WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 13, Issue 16, 729-753. Research Article ISSN 2277–7105

# STUDY OF ORGANISM PRODUCING BETA-GLUCAN AND LOOKING FOR ITS PREBIOTIC POTENTIAL

**Rutuja P. Gore and Savanta V. Raut***

Department of Microbiology, Bhavan's College, Munshi Nagar, Andheri (W), Mumbai-400058.

Article Received on
22 June 2024,
Revised on 12 July 2024,
Accepted on 02 August 2024
DOI: 10.20959/wjpr202416-33495

***Corresponding Author**
**Dr. Savanta V. Raut**
Department of Microbiology, Bhavan's College, Munshi Nagar, Andheri (W), Mumbai-400058.

## ABSTRACT

Research in food science focuses on understanding the microbiota's health benefits, particularly the role of dietary fibers like β-glucan in shaping gut microbiota. Its production involves complex enzymatic reactions in fungi and metabolic processes in bacteria. Extraction and quantification methods vary, with hot water extraction and acid base extraction is common. This study aims to isolate β-glucan-producing strains like *Saccharomyces cerevisiae*, *Aspergillus niger*, and any Bacterium, evaluate their growth in synthetic and crude media, and assess their prebiotic potential using *Lactobacillus rhamnosus* as control standard. Results contribute to understanding β-glucan's suitability as a prebiotic for promoting human health. Various growth parameters such as growth curve, pH change, and glucose levels were monitored to assess β-glucan production. *A. niger* exhibited the highest production of β-glucan, followed by *S. cerevisiae* and the soil isolate. Different crude media were tested for optimizing β-glucan production, with the mixed broth showing the most promising results. The soil bacterium on subjected to Vitek revealed that the organism is *Bacillus cereus.* The extracted β-glucan was tested for its prebiotic potential against *Lactobacillus rhamnosus*, with *A. niger*-derived β-glucan showing the highest prebiotic index i.e 2.8 compared to others have prebiotic index of less than 1. Additionally, the prebiotic activity score was calculated, with *A. niger*-derived β-glucan displaying a score1.21, indicating significant growth stimulation of probiotic bacteria. The study successfully isolated organisms capable of producing β-glucan, with *Aspergillus niger* showing the most promising results. Through rigorous experimentation in synthetic and crude media, *A. niger* consistently demonstrated robust growth and β-glucan production. Mixed

broth media emerged as particularly effective in enhancing β-glucan synthesis, suggesting the importance of media optimization for maximizing bioactive compound production. The study lays the groundwork for further research into harnessing the full potential of *A. niger*-derived β-glucan for various industrial, biomedical, and nutritional applications.

**KEYWORDS:** *Beta-glucan, Aspergillus niger, Saccharomyces cerevisiae, Bacillus cereus, Synthetic media, Crude media.*

## 1. INTRODUCTION

In the current decade, research in the field of food science pays attention to the study microbiota and their health benefits. There are as many as 10 times microorganisms within the gastrointestinal tract of humans. Diet plays an important role in maintaining and shaping the composition and activity of this microbiota. The diet should involve non-digestible long chain complex carbohydrates components particularly dietary fibers in addition to the necessary nutrients including vitamins, protiens, minerals and lipids, as it can strongly influence the composition of colonic microbiota and their metabolic products. Beta-glucan is considered as the common component of the human diet because of its positive health effects such as anti-tumour, anti-digestibility, immune modulating properties (Bin Du et al 2013). β-glucan is the principle fiber in barley, oat and is mainly found in microorganisms.

Beta-glucan is a non-starch polysaccharide composed of linear chain of β-D-glucose linked by β-(1→3), (1→4) and /or (1→6)-D-glycosidic linkage, predominantly consisting of over 25,000 D-glucose units which are either branched or unbranched. Beta-glucan has reported as a potential prebiotic mainly promoting the growth of beneficial intestinal microorganisms such as *Bifidobacterium* and *Lactobacilli* (Evdokia K et al 2009). Beta-glucan are of different conformational complexity from single helix to the triple helix and random coils. It seems to be the main active ingredient in barley. Owning to its importance Food and Drug Administration (FDA) allowed its incorporation in food products for improving human health.

Beta-glucan are of two types i.e water soluble and water insoluble. Salecan (β-glucan produced by *Agrobacterium sp.ZX09*) is water soluble whereas Curdlan is a water insoluble glucan. The solubility of glucan increases with temperature. Protein-bound glucans are insoluble. It is stated as the Generally Recognized as Safe (GRAS) category by the Food Drug and Administration it have no toxicity and side effects (Gemilang et al). FDA evaluated

several studies for the consumption of beta-glucan, on the basis of these studies a daily dose of 3g of beta-glucan from oat was recommended to decrease cholesterol levels (FDA, 1996; 1997).

Activity of β-glucan is influenced by conformation and molecular weight which affects the charecteristics, therefore β-glucan obtained from each source is different. Beta-glucan are generally found in the cell walls of some higher plants and also in some cereals like barley and oats. β -1,3-D-glucan and β-1,6-D-glucan are produced by molds and yeasts (Bhagavati et al). Cell wall of *Saccharomyces cerevisiae* is most abundant in β-glucan. This β-glucan is known to have various applications which include reduction in infection (Richard Fuller et al 2017), cancer inhibition. On the other hand cell walls of moulds belonging to the genus *Aspergillus* can also be used for β-glucan production species included are *A. oryzae.* In addition to this, bacteria can also synthesize β-glucan by producing extracellular products and polysaccharides (Gemilang Lara et al 2021). Bacteria like *Bacillus subtilis, B. natto* also has the potential to produce β-glucan. β-1,3-glucan from fungi are produced by complex enzymatic reactions (Douglas et al ). Fungi use glucose as a substrate which enters through active transport inside the fungal cells and starts to synthesize polysaccharides and produce β-glucan. Bacteria producing β-glucan are called as curdlan. Its production requires three steps which involve substrate absorption, metabolism and polymerization (Zhan X-B, et al). The substrate i.e glucose enters in cell cytoplasm via active transport, in substrate metabolism stage catabolism forms primary metabolites and precursors for the synthesis of Extracellular polymeric substrate (EPS). Phosphorylation with hexokinase starts glucose glycolysis with the release of ATP to form Glucose-6-phosphate which gets converted to Glucose-1-phosphate. The key precursor (UDP-glucose) then formed through the catalyst of UDP-glucose phosphorylase from UTP. Glucosyl-1-Phosphate from UDP-glucose containing D-glucose which is bound to lipid precursors (isoprenoid-lipid-phosphate) releases UDP which initiates polymerization of β-(1,3)-glycosidic bonds and stripped polymers from UTP by utilizing ATP from the tricarboxylic acid cycle or glycolysis. The cyclic process continues and synthesizes curdlan. Curdlan seems to have beneficial effects on the gut microbiome, show anti-tumour activity and also boost the immune system.

Extraction of β-glucan include a range of extraction processes and purification techniques which generally involves hot water extraction (Smiderle et al 2006), solvent extraction (Bhatty 1993), enzymatic extraction (Irakli et al 2004) and alkali extraction (Wei, et al 2006)

In humans the G.I tract shows the presence of trillions of organisms, various research was carried out which showed critical role of gut microbiota like immune maturation and homeostasis, providing energy biogenesis, preventing against pathogen overgrowth and biosynthesizing vitamins and neurotransmitters. The gut microiota may also lead to several diseases such as cardiovascular diseases, diabetes, inflammatory bowel disease. Investigations suggest that polysaccharides are expected to be used as potential prebiotics for improving gut health because of their good fermentability properties and can stimulate various beneficial gut microbiota.

## 2. MATERIALS AND METHODS

The study was carried out in the Department of Microbiology, Bhavan's College, Andheri (W), Mumbai, India. Three isolates were selected for the production of β-glucan in synthetic media and crude media. The study was conducted in January 2024 to March 2024.

### 2.1.Isolation of β-glucan producing strains

The study involves the use of *Saccharomyces cerevisiae*, *Aspergillus niger*, and *Bacillus subtilis.* Since *Bacillus subtilis* was not available therefore bacterium was isolated from soil. The working cultures were prepared by streaking loop full of stock culture from slants onto Potato dextrose agar for *A. niger* and *S. cerevisiae* while Nutrient agar used for the isolation of a bacterium and incubated for 48hr at room temperature. Note down the colony's characteristic of soil isolate

### 2.2.Production of β-glucan from isolated strain

Prepare a suspension of S. cerevisiae and Soil Bacterium containing $10^6$ cells/ml and a spore suspension of *A. niger* containing $10^6$ spores/ml count the spores using a Haemocytometer slide. Prepare Yeast extract glucose broth containing 5.2 g/l $K_2HPO_4$, 15 g/l glucose, 0.5 g/l yeast extract, 0.12 g/l $MgSO_4$, 0.54 g/l $NH_4Cl$ distribute into 3 flask each containing 100 ml of broth. Inoculate 1ml from each suspension in each flask and incubate for 96hr at 30 ℃ at an agitation speed of 200 rpm. 30 ℃ was used for incubation in order to avoid the effect of temperature on β-glucan yield. Measure the growth of each microorganism by using following parameters.

- **Growth of microorganisms**

From each growth media optical density of the cultures was measured by collecting 3ml of growth media. The optical density indicates the number of cell population (Geming Lara, et al) this is analyzed at an absorbance at 600 nm, evaluated at 0, 24, 48, 72 and 96 hr.

- **pH change**

Evaluation of pH of growth medium analyzed after every 24 hr starting from 0 h to 24, 48, 72 and 96 hr by collecting 2 ml of sample from each growth media in a beaker and measuring pH using calibrated pH meter.

- **Estimation of sugar level**

Presence or absence of the growth of microorganism is evaluated by measuring glucose levels after every 24 hr of incubation starting from 0 hr to 24, 48, 72 and 96 hr by using Dinitrosalicylic acid method. (Geming Lara, et al).

**2.3.Extraction and Quantification of β-glucan from isolates grown in synthetic media**

- **Extraction (Chaiyasut *et al* 2016)**

Incubated cell biomass was collected by centrifuging at 7500 rpm for 10 min at 4 ℃. Cells autolysed by adding 15 % of cell biomass in distilled water at pH 5.0 adjusted by using 1M HCL, incubated for 48 hr at 50 ℃ at an agitation speed of 120 rpm followed by additional incubation step for 15 min at 80 ℃ in a water bath. Centrifuge the samples at a speed of 7500 rpm at 4 ℃ for 10 min, dry the obtained pellets in hot air oven at 60 ℃. Mix the autolysed cells with 5ml of 1M NaOH and incubate for 2 hr at 80 ℃ with a stirrer. Subsequently centrifuge the cells at speed of 6000 g at 4 ℃ for 25 min. Dissolve the obtained pellet in 5 ml of 1M $CH_3COOH$ re-incubate at 80 ℃ with stirrer for 2 hr, centrifuge the pellet at a speed of 6000 g for 25 min at 4 ℃, air dry pellet. Mash the obtain samples using mortar till fine powder obtained at -20 ℃.

- **Quantification (Freimund *et al* 2003)**

Take dried weighed β-glucan (20 mg) into a glass tube. Add 3 ml of aqueous Trifluoroacetic acid 70% and seal of the tube, the mixture was stirred vigorously at room temperature until a homogenous suspension or solution was achieved. The tube was placed in a water bath for 3 hr and stirred. After the end of the hydrolysis time, the mixture was cooled to room temperature and completely transferred to a suitable container in which it was neutralised by addition of 1 N NaOH. Subsequently, the mixture was filtered through paper and an aliquot of the filtrate was taken for the determination of the monosaccharides.

Monosaccharides are determined using Dinitrosalicylic acid test (DNSA) and Glucose oxidase / Peroxidase test.

### 2.4.Optimizing the production of β-glucan using different crude media

- **Fruit pulp media**

For fruit pulp media, the fruit pulp was collected from local juice centre (Kanifnath Rasvanti Gruh). The pulp should consist of orange, pineapple, pomegranate, papaya etc in order to get greater amount of sugar. Sun dry the pulp for 4-5 days and make a powder. Add 10gm of powder/ 100ml of tap water, keep this overnight and filter the water using filter paper. Distribute in 3 flask each having a volume of 100ml.

In this add 0.1 gm ammonium sulphate and sterilize it by autoclaving. Inoculate 1% (v/v) each isolated sample suspension as done for synthetic media perform same steps and measure growth of each isolate after every 24 hr till 96 hr.

- **Millet powder broth**

For millet powder media, two millets namely Ragi (Finger Millet) and Jowar (Sorghum Millet) in equal amount were grounded in electric grinder till coarse powder is obtained. Add 10gm of powder/ 100ml of tap water, keep this overnight and filter the water using filter paper. Distribute in 3 flask each having a volume of 100ml to this add 0.1 gm ammonium sulphate and sterilize it by autoclaving. Inoculate each isolated sample suspension as done for synthetic media perform same steps and measure growth of each isolate after every 24 hr till 96 hr.

- **Mixed Broth (Fruit Pulp Broth+ Millet Powder Broth)**

For mixed broth take 5 gm of fruit pulp powder and 5 gm of millet powder / 100 ml of tap water, keep this overnight and filter the water using filter paper. Distribute in 3 flask each having a volume of 100ml to this add 0.1 gm ammonium sulphate and sterilize it by autoclaving. Inoculate each isolated sample suspension as done for synthetic media perform same steps and measure growth of each isolate after every 24 hr till 96 hr.

### 2.5.Determining Prebiotic potential of β-glucan extracted from each isolate (Palframan *et al* 2003)

- **Bacterial strains isolation**

One probiotic strains selected namely *Lactobacillus rhamnosus* (obtained from Bhavans Research Centre) was loop full containing $10^6$ cells/ml isolated on De Man-Rogosa-Sharpe (MRS) agar. The strains was stored at 4°C in MRS agar (Palframan *et al* 2003).

- **Growth kinetics of isolated microorganisms**

Microorganisms were cultivated in 10 mL culture medium at anaerobic condition for 24 h, with a shake rate of 50 rpm. Medium was inoculated with $10^6$ colony-forming units (CFU) of strain of lactobacilli (inoculated with 1% v/v of an overnight culture obtained from a single colony). The culture medium contained: 10 g/L, either lactose, used as control, one of the studied prebiotics (β-glucan from each isolate); 1 g/L, yeast extract; 5 g/L, casein peptone (Figueroa-Gonzalez et al 2019). Growth was measured at 0 h and 24 h, by absorbance at 650 nm with a colorimeter and the number of colony-forming units was determined by counting them in MRS agar plates performing Viable Count technique i.e spread plate incubating at anaerobic condition for 48 h.

- **Prebiotic index**

Prebiotic index (Ipreb) was calculated according to (Palframan et al 2003); it is the ratio of probiotic growth in the prebiotic to probiotic growth in a control carbohydrate. A prebiotic index higher than 1 means that the carbohydrate has a positive effect on the probiotic growth. If the prebiotic index is near to 1, indicates a low effectiveness of the evaluated carbohydrate.

The prebiotic index was calculated according to equation

Formula is as follows;

$$I_{preb} = \frac{\text{CFU of Probiotic in Prebiotic Carbohydrate}}{\text{CFU of Probiotic in Control Carbohydrate}}$$

**Prebiotic activity score**

Prebiotic activity scores (Apreb) were determined using the equation

$$Apreb = (LogP_{24} - LogP_0)_{prebiotic} / (LogP_{24} - LogP_0)_{lactose} - (LogE_{24} - LogE_0)_{prebiotic} / (LogE_{24} - LogE_0)_{lactose}$$

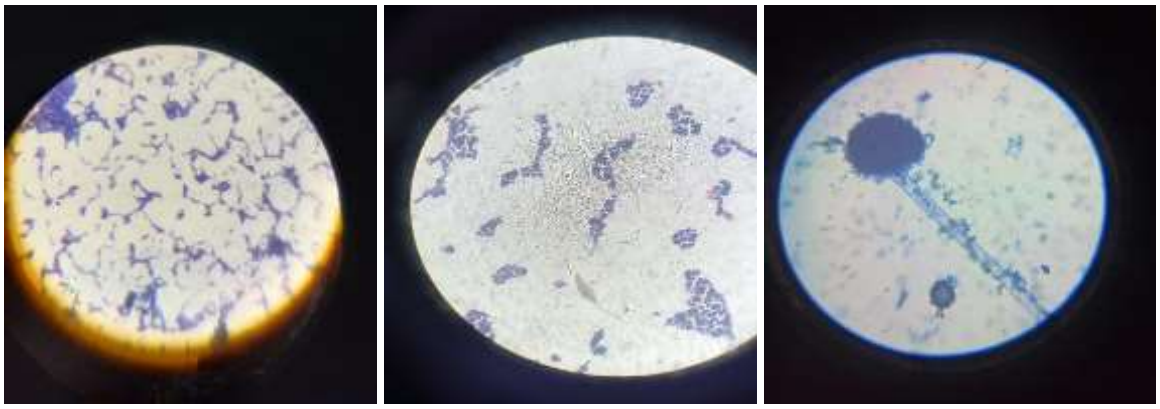
Where, Apreb is the prebiotic activity score;

Log P are the log of growth (CFU/mL) of the probiotic bacteria at 24 h (P24) and 0 h (P0) of culture on prebiotic and lactose.

Log E are the log of growth (CFU/mL) of *E. coli* at 24 h (E24) and 0 h (E0) of culture on prebiotic and lactose.

## 3. RESULTS AND DISCUSSION

In this study, we isolated different organisms. Based on literature review three organisms were selected for production of β-glucan. Out of this three organism two of which were known namely *Aspergillus niger* and *Saccharomyces cerevisiae* and one was isolated from soil (unkown). Two of which organism were isolated on Potato dextrose agar, whereas organism obtained from soil was isolated on Nutrient agar. All of the isolates were tested for production in different media. Growth of organism were checked using different parameters namely Growth curve, Change in glucose level and Change in pH, followed by extraction of produced β-glucan later on the production and quantification was done by performing Acid hydrolysis method. Further on the isolated β-glucan was tested for Prebiotic Potential.



**a) Soil Isolate.** **b) *Saccharomyces cerevisiae.*** **c) *Aspergillus niger.***

**Fig. 3.1: Gram staining of a) Soil Ioslate, b) *Saccharomyces cerevisiae* and Lactophenol cotton blue staining of c) *Aspergillus niger.***

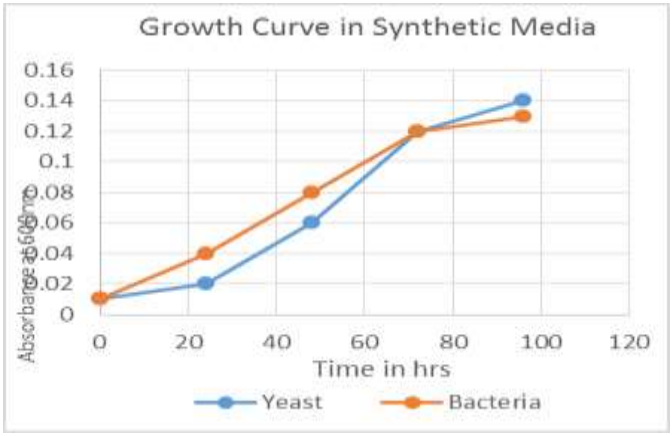



**Fig. 3.2: Growth curve of *S. Cerevisiae* and Isolate in synthetic media.**

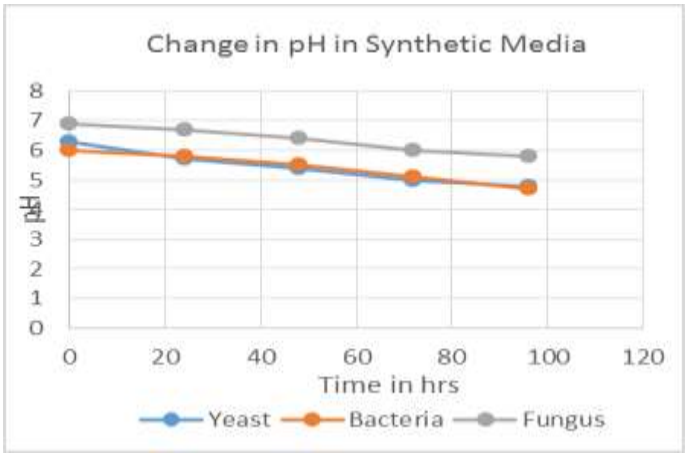



**Fig. 3.3: pH changes in synthetic media Soil.**

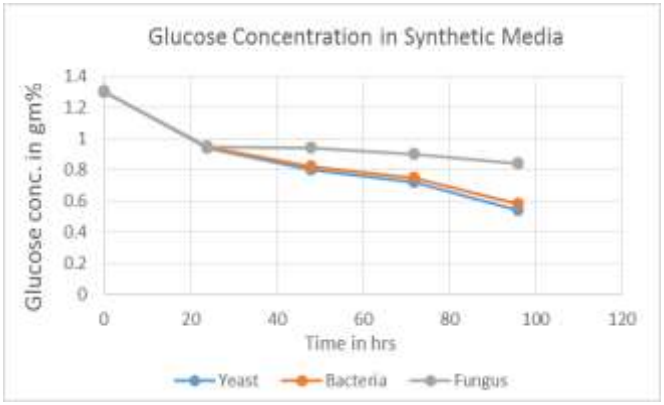



**Fig. 3.4: Change in glucose level in synthetic media.**

**Table 3.1: Microorganism with their cell mass weight in synthetic media.**

| Isolates | Wet weight (gm / 100ml) | Dry weight (gm / 100ml) |
|---|---|---|
| *S. cerevisiae* | 6.1 gm | 0.4 gm |
| *A. niger* | 1.7 gm | 0.62 gm |
| Soil Isolate | 0.68 gm | 0.06 gm |


**Fig. 3.5: Extracted β-glucan from *A. niger, S. cerevisiae* and Soil Isolate.**

**Table 3.2: Percentage of Reducing sugar per 100 gm of Cell Biomass in Synthetic Broth.**

| Isolates | Graphical conc (ug/ml) | Percentage of Reducing sugars |
|---|---|---|

| | | per 100 gm of cell biomass |
|---|---|---|
| *A. niger* | 833 ug/ml | 0.12 % |
| *S. cerevisiae* | 333 ug/ml | 0.08 % |
| Soil Isolate | 133 ug/ml | 0. 16 % |

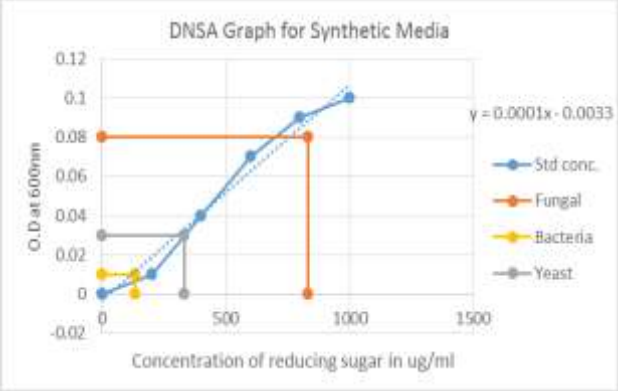



**Fig. 3.6: Content of reducing sugars in extracted β-glucan from synthetic media.**

**Table 3.3: Beta-Glucan yield from microbial cultures in synthetic broth media.**

| Isolates | Beta-Glucan Yield (mg %) |
|---|---|
| *A. niger* | 45.20 mg% |
| *S. cerevisiae* | 32 mg% |
| Soil Isolate | 24.5 mg% |

**Fig. 3.7: Crude media from dried fruit pulp.**

**Fig. 3.8: Crude media from millet powder.**

**Fig. 3.9: Mixed Broth (Fruit Pulp + Millet Powder).**

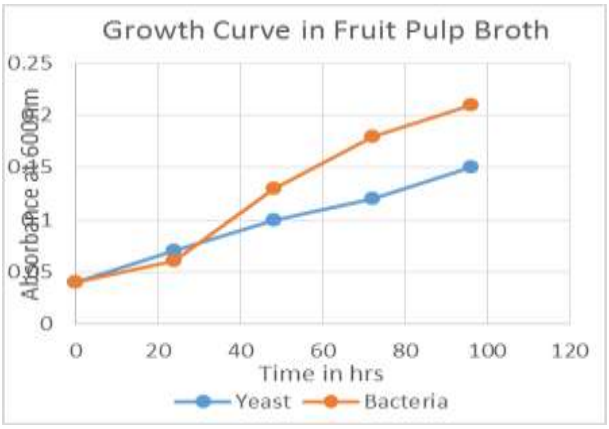



**Fig. 3.10: Growth curve in fruit pulp media.**

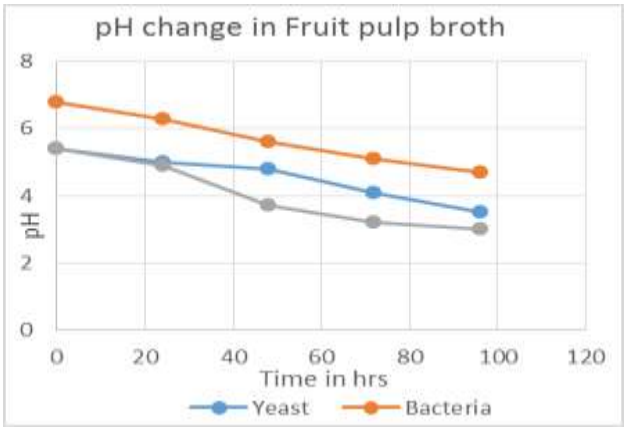



**Fig. 3.11: pH change in Fruit Pulp media.**

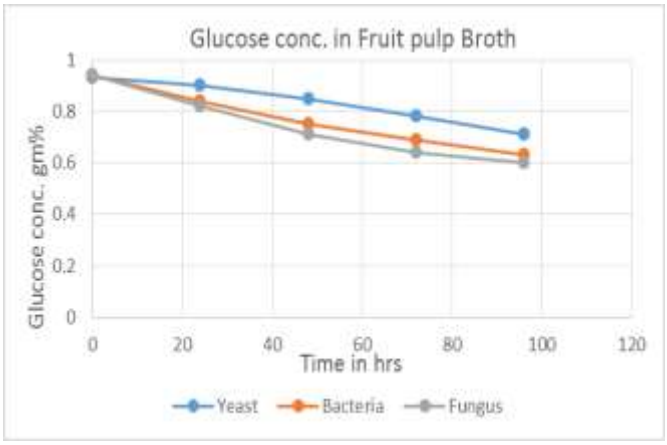



**Fig. 3.12: Change in glucose level in Fruit Pulp media.**

**Table 3.4*:* Microorganism with their cell mass weight in Fruit Pulp Broth.**

| Isolates | Wet weight (gm / 100ml) | Dry weight (gm / 100ml) |
|---|---|---|
| *S. cerevisiae* | 2.5 gm | 0.38gm |
| *A. niger* | 8.42 gm | 0.8 gm |
| Soil Isolate | 2.4 gm | 0.25 gm |

**Table 3.5: Percentage of Reducing sugar per 100 gm of Cell Biomass in Fruit Pulp Broth.**

| Isolates | Graphical conc (ug/ml) | Percentage of Reducing sugars per 100 gm of cell biomass |
|---|---|---|
| *A. niger* | 733 ug/ml | 0.08 % |
| *S. cerevisiae* | 433 ug/ml | 0.1 % |
| Soil Isolate | 233 ug/ml | 0. 06 % |

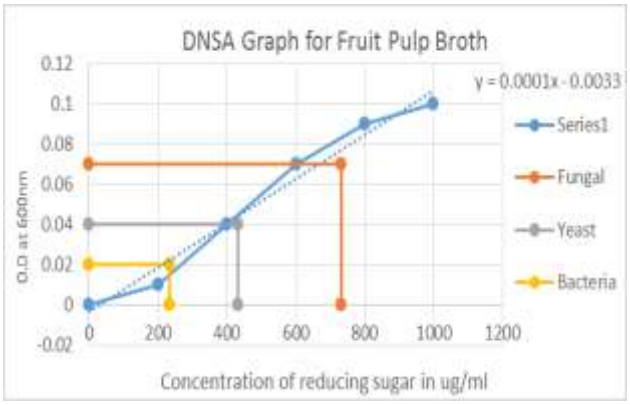



**Fig. 3.13: Content of reducing sugar in extracted β-glucan from Fruit Pulp Media.**

**Table 3.6: Beta-Glucan yield from microbial cultures in Fruit Pulp Broth.**

| Isolates | Beta-Glucan Yield (mg %) |
|---|---|
| *A. niger* | 45.20 mg% |
| *S. cerevisiae* | 26.4 mg% |
| Soil Bacterium | 16.9 mg% |

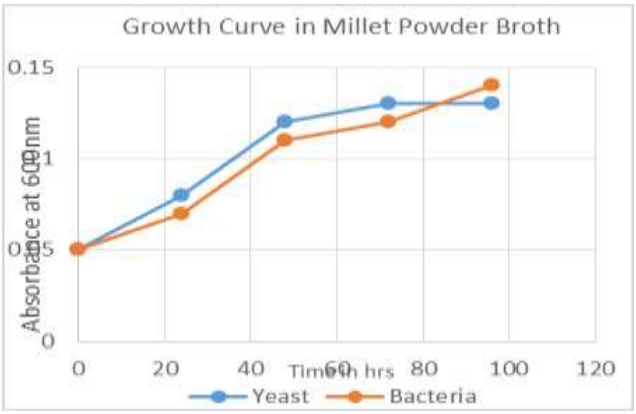



**Fig. 3.14: Growth curve in millet broth.**

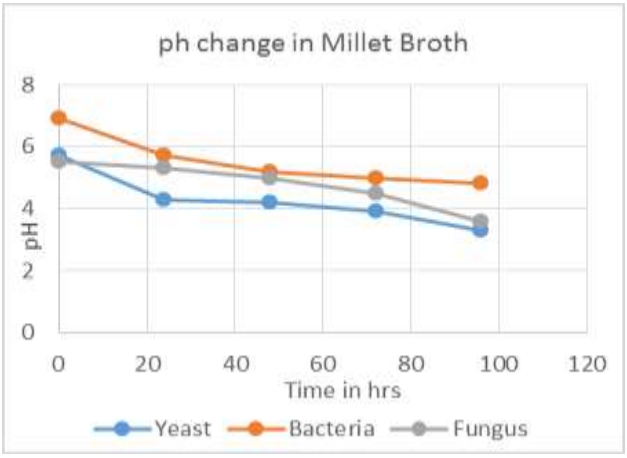



**Fig. 3.15: pH change in Millet Broth.**

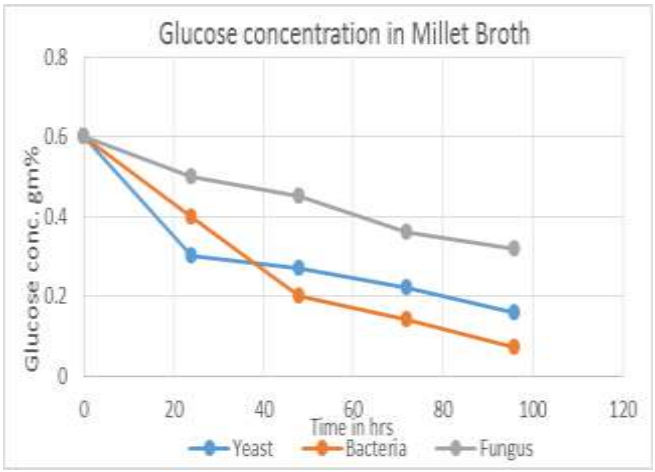



**Fig. 3.16: Change in glucose level in Millet Broth.**

**Table 3.7: Microorganism with their cell mass weight in Millet Broth.**

| Isolate | Wet weight (gm / 100ml) | Dry weight (gm / 100ml) |
|---|---|---|
| *S. cerevisiae* | 0.72 gm | 0.2gm |
| *A. niger* | 1.53gm | 0.08 gm |
| Soil Isolate | 0.47gm | 0.88 gm |

**Table 3.8: Percentage of Reducing sugar per 100 gm of Cell Biomass in Millet Broth.**

| Microbial culture | Graphical conc (ug/ml) | Percentage of Reducing sugars per 100 gm of cell biomass |
|---|---|---|
| Fungal | 1335 ug/ml | 1.6 % |
| Yeast | 2165 ug/ml | 1.0 % |
| Bacteria | 1165 ug/ml | 0.13% |

**Table 3.9: Beta-Glucan yield from microbial cultures in Millet Powder Broth.**

| Microbial cultures | Beta-Glucan Yield (mg %) |
|---|---|
| *A. niger* | 28.30 mg% |
| *S. cerevisiae* | 16.9 mg% |
| Soil Bacterium | 23 mg% |

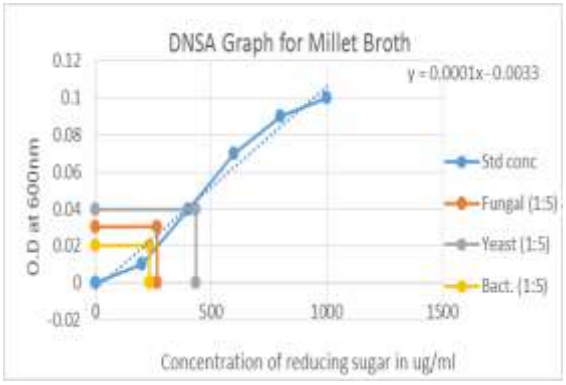



**Fig. 3.17: Content of reducing sugar in extracted β-glucan from Millet Broth.**

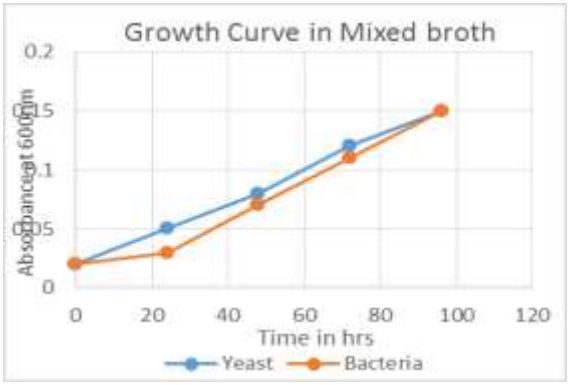



**Fig. 3.18: Growth curve in Mixed Broth.**

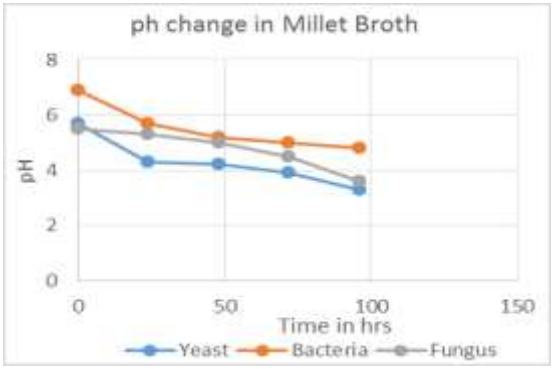



**Fig. 3.19: pH change in Mixed Broth.**

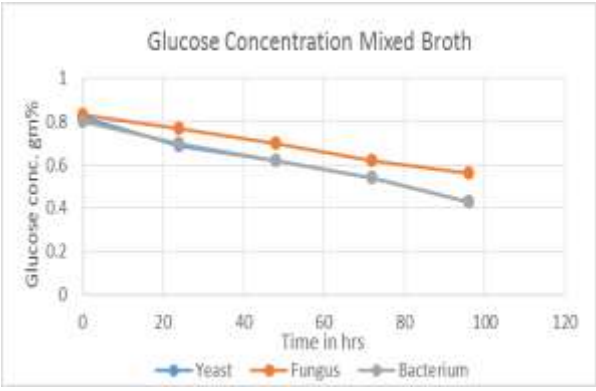



**Fig. 3.20: Change in glucose level in Mixed Broth.**

**Table 3.10: Microorganism with their cell mass weight in Mixed Broth.**

| Isolate | Wet weight (gm / 100ml) | Dry weight (gm / 100ml) |
|---|---|---|
| *S. cerevisiae* | 1.4 gm | 0.4gm |
| *A. niger* | 8.0 gm | 0.8 gm |
| Soil Isolate | 1.8 gm | 0.3 gm |

**Table 3.11: Percentage of Reducing sugar per 100 gm of Cell Biomass from Mixed Broth.**

| Isolate | Graphical conc (ug/ml) | Percentage of Reducing sugars per 100 gm of cell biomass |
|---|---|---|
| *A. niger* | 1335 ug/ml | 1.3 % |
| *S. cerevisiae* | 5330 ug/ml | 1.7 % |
| Soil Isolate | 2165 ug/ml | 7 % |

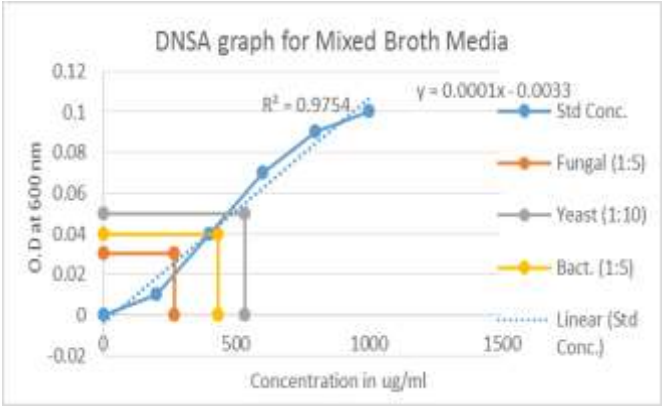



**Fig. 3.21: Content of reducing sugar in extracted β-glucan from Mixed Broth.**

**Table 3.12: Beta-Glucan yield from microbial cultures in Mixed Broth.**

| Isolate | Beta-Glucan Yield (mg %) |
|---|---|
| *A. niger* | 58 mg% |
| *S. cerevisiae* | 25 mg% |
| Soil Isolate | 40 mg% |

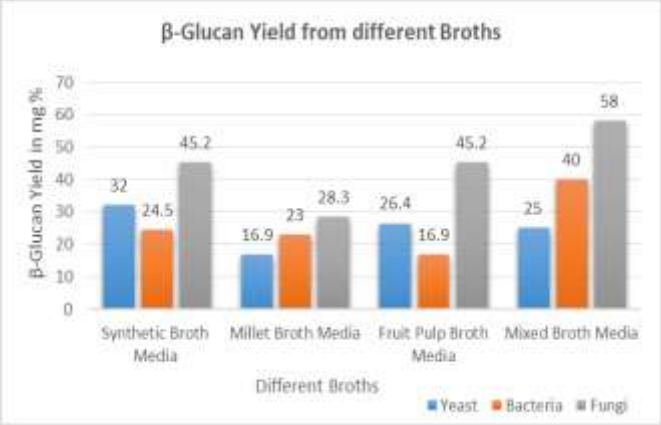



**Fig. 3.22: Yield of β-glucan from each broth.**

**Table 3.13: Optical density of Probiotic culture inoculated with β-glucan of each isolate.**

| Particulars | O.D at 0 hrs | O.D at 24 hr | Increase in O.D |
|---|---|---|---|
| Broth + Culture | 0.02 | 0.10 | 0.08 |
| Broth + 1% lactose | 0.03 | 0.32 | 0.29 |
| Broth + β-glucan of *A. niger* | 0.03 | 0.37 | 0.34 |
| Broth + β-glucan of *S. cerevisiae* | 0.02 | 0.09 | 0.07 |
| Broth + β-glucan of Soil bacterium | 0.03 | 0.31 | 0.28 |

**Table 3.14: Viable Count of *L. rhamnosus* inoculated with β-glucan of each isolate.**

| Particulars | Average CFU/ ml at 0 hr | Average FU/ml at 24 hrs | Increase in CFU/ml after 24hrs |
|---|---|---|---|
| Broth + Culture | $2.7 \times 10^6$ | $5.7 \times 10^9$ | $5.6 \times 10^8$ |
| Broth + 1% lactose | $1.6 \times 10^6$ | $2.0 \times 10^{10}$ | $1.9 \times 10^{10}$ |
| Broth + β-glucan of *A. niger* | $1.9 \times 10^5$ | $5.6 \times 10^{10}$ | $5.4 \times 10^{10}$ |
| Broth + β-glucan of *S. cerevisiae* | $2.7 \times 10^5$ | $3.6 \times 10^7$ | $3.5 \times 10^7$ |
| Broth + β-glucan of Soil bacterium | $1.2 \times 10^5$ | $3.1 \times 10^9$ | $3.9 \times 10^9$ |

**Table 3.15: Prebiotic Index of β-glucan extracted from each isolate.**

| Particular | Prebiotic Index | Inference |
|---|---|---|
| β-glucan of *A. niger* | 2.8 | >1 indicating positive effect |
| β-glucan of *S. cerevisiae* | 0.0018 | <1 indicating negative effect |
| β-glucan of Soil bacterium | 0.2 | <1 indicating negative effect |

Key – Ratio >1 indicates Prebiotic effect Ratio <1 indicates No Prebiotic effect

**Table 3.16: Optical density of *E. coli* inoculated with β-glucan of each isolate.**

| Particulars | O.D at 0 hrs | O.D at 24 hr | Increase in O.D |
|---|---|---|---|
| Broth + Culture | 0.02 | 0.09 | 0.07 |
| Broth + 1% lactose | 0.02 | 0.09 | 0.07 |
| Broth + β-glucan of *A. niger* | 0.03 | 0.06 | 0.03 |
| Broth + β-glucan of *S. cerevisiae* | 0.02 | 0.07 | 0.05 |
| Broth + β-glucan of Soil bacterium | 0.03 | 0.10 | 0.07 |

**Table 3.17: Viable Count of *E. coli* inoculated with β-glucan of each isolate.**

| Particulars | Average CFU/ ml at 0 hr | Average CFU/ml at 24 hrs | Increase in CFU/ml after 24hrs |
|---|---|---|---|
| Broth + Culture | $1.1 \times 10^7$ | $1.1 \times 10^8$ | $8.0 \times 10^7$ |
| Broth + 1% lactose | $2.5 \times 10^7$ | $1.7 \times 10^8$ | $1.1 \times 10^8$ |
| Broth + β-glucan of *A. niger* | $5.5 \times 10^6$ | $9.8 \times 10^7$ | $9.2 \times 10^7$ |
| Broth + β-glucan of *S. cerevisiae* | $2.1 \times 10^7$ | $8.2 \times 10^7$ | $6.1 \times 10^7$ |
| Broth + β-glucan of Soil bacterium | $4.4 \times 10^7$ | $1.3 \times 10^8$ | $8.6 \times 10^7$ |

**Table 3.18: Prebiotic activity score.**

| Isolate | Prebiotic activity score | Inference |
|---|---|---|
| β-glucan of *A. niger* | 1.21 | >1 indicating positive effect |

## DISCUSSION

Gram staining of *Saccharomyces cerevisiae* and soil bacterium was performed and Lactophenol cotton blue staining was performed for *Aspergillus niger*. The soil bacterium was found to be gram positive rod shaped bacilli which was further required to be confirmed and *Saccharomyces cerevisiae* was found to be gram positive with oval shaped morphology.

Fig. 3.2 shows the growth curve for *S. cerevisiae*, and Soil Isolate plotted using optical density value. Each microorganism displays a growth phase and significantly different absorbance value which exhibit the number of microbial cells. The greater the number of microbial cells indicates greater amount of β-glucan production. The absorbance value for *A. niger* was not obtained as growth was observed by formation of flocs. *S. cerevisiae* and Soil Isolate experienced an exponential phase or logarithmic phase at 24 hr when there was greater increase in number of cells, the greatest growth was seen for *S. cerevisiae*.

The change in the pH of media is because of fermentation by each microorganism for 96 hr which are displayed in Fig. 3.3. It can be seen that during initial hours the pH of each organism was decreased, the greater decrease was observed in *S. cerevisiae* compared with other microorganism the lowest pH of 4.8 was obtained at 96 hr. The pH plays an important role in the increased production of β-glucan. According to (Kalyanasundaram et al. 2012) the optimal pH for β-glucan production is 5.5-7.0. The decrease in pH in each medium is because of conversion of glucose into various organic acids which result into acidic medium. The acids could be pyruvic acid, lactic acid, butyric acids or fatty acids which renders the pH of medium.

The change in glucose level in medium for each culture was observed for 96 hr shown in Fig. 3.4. It was noticed that for each microorganism the glucose level was decreased after 24 hrs. The greatest decrease in glucose level was noticed for *S. cerevisiae* which was 0.54 gm% from original concentration i.e 1.4gm%.

The glucose uptake by microorganism is converted to various such metabolites which include ethanol, acetic acid, formic acid and $CO_2$. The production of β-glucan is dependent on glucose. The decrease in glucose level indicates absorption of glucose. Greater uptake of glucose by *S. cerevisiae* displays that it is highly dependent on glucose as a carbon source for its growth. Lower uptake of glucose was seen in *A. niger* although fungi has greater affinity towards glucose, lower uptake indicates inability to use glucose as a substrate in producing β-glucan. The soil isolate also showed decreased uptake in glucose level at 24 hr. Glucose is produced by bacteria which is used as a secondary metabolite in the form of β-glucan (Dhivya et al. 2014).

Table 3.1 shows the result for growth of *S. cerevisiae, A. niger* and Soil isolate in the form of cell biomass from synthetic media. Fig 3.5 represents extracted β-glucan from each isolate. The *S. cerevisiae, A. niger* and Soil Isolate were subjected to glucan extraction. 45.2 mg%, 32 mg% and 24.5 mg% of β-glucan yield was recorded in *A. niger, S. cerevisiae* and Soil Isolate respectively. The greater amount of β-glucan was obtained from *A. niger* this could be because presence of β-1, 3 and β-1, 6 bond obtained in cell walls of mycelium, whereas Soil isolate which is bacteria, are known to produced β-glucan from secondary metabolites and *S. cerevisiae* produces from its cell wall. In *Aspergillus*, β-glucan is produced via a membrane-bound complex using UDP glucose. *Saccharomyces cerevisiae* synthesizes β-glucan from glucose metabolism, converting it into UDP glucose. Bacteria produce glucans using UDP glucose and UMP as precursors. Decreased pH levels increase intracellular enzyme concentrations, promoting β-glucan synthesis via enzymes like β-glucanase and phosphoglucomutase.

The highest percentage of β-glucan i.e 45.2 mg% was obtained from *A. niger* compared to other two isolates (Table 3.3). It produces greater cell biomass although less of glucose was converted (Fig. 3.4). However the *Aspergillus* can also adapts its metabolism to produce more biomass even when sugar concentration are limited. This could be due to several factors like metabolic versatility where they can utilize different carbon sources other than glucose. Although more of the glucose is converted by Soil Isolate but less of glucan was produced

compared to *Aspergillus niger* may be because no greater level of decrease in pH (Fig 3.3) was seen which eventually depletes the level of enzymes required for synthesis of β-glucan.

*S. cerevisiae* commonly yields β-glucan at approximately 6-12 gm% (Zechner-Krpan et al. 2010), *A.niger* typically produces β-glucan ranginging from 9-11 gm% (Paulraj et al. 2010) and bacterium approximately 6-7 gm% (Kalyanasundaram et al. 2012) based on our experimental finding the results exhibit lesser yield however the optimization of β-glucan production is done using various crude media.

Three different crude media were prepared, Fig 3.7 represents crude media prepared from Dried Fruit Pulp, Fig 3.8 represents crude media prepared from Grounded Millets and Fig 3.9 represents a mixture of both the crude media (Mixed Broth).

For Fruit Pulp the exponential or the logarithmic phase for both the isolate was observed after 24 hr of incubation (Fig. 3.10), whereas no absorbance was taken for *A. niger* as growth was not seen in the form of turbidity because flocs were observed. The change in pH was seen during initial hours of incubation. Major decreased pH is observed in *A.niger* i.e of 3.2 at 96 hr indicating more conversion of sugar to various other organic acids, whereas the soil isolate also showed greater decrease in pH being in optimal range. The original glucose concentration of Fruit Pulp Broth was found to be 0.99 gm%. The uptake of sugar from fruit pulp broth was seen in each isolate by determining reducing sugar content after every 24 hrs. The major uptake of sugar and its conversion is seen in *A. niger* i.e 0.60 gm% of reducing sugar was observed after 96 hrs of incubation. This indicates that the organism is highly dependent on carbon sources for its growth. The sugar consumed by *A. niger* causes production of amino acids and organic acids like citric acid and gluconic acid during fermentation which eventually affects the acidity of the medium and thus results into change or decrease in pH. The glucose level in *S. cerevisiae* decrease after 48 hr depicted in Fig. 3.12. Observing the results of growth parameter we can confirm that Crude media prepared from Dried Fruit Pulp shows the presence of various sugars, minerals and other nutrients requires for microbial growth, all of which serves as a substrate for microorganism to metabolize and produce energy.

Table 3.4 shows the result for growth of *S. cerevisiae*, *A. niger* and Soil isolate in the form of cell biomass from fruit pulp media. From the findings it was observed that among all of isolates *A. niger* showed greater biomass of 0.8 gm (Table 3.4) although there is no such

major difference seen in other two isolates. However the biomass yield is lesser as compared to synthetic media. *A. niger* also showed the highest production of β-glucan (Table 3.6) i.e 45.20 mg% which was same as that of yield obtained from synthetic media indicating that though Fruit pulp media failed to increase biomass of isolate still higher amount of β-glucan is produced i.e less biomass but more of β-glucan except for other two isolate i.e *S. cerevisiae* and Soil isolate where less amount of β-glucan is obtained.

For Millet Broth the exponential phase for both the isolate was seen after 24 hr of incubation Fig. 3.14, whereas no absorbance was taken for *A. niger* as growth was seen in the form flocs. The change in pH was seen during initial hours of incubation except for *A. niger* where in initial hr no major decrease was seen. However the pH started decreasing after 72 hr. At 96 hr the lowest pH was seen for *A. niger* compared to other isolates i.e 3.6 indicating more conversion of sugar to various other organic acids, whereas the soil isolate also showed greater decrease in pH. The original glucose concentration of Millet Broth was found to be 0.6 gm% which was less as compared to crude media prepared from Fruit Pulp. The major uptake of sugar and its conversion is seen in Soil isolate i.e 0.07 gm% after 96 hrs of incubation. The bacterium utilizes sugars as a source of energy for their metabolic processes. Millets being rich in carbohydrates it provides bacteria with readily available sugar to fuel their growth. Bacteria break down these sugars through various biochemical pathways to generate ATP, the energy currency of cells, allowing them to carry out essential functions and proliferate. The glucose level in *S. cerevisiae* decrease rapidly after 24 hr depicted in Fig. 3.16. Millet powder supports the growth of each microorganism indicating presence of various sugars, minerals and other nutrients requires for microbial growth, all these nutrients serves as a substrate for microorganism to metabolize and produce energy.

Table 3.7 shows the result for growth of *S. cerevisiae*, *A. niger* and Soil isolate in the form of cell biomass from Millet Powder media. Among all of isolates Soil isolate showed greater biomass of 0.88 gm (Table 3.7) major difference seen in obtained biomass other two isolates. However the biomass yield is lesser as compared to synthetic media. *A. niger* also showed the highest production of β-glucan (Table 3.9) i.e 28.30 mg% which was less as compared to yield obtained from synthetic media. *S. cerevisiae* showed lower production of β-glucan compared to previous two media.

The mixture of two media (Mixed Broth) was prepared in order to determine the combine effect on biomass and β-glucan production from each isolate. The exponential phase for both

the isolate was seen after 24 hr of incubation (Fig. 3.18), whereas no absorbance was taken for *A. niger* as growth was seen in the form of flocs. The change in pH for *S. cerevisiae* was seen during initial hours of incubation except for *A. niger* where in initial hours no major decrease was seen. However the pH started decreasing after 72 hr. At 96 hr the lowest pH was seen for *A. niger* compared to other isolates i.e 3.6 indicating more conversion of sugar to various other organic acids, whereas the soil isolate also showed greater decrease in pH (Fig. 3.19). The original glucose concentration of Mixed Broth was found to be 0.82 gm%. The major uptake of sugar and its conversion is seen in both *A. niger, S. cerevisiae* i.e 0.43 gm% after 96 hrs of incubation. The glucose level in *A. niger* and *S. cerevisiae* decrease rapidly after 24 hr depicted in Fig. 3.20. Observing the results of growth parameter we can confirm that Mixed broth can also supports the growth of each microorganism indicating presence of various sugars, minerals and other nutrients requires for microbial growth.

Table 3.10 shows the result for growth of *S. cerevisiae*, *A. niger* and Soil isolate in the form of cell biomass from Mixed Powder broth. Extraction of β-glucan was done from each isolate. The experiment was carried out in triplicate and an average yield is presented in (Table 3.12) from the findings it was observed that the Mixed Broth resulted in greater production of β-glucan from *A. niger* and Soil isolate i.e 58 mg% and 25 mg% respectively (Table 3.12) compared to all of the previous media used. However the biomass yield is lesser as compared to synthetic media more of the yield is obtained from *A. niger* i.e 58 mg% which was greater as compared to yield obtained from synthetic media. From the finding one can conclude that that the mixture of Fruit Pulp Broth and Millet Broth showed a positive effect on production of β-glucan rather than alone thus providing promising result and can readily supports the growth and production of β-glucan from each isolate.

On comparing the yield obtained from synthetic media and other crude media the greater optimization in production of β-glucan was observed in Mixed Broth for *S. cerevisiae* and *A.niger* (Fig. 3.22). The obtained yield is somewhat less than standard values of β-glucan as discuss earlier indicating Mixed Broth can be used rather than synthetic broth for production of β-glucan.

Since all of the isolates are known to produce β-glucan the studied strains shows a greater potential to be used as a starter culture for production of β-glucan in various fermented food.

The extracted β-glucan was subjected for analyzing Prebiotic Potential from each isolate i.e *S. cerevisiae*, *A. niger* and Soil Isolate against probiotic culture namely *Lactobacillus rhamnosus*. The growth behaviour of *L. rhamnosus* in presence of β-glucan extracted from *S. cerevisiae*, *A. niger* and Soil Isolate is shown in Table 3.13 and 3.14. The probiotic culture shows growth in broth containing the tested β-glucan which is suspected to be having a prebiotic potential. Results exhibit that *L. rhamnosus* shows the greatest growth in presence of β-glucan obtained from *A. niger* and Soil Isolate i.e $5.4 \times 10^{10}$ and $3.9 \times 10^{9}$ respectively, whereas decreased growth was observed in presence of β-glucan obtained from *S. cerevisiae* seems like not supporting the growth of probiotic. The extracted β-glucan from Soil Isolate and *S. cerevisiae* seems to be less efficient on comparing the growth of probiotic in lactose. The positive control was set up i.e lactose since it is the natural substrate in dairy food and also the lactobacilli are adapted to use this as a carbon source.

Prebiotic index obtained for β-glucan with different isolates are shown in Table 3.15. The prebiotic index serves as a quantitative measure for comparing various prebiotic carbohydrates. When the ratio derived from the values exceeds 1, it indicates that the growth of microorganism is enhanced by the tested prebiotic in comparison to control carbohydrate. The results obtained for β-glucan derived from *A. niger* is greater than 1, indicating that the tested β-glucan shows prebiotic potential against probiotic. The β-glucan from other two isolate failed to show prebiotic activity indicating low effectiveness on probiotic culture. The prebiotic indexes from the obtained result showed a positive effect of the tested β-glucan from *A.niger* over the growth of lactobacilli strain.

For determining prebiotic activity a reference strain used was *Escherichia coli.* The extracted β-glucan was subjected for analyzing prebiotic activity score from each isolate i.e *S. cerevisiae*, *A. niger* and Soil Isolate against reference strain namely *E.* coli. The prebiotic activity score is shown in Table 3.18. The β-glucan isolated from *A. niger* showed a prebiotic score of greater than 1 i.e 1.21. According to Huebner et al. 2007, the prebiotic activity score serves as an indicator of the growth stimulation of tested strain by specific prebiotic compared to control carbohydrates or reference bacteria like *E. coli.* A Prebiotic activity score lower than 1 or negative suggests reduced growth on a particular prebiotic compared to the control carbohydrate or lower growth compared to reference bacteria.

On comparing prebiotic activity score with standard prebiotics like Frutafit, lactulose, Oligomate 55 having prebiotic activity score 2.7, 1.9 and 6.68 (Figueroa-Gonzalez et al 2019)

which was greater from experimental findings. Given the diverse metabolic capabilities of *Lactobacilli*, it is reasonable to except significant variation in prebiotic activity scores among different prebiotics used by single probiotic strain. For instance *L. rhamnosus* exhibited notably higher score on D-Oligomate 55 compared to Frutafit and Lactulose. The utilization of specific prebiotics by probiotic relies on the presence of particular hydrolysis and transport system, which are encoded by specific genes. These genetic differences may lead to varied prebiotic activity score among different strains (Gopal et al 2001; Goh et al 2007; Kniefel et al 2000).

The obtained soil isolate on performing VITEK was identified as *Bacillus cereus* being an efficient isolate for producing β-glucan.

## 4. CONCLUSION

Based on the experimental results, the study focused on isolating organisms capable of producing β-glucan, a polysaccharide known for its various health benefits, including immune modulation and prebiotic properties. Three organisms were selected for investigation: *Aspergillus niger*, *Saccharomyces cerevisiae*, and an unidentified organism obtained from soil, which was later based on VITEK result was known to be *Bacillus cereus*.

The production of β-glucan by these isolates was assessed in synthetic media and various crude media formulations, including those derived from dried fruit pulp, grounded millets, and a combination of both. The growth of the organisms was monitored through various parameters such as optical density, pH changes, and glucose consumption over a 96-hour period.

*Aspergillus niger* demonstrated robust growth and β-glucan production across different media, with notable variations observed in pH changes and glucose utilization among the isolates. As per standard results where *A.niger* typically produces β-glucan ranging from 9-11 gm% (Paulraj et al 2010) the experimental result were quiet less therefore further research could focus on optimizing the cultivation conditions for *A. niger* to maximize β-glucan production. This could involve fine-tuning parameters such as pH, temperature, carbon source concentration, and agitation rate to enhance the yield of β-glucan. Following growth assessment, β-glucan extraction and quantification were conducted using acid hydrolysis methods. *A. niger* consistently yielded the highest amount of β-glucan, suggesting its suitability for industrial-scale production.

Interestingly, the study also explored the prebiotic potential of the extracted β-glucan using *Lactobacillus rhamnosus* as model organisms. *A. niger*-derived β-glucan exhibited promising prebiotic activity, indicating its potential as a functional ingredient in promoting gut health although on comparing prebiotic activity score with standard prebiotics like Frutafit, lactulose, Oligomate 55 having prebiotic activity score 2.7, 1.9 and 6.68 (Figueroa-Gonzalez et al 2019) the obtained prebiotic score for *A. niger*-derived β-glucan was low although indicating less efficient, future research could delve deeper into the mechanisms underlying the interaction between *A. niger*-derived β-glucan and gut microbiota. This could involve in vitro and in vivo studies to elucidate the effects of β-glucan on microbial composition, fermentation patterns, and host health.

Furthermore, the study evaluated the efficacy of different media formulations in enhancing β-glucan production. Mixed broth media, combining dried fruit pulp and grounded millets, emerged as particularly effective in promoting β-glucan synthesis, especially from *A. niger*. This finding underscores the importance of media optimization for maximizing the production of bioactive compounds. Continuation of research into novel media formulations could lead to the discovery of alternative substrates that promote higher β-glucan production. Investigating the use of agricultural by-products or waste streams as inexpensive and sustainable media sources may be particularly fruitful

The study possess several drawbacks to consider like contamination risk of beta-glucan with by-products which can affect product purity, gastrointestinal discomfort such as bloating or diarrhea, allergic concerns, structural specificity, regulatory hurdles.

Overall, the study highlights the potential of *A. niger* as a prolific producer of β-glucan with promising prebiotic properties. The findings suggest avenues for further research into optimizing cultivation conditions and exploring the therapeutic potential of β-glucan-rich extracts in functional foods and pharmaceuticals.

## 5. REFERENCES

1. CHAIYASUT, C., PENGKUMSRI, N., SIVAMARUTHI, B. S., SIRILUN, S., KESIKA, P., SAELEE, M., PEERAJAN, S. Extraction of β-glucan of *Hericium erinaceus*, *Avena sativa* L., and *Saccharomyces cerevisiae* and in vivo evaluation of their immunomodulatory effects. Food Science and Technology, 2018. ISSN 0101-2061.

2. Chen, G., Xie, M., Wan, P., Chen, D., Ye, H., Chen, L., Liu, Z. Digestion under saliva, simulated gastric and small intestinal conditions and fermentation in vitro by human intestinal microbiota of polysaccharides from Fuzhuan brick tea. Food Chemistry, 2018; 244: 331–339.
3. Figueroa-Gonzalez, I., RODRÍGUEZ-SERRANO, G., GÓMEZ-RUIZ, L., GARCÍA-GARIBAY, M., & CRUZ-GUERRERO, A. Prebiotic effect of commercial saccharides on probiotic bacteria isolated from commercial products. Food Science and Technology, 2019.
4. Freimund, S., Sauter, M., Käppeli, O., & Dutler, H. A new non-degrading isolation process for 1,3-β-d-glucan of high purity from baker's yeast Saccharomyces cerevisiae. Carbohydrate Polymers, 2003; 54(2): 159–171.
5. Kalyanasundaram, G.T., Doble, M., Gummadi, S.N., Production and downstream processing of (1-3)-b-D-glucan from mutant strain of Agrobacterium sp ATCC 31750. AMB Exp, 2012; 2(1): 31.
6. Kutcharlapati Sunita Raju Alternative Culture Media for Cultivation of Bacteria. Volume, 2020; 9: 3.
7. Lara Utama, G., Dio, C., Sulistiyo, J., Yee Chye, F., Lembong, E., Cahyana, Y., Singh, S. Evaluating comparative β-glucan production aptitude of *Saccharomyces cerevisiae, Aspergillus oryzae, Xanthomonas campestris*, and *Bacillus natto*. Saudi Journal of Biological Sciences, 2021.
8. Palframan, R., Gibson, G. R., & Rastall, R. A. Development of a quantitative tool for the comparison of the prebiotic effect of dietary oligosaccharides. Letters in Applied Microbiology, 2003; 37(4): 281–284.
9. Ren, Y., Xie, H., Liu, L., Jia, D., Yao, K., & Chi, Y. Processing and Prebiotics Characteristics of β-Glucan Extract from Highland Barley. Applied Sciences, 2018; 8(9): 1481.
10. Russo, P., López, P., Capozzi, V., de Palencia, P. F., Dueñas, M. T., Spano, G., & Fiocco, D. Beta-Glucans Improve Growth, Viability and Colonization of Probiotic Microorganisms. International Journal of Molecular Sciences, 2012; 13(5): 6026–6039.
11. Wang, H., Chen, G., Li, X., Zheng, F., & Zeng, X. Yeast β-glucan, a potential prebiotic, showed a similar probiotic activity to inulin. Food & Function, 2020; 10386–10396.