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Production and Characterization of Exopolysaccharide extracted from bacteria isolated in homemade and packed Idli batter

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Abstract: ***Diverse exopolysaccharide (EPS)-producing isolates were isolated from an Indian acidic fermented food (Idli) based on the colony morphology. Lactic acid bacteria are non-pathogenic organism widely distributed in nature typically involved in a large number of spontaneous food fermentation. One of the EPS-producing micro flora was selected for further characterization using FT-IR, SEM, TGA and XRD analysis. The effect of pH, salt and temperature on the yellow pigment were studied and these parameters are gently influence the growth of bacterial isolates. In pH the maximum yield of biomass were obtained at pH (8) . In temperature maximum yield at 40 C and in salt concentration (Nacl) the maximum yield were obtained with 2%. Effect of total phenolic content, antioxidant activity and reducing power assay influence the growth biomass of the bacteria. In the structural characterization, the FT-IR spectroscopy revealed the α-d-glucose nature of the EPS. The SEM showed smooth surfaces and compact structure. TGA results showed higher degradation temperature of 272.01° C. XRD analysis proved the 33.4% crystalline nature of the EPS. All the above characteristics of the EPS produced by L. lactis showed that the EPS is of a good-quality polysaccharide with potential applications in the food industry.***

Keywords: Idli batter, Exopolysaccharide, homemade and Packed

I. INTRODUCTION

Lactic acid bacteria (LAB) are known to produce several industrially important biomolecules including exopolysaccharides (EPS) from fermented foods (Patel and Prajapathi, 2013).EPS production is well documented among various species of LAB belonging to *Leuconostoc spp., Lactobacillus spp., Lactococcus spp.* and *Streptococcus spp.* (Navarini *et al.,* 2001). Homopolysaccharides secreted by *Lactobacillus spp.* contain glucose or fructose as sole monosaccharides and are classified as glucans (dextrans) and fructans, respectively (Kralj *et al.,* 2005). EPS are profusely used as bio-absorbants, bio-flocculants, encapsulating constituents, heavy metal confiscating agents, drug distribution agents and ion-exchange resins (Ismail *et al.,* 2010). Bacteria produce biofilms to defend the microbial community against the environmental stress (Ciszek-lenda *et al.,* 2011). Bacterial exopolysaccharides are the most important constituent of extracellular polymer forming biofilm and mediate most of the cell-to-cell and cell-to-surface connections and stabilization in the intestine (Flemming *et al.,* 2010). The EPS produced by LAB act as an immuno-stimulator, anti-tumour agent and blood cholesterol-lowering agent. EPS are used as drug conjugates, coatings, and matrix agents to develop the specificity of drug release in colon cancer treatment (Vandamme *et al.,* 2002). Current research is mainly focussed on developing polymeric matrices with tuned characteristics such as transparency, barrier and mechanical properties, biocompatibility and bioactivity. The *in vitro* manipulations of polymer have been carried out to form structured materials (e.g. nanoparticles, scaffolds or hydrogels) that can be used for numerous industrial and medical applications and also as edible coatings for numerous food products (Lavanya *et al.,* 2011, Ruas-Madiedo *et al.,* 2002b, Bounaix *et al.,* 2009). Some of the EPS (exopolysaccharide) producing LAB species isolated from *idli* batter are *Leuconostoc mesenteroides, Weissella confusa, Weissella cibaria, Pediococcus parvulus* (Patel *et al.,* 2014; Sawale and Lele, 2010). Furthermore EPS was used as a bio-floccuants, stabilizing, emulsifying, bio-absorbants, and act as drug delivery, heavy metal removing agents (Liu *et al.,* 2010). Dextrans are produced through various LAB especially *Leuconostoc* and *Streptococcus* species (Leathers, 2002). *Leuconostoc spp* produced α-1-6 and α-1-4 linkages polysaccaharide (Dextran) can be used as an anti-tumor agent (Sawale *et al.,* 2010).

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Glucan was produced by *Leconostoc dextraniucm* exhibited a highly linear structure with about 96% of α 1-6 and α-1 -4 linkages (Majumder *et al.,* 2009). α-glucans produced from *Streptococcus mutans* and *Streptococcus sobrinus* contains 90% of α-1-3 linkages. *Leuconostoc mesenteroides* produced high molecular weight α-glucans predominantly α-1-6 linkages and also contains α-1-4 and α-1-3 linkages (Cerning, 1990). Glucan produced by *Leconostoc garium* PR contains 95% of α-1-6 glucopyranose linkage carrying low content branches of α- 1-2, α-1-3 and α-1-4 linkage (Capek *et al.,* 2011).The polysaccharide from *Pantoea agglomerans* KFS-9 showed highly scavenging activity, superoxide radicals and hydroxyl radical‟s activity were isolated from mangrove forest (Wang *et al.,* 2008). Exopolysaccharides having the antioxidant capacity can securely interact with free radicals and terminate the chain reaction previous adverse deterioration arises (Ye *et al.,* 2012). *Keissleriella* sp. YS 4108 and *Penicillium* sp. F23-2, produced polysaccharides revealed good free-scavenging activities, particularly scavenging capabilities on superoxide radicals and hydroxyl radicals (Sun *et al.,* 2004:2009). EPS was intensively used as food additives to improve the texture which impact on the development of innovative food products with enhanced appearance, mouth feel, firmness, and rheological properties (De Vuyst *et al.,* 2001). EPS-producing cultures in dairy foods are known to promote alleviating and water-binding functions (Lavanya *et al.,* 2011). EPS-producing probiotic microflora has been suggested as effective functional starter cultures for preparing fermented foods (Ruas-Madiedo *et al.,* 2002). The exopolysaccharide (Dextran) produced by LAB which enhance the texture and sensory properties in many Indian fermented foods (Patel and Prajapati, 2013). This chapter is planned to screen and identify the EPS-producing microorganism and characterize the EPS produced by a selected strain. Fermentation is a specific type of heterotrophic metabolism. This is a metabolic process which is induced by a microorganism and characterized by the anaerobic breakdown of carbohydrates to alcohol or organic acids. Fermentative organisms are very important in industry and are used to make many different types of food products. The different metabolic end products produced by each specific bacterial species are responsible for the different taste and properties of each food. The quality of the end product mainly depends on the microbial composition and raw material of the food. Fermentation through natural microflora helps to enhance food properties such as taste, flavour, texture, nutritional value and shelf life (Ali *et al.,* 2011).Idly batter is a fermented product consumed widely in the Indian subcontinent. Idly is made by steaming a batter comprise of fermented black lentils (de-husked) and rice. The fermentation process breaks down the starches so that they are more partially metabolized by body. Lactic acid bacteria are the major group of organisms involved in the idly batter fermentation. *Leuconostoc mesenteroides and streptococcus* thermophilus in grains. These microorganisms divide and produce lactic acid and carbon dioxide that makes the batter anaerobic condition and leaven the product. Lactic acid bacteria and yeasts play an important role in numerous natural food fermentations such as curd, cheese, pickles and various other traditional foods. Lactic Acid Bacteria associated with fermented foods includes species of the Genera *Enterococcus, lactobacillus, lactococcus, leuconostoc, pediococcus, streptococcus, Tetragenococcus, Vagococcus and Weisella*. These organisms have also gained popularity as probiotics. Probiotics are live microorganisms when take in adequate amount beneficially affect the host by improving the intestinal microbial balance. Among these microorganisms, Lactic Acid Bacteria is regarded as a major group of probiotic bacteria. Probiotic microorganisms are typically members of the genera *Lactobacillus, Bifido bacterium and Streptococcus*. These bacteria are fermentive, obligatory, or facultative anaerobic organisms, which are typically non motile and of varying shapes. They typically produce lactic acid. Their inherent biological features enable them to predominate and prevail over potential pathogenic microorganisms in the human digestive tract. EPS produced by Lactic Acid Bacteria are in a great variety, depending on the type of lactic acid bacteria strains, culture conditions and medium composition (Looijesteijn and Hugenholtz 1999). EPS-Extracellular polymeric substances are natural polymers occur in nature of high molecular weight secreted by microorganisms into their environment. EPS's establish the functional and structural integrity of bioflim are considered the fundamental components that determine the physiochemical properties of a bioflim. EPS are mostly composed of polysaccharides (exopolysaccharides) and proteins, but include their other macro molecules such as DNA, Lipids and Humic substances.EPS's are the sugar based parts of EPS's. It is generally consisting of monosaccharide and some of non carbohydrate substituents (such as acetate, pyruvate, succinate and phosphate).Occurring to the wide diversity in composition exopolysaccharides has found diverse applications in various food and pharmaceutical industries. Many microbial EPS sugars provide properties that are almost identical to the gums currently in use with innovative approaches; Moreover, considerable progress has been made in discovering and developing new microbial EPS sugars that posse's novel industrial application.Biofilms are composed of microorganisms and extracellular polymeric substances (EPS) (Flemming and Wingender, 2010). A thorough analysis of EPS is prerequisite to study the structure and stability of biofilm. Analyzing EPS is performed to understand the biofilm composition and biofilm adjustment to environmental changes. Bacteria produced biofilm to defend the microbial community against the environmental stress. Bacterial exopolysaccharides are the essential constituent extra cellular polymer form biofilm and mediate most of the cell - to- cell surface connections and stabilization in intestine (saravanan *et al*., 2014- 2015).

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Exopolysaccharides are long chain polysaccharides that are secreted mainly by bacteria and micro algae into the environment during growth that are not permanently attached to the surface of the microbial cells.It can be used as bioadhesives, bioflocculants, biosorbents, gelling agents, stabilizers and thickeners.EPS has additional hypothesized physiological benifit is that it will remain for longer in the gastrointestinal tract , thus enhancing colonization by probiotic bacteria (German et al.,1999). The human gastrointestinal tract is densely colonized by broad spectrum of microorganisms that live in symbiosis with their host (Berg, 1996, Proctor et al., 2019).

II. MATERIALS AND METHODS

A. *Isolation of bacteria from idli batter*

Samples were collected at different preparation of idli batter from household and Packed idli batter in Madurai. All the samples were collected in sterile containers with proper hygienic conditions. Collected samples were brought to the laboratory for further analysis. The samples were subjected to a microbiological analysis to observe the dynamic changes in the population of idli batter. One gram of batter sample was added to the stock solution in a sterile distilled water to get a uniform suspension. They were further serially diluted and plated on MRS agar for anaerobes in an anaerobic jar at 30 °C for 24 h and nutrient agar for aerobes incubated at 30°C for 24 h. The colonies that appeared after incubation were counted as colony forming unit (cfu) per gram of batter on dry weight basis. Characteristics of the colony were observed and representative single colonies were isolated, sub cultured on agar slopes of the respective media.

B. *Screening of Probiotic Bacteria*

1) *Hemolytic Assay:* MRS Agar with 5% blood was used for checking the hemolytic activity of the isolated bacteria. The bacteria were streaked on the MRS blood agar plates, incubated at 37 degrees Celsius for 48 hours.

C. *Molecular Characterization of the Isolates*

1) *DNA isolation and amplication by PCR:* The MRS and nutrient agar plates was streaked with selected isolates and incubated at 30 °C for 24 h, the single colony from plate was suspended in 200 µl sterile distilled water and then 10 µl proteinase K was added and heated at 95 °C for 5 min. Then centrifuged at 8000 rpm for 5 min and supernatant was collected and used for PCR amplification. The total bacterial Universal-16S rRNA was amplified with the primer U1F AGAGTTTGATCCTGGCTCAG and U1R GGTTACCTTGTTACGACTT, 1F 5′ - AGAAGAGGACAGTGGAAC- 3′ and 1R 5′ - TTACAAACTCTCATGGTGTG - 3′ (Singh and Ramesh, 2009). The PCR reaction mixture were performed in 50 µL containing 5 µL DNA, 5 µL 10x PCR buffer, 0.5 µL Taq polymerase [5U/µL] (all Sigma aldrich), 2µL of each primer [10pmol/µL]. The amplification was started at 94 °C for 5 min, and then followed by 30 cycles of 94 °C for 1 min, 55 °C for 1 min, 72 °C for 2 min, and a final extension step at 72 °C 10 min (Singh and Ramesh, 2008). The PCR products (approx. 1400 bp) were separated on a 1% (wt/vol) agarose gel at a persistent power of 120 V in 1×TAE-buffer for 30 min. The amplified products were visualized under UV transilluminator for identification. . Muscle online tool was used for multiple sequence alignment of all sequence.

D. *Confirmation of EPS- producing Bacteria by Congo Red*

The agar medium was flooded with an aqueous solution of Congo Red (1 mg/ml) for 15 min. Then add 1 M NaCl for 15 min. Visualized zones of hydrolysis could be stabilized for at least 2 weeks by flooding the agar with 1 M HCL. Dye color changes to blue and inhibits further enzyme activity.

E. *Extraction and Purification of EPS*

Fifty millilitres of cell suspension was grown in MRS medium supplemented with sucrose under shaking condition of 100 rpm at 30 °C for 24 h. The cell suspension was heated to 100°C for 10 min for inactivating the enzymes, and then, the suspension was cooled to room temperature and centrifuged at 4100 g for 20 min to remove the biomass. The crude solution was further treated with Sevage reagent (chloroform: n-butanol at 5:1 v/v) three times to remove the proteinaceous materials. EPS was precipitated with cold ethanol (three times of the volume) and left overnight. The precipitate was collected through centrifugation at 19200 g for 15 min and dissolved in Milli Q water. Afterwards, it was encased in a dialysis bag (12- 14 KDa) and dialysed at 4 °C with Milli Q water for 48 h for partial purification (Liu et al., 2010). The fraction of EPS was carried out for purification in a DEAE-52 anion-exchange chromatography column (1.5 cm - 50 cm) with consecutive elution using distilled water and Nacl2 solution in a linear gradient of 0.2-1M Nacl2 at the flow rate of 1 mL/5min.The main fraction was further purified over a Sephadex G-100 column (2.6 cm- 50 cm) and the column was eluted through 0.01M Nacl2 solution at a flow rate of 1mL/min.

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F. Growth Optimization of Bacteria in Different pH, Salt and Temperature

1) *Effect of Ph:* Nutrient broth medium with 2% salt were prepared and transferred into a 5 test tubes. Adjust the ph of the medium to 2, 4, 6, 8 and 10 respectively. Inoculated the tubes with overnight bacterial culture and incubate the tubes for 24 hours. The optical density was measured at 540nm (Goswami et al., 2020).
2) *Effect of Different Temperature:* Nutrient broth were prepared and transferred into test tubes. The test tubes were inoculated with bacterial culture and incubate the tubes for 24 hours. The optical density was measured at 540nm (Goswami et al., 2020).
3) *Effect of Salt Concentration:* Nutrient broth were prepared and transferred into 5 test tubes. Add NaCl at different concentrations (2, 4, 6, 8 and 10).Inoculate the tubes with bacterial culture and incubate at 24 hours. The optical density was measured at 540 nm (Goswami et al., 2020).

G. Structural Characterization of EPS

1) *Fourier Transforms infrared Spectroscopy Analysis:* The purified EPS powder was prepared with dry potassium bromide (KBr) followed by pressing the combination and compressed them into a pellet form. The spectrum was recorded between the wavelength ranges of 400 and 4000 cm-1 in Fourier transform infrared (FT-IR Model: 6700) (Kanmani et al., 2011).
2) *Thermogravmetic Analysis:* TG-DTA analysis of EPS was conceded with the thermal system (TG-DTA/DSC Model: Q600 SDT). About 10 mg of dried sample was used for the TG-DTA experiment. TG-DTA thermograms were attained in the range of 0–400 ºC under the flow of nitrogen air at the rate of 10 ºC min−1. Their individual graphs were plotted with weight (percentage) loss and heat flow against temperature (Wang et al., 2010).
3) *X-ray Diffraction Analysis:* To study the physical characterization of EPS, X-ray diffraction scan at diverse ranges of two-theta angles (10-70 °C) was performed. XRD was accomplished on X-ray powder diffractometer (Philips X'pert pro, the Netherlands) with a Cu tube X-ray produced at 40 kV and 30 mA with PW3011/20 proportional detector. Crystallinity index was measured from the area under crystalline peaks standardized with equivalent to total scattering area (Ricou et al., 2005).
4) *Scanning Electron Microscopy Analysis:* The EPS (2–3 mg) was dissolved in Milli Q water and stirred constantly to get uniform dispersion in a water bath at 40 °C and cooled to room temperature. Then the solution was diluted to 0.1 mg/mL. About 5–10 µl was dispersed on a mica disc (Pelco mica disc 10 mm), and absolute ethanol was dropped over the sample to fix it on the mica disc. Then the mica sheet was air-dried to remove the residual ethanol. Subsequently, the AFM images were captured by scanning probe microscope (Brukers MM8) in tapping mode. The cantilever oscillated at its appropriate frequency (158 kHz), and the ambitious amplitude was 0.430 V. Then the EPS was fixed above the aluminium stub and then examined through SEM (Hitachi, Model: S-3400N) (Ahmed et al., 2013).
5) *Total Phenol Content:* The total polyphenols content was determined calorimetrically by Folin Ciocalteu procedure. To 0.5ml of EPS extract, add 5ml of Folin Ciocalteu reagent (adjusted with distilled water) was added. After 5 minutes 1ml of aqueous sodium carbonate solution was added and kept it for 40 minutes. After 40 minutes the absorbance was measured at 725nm against blank. The gallic acid stock solution was prepared by dissolving 0.5gm in 10ml in ethanol or methanol and it was diluted to 100ml with distilled water. The phenol content was determined by means of a calibration curve prepared with gallic acid and expressed as mg of gallic acid equivalent. This method was based on a modified protocol of Henriques et al., (2007).
6) *Antioxidant Activity Determination by DPPH Assay:* This method is based on the modified protocol of Sasidharan et al., (2013). The percentage of antioxidant activity was determined by DPPH assay method. Methanolic DPPH 0.1mM of methanolic DPPH radical solution was prepared and 0.2ml of extract was added to 1.9ml methanol solution of DPPH. Shake the mixture and mix well, left to stand at room temperature dark for 30 minutes. The absorbance was measured at 517nm against the methanol as blank. The scavenging activity (AA %) was determined according to Mensor et al., (2001). AA% = 100- [(Abssample – Absblank) X 100/Abscontrol
7) *Reducing Power Assay:* In this method, standard solution of ascorbic acid were prepared (0.1-0.5mg/ml). Add 1ml of extract into one test tube(t1) and add equal volume of phosphate buffer &1%of potassium ferricyanide and incubated at 500C for 15 minutes. After 5min add 1ml 10% TCA [trichloro acetic acid] to the test tubes to stop the reaction and then the tubes were centrifuged at 2800rpm for 10 min .After that 1ml of supernatant were mixed with distilled water and 0.1%Fecl3 (1ml). Then the absorbance was measured at 700nm. This method is based on the modified protocol of Roe et al., (1974).
8) *Antibacterial Activity:* The bacterial strains were inoculated into the nutrient broth and incubated at370C for 24 hours. Muller Hinton agar plates were prepared. In the plates wells were made and spread plating was done with the 24 hours grown pathogens which were to be tested such as Escherichia coli, Serratia spp Bacillus spp., and Pseudomonas spp. Then 100µl of EPS extract was added to the wells and incubate the plates at 300C for 24 hours. The results were observed by the presence of a clear zone around the disc in the medium. This method is based on the modified protocol of August et al., (2000).

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III. RESULTS

A. Isolation of Bacteria from idli batter

Among the samples collected, five isolates were isolated from homemade and readymade idli batter. From this showed EPS were selected for characterization.

Figure.1. Packed spread plate

Figure.2.Homemade spread plate

B. Screening of Probiotic Bacteria

Strains were isolated from idli batter. Samples were inoculated in MRS agar plates and incubated for 2days at 30 c. Total 2 pure cultures were obtained which were screening

Figure.3.Isolation of strain - Packed

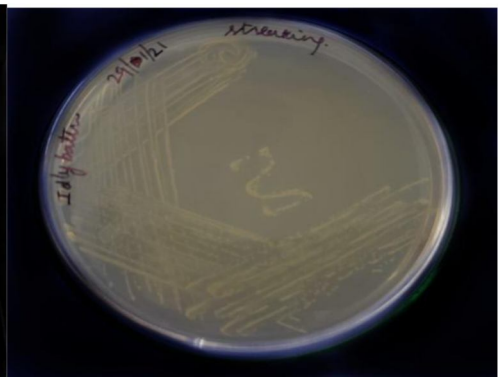

Figure.4.Isolation of strain - Homemade

C. Hemolytic Assay

The above two isolated pure cultures were screened for hemolytic activity. Out of which two cultures showed the hemolytic activity and only one was found to be non-hemolytic which was taken for further characterization.

Figure.5. Hemolytic assay

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D. Confirmation of EPS- producing bacteria by Congo Red

The Congo red agar method confirmed the formation of biofilms after 48 h indicated by the growth of black colour colonies.

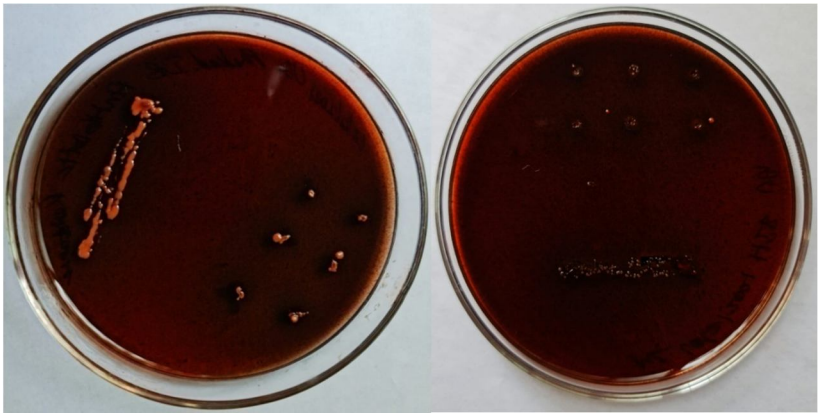

Figure.12. Congored packed Figure.13.Congo red homemade

IV. GROWTH OPTIMIZATION OF BACTERIA IN DIFFERENT PH, SALT AND TEMPERATURE

A. Temperature

The bacterial isolates were cultivated at different temperatures and the maximum production of Idli batter was obtained at temperature. A standard graph was plotted with temperature in y-axis and cell density in x-axis.

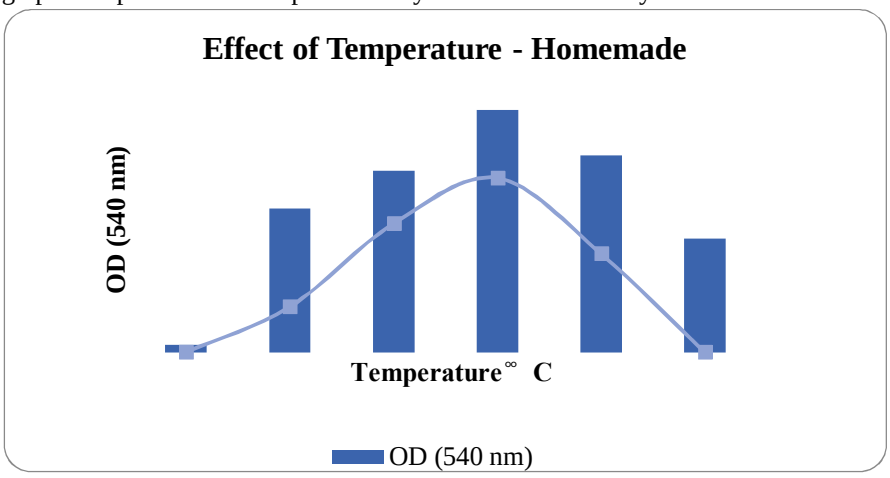

Figure.22.Effect of Temperature Homemade

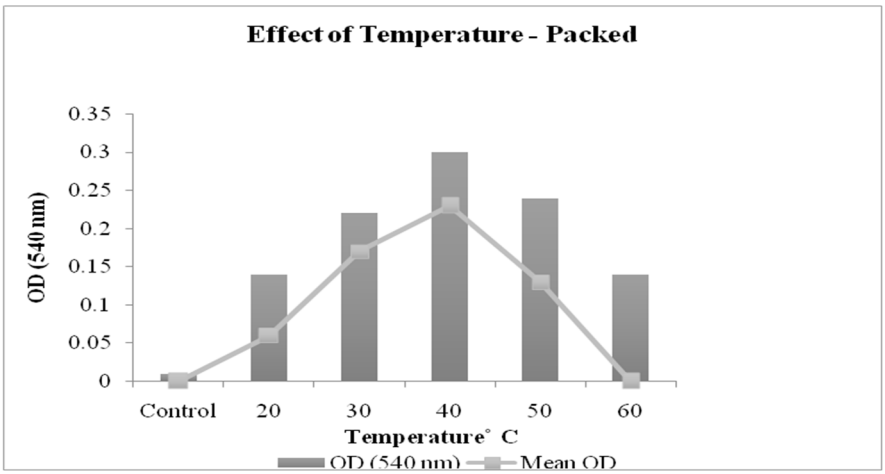

Figure.23.Effect of Temperature Packed

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B. pH

The bacterial isolates were cultivated in different pH (2,4,6,8,&10) and the maximum production of Idli batter was obtained at the pH 8. A graph was plotted with pH in the y-axis and the cell density, EPS production in x-axis.

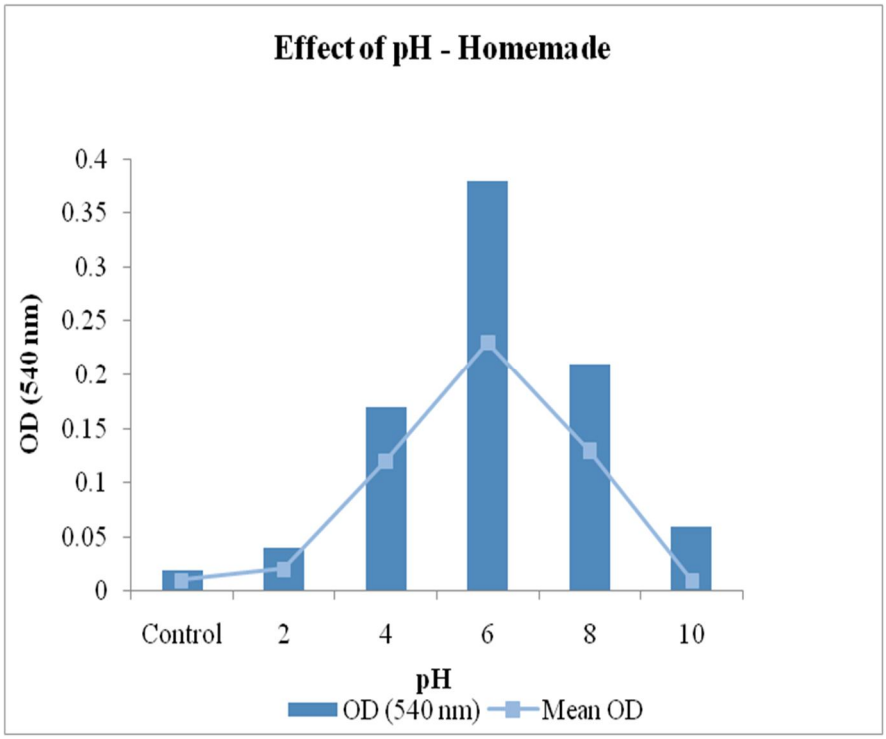

Figure.24.Effect of pH Homemade

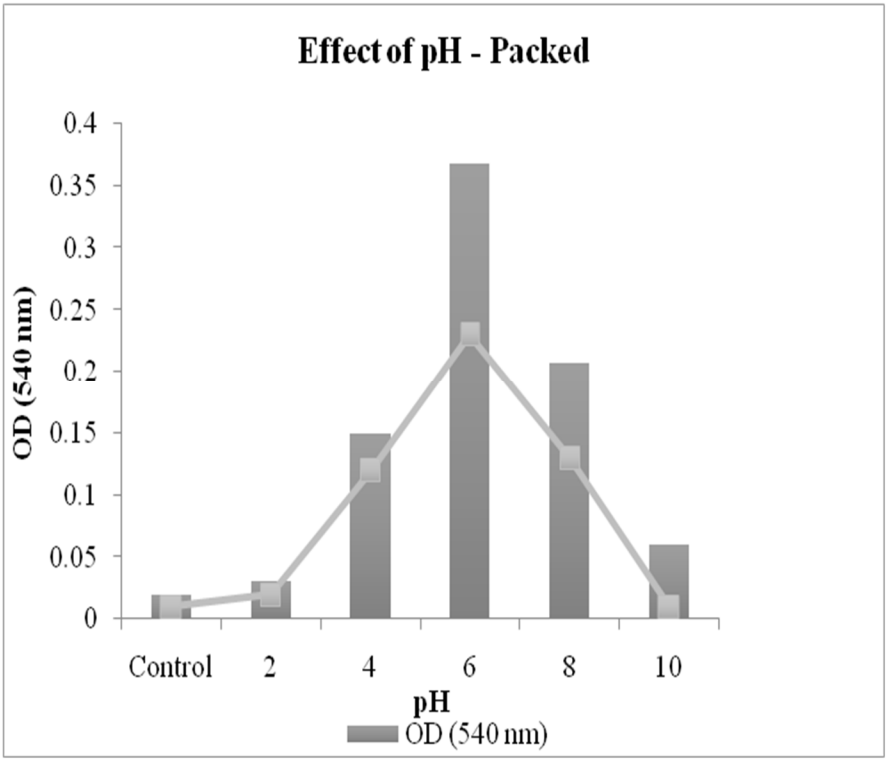

Figure.25.Effect of pH Packed

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C. Salt

Bacterial isolates were cultivated in different Nacl concentrations (2%,4%,6%,8%&10%)and the maximum production of Idli batter was obtained at the concentration of Nacl. A standard graph was plotted.

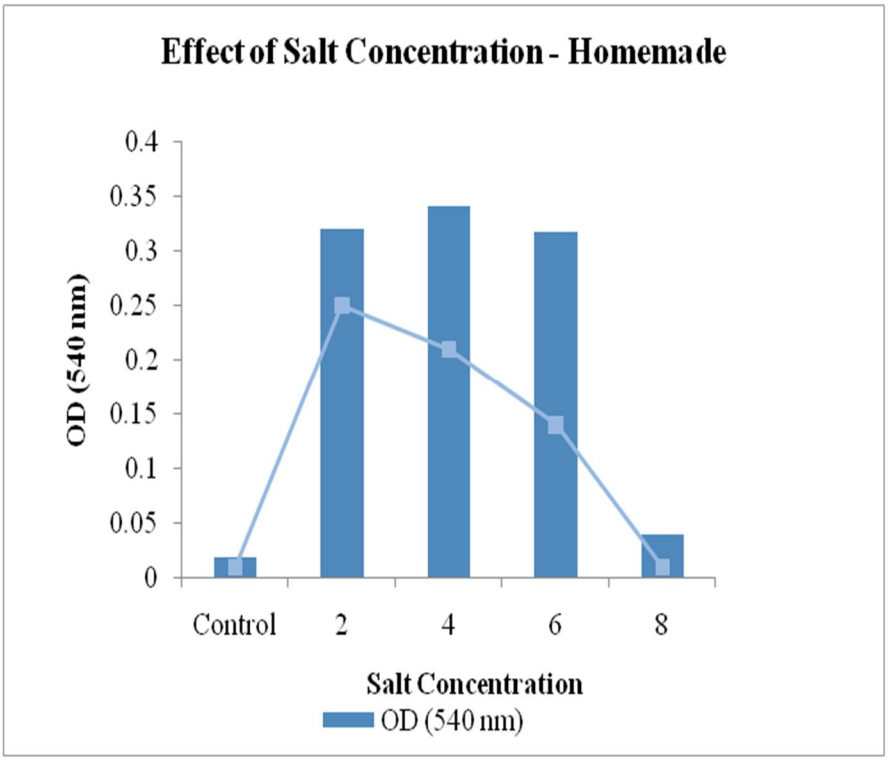

Figure.26.Effect of salt concentration Homemade

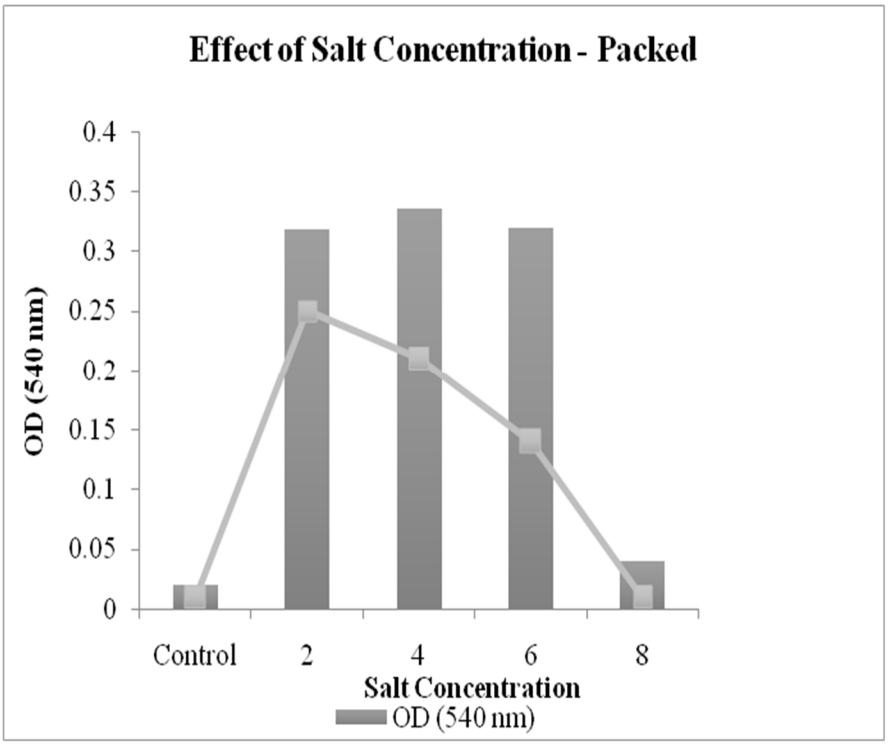

Figure.27. Effect of salt concentration Packed

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V. ANTIOXIDANT ACTIVITY

A. DPPH free Radical Scavenging Assay

DPPH, a relatively stable organic radical has been widely used in the determine of antioxidant activity. Ability to scavenge hydrogen radical is one of the important mechanisms for antioxidant activity. The EPS isolated showed of radical scavenging activity and *Lactobacillus* showed radical scavenging activity.

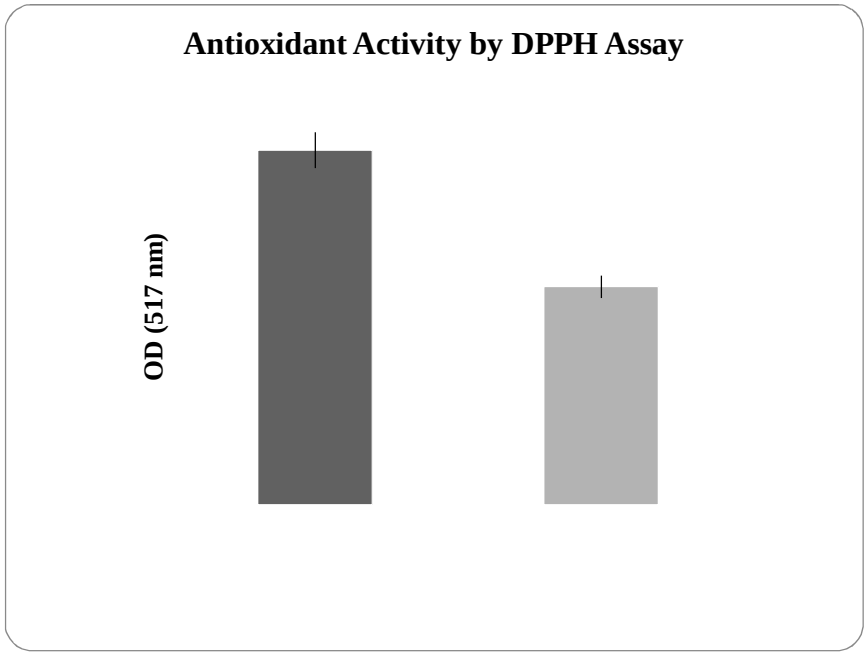

Figure.28.Antioxidant activity by DPPH Assay Homemade

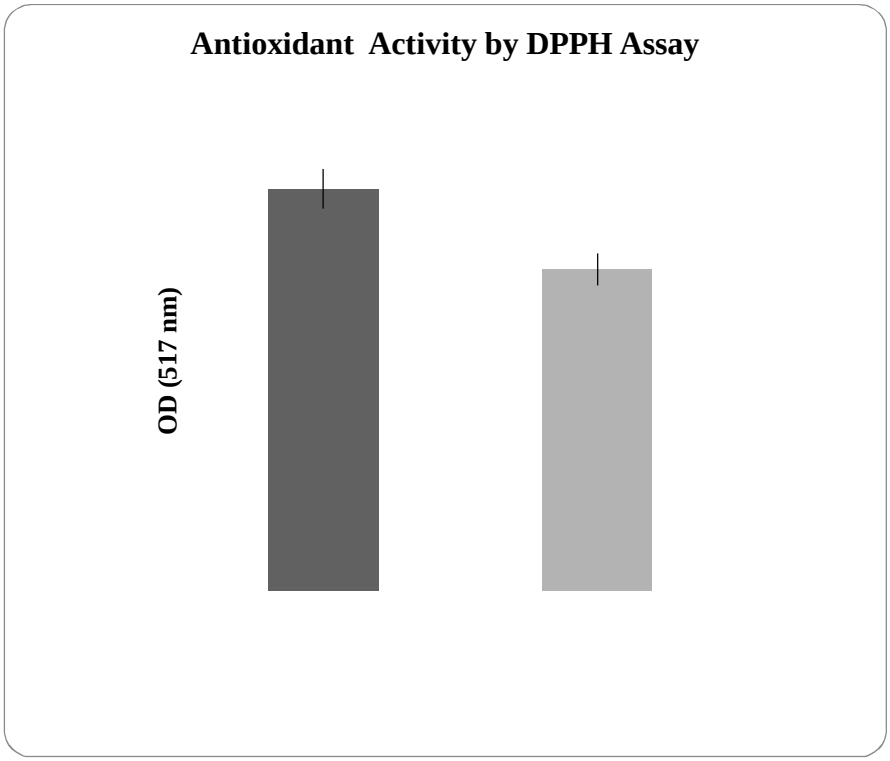

Figure.29.Antioxidant activity by DPPH Assay Packed

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B. Total Phenol Content

The total polyphenolic content of bacterial yellow pigment (methanol extract) was determined by Folin-Ciocalteau procedure. It was obtained at a absorbance of 725nm is 0.177 and a standard graph was plotted

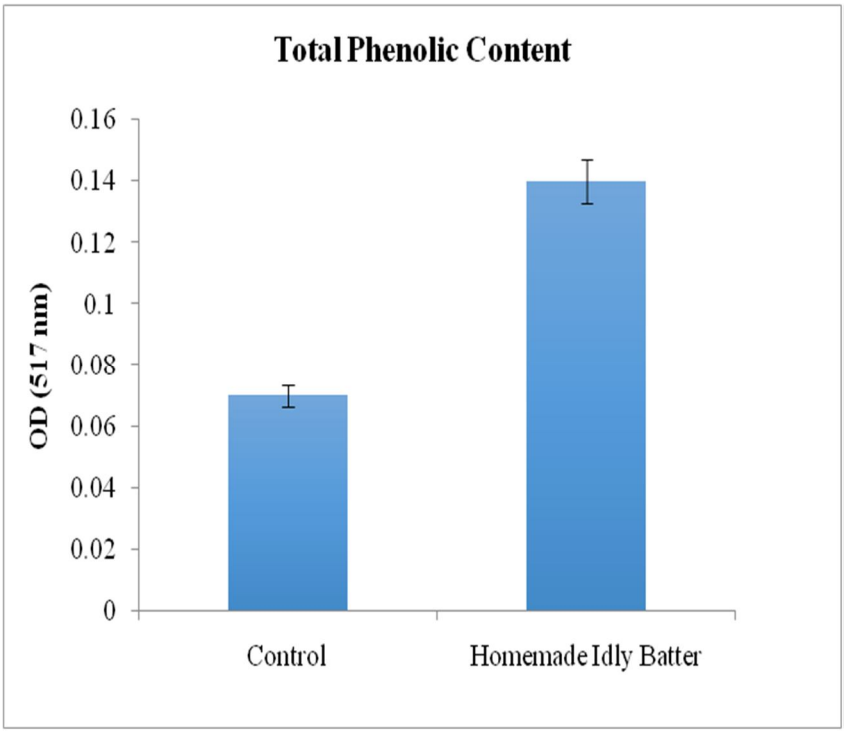

Figure.30.Total phenol content Homemade

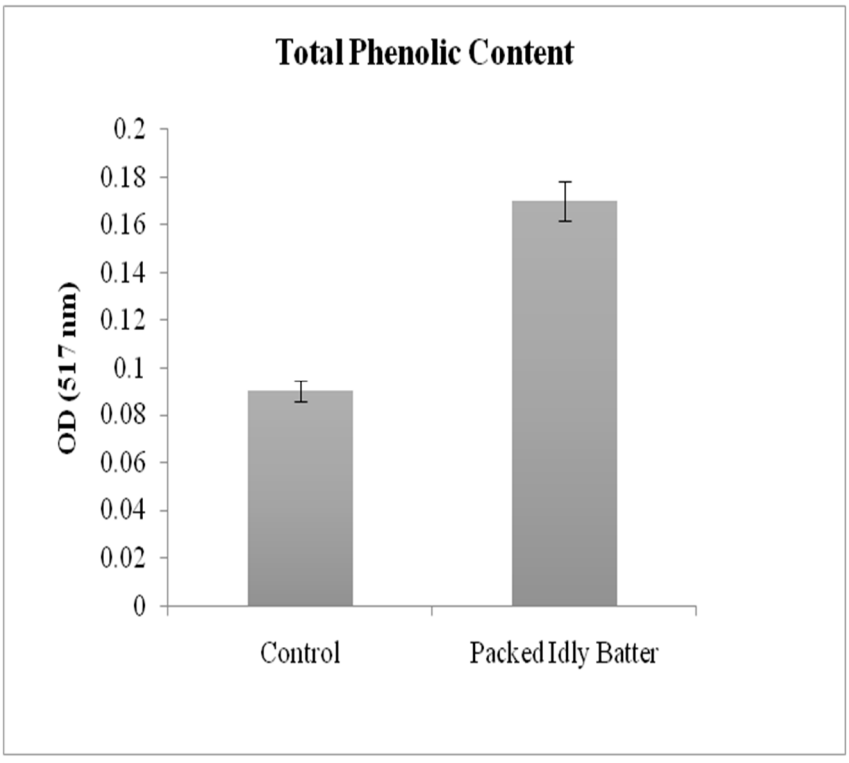

Figure.31.Total phenol content Packed

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C. Reducing Power Assay

Reducing power assay measures the ability of the CFE against oxidative effect of reactive oxygen species (ROS). The EPS isolated showed the reducing activity, but the Lactobacillus showed reducing activity.

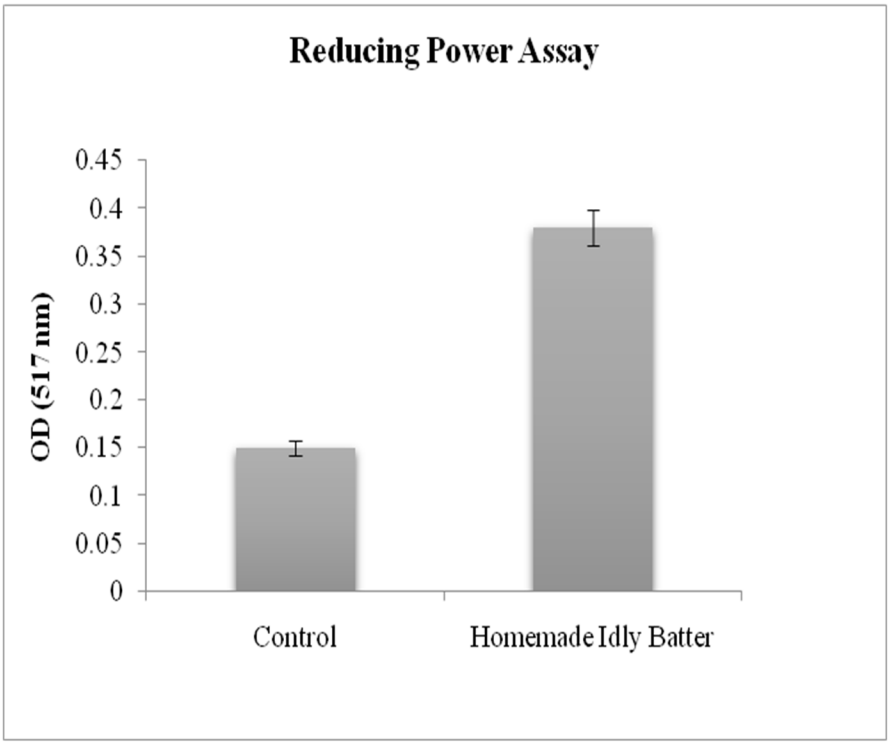

Figure.32.Reducing power assay Homemade

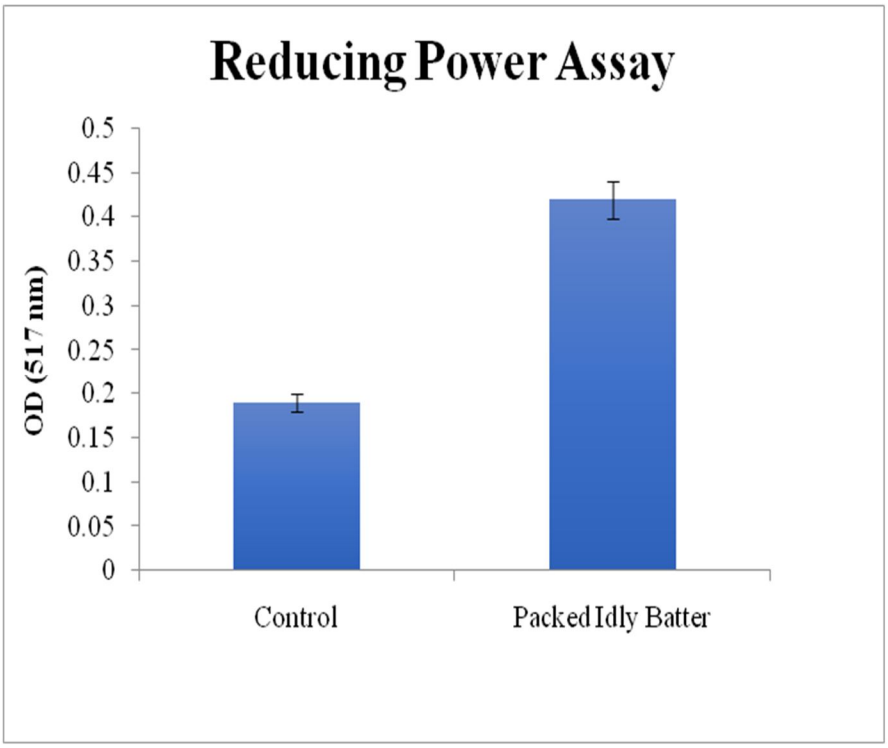

Figure.33.Reducing power assay Packed

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D. Antibacterial activity

The strain ACFM05 was sensitive to all antibiotics tested (ampicillin, teracycline, vancomycin, kanamycin and gentamycin).

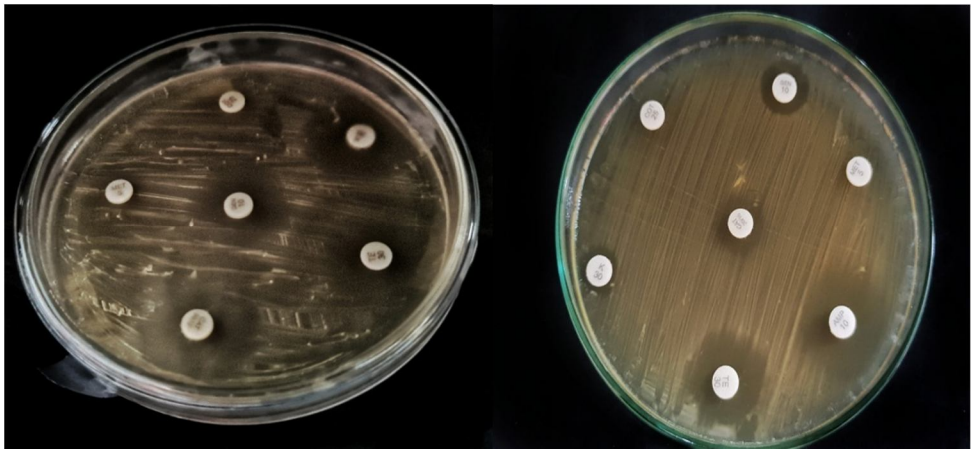

Figure.34.Antibiotic packed

Figure.35.Antibiotic Homemade

VI. STRUCTURAL CHARACTERIZATION OF EPS

A. Fourier Transforms Infrared Spectroscopy Analysis

The FT-IR spectrum of purified EPS showed numerous peaks from 3441 cm−1 to 516 cm−1. Presence of high levels of hydroxyl groups (O–H) was indicated by broad absorption peak around 3250–3500 cm−1, confirming the polysaccharide nature of the material.

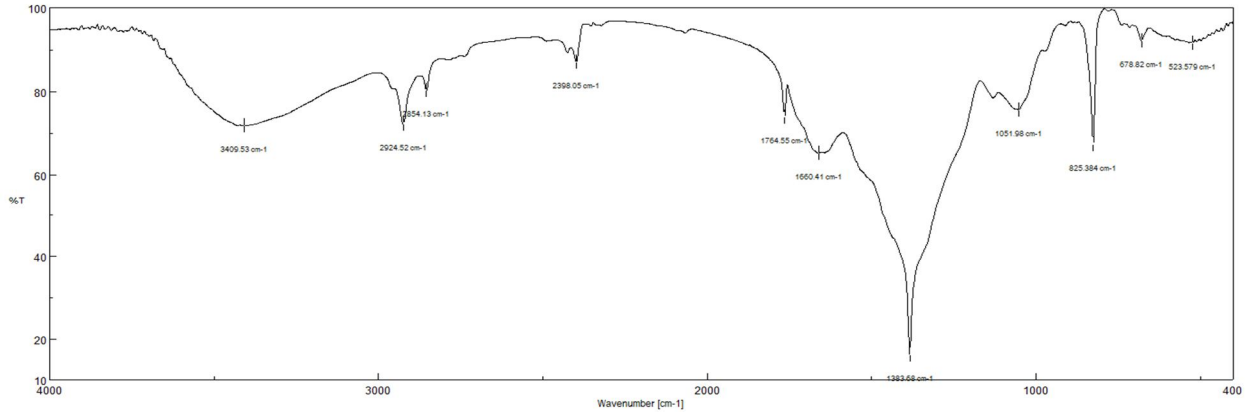

Figure.36.PACKED

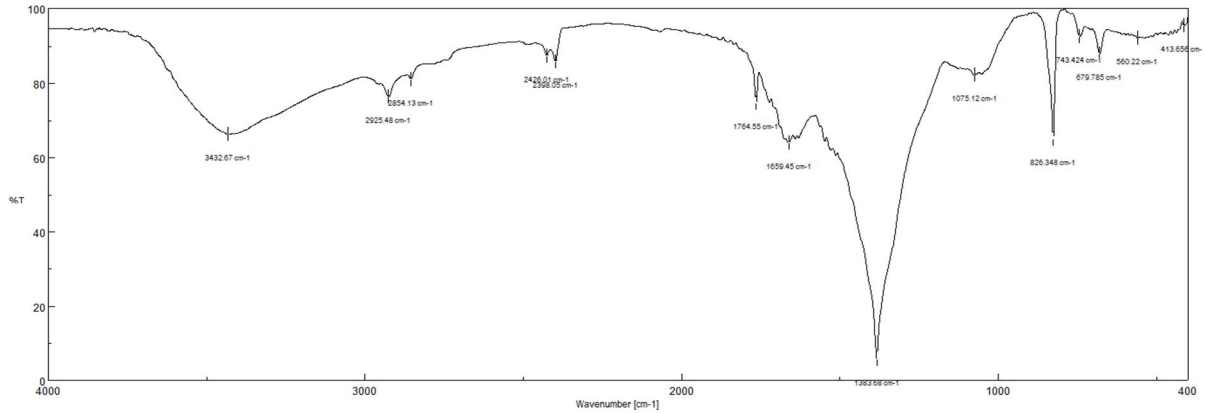

Figure.37.homemade

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B. Thermogravimetric Analysis

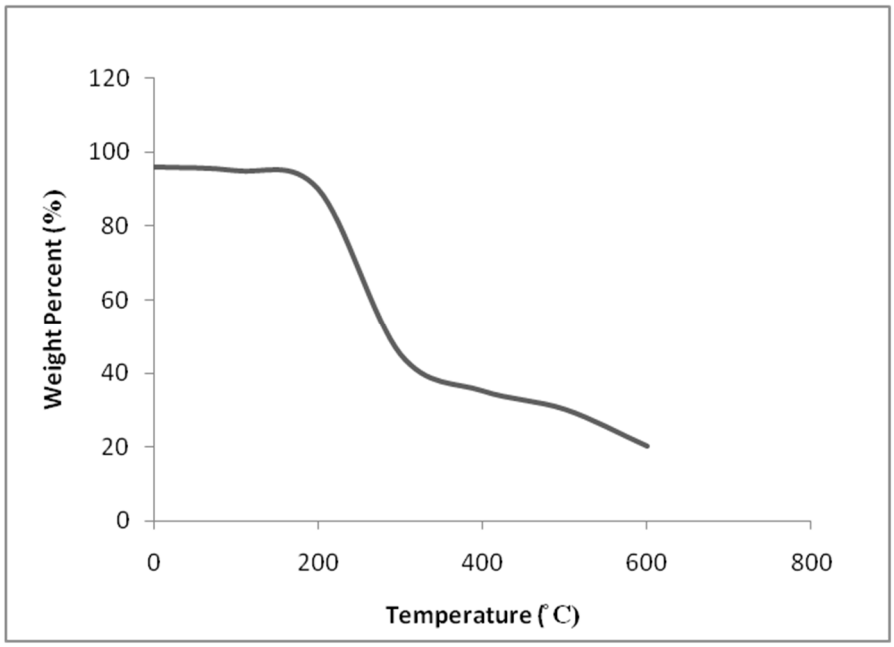

Figure.38.Thermogravimetric analysis

TG-DTA Analysis, The weight loss of EPS was up to 15% from 0 °C to 100 °C, due to moisture. Total weight loss of EPS occurs at the temperature of (Tc) 241.14 ºC. It is safe to be used in dairy industry when process temperature seldom overpasses 150 °C

C. X-ray Diffraction Analysis

From XRD pattern it was found that crystalline peaks were superimposed in the amorphous phase of the EPS imfering a partial crystalline (33.4%).

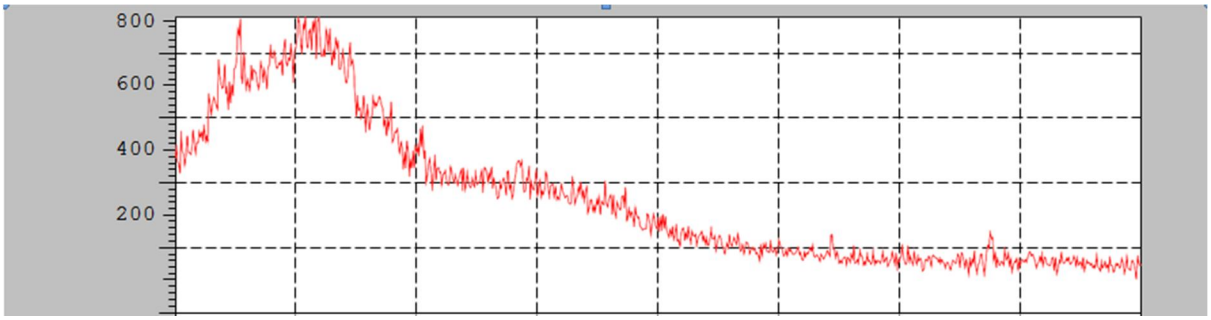

Figure.39.XRD Homemade

Figure.40.XRD Packed

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D. Scanning Electron Microscopy Analysis

At 5000 X magnifications EPS showed smooth, consistent polymeric matrix indicating the structural reliability important for bio based films formation. Much of the SEM properties of EPS are comparable to the characteristics of polymers. SEM images of the EPS magnifications showed smooth, consistent polymeric matrix indicating the structural reliability important for bio-based films formation.

E. Cell Surface topography of EPS was observed by SEM image

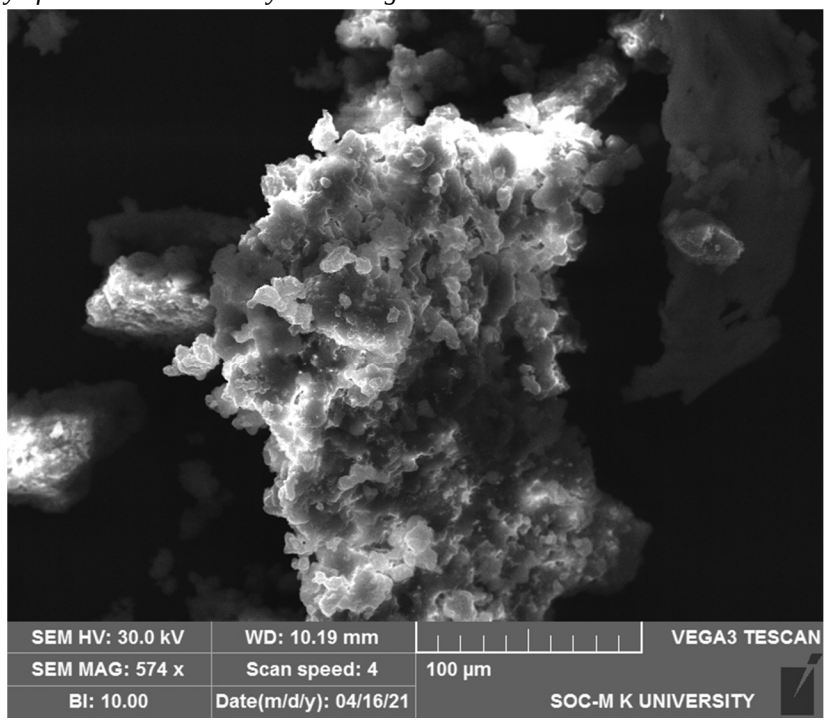

Figure.41.Scanning Electron Microscopy Homemade

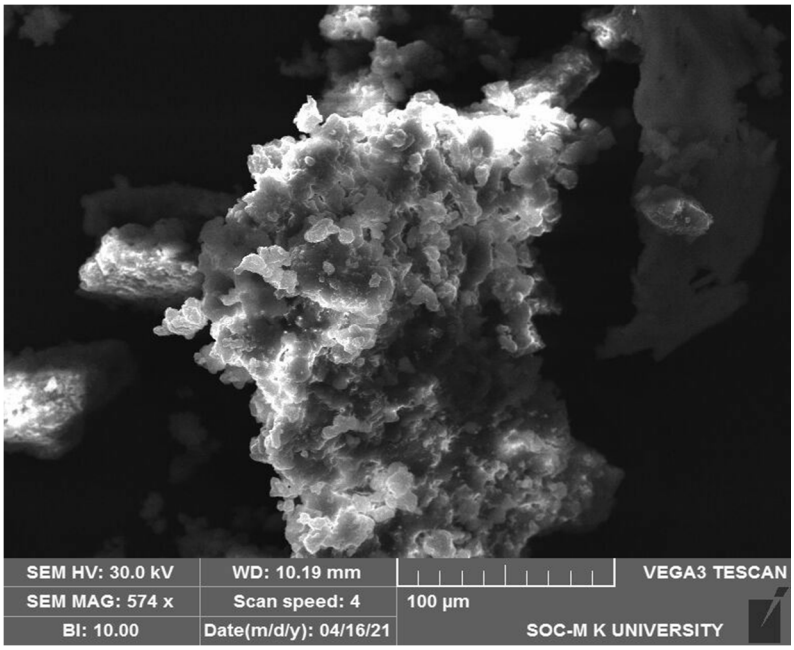

Figure.42.Scanning Electron Microscopy Packed

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VII. DISCUSSION

In the contemporary analysis, many possible strains of probiotic bacteria has been isolated from homemade and readymade idli batter. The probiotic bacteria is then further specified screening test were implemented by comparing with Lactobacillus. Gram staining appears that the isolates of packed and homemade. Effect of pH, Salt and temperature of the bacterial isolates were studied and influence the growth of bacterial isolates can be determined. After 24 hours incubation, the maximum optimization of EPS were obtained at the temperature at and maximum amount of pH were obtained at and the Salt concentration at can be observed graphs were been plotted with the particular values with temperature, pH and Salt concentration on the x-axis and absorbance on the y-axis. To identify the presence of EPS in idli batter we used congo red method and it results the presence of EPS in 48 hours. The molecular characterization of exopolysaccharides the DNA shows the presence of bacteria in genomic DNA. The total genomic DNA was extracted using enzymatic method and loaded with 0.2% of agarose gel in electrohorosis gel flow from negative charge to positive, to show the presence of DNA.Biochemical characterization is done to identify the positive and negative result of bacteria. We done six major biochemical analysis oxidase, Indole, Methyl red, Voges proskauer, Citrate and starch and we got three positive results in methyl red, Voges proskauer and citrate test.

VIII. CONCLUSION

Isolation and characterization of exopolysaccharides from have been further characterized to give the following details. The EPS further confirm to contain α-D-Glucose and glycosidic linkage as shown by FT-IR. The superior quality of EPS with only 33.4% crystalline nature was proved by XRD. EPS thermostable characteristics play an important role in the food industry especially in rheological properties as the manufacturing and processing of numerous food preparations are carried out at higher temperatures. The EPS was shown to compact structure, smooth surface indicating structural stability as indicated in SEM. Strongly packed and obligate irregular shaped molecules signify strong attraction for water molecules and have pseudo plastic property. EPS showed high thermal stability as shown by TGA and suggesting that the EPS is potential to be used as an encapsulating material in food industry. Antioxidant properties of EPS shown similar trend in DPPH and Hydroxyl radical activity as compare the Control and less in Metal chelating activity. However, the metal chelating activity is considerably high at higher concentrations. These EPS can further be optimized for commercial production and utilization food and pharmaceutical industry.

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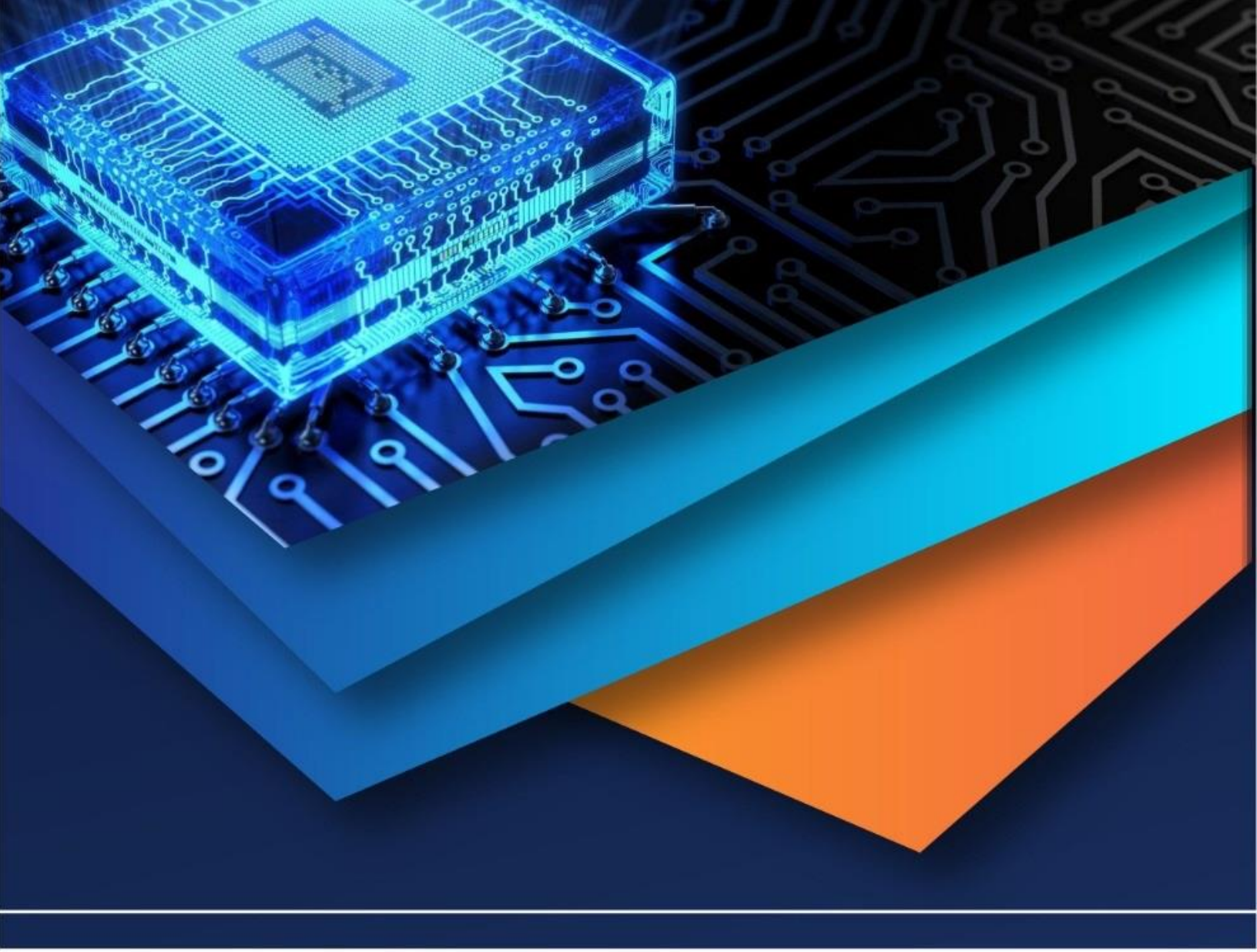

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