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# Enhanced exopolysaccharide production from food waste as a substrate through fed-batch FMN: An exploratory investigation of fluoride resistant bacteria

**R Vijay, K Srinivasan, Arunkumar Anandharaj, N Baskaran, Ashish Rawson and S Vignesh**

**Abstract**
Fluoride ($F^-$) is a non-biodegradable pollutant that is relatively persistent in nature and poses serious threats even at low contamination levels. The drastic increase in urbanization, industrialization, and population resulted in alarming $F^-$ ion pollution and intensive search for effective and inexpensive measures to combat its consequences. This study aims to identify $F^-$ resistant bacteria out of 80 strains isolated from fruit and dairy wastes and their tolerance across a range of sodium fluoride concentrations (10 mM to 500 mM) through plate diffusion and tube dilution methods. This study was also navigated to investigate the exopolysaccharides (EPS) production capacity of strains identified for a high degree of $F^-$ resistance. The fed-batch fermentation method was employed to bio-convert the food waste through supplementation of additional nitrogen sources. The yield of microbial EPS ranges from 1 - 10 mg/ 250 mL, and the FT-IR analysis confirmed their presence. This paper describes the potential of food waste as a valuable resource for producing biologically important polymers with commercial applications and mitigation of wastewater metals.

**Keywords:** fluoride resistance bacteria, fed-batch fermentation, exopolysaccharides, food waste

## 1. Introduction
Globally, around one-third of the total food produced (rich source of macro and micronutrients) is wasted every year (equivalent to around 1.3 billion tons (Kim *et al.*, 2021) [14]. Owing to their perishability and short shelf-life, the significant contributor of these food waste biomass are fruits, vegetables, dairy, and meat products. The percentage of food portion wasted in fruits and vegetables (21.6% of the total food) is much higher than meat and animal products (11.9%) and cereals and pulses (8.6%). The typical nutritional composition of these food waste ranges from 35.5 – 69.0% of total sugar and 3.9 – 21.9% of protein and other organic compounds (Uçkun Kiran *et al.*, 2014) [22]. This waste can also be categorized for its poor biological and oxidative stability and high enzyme activity. Likewise, the dairy effluents (waste generated in the dairy manufacturing process) possess distinguished organic content such as fat, nitrogen, phosphorus, and trace elements. Interestingly, these food wastes are also rich in microbial biomass with the potential to produce value-added components through a fermentation-like bioconversion process.

Fluoride ($F^-$) is a trace element that cannot be mineralized to a less toxic state through the routine desorption processes due to its inherent atomic structure. This trace element occurs naturally in soil and water and causes pollution and toxicity if the concentration is above 1.5 ppm. Moreover, it easily finds its way on the surface of plants like fruits and vegetables through groundwater, cultivation, and post-processing. Meanwhile, the chemical decontamination methods such as ion exchange and precipitation of cations pose limitations such as the chemicals would itself become contaminants. Similarly, though advanced technologies such as nano science and material chemistry-based techniques are developed to combat the $F^-$ pollution, bioremediation using $F^-$ resistant bacteria remains a viable solution considering its low cost and effective outcomes. In recent years, enormous studies investigated the potential of metal resistant bacteria from diverse environmental samples and their role in the bioremediation of inorganic pollutants. The scientific fact that the extracellular polysaccharides (EPS) on the bacterial surface has implications for heavy metal bio-sorption. The presence of heavy metal resistance can increase the bacterial EPS composition holds better promise for newer solutions and gains prominence (Gupta & Diwan, 2017) [9].

Literature evidence also suggests that the EPS produced on the peripheral surface of bacterial cells offers a defensive mechanism from the toxicity of metal ions. Further, the composition and structure of microbial polymer render the capacity for sequestration of metal ions (Muthu *et al.*, 2017).
These microbial polysaccharides are increasingly used in food, nutraceutical, pharmaceutical, and cosmetic industries for improving the quality, texture and flavor as an emulsifier, thickeners, stabilizer and gelling agent (Jindal & Singh Khattar, 2018) [12]. Several agricultural wastes and their by-products are experimented as substrates for the production of polysaccharides. While the key advantage in utilizing microbial polysaccharides lie in the inexpensive nature of the process, the ability to alters the functional properties of those sugar compounds through varying growth conditions of the strains, chemical composition of the medium and ease of downstream processing supports its products and applications (Freitas *et al.*, 2014) [8].
Many researchers put significant efforts to find and optimize food waste to maximize microbial polymers production in the past decade. The concentration of accessible carbon and nitrogen have direct links with the production of bacterial EPS. Several authors reported suitable conditions like (pH, temperature) and supplements (specific proteins) for maximum production of desired bacterial polymer (Singh *et al.*, 2017) [21]. *Pseudomonas aeruginosa Al-Dhabi144* was found to be resistant to $Cu^{2+}$, $Cd^{2+}$, $Pb^{2+}$, $Co^{2+}$ and $Zn^{2+}$ and used to produce polysaccharides in submerged fermentation with palm juice wastewater used as feedstock. This bacteria had a threefold increase in EPS production with optimised conditions and effectively removed lead in the food medium (Al-Dhabi *et al.*, 2020) [2]. However, no studies decoded the food waste-associated bacteria resistant to $F^-$ and its ability to sustain and produce microbial polymers during the fed-batch fermentation process in the nutrient-rich food waste medium.
Therefore, the present study was undertaken with the objectives (i) to isolate and screen bacterial strains that are resistant to $F^-$ ions from fruits and dairy waste; (ii) to prepare and optimize the growth characteristics of the selected $F^-$ resistant cultures under various pH and temperature in food waste medium; (iii) to evaluate the capacity of bacterial EPS production using $F^-$ resistant strains through the fed-batch fermentation process.

## 2. Material and Methods

### 2.1 Sample collection and materials

The fruit and dairy waste were collected from waste bins and the dairy industry in and around Thanjavur district, Tamil Nadu, India. The solid and liquid waste were collected in sterile containers, immediately stored in an icebox, carried to the laboratory, and stored in a refrigerator before 4 °C until further analysis. Nutrient agar, De Man, Rogosa and Sharpe (MRS) agar, Mannitol Egg Yolk Polymyxin (MYP) Agar, nutrient broth (NB), yeast, and beef extract were hi-grade purchased from Himedia Pvt. Ltd, Mumbai, India. Sterile ultrapure Milli Q water was used in the entire study.

### 2.2 Isolation and identification of bacteria

About 1 g of the sample was suspended in 100 ml of sterile saline solution and mixed through a rotary shaker for 30 min. Then, the serially diluted sample was inoculated into nutrient agar for the total viable count and selective media such as MYP Agar for *Bacillus* species, and MRS agar for *Lactobacillus* species. The plates were incubated at 37±2 °C for 24 hrs. Discrete colonies were picked and sub-cultured until pure colonies were obtained. The pure bacterial cultures were freshly prepared in nutrient broths before every experiment. The bacteria isolated from the selected media were referred to as like organisms (LO) of the selective bacterial group (Vignesh *et al.*, 2015) [23] and were further confirmed by biochemical characterization assays. The bacterial isolates were stored at 4º C on respective media for short-term maintenance and 40% glycerol at -20º C for long-term storage.

### 2.3. Determination of fluoride resistant strains

For the selective screening of $F^-$ resistant bacteria from the isolated cultures of food waste samples, plate diffusion and dilution methods were used to measure the $F^-$ tolerance level accurately. The $F^-$ stock solution of 1000 mM was prepared using analytical grade sodium fluoride (NaF) using double-distilled Milli Q water and autoclaved at 121° C for 15 mins. The concentration of NaF solution was prepared in ranges of 10, 50-, 100-, 250- and 500-mM from the stock solution and stored at 4 °C until further analysis.

#### 2.3.1. Plate Diffusion method

For the plate diffusion method, 300µl of different concentrations of $F^-$ solutions were pipetted in a central well of 4 mm depth and 1 cm in diameter in nutrient media and allowed to diffuse for 24 h to obtain the concentration gradient of the element. On each plate, 8 strains were streaked radially and incubated at 37±1 º C for 48 hours. All the trials were performed in duplicate. After incubation, the inhibition of bacterial growth is measured through measuring ratio: length of growth in mm to length of total inoculated length in mm (Hassen *et al.*, 1998; Vignesh *et al.*, 2016) [11, 24].

#### 2.3.2. Tube dilution method

The lowest metal concentration that completely inhibits microbes' growth is termed "Minimum inhibitory concentration" (MIC). For tube dilution, optical density (OD) at 600 nm was utilised for growth indication. The media and $F^-$ solution are separately sterilised at 121 °C for 15 min. An appropriate amount of $F^-$ solution and 200 µl of standardized (adjusted to 0.5 McFarland standards for $10^8$ CFU/mL) bacterial isolates were added to the test tube and made 10 ml using the sterile nutrient broth. The sterile nutrient broth was considered negative control. The growth was monitored after incubation at 37±1 °C for 24 h (Hassen *et al.*, 1998) [11]. The maximum concentration at which the bacteria can grow was identified as maximum tolerance concentration.

### 2.4. Effect of pH and temperature on bacterial growth kinetics

0.1 mL of standardised (using 0.5 McFarland standard for adjusting the microbial culture - $10^8$ CFU/mL) culture was inoculated in 10 mL of nutrient broth and incubated at 20, 25, 30, 35 and 40 ºC for determining the suitable growth temperature. The UV visible spectrophotometer (OD at 600nm) was used for analysed growth rate of the microorganism. To optimize the pH, 10 ml of nutrient broth was adjusted to different pH levels (4, 5, 6, 7, 8 and 9) for the determination of an effective growth rate of 37±1 °C at 120 rpm. After 24 hours of incubation, the growth rate was measured using a UV visible spectrophotometer at 600nm.

### 2.5. Preparation and optimization of food waste as a fermentation medium

With the objective of utilizing the food waste (fruit and dairy in this study) to produce value-added components out of the waste, the food waste medium was optimized for bacterial growth. The fruit and dairy waste were pulverised separately, blended into a thick paste, centrifuged at 9000 rpm for 10 min, and supernatant autoclaved at 121 °C and stored at 4 °C. Protein and reducing sugar content was determined using Kjeldahl and Dinitro salicylic acid methods following (AOAC,2019) methods. In fruit waste, 8 g of sample was blended with 40 mL of water and supernatants were obtained after centrifugation at 14000 rpm for 10 min and autoclaved at 121 °C and stored at 4 °C which was considered as fermentation medium 1(FM1). Using similar conditions, dairy waste 40mL was centrifuged and supernatant collected and supernatant autoclaved at 121 °C termed fermentation medium 2(FM2).

Parallel experiments were carried out to optimize the bacterial growth rate in the food medium by the addition of supplements in comparison with the promising standard nutrient broth medium. (i) The growth rate of bacterial isolates in different compositions of FM1, FM2 (ratio of 20 – 100%) were measured by UV reading at 600 nm in 24 and 48 hrs. (ii) pH adjusted and the addition of soy protein supplement at 0.002 g to 1:0 and 1:1 ratios of FM1 and FM2, the growth rate of bacterial culture (200 µl) inoculated and incubated at 37 °C in 10 rpm for 24 h were read at 600 nm in 24 and 48 h. (iii) The bacterial growth rate in the food medium is also optimized with varying pH and temperature.

### 2.6. Fed-Batch Fermentation to produce bacterial EPS

The production of EPS by the $F^-$ resistant strains using food waste medium in a fed-batch fermentation process was carried out given their industrial application. The fed-batch fermentation was carried out in a 500 ml Erlenmeyer flask and the initial working volume of 250 ml. The pH adjusted FM1, FM2 and water were mixed in a ratio of (1:1:2) in a 500 mL conical flask. In the FM1 and FM2, 2.5 mL of standardized cultures (McFarland standard 0.5) was inoculated and kept in a shaking incubator at 120 rpm for 48 hrs at 37±1 °C. After fermentation, fed-batch fermentation was performed for 48 h. Initially, the feeding medium contained 5 ml of FM1 and FM2, and 0.1 g of soy protein isolate was continuously added every 6 h as 5 mL of supplements from 12 h to 48 h. All experiments were performed in duplicate.

### 2.7. Isolation and purification of EPS

The extraction and purification of bacterial EPS was achieved through centrifugation (10000 rpm, 10 min) and collection of supernatant along with the cold ethanol (ratio 2:1) at a temperature of 4 °C (Abou-taleb *et al.*, 2015) [1] for 24 h. After 24 h, the supernatant was centrifuged at 6000 rpm for 20 min at 4 °C, and the pellet was collected. In milli Q water, the crude polysaccharide was dissolved, and for removal of unfermented sugars and small molecules, the solution was dialyzed for pure polysaccharides. Using 2:1 volume of 100% ethanol, the pellet was precipitated, and the precipitate was dried at 37 °C for 24 h (Abou-taleb *et al.*, 2015) [1]. The isolation and purification of the pellet were done at both 24 h and 48 h. All the pellets were concentrated and freeze-dried for further experiments.

### 2.8. FT-IR spectra analysis

ATR-FTIR (Attenuated total reflectance -Fourier transformed infrared spectroscopy) spectrum was used to determine the functional group of dialyzed polysaccharides. The IR SHIMADZU Affinity-1S FTIR spectrophotometer with deuterated L-alanine doped triglycine sulphate (DLATGS) detector was used to obtain spectrum by Lab solution IR software. The resolution of the spectrum was 4 $cm^{-1}$ collected from 500-5000 $cm^{-1}$ with 45 scans for a single spectrum. The graph was plotted with the x-y axis as wavenumber and transmittance.

## 3. Results and Discussion

### 3.1 Isolation and selection of $F^-$ resistance bacteria

A total of 80 bacterial strains were isolated from fruit waste (40 nos.) and dairy waste (40 nos.). All the strains were screened for fluoride toxicity by plate diffusion and tube dilution methods. Among 80 strains, 30, 30, 20 strains from PCA, MYP and MRS were isolated respectively. The strains FVV1, FVV2, FVV5 were isolated from PCA, DVV3 from MYP and FVV4 from MRS. The bacterial colonies were patched on 10 mM NaF containing nutrient agar plates, and from that, the $F^-$ resistance colonies were obtained and streaked on nutrient agar plates containing varying concentrations of NaF from 10 mM to 500 mM. The $F^-$ resistance profile is tabulated in Tables 1 and 2.

**Table 1:** Percentage of Fluoride resistant strains from fruit waste

| Percent of growth | Fruit waste (n = 40) Fluoride ($F^-$) resistant strain | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 10 mM | | 50 mM | | 100 mM | | 250 mM | | 500 mM | |
| | N | % | N | % | N | % | N | % | N | % |
| 0 – 10 | - | - | - | - | - | - | - | - | - | - |
| 11 – 20 | - | - | - | - | - | - | - | - | - | - |
| 21 – 30 | - | - | - | - | - | - | - | - | - | - |
| 31 – 40 | - | - | - | - | - | - | - | - | - | - |
| 41 – 50 | - | - | - | - | - | - | - | - | - | - |
| 51 – 60 | - | - | - | - | - | - | - | - | - | - |
| 61 – 70 | - | - | - | - | - | - | - | - | - | - |
| 71 – 80 | - | - | - | - | - | - | 3 | 7.5 | 3 | 7.5 |
| 81 – 90 | - | - | 2 | 5 | 6 | 15 | 7 | 17.5 | 8 | 20 |
| 91-100 | 40 | 100 | 38 | 95 | 34 | 85 | 30 | 75 | 29 | 72.5 |

**Table 2:** Percentage of Fluoride resistant strains from dairy waste

| Percent of growth | Dairy waste (n = 40) Fluoride ($F^-$) resistant strain | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 10 mM | | 50 mM | | 100 mM | | 250 mM | | 500 mM | |
| | N | % | N | % | N | % | N | % | N | % |
| 0 – 10 | - | - | - | - | - | - | - | - | - | - |
| 11 – 20 | - | - | - | - | - | - | - | - | - | - |
| 21 – 30 | - | - | - | - | - | - | - | - | - | - |
| 31 – 40 | - | - | - | - | - | - | - | - | - | - |
| 41 – 50 | - | - | - | - | - | - | - | - | - | - |
| 51 – 60 | - | - | - | - | - | - | - | - | - | - |
| 61 – 70 | - | - | - | - | - | - | - | - | - | - |
| 71 – 80 | - | - | - | - | - | - | 2 | 5 | 3 | 7.5 |
| 81 – 90 | - | - | - | - | 4 | 10 | 4 | 10 | 6 | 15 |
| 91 – 100 | 40 | 100 | 40 | 100 | 36 | 90 | 34 | 85 | 31 | 77.5 |

In plate diffusion method more than 50% of strains easily tolerated higher concentrations. At a minimum concentration of 10mM of $F^-$, 100% showed a 91-100% growth rate. At 500 mM of $F^-$ concentration, 72.5% of the population showed a 91-100% growth rate. Table 3 shows the different growth percent and their MIC in varying concentrations of fluoride. The minimum inhibitory concentration (MIC) confirmed that only one organism survived in 250 mM concentration out of

the 40 strains isolated from the fruit waste. 50% of strains were resistant at minimum concentration level (10mM), whereas 2.5% of strains grew at 250mM concentration. This strain belonged to *Bacillus* family.

For dairy waste in plate diffusion method, at a minimum concentration of 10mM, 100% of the population showed growth at 91-100%. At 500mM of $F^-$ concentration, 77.5% of the population showed growth at 91-100%. Likewise, only one organism survived in 250 mM concentration in MIC assay on the bacteria isolated from dairy waste. Based on the media, sample, and fluoride toxicity, the resistant strains were named FVV1, FVV2, FVV4, FVV5 and DVV3 isolated from fruit and dairy waste, respectively. These resistant strains belonged to *Lactobacillus* family.

**Table 3:** Minimum inhibitory concentration for food waste

| Concentration of metals (mM) | Minimum inhibitory concentration for Fruit waste and dairy waste | | | |
|---|---|---|---|---|
| | Fruit waste | | Dairy waste | |
| | N | % | N | % |
| 10 | 20 | 50 | 17 | 42.5 |
| 50 | 13 | 32.5 | 18 | 45 |
| 100 | 6 | 15 | 4 | 10 |
| 250 | 1 | 2.5 | 1 | 2.5 |
| 500 | - | - | - | - |

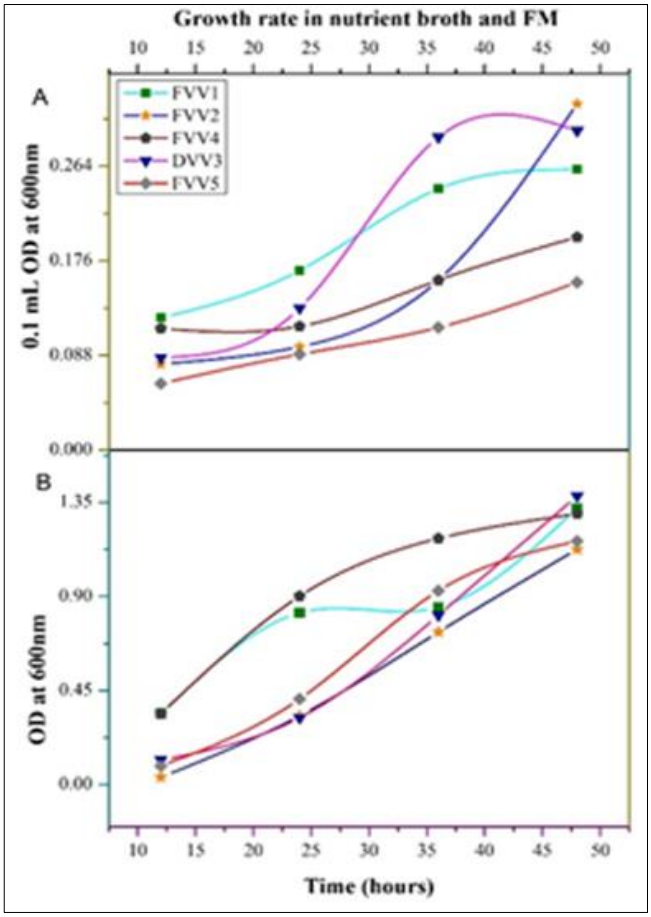


**Fig 1:** Growth rate of strain at different composition of FW and DW

### 3.2 Optimization of bacteria at pH and temperature

The optimum pH and temperature for fluoride resistant strains were seen at pH 6.5 and 35 °C for all strains.

### 3.3 Preparation and optimization of fermentation medium

To effectively utilize the food waste medium for value-added products, the food waste composition was modified and optimized. To obtain the original composition of carbon to nitrogen ratio (1:2) in a standard reference medium such as nutrient agar, the food waste medium was formulated through supplementation of a nitrogen source "soy protein". The fermentation medium was prepared in the same combination of nutrient broth. Fig 1 shows the growth rate of FVV1 at different composition for FW and DW upto 48 h.

In comparison with nutrient broth, 10-fold dilution was made for all 5 strains for OD at 600 nm. For fruit waste the growth rate decreased with an increase in concentration for 24 h, while showed higher growth for 8, 6 mL at 48 h. In dairy waste, the higher population was seen in 10,6,4 mL in 24 h, and for 48 h the growth rate decreased with decreasing concentration. The concentration (1:1, 1:0) was used as FM and the growth rate plotted in Fig 2. The same concentration with pH adjusted to 6.5 and influence of soy protein isolate in growth rate was shown in graph 3. The growth rate of strain DVV3 at optimum composition with fed-batch fermentation was compared against nutrient broth upto 2 days and plotted in Fig 3.

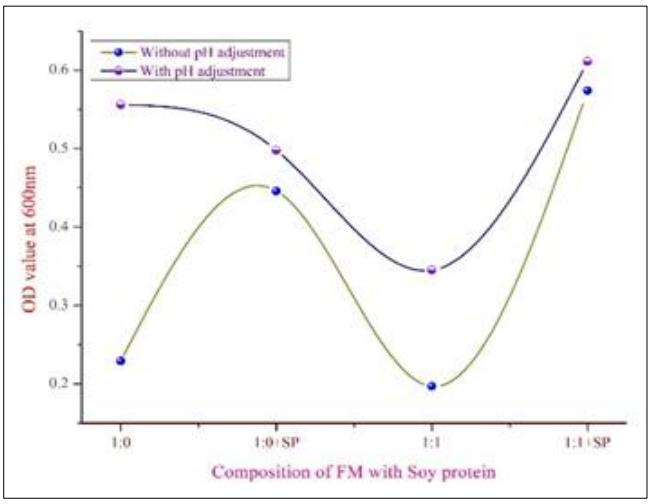


**Fig 2:** Growth rate of strain DVV3 at different composition of FM with soy protein

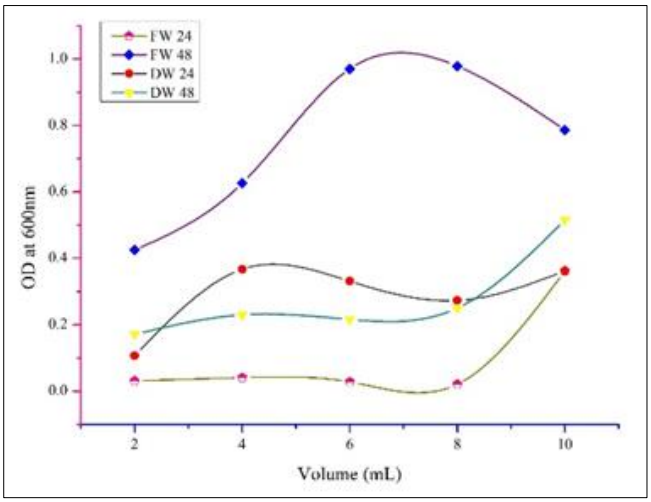


**Fig 3:** Growth rate of strain DVV3 in nutrient broth and FM

### 3.4 Fed-batch fermentation

The yield of polysaccharides at 24 h and 48 h are shown in Table 4 and Fig 4. The yield of polysaccharides increased from 24 to 48 h the yield was higher in strains FVV1, FVV4

in both FM and NB while DVV3 gave more yield in NB compared to FM. The yield of polysaccharide by each strain ranged between 1-10 mg/250mL. The maximum yield of polysaccharide was by strain FVV1.

**Table 4:** Yield of polysaccharide in nutrient broth and fermentation medium at 24 and 48 h interval

| Fermentation medium with culture | Yield of polysaccharide (mg/250mL) at 24 h | Yield of polysaccharide (mg/250mL) at 48 h | Total yield (mg/200mL) |
|---|---|---|---|
| NB FVV5 | 0.12 | 1.9 | 2.02 |
| NB FVV4 | 0.17 | 0.8 | 0.97 |
| NB DVV3 | 0.11 | 9.6 | 9.71 |
| NB FVV2 | 0.09 | 1.3 | 1.39 |
| NB FVV1 | 0.1 | 9.6 | 9.7 |
| FM FVV5 | 0.11 | 2.2 | 2.31 |
| FM FVV4 | 2.5 | 4.4 | 6.9 |
| FM DVV3 | 1 | 1.7 | 2.7 |
| FM FVV2 | 0.7 | 0.9 | 1.6 |
| FM FVV1 | 4.4 | 5.1 | 9.5 |

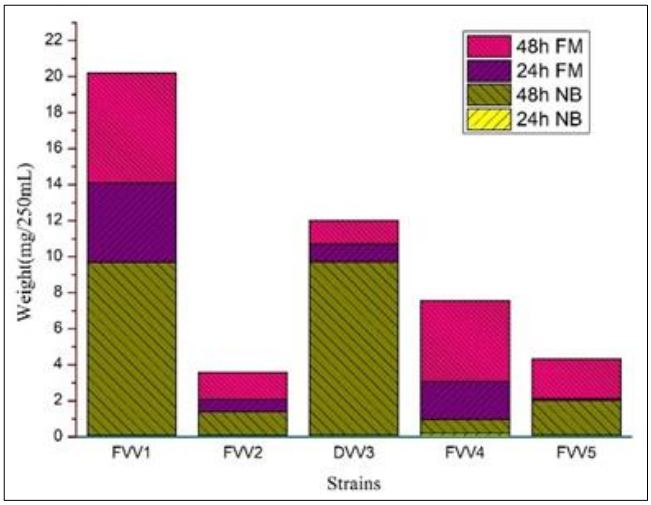


**Fig 4:** Yield of polysaccharide in FM and nutrient broth

### 3.5 UV-Analysis for confirming the purity of polysaccharides

There was no visible peak of polysaccharides at 280 and 260nm in the UV spectrophotometer, indicating no presence of protein and nucleic acids in polysaccharides (Li *et al.*, 2013) [15].

### 3.6 FT-IR

The sample peaks are represented in Fig 5. The peaks between 900-1200cm$^{-1}$ are considered as fingerprint for polysaccharides (Fellah *et al.*, 2009) [7]. A carbon-hydrogen stretching was identified at 2929.22cm$^{-1}$ and hydroxy group vibration exhibited at 3275cm$^{-1,}$ confirming fructose presence (Barone & Medynets, 2007) [6]. The bands at wavenumber 1541.12cm$^{1}$ and 1600-1650cm$^{-1}$ correspond to the amide group, which may be due to residual microbial biomass not being completely removed during purification. The presence of 3270-3280cm$^{-1}$ peaks are characteristics of beta-cellulose, showing that the 3 strains can be used to produce cellulose. The peaks at 1610-1630 and 1430-1455cm$^{-1}$ indicates the presence of uronyl residues in sample. It confirms the probable presence of pectin and hemicellulose (Andritsou *et al.*, 2018) [3]. The peaks around 2930 and 2360cm$^{-1}$ indicate the absorption of C-H and aliphatic C-H bonds. The peak at 1651.07 and 2358 are absorption peak of nitrile and amide group. The peaks at 1010-1100cm$^{-1}$ indicate the possibility of sugar ring in pyranose rings (Qiao *et al.*, 2010) [18]. The broader group at 3270-3300cm$^{-1}$ indicates the O-H stretching and peak around 2922-2930cm-1 associated with the amide group (Sánchez-León *et al.*, 2020) [19]. The weak band at 875.06cm$^{-1}$ associate to C-1-H (β) (β-configuration) (Muhidinov *et al.*, 2020) [16]. The absorption peak at 2929.04, 2929.87, 2922.16cm$^{-1}$ indicates the stretching of C-H bonds. The carbonyl group stretching was observed around 1651.07 and 1633.7cm$^{-1}$. The absorbance peak at 1475-1300cm$^{-1}$ was associated with the vibration of C-H. The stretching of C-OH and C-O-C were associated with a peak around 1200-1000cm$^{-1}$(Wang & Guo, 2020). Different metabolites may be produced, which can be characterized with further studies and research.

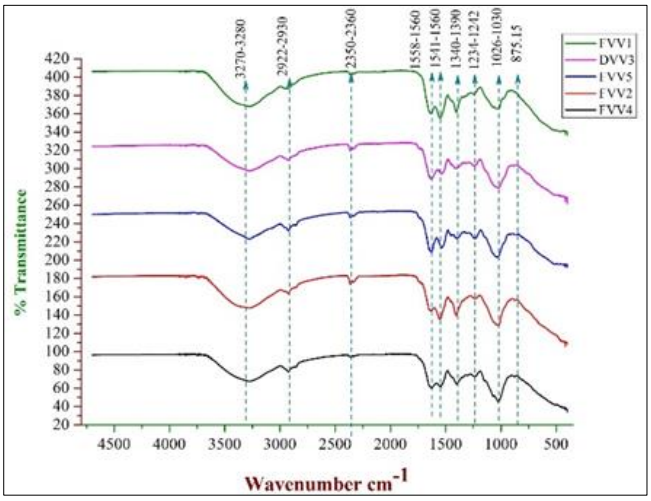


**Fig 5:** FTIR spectrum of polysaccharide

### 4. Discussion

Among the food wastes, a higher fluoride resistant bacterial population was observed in dairy waste isolates compared to fruit waste. In fruit waste, the contaminated groundwater and environment might contaminate fruit, resulting in wastes. These strains might have evolved resistance due to various contamination from ground and surface water, raw materials, and the environment by F$^{-}$. The analyses of the bacterial population in the environment might give information about the impact of F$^{-}$ on environmental health. The reducing sugar and protein content in nutrient broth was in a ratio of 1:2. The fermentation medium was prepared in the same combination of nutrient broth.

The growth rate of strains in fruit waste reduced with an increase in water, while dairy waste showed a higher growth rate at 4, 6 mL for 24 h compared to fruit waste. While strains in fruit waste showed higher growth rate at 8,6 mL and dairy

waste showed at 10mL and gradually decreased for 48 h. Upto 24 h, the dairy waste showed better growth compared to fruit, this might be due favorable conditions, nutrients, and doubling time. The depletion of the available nutrient might have slowed down the growth rate for 48h, while fruit waste the growth increased due to the higher amount of reducing sugar, whey, soluble solids, and strains doubling time in fruit waste compared to dairy waste due to preference of carbon sources. Parameters like pH and temperature played a vital in the growth of the organism in the fermentation medium. All the organisms might belong to the mesophilic category as optimum growth conditions belonged in the range of 30-40 °C.

Bacterial cellulose was produced using molasses, are carbon source with fed-batch and batch fermentation yielding 7.82 g/L and 5.3 g/L, respectively (Bae & Shoda, 2004) [5]. The production of polysaccharide, biomass, and ganoderic acid in fed-batch fermentation was significantly higher than batch fermentation by *G. lucidium* (Shih *et al.*, 2008) [20]. Bioplastic was produced from potato waste as carbon alternative and technical oleic acid, soybean waste free acid fatty, waste frying oil and glucose from *Acetobacter xylinum BPR2001 and Pseudomonas aeruginosa 42A2* respectively (Haas *et al.*, 2008; Fernández *et al.*, 2005) [10]. It was reported in the literature by (Mummaleti *et al.*, 2020) [17] that fresh coconut inflorescence sap can be used as low-cost substrate for production of levan using *Bacillus subtilis* and through optimization with a carbon source (yeast extract and sucrose) used as fed-batch fermentation, the yield was 62.1 g/L. In the present study, the fruit and dairy waste have good nitrogen and carbon sources for bacterial growth, producing polysaccharides. These results indicate the agricultural waste can be used to produce polysaccharides and other metabolites using microbes.

Mango peel extract as a fermentation medium with yeast *Pichia pinus* produced 4.8-5 g/L EPS, proving citrus fruits peel wastes can be used as a fermentative medium as a source of carbohydrates (Jwanny *et al.*, 1989) [13]. The quantity of EPS the strains FVV1, FVV2, DVV3, FVV4 and FVV5 yielded were 9.5, 1.6, 2.7, 6.4 and 2.31 (mg/250mL). The strains FVV1 and DVV2 produced more polysaccharides, this may be due to the strain might have more adaptability and favorable conditions in the fermentation medium compared to other strains. The strain DVV3 was isolated from dairy waste using selective MRS agar produced more yield proving the capacity to grow well in a fermentation medium that contains dairy waste. This result proved that all 5 strains can produce polysaccharides and revealed an advantage of using food waste and avoiding expensive disposal processes and materials. There is another possibility of using same FM to produce single-cell protein since higher biomass and growth rate is found.

The FT-IR spectrum revealed the possibility of different polysaccharides and other products that must be researched further. The polysaccharide yield ranged 0.9-10 mg/250mL, which is considerably high when low investment cost is required for raw materials. The yield can be effectively increased by increasing the concentration, time duration and interval, different strain, optimizing conditions and composition in fed-batch fermentation. This study proves that food waste can effectively be used as a source for varied products from microbes. Compared to other agricultural and other wastes, the food waste is cheap, high availability and available in large quantity, which can be used as an alternative substrate for polysaccharide production with better modifications.

## 5. Conclusion

This study highlights the potential of utilizing food waste as a base medium for producing polysaccharides with necessary modifications. These $F^-$ strains can also be used for the bio sorption efficacy of fluoride, providing multi-functionality. The strains can adsorb fluoride and produce polysaccharides of commercial interest. Moreover, with the perspective of food waste valorization, fluoride resistant strains can be used for metabolite production with improved industrial applications.

## 6. Acknowledgment

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## 7. Reference

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