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PRODUCTION AND CHARACTERIZATION OF DEXTRAN FROM LEUCONOSTOC MESENTEROIDES SSP. MESENTEROIDES ISOLATED FROM IRAQI FISH INTESTINE

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ABSTRACT
In this study, production of dextran by *Leuconostoc mesenteroides* ssp. *mesenteroides* isolated from Iraqi fish intestine of *Barbus grypus* was studied. For maximum yield of dextran, effects of various parameters such as temperature, incubation period, pH, inoculum size and aeration were studied. *L. mesenteroides* ssp. *mesenteroides* produced maximum dextran after 24 hours of incubation at 30^{o}C with 4% inoculum size at pH 7.0 under anaerobic condition. Purification and characterization of dextran were done, the color, texture**,** total protein, total carbohydrates and functional group of dextran were determined. Total amount of dry weight of purified dextran was about 1 g/L, the dextran was observe to be whitish , granular and easily soluble in water with a content of total protein 55.8 g/L and total carbohydrate of 80%. The Fourier Transform Infrared Spectroscopy (FTIR) analysis of dextran contains both α(1-6)glyosidic and α(1-3) D-glucan linkages .

KEYWORDS: *Leuconostoc mesenteroides*, Dextran, Production, Purification, characterization, FTIR.

INTRODUCTION
One of the most important source of polymeric materials are microbial polymers which have great potentials for use in many applications include food, pharmaceutical, agricultural, industries and in medicine. Dextran is an example of these biopolymers which is mostly produce by *Leuconostoc* spp. especially *Leuconostoc mesenteroides* (Onilude *et al*.,2013).

Leuconostoc spp. regard one of the most economically important genus belong to group of lactic acid bacteria and characterized by their ability to produce a wild antimicrobial agents such as bacteriocins, organic acids, diacetyl, hydrogen peroxide and biosurfactant (Holzapfel and Wood, 2014; O'Bryan *et al*., 2015), beside these antimicrobial agents *Leuconostoc* spp. can produce biopolymers such as dextran which is homopolysaccharide chain linked by α-(1-6) glycosidic bonds with different amount of branched linkages such as α-(1-2), α-(1-3) and α-(1-4) (Galle *et al*., 2010). Dextran have a basic chemical formula of H $(C_6H_{10}O_5)_n$ OH and produced from sucrose by the action of the bacterial extracellular enzyme dextransucrase(Malunga *et al*., 2012). It is considered nontoxic, biocompatibility, high biodegradability, wide availability (Kothari *et al*., 2015). Dextran can act as potential prebiotics, viscosifying, texturizing, gelling agent and be used as food supplement for health benefits (Aman *et al*., 2012; Rao *et al*., 2014; Dertli *et al*.,2016). Also dextran used in medicine as plasma/blood volume expanders , blood plasma substituent and drug delivery vehicle for variety of drugs for its excellent biocompatibility, biodegradability, wide availability and relative low cost modification through its reactive hydroxyl chemistry (Almeida *et al*.,2013).

This study was aimed to production, purification, characterization of dextran from *Leuconostoc mesenteroides* ssp. *mesenteroides* isolated from Iraqi fish intestine.

MATERIALS AND METHODS
Leuconostoc mesenteroides ssp. *mesenteroides*
Leuconostoc mesenteroides ssp. *mesenteroides* was isolated from Iraqi fish intestine of *Barbus grypus*, then identified through out cultural, microscopical and biochemical test according to Garvie (1986) and Vitek 2 system.

Detection of dextran production
Mucoidy and Ropiness
Mucoidy and ropiness test was carried on MRS agar medium, inoculation of *L. mesenteroides* ssp. *mesenteroides* was performed by spotting 2 µl of bacterial suspension on MRS agar. After incubation at 30^{o}C for 24-48 h, The mucoidy colonies were determined by visual appearance, and ropiness was

determined by touching them with sterile loop (Mostefaoui *et al*., 2014).

Ethanol precipitation method

Discs of sterile filter paper (5mm) were placed on MRS agar plate and were inoculated with 5 µl of cultural broth of *L. mesenteroides* ssp. *mesenteroides*, incubated at 30^{o}C for 2-3 days in order to detect the ability of isolate for dextran production (Paulo *et al*, 2012a). Dextran production was assessed on the basis of mucoid colony around these discs, the ability of dextran production of isolate was confirmed by mixing a mucoid substances in 2 ml of absolute ethanol (Paulo *et al.*, 2012a; Chun-lei *et al.*, 2014).

Dextran production

For dextran production, inoculum of *L. mesenteroides* ssp. *mesenteroides* was prepared by inoculating 10 ml of sucrose broth with a loop full of 24 h old culture of isolate above. After incubation for 24 h at 30^{o}C, 1 ml of this containing (9×10^{8}cfu/ml) was added to 100ml of the medium described by Sarwat *et al*. (2008) which contained (g/l): (sucrose 150 g, peptone 5.0 g , K_2HPO_4 15.0, $MnCl_2.4H_2O$ 0.01, yeast extract 5.0, NaCl 0.01, $CaCl_2$ 0.05). Incubation was done at 30^{o}C for 24 h(Onilude *et al*., 2013 with some modification).

Precipitation of dextran

The culture medium after 24 h was precipitated using equal volume of chilled ethanol, shaken using vortex, centrifuged at 8000 rpm for 20 min and the supernatant was decanted. This step was repeated twice (Sawart *et al*., 2008 with some modification). The precipitated dextran was dried in oven approximately at 40^{o}C for 45 minutes. Finally the dextran yield was calculated on dry weight (Mohanasrinivasan *et al*., 2014).

Determination of optimum condition for dextran production by *L.mesenteroides* ssp. *mesenteroides*

Effect of temperature of incubation

The influence of temperature on dextran production was studied by incubation of *L. mesenteroides* ssp. *mesenteroides* culture at temperature of (25, 30, 35) ^{o}C for 24 h and precipitation was done. The mount of dextran was estimated for each temperature.

Effect of incubation time

The effect of incubation time on dextran production was done at the best temperature for (24, 48, 72) h. Dextran produced at different incubation time was precipitated and the amount of dextran was estimated.

Effect of initial pH on Dextran production

For studying the influence of the initial pH of the culture medium on dextran production, the pH of the medium was adjusted to 6.0, 7.0 and 8.0 separately, before sterilization. Then incubated at the best temperature and incubation time, precipitation was done and amount of dextran was estimated.

Effect of aeration

The influence of oxygen on dextran production was studied, under aerobic and anaerobic condition. The medium was incubated at the best temperature, incubation time and pH. Precipitation was done and the amount of dextran was estimated for aerobic and anaerobic condition.

Effect of inoculum size

The effect of inoculum size on dextran production was studied by inoculating (1, 2, 4, 6) % of inoculum (9×10^{8} CFU/ml), the pH of the broth medium was adjusted to best pH before sterilization and the cultivation media were incubated at the optimum conditions, then precipitation was done for each inoculum size and the amount of dextran was estimated .

Purification of dextran

The purification of dextran obtained from precipitation at optimum condition was done as method described by Sawart *et al*. (2008), the precipitated dextran was dissolved in distilled water then the dextran slurry that gained was precipitated with equal volume of chilled ethanol. This procedure of re-dissolving, precipitation and washing was repeated three times to eliminate cells debris. Purified dextran was dried by using electrical oven at 40^{o}C for 45 minutes, then dried dextran was calculated on dry weight basis.

Characterization of dextran produced by *L.mesenteroides* ssp. *mesenteroides*

The characterization of dextran produced by *L. mesenteroides* ssp. *mesenteroides* was done by taking into consideration the **color and texture**.

Total protein content was determined using Biuret reaction, as described by Gronall *et al*.(1949).

The total carbohydrate was determined by phenol sulfuric acid method using glucose as a standard (Sadasivam and Manickam, 2005).

Fourier transform infrared spectroscopy (FTIR) have been carried out using (Bruker – Tensor 27 with ATR unit) in the Physics department \ Collage of Sciences\Al-mustansiriyah university. The instrument operates in the wave number range of (400-4000 cm^{-1}) that measures the amount of IR radiation reflected or transmitted through a sample. The output data is in the form of graphical chart, in which the X-axis represents the wave number, while Y-axis represents the transmittance (%). FTIR was used for determination of functional group of dextran.

RESULTS AND DISCUSSION

Detection of dextran production

- **Mucoidy and ropiness method**

The detection of dextran production were recorded according to mucoidy and ropiness of colonies on the surface of MRS agar. Results showed that *L.mesenteroides* ssp. *mesenteroides* was produced

mucoidy and ropniss colonies, with strong viscosity when introduce a loop into the colony. Bunkoed and Thaniyavarrin (2014) studied mucoidy and ropiness of lactic acid bacteria isolated from milk, they observed that EPS-producing bacteria were mucoid and ropiness appearance on MRS agar. The presence of mucoid colony is indicative of exopolysaccharide (EPS) production potential (Paulo *et al*., 2012b), also Torres-Rodríguez *et al*. (2014) used the same method for EPS-producing colonies detection by visual analysis to recognized between compact colony (not EPS producer) and creamy colony (EPS producer), while Rühmann *et al*.(2015) used visual analysis (mocoidy) of colonies as method of choice for detected EPS producing microbes.

Ethanol precipitation method

Ethanol precipitation method was used to confirm dextran producing isolate. Results showed that *L. mesenteroides* ssp. *mesenteroides* was producer isolate when cultured filter paper was precipitated by absolute ethanol (Figure 1). Results also showed that the better method for detection of dextran production is ethanol precipitation. The Ethanol precipitation method was used to confirmed polymers producing colony in absolute alcohol, the precipitate formation indicated the presence of EPS (Paulo *et al*., 2012b). Mostefaoui *et al*. (2014) used ethanol precipitation method to confirmed EPS producing strains, also Rühmann *et al*. (2015) used as common method for EPS detection, precipitation and purification.

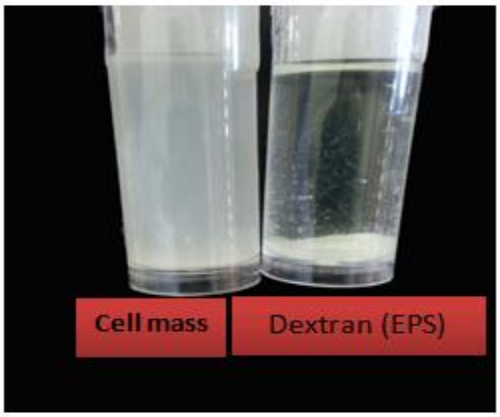

Figure: (1) Confirmation of dextran production by ethanol precipitation method

Dextran production

The result of the amount of dextran produced by *L.mesenteroides* ssp. *mesenteroides* was 309 mg\100ml. The production of exopolysaccharides depends on the strains tested and on the enzymatic metabolism of strains (Ruas-Madiedo and De Los Reyes-Gavilan , 2005). Dextran is an exopolysaccharide needs the dextran sucrase for production (Srinivas and Padma, 2014). Polak-Berecka *et al*. (2014) optimized medium for produced 210.28mg/L of EPS from *Lactobacillus rhamnosus* at optimum conditions while Sanhueza *et al*. (2015) reported that the amount of EPS produced by *Lactobacillus salivarius* was 450 mg/L. Furthermore Joshi and Koijam (2014) estimated 340.82 mg/L of EPS produced by *Leuconostoc lactis* isolated from fermented beverage. Moreover Yang *et al*. (2015) mentioned that the amount of dextran produced by *Leuconostoc citeum* which isolated from sauerkraut was 23.5g/L.

Determination of optimum condition for dextran production by *Leuconostoc mesenteroides* ssp. *mesenteroides*

Effect of temperature

After incubation of *L. mesenteroides* ssp. *mesenteroides* at different temperature, the dry weight of dextran was measured for each temperature . Results showed that the optimum temperature for dextran production was at 30^{o}C with dry weight 309 mg/100ml and at 25^{o}C the dry weight for dextran was 267 mg/100ml while at 35^{o}C dry weight was 284 mg/100ml (Figure 2). In contrast, Sawrat *et al*.(2008) studied the effect of temperature on dextran production from *Leuconostoc mesenteriodes* in various temperature and they observed the optimum temperature for dextran production was at 30^{o}C while Onilude *et al*. (2013) concluded that the production of dextran would be decrease gradually when the temperature below 25^{o}C.

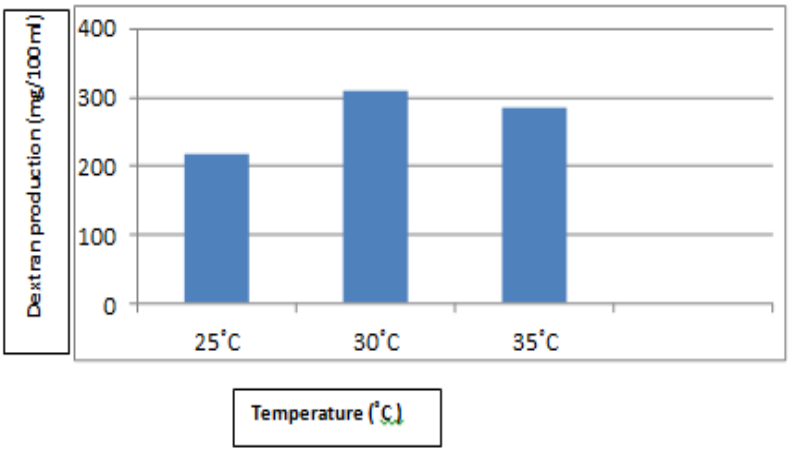

Figure: (2) Effect of Temperature on dextran dextran production from *Leuconostoc mesenteroides* ssp. *mesenteroides*

Effect of incubation time

After optimum temperature for dextran production, the effect of incubation time was studied by incubated of bacterial isolate *L. mesenteroides* ssp. *mesenteroides* at different incubation times (24 ,48 , 72)h. Results showed that the optimum incubation time for dextran production was 24 h which at this time the dry weight of dextran was 309 mg/100ml while at 48 h the dry weight was 299 mg/100ml and at 72 h the dry weight was 218 mg/100ml (Figure 3). The effect of incubation time on dextran production by *Leuconostoc* spp. was studied by Sarwat *et al*. (2008) mentioned that the best incubation time for unique dextran production was at 20 h in which dextransucrase increase with time reached maximum enzyme activity and production, after that the production decreased attributing for altering pH of the fermented broth from 7.5 to 4.5 . Also Onilude *et al*.(2013) reported that the production was gradually increased when incubation time increase from 4 h to 8h and reached the maximum production at 20 h after which gradually decrease of dextran production.

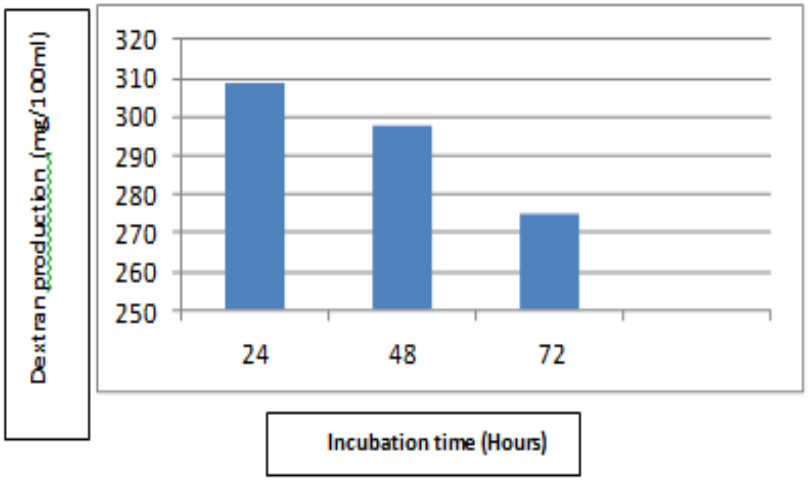

Figure (3): Effect of incubation time on dextran production from *Leuconostoc mesenteroides* ssp. *mesenteroides*

Effect of pH

The optimum pH for dextran production from *L. mesenteroides* ssp. *mesenteroides* was 7.0 which in this number the dry weight of dextran was 309 while at pH (6.0) was 205mg/100ml and at pH (8.0) was 260mg/100ml (Figure 4). In contrast Sawrat *et al*. (2008) found that the optimum pH for dextran production from *Leuconostoc mesenteroides* was at 7.0 while Onilude *et al.* (2013) studied the effect of different pH on dextran production and found that the production was progressively increased until reached maximum production at 6.5, then after that an decrease in production would be recorded. Mohanasrinivasan *et al*. (2014) observed that the optimum pH was at 6.8 for dextran production from *Leuconostoc mesenteroides*. On other studies Joshi and Koijum (2014) reported that pH 6.5 is the best for EPS production from *Leuconostoc lactis*. Further studies by Dogan *et al*.(2015) reported that the best pH level for EPS production from *Bacillus licheniformis* was at 6.0-7.0 . Moreover, Tayuan *et al*.(2011) mentioned that the best pH for EPS production from *Weissella* spp. was at 7.0.

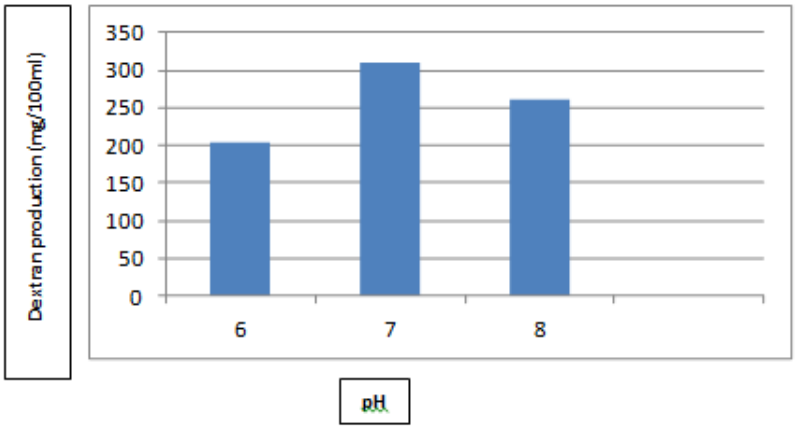

Figure (4): Effect of pH on dextran production from *Leuconostoc mesenteroides* ssp.*mesenteroides*

Effect of aeration

The production of dextran under anaerobic condition reached 309 mg/100ml, more than that gained under aerobic condition 282 mg/100ml (Figure 5).

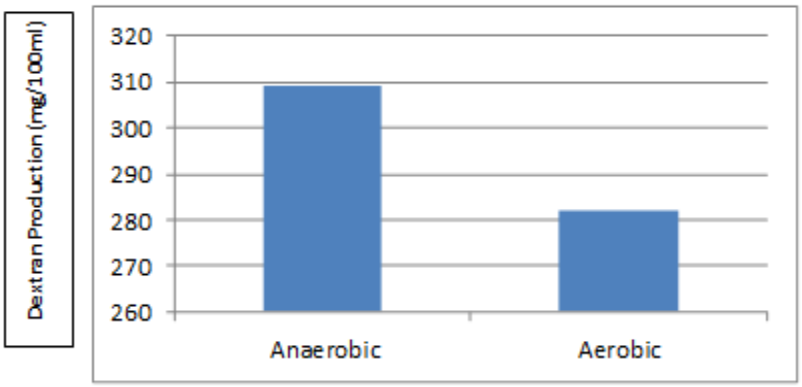

Figure: (5) Effect of aeration on dextran production from *Leuconostoc mesenteroides* ssp. *mesenteroides*

Effect of inoculum size

The best inoculum size for dextran production from *L. mesenteroides* ssp.*mesenteroides* was at 4% , which the dry weight of dextran reached to 369mg/100ml where in 6% the dry weight was 336mg/100ml while in 2% it was 317mg/100ml and in 1% the dry weight was 309 mg/100ml (Figure -6-). Onilude *et al*.(2013) studied the effect of inoculum size on dextran production from *Leuconostoc* spp. , they observed that the production was gradually increased with increasing inoculum size from 2% to 4% and the production gradually decrease when inoculum size reached 8% and 10%.

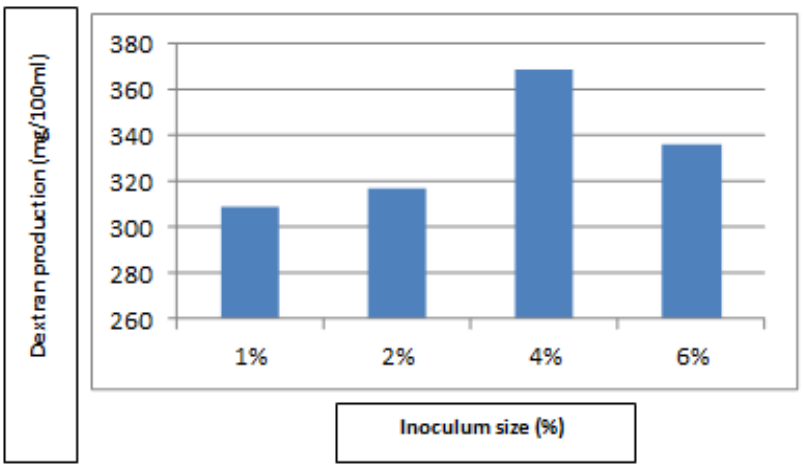

Figure: (6) Effect of Inoculum size on dextran production from *Leuconostoc mesenteroides* ssp. *mesenteroides*

Purification of dextran produced by *L. mesenteroides* ssp. *mesenteroides*

Dextran produced by *L.mesenteroides* ssp.*mesenteroides* was purified after production under optimum conditions (temperature, incubation time, pH, inoculum size and aeration). The total dry weight of dextran after purification was 1 gram /1L. Ethanol is quite miscible with water, therefore, when it added to dextran solution, ethanol disrupts the interactions between dextran molecules and water molecules, also any charged ions in the solution where ethanol disrupts polysaccharide hydration in the solutions which allowed dextran molecules to come closer and aggregated to form clumps which are too large to be suspending in the solution and that made easier to collect by centrifuge process (Kotz *et al.*, 2011).

Characterization of dextran produced by *L.mesenteroides* ssp. *mesenteroides*

Purified dextran characterized as granular, whitish and highly soluble in water.Total protein in purified dextran was 55.8 g\L, total carbohydrate was 80%. Dextran molecule consist of polymers of D-glucose with α-(1→6) linage linkage and different -(1→2), α-(1→3) and α-(1→4) branched linkages where the activities of it determined by their monosaccharide composition, glycosidic linkages and polymerization degrees (Delattre and Vijayalakshmi, 2009) . Sawart *et al.*(2008) found that total carbohydrate percentage of dextran produced by *Leuconostoc mesenteroides* was 79% while Onilude *et al*. (2013) determined total carbohydrate amount from dextran as 48%.

The FTIR results of produced dextran was showed that the band in the region of 3271.18^{cm-1} is due to the hydroxyl stretching vibrations of polysaccharides while the band in region of 2965.13^{cm-1} is for (C – H) stretching vibration , the band region of 1635.77cm^{cm-1} due to carboxylic group while the band region found in the 1217.08^{cm-1} and 1052.20^{cm-1} due to the (C – O) and (C – C) bonds respectively, the bond region in 1217.08^{cm-1} is due to (C – O – C) bond and glycosidic bridges while the bond region in 1052.20^{cm-1} is due to great chain flexibility present in dextran around the glycosidic bonds , showed contain α – (1 – 6) glycocidic bonds and the bond region in 862.62^{cm-1} is due to (1 – 3) – α – D glucan. FTIR spectra analysis of dextran produced by *L.mesenteroides* ssp. *mesenteroides* contain both α (1 – 6) and α (1 – 3) linkages (Figure -7-) and that agree with FTIR spectra analysis of dextran from *L.mesenteroides* NRRL B-1149 which contain both α (1–6) and α (1 – 3) linkages (Shulka *et al*.,2011) . Nikolic *et al*. (2009) showed that dextran is a polysaccharide consisting of long branched chains, mainly through the α – (1 – 6) and partly through the α (1–3) glycocidic linkages . Paul *et al*. (2012b) mentioned that the main bond which characterized dextran produced by *Leuconostoc* spp. is α-(1 – 6),on other study Mohanasrinivasan *et al*.(2014) reported that the main characteristic band found in dextran were due to C-C and C-O bonds , the band belonged to C-O-C and glycosidic bridges and also detected that the band regions 916.19 cm^{-1} and 862.18 cm^{-1} were characterized of (1-3)-α-D glucan. In conclusion, locally isolate of *Leoconostoc* had ability to produce dextran.

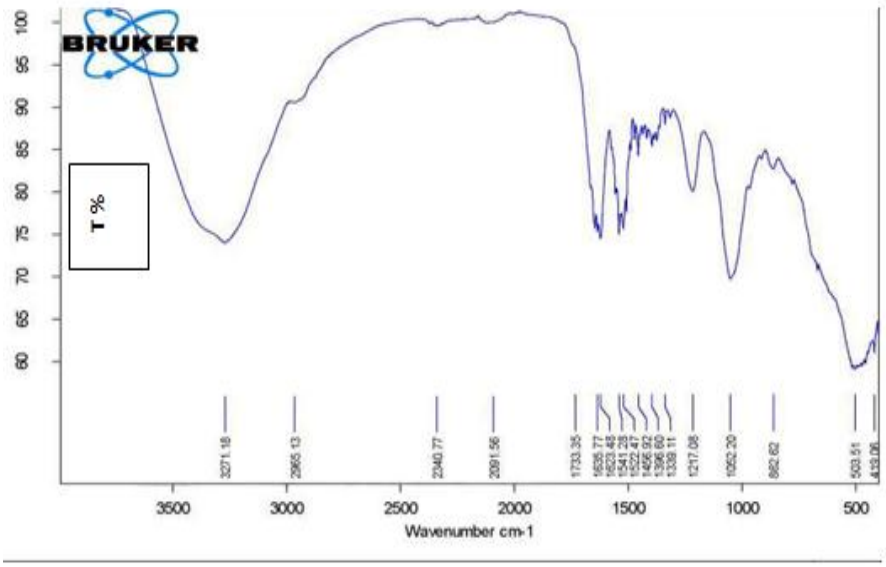

Figure: 7 FTIR spectra of dextran produced by *Leuconostoc mesenteroides* ssp. *mesenteroides*

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