contaminated feed or aspergillosis through invasive growth **Fig. 6.**

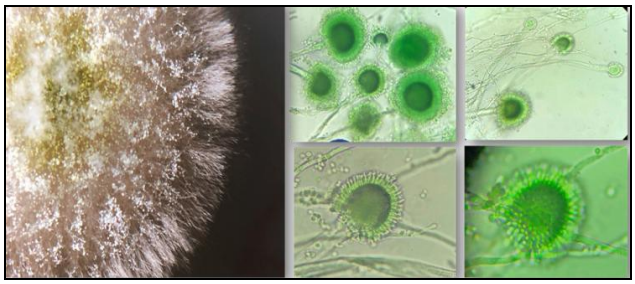

FIG. 6: SHOWN IN KRV_003 ASPERGILLUS FLAVUS (A-PLATE MORPHOLOGY AND B-E MICROSCOPIC VIEW)

***Penicillium sp*:** *Penicillium chrysogenum* or *Penicillium sp.* (formerly) is a species of fungus in the genus *Penicillium*. It is common in temperate and subtropical regions. It can be found on salted food products, but it is mostly found in indoor environments, especially in damp or water-damaged buildings **Fig. 7.**

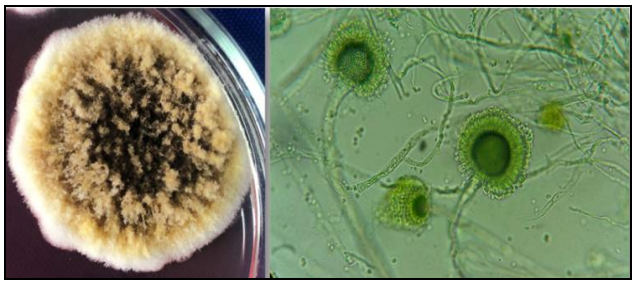

FIG. 7: SHOWN IN KRV_004 PENICILLIUM NOTATUM (A-PLATE MORPHOLOGY AND B-E MICROSCOPIC VIEW)

***Alternaria alternate*:** *Alternaria alternata* is one of the most common pathogens found in a variety of natural food products, including fruits and vegetables, cereal plants, seeds, and other plant organs, which is similar to fruits and vegetables that may be consumed as whole natural products. Although *A. alternata* has been regarded as the major mycotoxin-producing species, other species,

such as *Alternaria citri, Alternaria solani, Alternaria longipes* and *Alternaria tenuissima,* and other species may also produce the characteristic *Alternaria mycotoxins*. Various *Alternaria* species or strains can produce different *Alternaria* toxins, including alternariol, alternariol methyl ether, tenuazonic acid, and altertoxins. The production of *Alternaria mycotoxins* has been recorded in various naturally infected fruits and vegetables, such as tomatoes, apples, grapes, blueberries, oranges, lemons, mandarins, and olives [5]. *Alternaria mycotoxins* are not a major problem in strawberries, whereas Botrytis and *Rhizopus species,* the common strawberry pathogens, overgrow the slow-growing *Alternaria.* The most efficient way to cope with the mycotoxin problem in fresh produce is to prevent fungal growth by chemical, physical, or biological means or by an integrated approach **Fig. 8.**

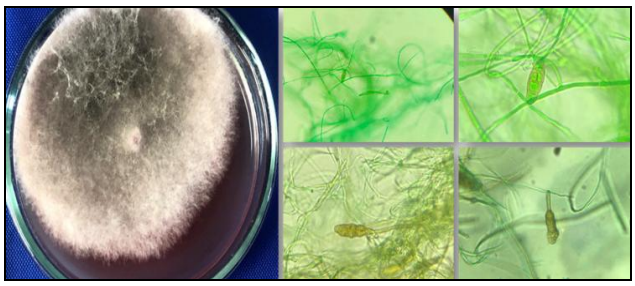

FIG. 8: SHOWN IN KRV_005 ALTERNARIA LONGIPES (A-PLATE MORPHOLOGY AND B-E MICROSCOPIC VIEW)

***Trichoderma polysporum*:** Trichoderma is a genus of fungi in the family Hypocreaceae, which is present in all soils, where they are the most prevalent culturable fungi. Many species in this genus can be characterized as opportunistic avirulent plant symbionts. **Fig. 9** refers to the ability of several *Trichoderma species* to form mutualistic endophytic relationships with several plant species.

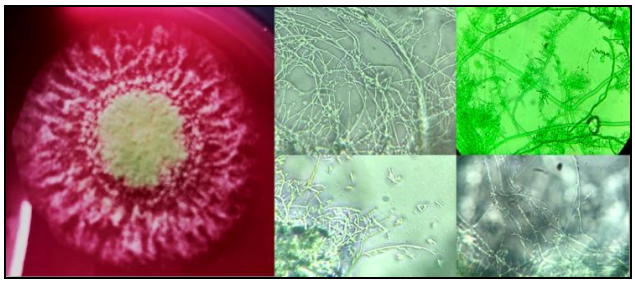

FIG. 9: FIG 4 SHOWN IN KRV_006 TRICHODERMA POLYSPORUM (A-PLATE MORPHOLOGY, AND B-E MICROSCOPIC VIEW)

Cultures are typically fast-growing at 25–30 °C, but some species of Trichoderma will grow at 45 °C. Colonies are transparent at first on media such as cornmeal dextrose agar (CMD) or white on richer media such as potato dextrose agar (PDA).

Mycelium is not typically obvious on CMD; conidia typically form within one week in compact or loose tufts in shades of green or yellow or less frequently white. A yellow pigment may be secreted into the agar, especially on PDA. Some species produce a characteristic sweet or 'coconut' odor. Conidiophores are highly branched and thus difficult to define or measure, loosely or compactly tufted, often formed in distinct concentric rings or borne along the scant aerial hyphae. The main branches of the conidiophores produce lateral side branches that may be paired or not, the longest branches distant from the tip and often phialides arising directly from the main axis near the tip. The branches may branch, with the secondary branches often paired and the longest secondary branches being closest to the main axis. All primary and secondary branches arise at or near 90° concerning the main axis. The typical Trichoderma conidiophore, with paired branches, assumes a pyramidal aspect.

Typically the conidiophore terminates in one or a few phialides. In some species (*e.g. T. polysporum*) the main branches are terminated by long, simple or branched, hooked, straight or sinuous, septate, thin-walled, sterile, or terminally fertile elongations. The main axis may be the same width as the base of the phialide or it may be much wider.

Enzyme activity = Amount of reducing sugar (X) × 1000 / dilution / Molecular weight of Glucose × Time

Protein Determination: Protein concentrations in the culture filtrate were estimated by the Bradford method [4]. Triplicates of samples were analyzed in a spectrophotometer at 595 nm. Enzymatic assays - For all assays, one unit of enzymatic activity (U) was released 1 µmoL of the corresponding product (glucose, xylose, galacturonic acid, ρ-nitrophenol, and oxidized syringaldazine), per minute under the assayed conditions. Estimation of alpha-amylase enzyme (in the liquid sample) by all the fungal isolates was determined, and the results are presented in **Table 2 Fig. 9 A-C.**

TABLE 2: EFFECT OF DIFFERENT SUBSTRATE USING ENZYME ACTIVITY (IN THE LIQUID SAMPLE) BY ALL THE FUNGAL ISOLATES

Isolate No	Name of the fungi	Enzyme activity (IU)					
		Water	Phosphate buffer pH7.0	Tris-cl pH8.0	Triton X100	Tween 20	Tween 80
KRV_001	*Chaetomium oryzae*	40.11	40.00	49.94	144.61	121.44	151.76
KRV_002	*Penicillium citrinum*	57.09	55.55	62.66	145.71	126.51	144.66
KRV_003	*Aspergillus flavus*	21.12	23.78	21.00	96.41	80.31	121.44
KRV_004	*Penicillium sp*	10.02	20.00	16.35	101.31	87.86	126.61
KRV_005	*Alternaria logipes*	68.78	54.90	69.94	144.66	131.56	151.76
KRV_006	*T. polysporum*	30.34	30.30	31.78	111.44	83.36	88.10

Estimation of α-amylase activity (U/gm) from the data in **Table 2**, it could be noticed that the highest activity was observed with *Chedomium oryzoe*, *Alternaria longipes* (151.76 U/gm) followed by *Penicillium citrinum* (144.76 U/gm). [4] found that *Aspergillus species* produce extracellular enzymes. These results are in agreement with [33]. Who mentioned that the two isolates that produced the highest concentration of amylase with higher activity were detected for mycotoxins formation. The result showed that no toxins were produced by the tested fungus, indicating safety to apply later in bread making. The fungal sources of the enzyme are regarded as safe. In liquid fermentation, the secreted proteins are released into the culture medium resulting in substrate degradation in the whole culture [15].

Effect of pH and Temperature on Activity α-Amylase Enzyme Production: The use of amylase in many industries has made a very important process to achieve optimize production with maximum yields. The effect of pH values and temperature on α-amylase production was determined and the results are shown in **Fig. 10** and **11.**

The amount of α- amylase increased significantly by increasing the pH value up to 9.0 was found in *Chaetomium oryzae* (160.56U/gm) in contrast, *Alternaria logipes* was expressed at low pH 5.0 (144.98 U/gm) Also, the results showed that increasing the temperature to 37 °C caused elevation in α- amylase production and recorded the highest value (60.66 U/gm)) and then decreased. These results are in agreement with those obtained by [35]. They reported that the production of α- amylase was observed at 50 °C and pH 6.0. As well [14] demonstrated that the maximum enzyme activity was 8.74 µ mol/mg at 40 °C and pH 7.1. Conversely, [18] reported that the production of α-amylase by *A. niger* was found to be the best at pH 5 and 30oC after 5 days incubation. Below and above this pH value, the production of α-amylase was significantly lower. Also [26] who found that Bacillus megaterium is a good producer of extracellular amylase at high temperature (60 °C) and pH 7 and also [6] who reported that the maximum production of α-amylase by the fungus *Preussia minima* was obtained at 25 °C and pH 9.

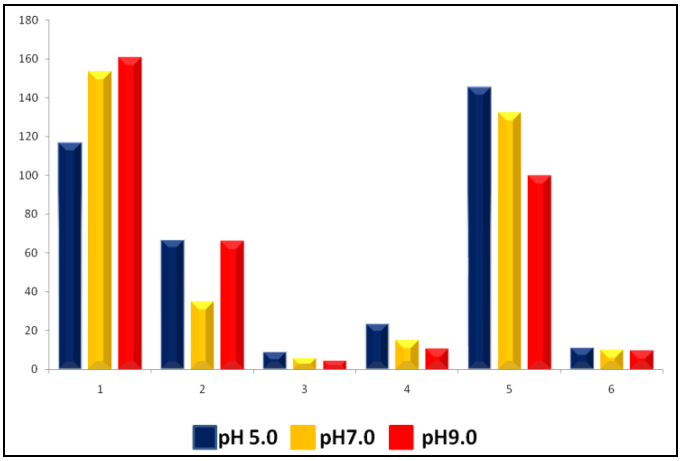

FIG. 10: SHOWN IN GRAPHICAL DATA OF DIFFERENT PH *A-AMYLASE* PRODUCING FUNGAL ISOLATES

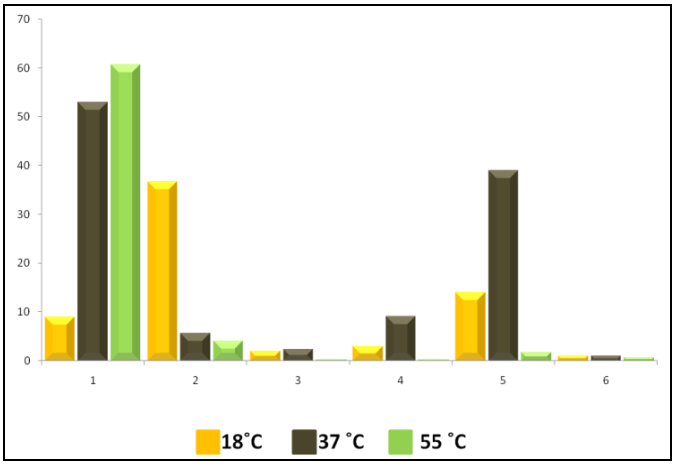

FIG. 11: SHOWN IN GRAPHICAL DATA OF DIFFERENT TEMPERATURE *A-AMYLASE* PRODUCING FUNGAL ISOLATES

Characterization of the purified *α-Amylase* Enzyme Molecular mass of the Purified *α-amylase* Enzyme: Determination of the purified α-amylase enzyme using SDS-PAGE electrophoresis with coomassie brilliant blue staining as **Fig. 12** considered not only for determining the molecular mass of the purified enzyme but also a method to indicate the enzyme purity. The molecular mass of the purified a-amylase enzyme was estimated to be about 55kDa. Similar results have been done using the technique is applicable to reveal amylase activity in a wide range of biological samples [36]. The method is not useful for enzymes sensitive to SDS and for high molecular mass amylases.

TABLE 3: SHOWS MEASUREMENT OF ANTIBACTERIAL ACTIVITY OF DIFFERENT FUNGAL ISOLATE METHANOLIC EXTRACT AGAINST *S. AUREUS* MTCC 96

Measurement	In mm			
	Area	Perimeter	Length	Radius
Sample_Positive control	74347.67	966.5818	309.760	153.8363
Sample _KRV-001	49603.78	789.5185	247.410	125.6558
Sample _KRV-002	-	-	-	-
Sample _KRV-003	-	-	-	-
Sample _KRV-004	-	-	-	-
Sample _KRV-005	-	-	-	-
Sample _KRV-006	8085.556	318.7571	97.983	50.73177

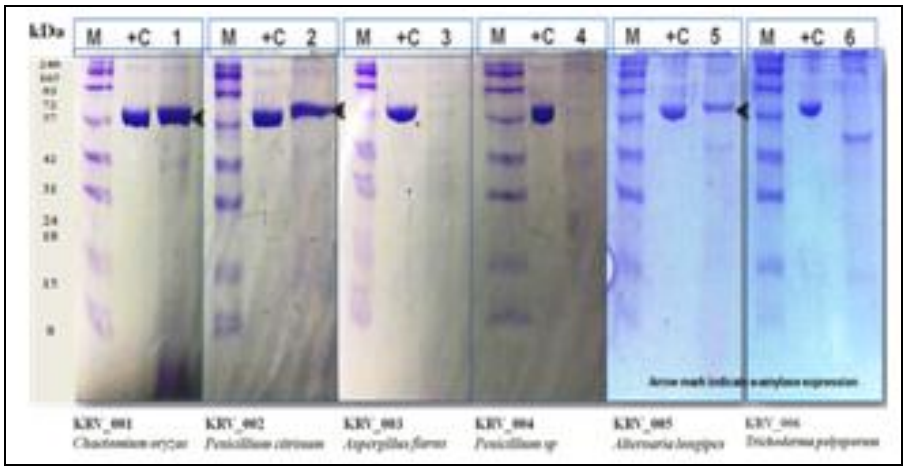

FIG. 12: SHOWN IN SDS-PAGE ANALYSIS OF α-AMYLASE PRODUCING FUNGAL ISOLATES

Our results showed that *Chaetomium oryzae*, *Penicillium citrinum, Aspergillus flavus, Penicillium sp, Alternaria longipes* and *Trichoderma polysporum*. Among them, *Chaetomium oryzae, Penicillium citrinum, Alternaria longipes*, was grown in sugarcane bagasse as the sole source of carbon, and maximum enzymatic activity was found.

This fungus showed the highest activity of α-amylase activity expressed in *Chaetomium oryzae, Penicillium citrinum,* and *Alternaria longipes* 30 days of culture, respectively. This fungal isolate was maximum activity of α-amylase at 37 °C and 55 °C low temperature compared to other studies. Therefore, *Chaetomium oryzae, Penicillium citrinum* and *Alternaria longipes* improved the enzymatic producer under the sugarcane bagasse substrate and its potentially important enzyme production.

Antibiotic Compounds: KRV_001Chaetomium oryzae has marked antimicrobial activity and little less activity of KRV_006 *Trichoderma polysporum* against *S. aureus* MTCC96, than other isolates of fungus strains. The fungi can produce alpha amylase and xylanase enzyme protease. Methanol extract exhibited antifungal activity whereas the water extract displayed antibacterial activity.

The organism produces volatile and water soluble antibiotics and shows significant inhibition in the plate **Fig. 13 Table 3**. The similar result was found in the organism produces volatile and water soluble antibiotics and shows significant inhibition on the formation of sclerotia of *Rhizoctonia solani* [29].

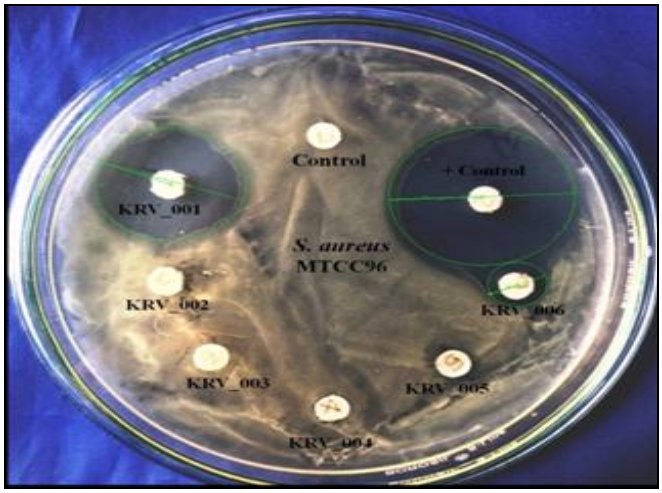

FIG. 13: SHOWS ANTIBACTERIAL ACTIVITY OF DIFFERENT TYPES OF FUNGAL EXTRACT AGAINST S.AUREUS MTCC96

Mechanism of Antibacterial Compounds: The mechanism of the antibacterial activity is presented in **Fig. 14**. The excellent antibacterial activity can be described as the presence of fungal extracellular enzymatic components present in the KRV_001 Chaetomium oryzae has marked antimicrobial activity and little less activity KRV_006 *Trichoderma polysporum*. It is well known that the + ions and as well as antibiotic function to prevent cell respiration and punch holes in the bacterial cell membrane and thus destroy the DNA and RNA inside. The reaction with + ions forms reactive oxygen, attacking and damaging the microorganisms in multiple areas [3].

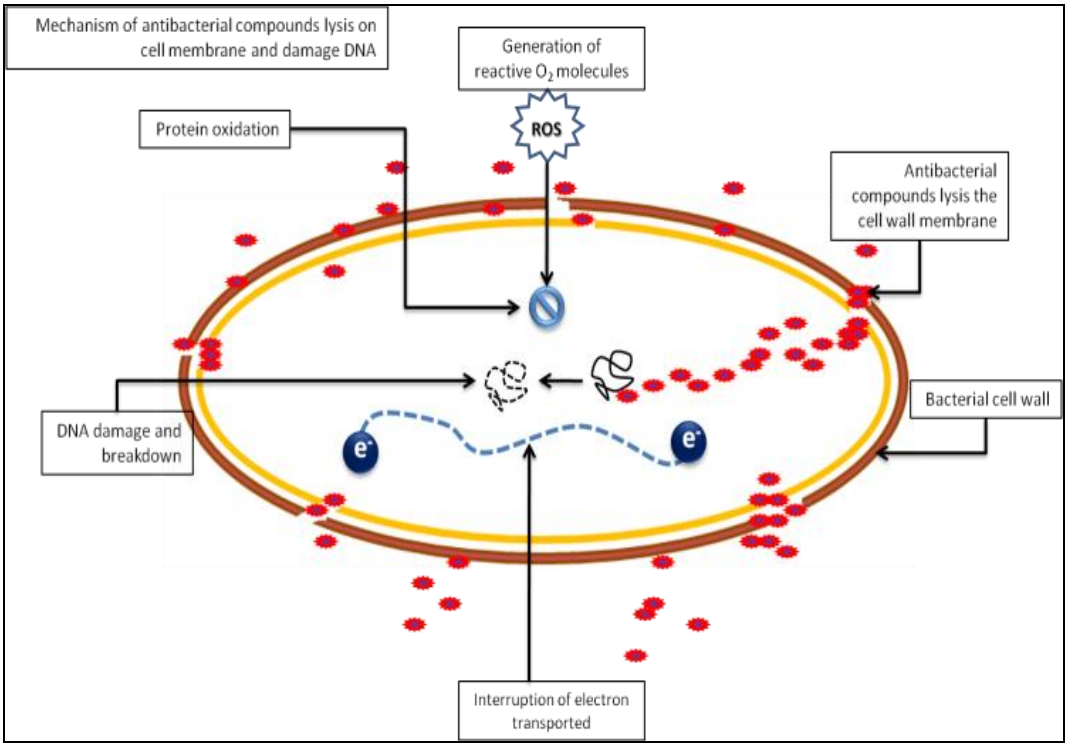

FIG. 14: SHOWS MECHANISM OF ANTIBACTERIAL ACTIVITY FUNGAL COMPOUNDS

CONCLUSION: The results obtained in the present paper reveal the potential of sugarcane bagasse as an alternative and cheap substrate for the production of a-amylase enzymes and antibacterial compounds by fungus. The different fungal species was KRV_001Chaetomium oryzae, KRV_002 *Penicillium citrinum*, KRV_003 *Aspergillus flavus*, KRV_004 *Penicillium notatum*, KRV_005 *Alternaria longipes* and KRV_006 *Trichoderma polysporum*. Among them, KRV_001 *Chaetomium oryzae*, KRV_002 *Penicillium citrinum*, KRV_005 *Alternaria longipes* were maximum expressed α-amylase based on the plate assay by different pH, temperature, and different substrate using amylase activity, SDS-PAGE, Antimicrobial activity has been done in Methanol extract exhibited antibacterial activity against S. aureus MTCC96 whereas the water extract displayed antibacterial activity. The organism produces volatile and water-soluble antibiotics and shows significant inhibition as the potential value of KRV_001Chaetomium oryzae and little less activity of KRV_006 *Trichoderma polysporum* among the isolates. From the above investigation, it

can be concluded that sugarcane bagasse is a very good substrate for the production of α-amylase enzyme and synthesizing the antibacterial compound from the substrate. This study is a pioneering work, and further study should be carried out for the development of the new drug leads for future and further study

ACKNOWLEDGMENTS: We are sincerely thankful to Vice President, Chancellor, and Vice-Chancellor, Kalasalingam University, Krishnankoil, Viruthunagar District, Srivilliputhur – 626 126 Tamil Nadu.

CONFLICTS OF INTEREST: The authors declare that they do not have any conflicts of interest.

Financial Support: This study was done by students.

Ethics Statement: None.

REFERENCES:

1. Amike S and Keller NP: *Aspergillus flavus* Annual Review of Phytopathology. 49: 107-33. PMID 21513456 DOI: 10.1146/annurev-phyto-072910-095221.
2. Abbas HK, Accinelli C, Zablotowicz RM, Abel CA and Bruns HA: Dynamics of mycotoxin andAspergillus flavuslevels in aging Bt and non-Bt corn residues under Mississippi no-till conditions. J Agric Food Chem 2008; 56: 7578-85.
3. Anthony A, Dalziel NRG and Fitzgerald RJ: Mechanisms of cell and tissue damage. Mims' Pathogenesis of Infectious Disease 2015; 171–231.
4. Bradford MM: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 1976; 72: 248-54.
5. Barkai-Golan R, Barkai-Golan, R and Paster N: *Alternaria mycotoxins* in (Eds.) Mycotoxins in fruits and vegetables. Elsevier San Diego CA USA 2008; 185-203.
6. Zaferanloo D, Quang BT, Daumoo S, Ghorbani MM, Mahon PJ and Palombo EA: Optimization of protease production by endophytic fungus, *Alternaria alternata*, isolated from an Australian native plant. World J Microbiol Biotechnol DOI 10.1007/s11274-014-1598-z.
7. Bocchini DA, Damiano VB, Gomes E & Silva R: Effect of *Bacillus circulans* D1 Thermostable Xylanase on Biobleaching of Eucalyptus Kraft Pulp. Applied Biochemistry and Biotechnology 2003; 105: 393-401.
8. Buaban B, Inoue H, Yano S, Tanapongpipat S, Ruanglek V, Champreda V, Pichyangkura R, Rengpipat S & Eurwilaichitr L: Bioethanol Production from Ball Milled Bagasse Using an OnSite Produced Fungal Enzyme Cocktail and XyloseFermenting Pichia Stipitis. Journal of Bioscience and Bioengineering 2010; 110: 18-25.
9. Cherry J. & Fidantsef AL: Directed evolution of industrial enzymes: an update. Current Opinion in Biotechnology 2003; 14: 438-443.
10. Canilha L, Chandel AK, Milessi TSS, Antunes FAF, Freitas WLC, Felipe MGA & Silva SS: Bioconversion of Sugarcane Biomass into Ethanol: An Overview about Composition, Pretreatment Methods, Detoxification of Hydrolysates, Enzymatic Saccharification, and Ethanol Fermentation. Journal of Biomedicine and Biotechnology 2012; 1-15.
11. Chatterjee AK, Chakraborty R and Basu T: Mechanism of antibacterial activity of copper nanoparticles. Nanotechnology 2014; 25: 135101.
12. Cole SW: The determination of reducing sugars by titration of ferricyanide. Biochemical Journal 1933; 27: 723–726.
13. Dibyangana Raul, Tania Biswas, Suchita Mukhopadhyay, Shrayan Kumar Das and Suvroma Gupta: Production and Partial Purification of Alpha Amylase from Bacillus subtilis (MTCC 121) Using Solid State Fermentation, 2014, Biochemistry Research International Volume 2014, Article ID 568141, 5 pages http://dx.doi.org/10.1155/2014/568141.
14. Dibyangana Raul, Tania Biswas, Suchita Mukhopadhyay, Shrayan Kumar Das and Suvroma Gupta: Production and Partial Purification of Alpha Amylase from Bacillus subtilis (MTCC 121) Using Solid State Fermentation, Biochemistry Research International Volume 2014, Article ID 568141, 5 pages http://dx.doi.org/10.1155/2014/568141
15. Ellaiah P, Adinarayana K, Pardhasaradhi S and Srinivasulu B: Isolation of alkaline protease producing bacteria from Visakhapatnam soil. Indian J of Microbi 2002; 173-175.
16. Gamez S, Gonzalez-Cabriales JJ, Ramirez JA & Garrote G: Study of the hydrolysis of sugar cane bagasse using phosphoric acid. J of Food Engineering 2006; 74: 78-88.
17. Ganga S, Ritwick EK, Snehamol Xavier, Mohamed Haroon AR and N Hariram A: Novel and simple rapid indicator staining for observation of arbuscular mycorrhizae. In Root Samples of Eleusine Coracana J Ecobiol 2014; 33(3): 161-67.
18. Gupta VK, Kubicek CP, Berrin JG, Wilson DW, Couturie M, Berlin A, Ferreira Filho EX & Ezeji T: Fungal enzymes for bio-products from sustainable and waste biomass. Trends in Biochemical Sciences 2016; 41: 633-645.
19. Hernández MS, Rodríguez MR, Guerra NP and Rosés RP: Amylase production by *Aspergillus niger* in submerged cultivation on two wastes from food industries. J Food Process Eng 2006; 73: 93–100.
20. Hagihara H, Igarashi K, Hayashi Y, Endo K and Ikawa-Kitayama K: Applied Environ. Microbi 2001; 67: 1744-50.
21. Khan JA and Priya R: A study on partial purification and characterization of extracellular amylases from *Bacillus subtilis.* Adv Appl Sci Res 2011; 2(3): 509519.
22. Knox AM, Preez, JC and Kilian SG: Starch fermentation characteristics of *Saccharomyces cerevisiae* strains transformed with amylase genes from *Lipomyces kononenkoae* and *Saccharomy copsis* fibuligera. Enzyme Microb. Technol 2004; 34(5): 453-460.
23. Karnwal A and Nigam V: Production of amylase by isolated microorganisms and its application. Int J Pharm Bio Sci 2013; 3(4): 354-60.
24. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ: Protein Measurement with the Folin Phenol Reagent. J Biol Chem 1951; 193: 265-75.
25. Martins DAB, Prado HFA, Leite RSR, Ferreira H, Moretti, MMS, Silva R & Gomes E: Agroindustrial wastes as substrates for microbial enzymes production and source of sugar for bioethanol production. Ed Integrated Waste Management 2011; 319-60.

26. Oyeleke S B, Auta SH and Egwim EC: Production and characterization of amylase produced by Bacillus megaterium isolated from a local yam peel dumpsite in Minna, Niger State Journal of Microbiology and Antimicrobials 2010; 88-92.
27. Parameswaran B, Nigam PSN, P, Pandey A: Sugarcane Bagasse. In Ed Biotechnology for Agroindustrial Residues Utilisation-Utilisation of Agroresidues Springer 2009; 239-52.
28. Pitt J: A Laboratory Guide to Common Penicillium Species. Commonwealth Scientific and Industrial Research Organization, Food Research Laboratory NSW Australia 1991.
29. Ranganathan Kapilan and Vethanayagam Celesty Anpalagan: Antimicrobial activity and amylase production by Rhizophus microsporus strain isolated from germinating maize seed. Asian Journal of Plant Science and Research 2015; 5(10): 1-8.
30. Sivaramakrishnan S, Gangadharan D, Nampoothiri KM, Soccol CR and Pandey A: Alpha amylases from microbial sources. An overview on recent developments. Food Technol Biotechnol 2006; 44: 173-84.
31. Sanghvi GV, Koyani RD and Rajput KS: Isolation, optimization and partial purification of amylase from Chrysosporium asperatum by submerged fermentation. J Microbiol Biotechnol 2011; 21: 470-476.
32. Skolko J & Groves JW: Notes on seed-borne fungi VII. Chaetomium. Can J Bot 1953; 31: 779-809.
33. Sindhu R, Binod P and Pandey A: Biological pretreatment of lignocellulosic biomass - an overview. Bioresource Technology 2016; 199: 76-82.
34. Skolko AJ and JW: Groves, Notes On Seed-Borne Fungi: Vii. Chaetomium. Canadian Journal of Botany November 1953 https://doi.org/10.1139/b53-060
35. Saxena R and Singh R: Amylase production by solid-state fermentation of agro-industrial wastes using Bacillus sp. Brazilian Journal of Microbiology 2011; 42(4): 1334-32.
36. Martinez TF, Alarcon FJ, Diaz-Lopez M and Moyano JF: Improved detection of amylase activity by sodium dodecyl sulfate-polyacrylamide gel electrophoresis with copolymerized starch. Electrophoresis 2000; 21: 2940-43.
37. Tsukagoshi N, Kobayashi T & Kato M: Regulation of the amylolytic and (hemi) cellulolytic genes in aspergilli. The J of General and Applied Microbiology 2001; 47: 1-19.
38. Tallapragada P and Venkatesh K: Isolation, identification and optimization of xylanase enzyme produced by Aspergillus niger under submerged fermentation. J of Microbiology and Biotechnology Res 2011; 1: 137-47.

How to cite this article:
Kalaiyarasan A, Roobamathy S, Vijayaraghavan B, Sundaram RL and Hariram N: Effect of Sugarcane bagasse as a source of carbon for *α-amylase* enzyme and antibacterial compound producing different types of wild Filamentous Fungi. Int J Pharm Sci & Res 2022; 13(4): 1695-07. doi: 10.13040/IJPSR.0975-8232.13(4).1695-07.