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Screening and Characterization of Exopolysaccharide Producing Mesophilic *Lactobacillus acidophilus* Isolated from Human Dental Caries

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Abstract: *Dental caries is an infectious, communicable disease that acid-forming bacteria of dental plaque can destroy tooth structure in the presence of fermentable carbohydrates such as sucrose, fructose, and glucose. It continuous to be a major problem in dentistry affecting the mankind even today. In this present investigation EPS producing cariogenic Lactobacillus acidophilus were isolated from various dental clinics were analyzed and characterized by phenotypic and 16SrDNA gene sequence for the production of exopolysaccharides. Further, the optimal conditions of temperature (30°C - 45°C) were determined. Lactobacillus acidophilus were identified and screened on the basis of the molecular weight of its plasmid DNA (100 kb). The EPS production by Lactobacillus acidophilus was confirmed by thin layer chromatography (TLC) analysis. Optimization of culture condition for mass EPS yield was studied. When the strains were grown in 10% (W/V) reconstituted with skim milk under different culture conditions. The result showed that culturing of Lactobacillus acidophilus strain - LACVG02, LACVG06, LACVG17, LACVG36, LACVG38, LACVG75, LACVG85 and LACVG92 at temperature (37°C) and pH (6.5) optimal for growth was also favourable for EPS production. Supplementation in 10% (w/v) skim milk with different concentration (1%,2%,3%,4% and 5%) of carbon sources (glucose, sucrose, maltose, dextrose and fructose) increased EPS production; glucose being more effective than other sugars. Supplementation with different concentration of nitrogen sources (Peptone, Beef, Yeast, Urease, Gelatin, Ammonium Ferric Citrate, Ammonium Molyptate, and Ammonium per Sulphate, Ammonium Acetate) at 1% - 5% (w/v) resulted in about two-fold increase in EPS production. The potentiality of Lactobacillus acidophilus in EPS production was assessed. EPS production by Lactobacillus acidophilus is partially growth associated. A maximum of 1.99 gm of EPS/ml was synthesized by LACVG06 in the skim milk medium supplemented with 5% (w/v) glucose and 5% (w/v) of different nitrogen sources increased in EPS production; ammonium acetate being more effective than other nitrogen sources by LACVG06 in skim milk production medium produce a maximum of 1.98 gm of EPS/ ml was synthesized at 37°C and at initial pH 6.5.*

***Keywords*:** *Dental caries, Exopolysaccharide (EPS), Lactobacillus acidophilus, Plasmid analysis and Thin Layer Chromatography (TLC).*

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I. Introduction

Dental caries is one of the most prevalent infectious diseases of humans. It is preceded by accumulation of dental plaque. Dental plaque is a complex biofilm formed on teeth surface composed of self-produced extracellular polymeric substances (EPS), mainly glucans (Costerton JW, 1999). The fermentation of dietary sugars by acidogenic oral micro biota plays key role in the development of caries. The dissolution of mineral in enamel and dentin due to acids released by these microorganisms cause carious lesions (Arends and Christoffersen, 1986) and extra cellular polysaccharides such as glucan and fructan was shown repeatedly in clinical studies (Zero DT, 2004). Recent studies have demonstrated that the micro biota of children with severe ECC differs significantly from that of their caries-free counterparts (Tanner et al., 2011) and that *Lactobacilli* comprise a significant portion of the cariogenic biota (Callaway et al., 2013). High prevalence of *Lactobacilli* in caries lesions and their ability to generate a low *p*H environment, as well as to survive in it, suggest that *Lactobacilli* are key determinants underlying the development and severity of caries, particularly in caries progression (Beighton, 2005). Further, the *Lactobacilli* are generally considered to be non-pathogenic except in dental caries (Attebery and Finegold, 1970). Historically *Lactobacilli* were the first microorganisms implicated in dental caries development. This genus is involved in the progression of carious and caries dentin is the main ecological site of *Lactobacilli*. They appear during the first years of child's and are present in high numbers in saliva, on the dorsum of the tongue, mucous membranes, the hard plate, in dental plaque and few numbers, on tooth surface (Straetemans et al., 1998).

During the last fifteen years, the *Lactobacillus* genus has subjected to numerous taxonomical changes and includes at present more than 80 species (Loesche et al., 1984), some of which having been found in the oral cavity. *Lactobacilli* seem to be mostly transient in the oral cavity of small children, and only later a resident *Lactobacillus* flora is established (Badet and Thebaud, 2008). Analysis of *Lactobacilli* by culture under micro aerophilic conditions in 65 deep caries samples indicated that *Lactobacillus acidophilus* was numerically dominant, although *Lactobacillus paracasei*, *Lactobacillus rhamnosus*, and *Lactobacillus fermentum* were also present in many samples (Martin et al., 2002). Efforts to precisely identify LB to the species level have been difficult due to the lack of reliable and precise taxonomical identifiers. This difficulty is further intensified using biochemical reactions for species identification, up to 17 phenotypic tests sometimes being required for speciation (Hammes and Vogel, 1995). Because of the difficulties associated with accurate characterization, most clinical studies report simply counts of 'lactobacilli' on selective media. With the advent of molecular and genetic typing methods, however, including species-specific primers and distinctive 16S rDNA segments (Chhour et al., 2005); the task of identifying the LB has become more achievable. This is important because if the specificity exists between a few species of *Lactobacillus* and caries, those species could be further characterized and their natural history better understood so as to gain insight into their natural reservoirs, perhaps devising methods to prevent their transmission and acquisition.

Currently the infectious diseases are the most significant threat for human beings with high morbidity and mortality throughout the world, due to the rapid emergence of multiple drug resistance (MDR) and it creates a major health illness in the medication of infectious diseases spread by pathogenic microbes (Thirumalairaj et al., 2015). The main factor contributing to microbial resistance is the EPS produced by the microbes that allow them to with stand extreme environmental conditions and antimicrobial agents. The exopolysaccharide forming bacteria are resistant to antimicrobial agents due to the lack of penetration of antimicrobial agents. For this reason previous studies have shown that EPS production by LAB can be improved by manipulating medium composition (Degeest et al., 2002; Duenas et al., 2003; Lee et al., 2007), such as carbon source (Looijesteijn et al., 1999; Cerning et al., 1994), nitrogen source (Degeest and De Vuyst, 1999; Zisu and Shah, 2003), and environmental conditions, that is, temperature (Nichols et al., 2005; Vaningelgem et al.,2004), *p*H (Kimmel et al., 1998; Degeest et al., 2002), oxygen tension (Gamar-Nourani et al., 1998) and incubation time (Pham et al., 2000; Lin and Chang Chien, 2007). For some EPS-producing LAB strains, such as those from *Streptococcus* (Escalaute et al., 1998; Urshev et al., 2006; Faber et al., 2001), *Lactococcus* (Ramos et al., 2001; Dabour et al., 2005) and *Lactobacillus* (Desai et al., 2006; Rodríguez-Carvajal et al., 2008; Briczinski and Roberts, 2002; Torino et al., 2005), manipulating their physiological conditions could result in increased EPS biosynthesis.

In the search of EPS-producing LAB strains for potential ability to avoid antimicrobial applications, for this reason we found that culturing of all *Lactobacillus acidophilus* strains - LACVG02, LACVG06, LACVG17, LACVG36, LACVG38, LACVG75, LACVG85 and LACVG92 isolated from caries of pre - school children's in Tirupur produced a viscous EPS when grown in skim milk. In order to further understand the EPS biosynthesis properties of all *L. acidophilus*, growth and EPS production by these strains in skim milk were investigated under different culture conditions. So hence the present study has made an attempt to point out the EPS production LAB was isolated, purified and characterized with respect to monosaccharide composition, molecular mass and viscosity properties of the polymer.

II. Materials And Methods

2.1.Sample collection

A total of 50 different decay samples were collected from various dental clinics at different areas in and around Tirupur District.

2.2.Laboratory analysis

Each decay sample was placed individually in to 5 ml of a sterile physiological saline (0.9% (w/v) NaCl) solution contained in sterile sample container for enrichment it was kept in an incubator at 37°C for 24 hours. The processed samples were used for cariogenic *Lactobacillus acidophilus* isolation. Spread plate method was performed to isolate pure culture of *Lactobacillus acidophilus* and for viable cell counting. For each sample, successive decimal dilutions were made in sterile physiological saline (0.9% (w/v) NaCl) solution and 0.1ml of the appropriate dilution was spread plated on MRS agar plated by using a sterile L-rod. Plates were incubated at 37°C for 24 hours. After the incubation period the number of colonies formed on the surface of MRS agar was counted from that the number of viable cells (in colony forming units: CFU / ml) present in 0.1 ml of each sample were determined. For further studies the working cultures were stored on nutrient agar slant at 4°C and sub culture prepared every 2 weeks. Identification was carried out based on the following main characteristic features: Microscopic examination of morphology and mobility, Gram staining, Catalase test, Oxidase test, Fermentation ability on different carbon sources and genotypic characterization.

2.3. Effect of temperature on the growth of Lactobacillus acidophilus

Prepare MRS broth and pour in to test tube. Sterilize the medium at 121^{o} C for 15 mins. Test organism was inoculated into sterile tubes. All tubes were incubated at various temperatures (30°C, 35°C, 40°C and 45°C) at 24 hours. After completion of incubation period observe the growth of bacteria and measure optical density at 600 nm uing spectrophotometer.

2.4. Screening of exopolysaccharide production

MRS broth contained in a conical flask (250ml) was autoclaved (121°C for 20 minutes) and cooled to hand bearable warmth. The investigated strains were grown in 50 ml of MRS broth for 48 hours at 37°C.

2.5. Determination of biomass dry weight

After incubation the cells were removed by centrifugation (800 rpm for 15 minutes). The cell pellet was washed twice with 500ml of 1 % PBS buffer and dried at 37°C for overnight. The dry weight of the biomass was obtained by calculating the difference between the dry weight of the empty vial and the dry weight of the vial with biomass.

2.6. EPS Isolation and Purification

EPS were isolated and purified according to the method of (Cerning et al., 1992). The cultures were heated at 100°C for 15 min to inactive enzymes. Afterward, samples were treated with trichloroacetic acid (10% (w/v) with agitation for 30 min and centrifuged at 8000 rpm for 15 min for cell and protein removal. The EPS in the supernatant were concentrated by adding three fold volume of cold ethanol and incubated at 4°C for overnight. The precipitate was collected by centrifugation at 5000 rpm for 15 min and dissolved in 500µl of 1% PBS buffer.

2.7. Estimation of Total Sugar Concentration

Total sugar concentration was determined by the phenol sulphuric acid method using glucose as a standard.

2.8. Thin Layer Chromatography (TLC)

The hydrolysed samples were analyzed by thin layer chromatography using TLC sheet. The solvents used for the TLC were Methanol, Acetic acid and Benzene in the ratio 3:1:1.

2.9. Characterization of EPS production by cariogenic pathogen *Lactobacillus acidophilus*

2.9.1. Isolation of Plasmid DNA from *Lactobacillus acidophilus*

Plasmid were isolated from cariogenic EPS producing *Lactobacilli*, using Phenol Chloroform Method and the presence of C-DNA was checked by 0.7% agarose gel was with visualized under UV light on transilluminator and photographed. Size of the plasmids was determined with the help of a calibration curve prepared using log MW (kb) of the standard molecular marker.

3.0. Optimization of EPS Production

Influence of different carbon sources, varies *p*H, temperature and nitrogen sources in the EPS production was analysed by standard methods.

3.1. Fermentation experiments

3.1.1. Effect of initial *p*H and Temperature on EPS production

The effect of initial *p*H of the medium and temperature on the growth and EPS production by *Lactobacillus* spp. was grown at various initial *p*H (5.0, 5.5, 6.0, 6.5 and 7.0) at 37°C and temperatures (25°C, 30°C, 37°C and 45°C) at *p*H 6.5 in 10% (W/V) of skim milk (Plate. 3).

3.1.2. Effect of carbon source on EPS production

The effect of carbon sources on EPS production was studied by supplementation in the skim milk with different concentration of 1% - 5% (W/V) of different sugars (Glucose, Sucrose, Lactose, Fructose, Galactose, Raffinose and Maltose) (Plate: 4).

3.1.3. Effect of nitrogen source on EPS production

The effect of nitrogen sources on EPS production was studied by supplementation in the skim milk with 0.5% - 1.0% (W/V) of different nitrogen sources (Peptone, Beef, yeast, Urease, Ammonium Ferric Citrate, Ammonium Molyptate, Ammonium Per Sulphate, Ammonium Acetate and Gelatin) (Plate: 5).

The total EPS concentration was determined by Phenol Sulphuric Acid Method (Dubois et al., 1956).

III. Results And Discussion

In total of fifty decay samples collected from various dental clinics at different areas in Tirupur District. Further the isolates were identified as *Lactobacillus acidophilus*. According to Bergy's manual of Bacteriology and 16SrDNA gene sequencing. The isolated cariogenic *Lactobacillus acidophilus* was used for the production of exopolysaccharides *invitro*, since it is pathogenic, decay grade organism, possess the status of extracellular polysaccharide formation plays a key role in the pathogenesis of infections in the oral cavity. Bacteria implicated in the accumulation of dental plaque, the precursor of gingivitis and periodontitis, are embedded in a matrix of bacterially derived exopolysaccharide that largely determines the structural integrity and diffusion properties of plaque biofilm (Palmer et al., 2003).

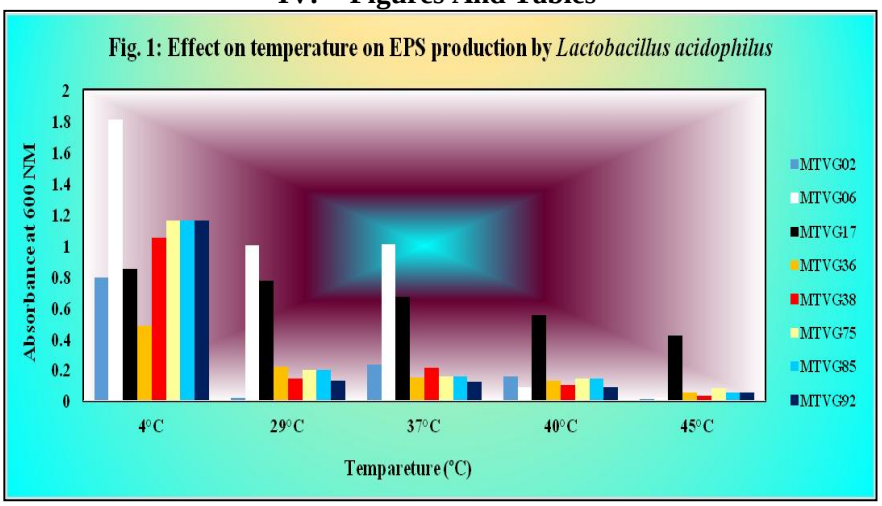
The exopolysaccharides (EPS) produced by *Lactobacillus acidophilus* have received increased interest, mainly because of their environmental factors contributing to microbial resistance. *Lactobacillus acidophilus* is identified on the basis of morphological, biochemical tests the results are illustrated in table 1. The isolates from decay sample displayed as white, opaque, mucoid shape with glittering and slimy appearance on MRS agar medium. Both the isolates were found to be gram positive, rod, non-spore forming, Catalase and oxidase negative and able to ferment different sugars such as glucose, sucrose, fructose, maltose and Mannitol. The colony forming units (CFU/ml) of *Lactobacillus acidophilus* on MRS agar were determined after incubation at 37˚C for 24 hours and the results are shown in (Table 2). Colonies showing white, opaque appearance with waxy growth were considered as *Lactobacillus acidophilus* and used for further investigation. The plasmid DNA was isolated by boiling preparation method of Holmes & Quigley, 1981; modified by Riggs & McLachlan, 1986 from the cariogenic *Lactobacillus acidophilus* and its molecular weight was found out by running in agarose gel electrophoresis along with the DNA ladder. When comparing all the separated plasmid DNA with ladder DNA indicating that the plasmid DNA from all the seven isolates (LACVG02, LACVG06, LACVG17, LACVG36, LACVG38, LACVG75, LACVG85 and LACVG92) are above 100 kb in size (Plate: 7). The identified isolates were subjected to EPS production at 37˚C for 24 hours (Plate: 1). After incubation, the EPS production was determined by measuring the total carbohydrate content by Phenol sulphuric acid method (Dubois et al., 1956). The quality of EPS product was studied in thin layer chromatography (TLC) with the presence of standard sugars such as glucose, sucrose, fructose and maltose. Brown colored spots were appeared on TLC plate when spraying with α- Napthol reagent (Plate: 2).

Several parameters, such as carbon sources, nitrogen sources, temperature and *p*H of different concentration were tested to determine the optimal conditions for the production of EPS (Fig: 1, 2, 3 and4). The influence of carbon sources on growth and EPS production were tested and the results are summarized as follows. Glucose provided the highest EPS yield followed by sucrose whereas fructose supported the lower EPS synthesis. No significant difference is observed between dextrose and maltose. The biomass concentration was maximum in both sucrose and fructose in MRS medium and it is more or less similar in remaining sugars. When comparing the growth and EPS yield from different sugars, revealed that no correlation between the biomass and EPS yield. The effect of various concentration of glucose on EPS yield was tested and results were shown in (figure: 1). The maximum EPS yield was attained with glucose at 5gm/L but no significant differences were obtained between glucose at 1 and 4gm/l. The sugar concentration of 5gm/l is the optimal value for the high growth and EPS yield. To determine the optimal temperature for maximum yield of EPS, batch cultures were performed with glucose (5gm/L) as the carbon source at five different incubation temperatures for 24hours. The growth temperature of 37˚C was also optimal for EPS yield.The influence of *p*H on EPS synthesis was determined in batch cultures performed with glucose (5g/l) as the carbon source at 37˚C for 24hours. The maximum EPS yield and biomass was attained at *p*H 6.5. The optimal conditions for the maximum growth and EPS yield were found to be glucose 5g/ml, 37˚C, *p*H 6.5. There was a correlation between biomass and EPS yield at optimal conditions. However, it changes significantly at remaining conditions. The maximum viable count did not correspond with the maximum EPS production present results are also in accordance with Anderson *et al.,* (1998) who reported that the brown colored spots were obtained when spraying the TLC by α-Napthol reagent which indicates the presence of carbohydrates in the isolated EPS. Present results are correlated with the report of Anderson *et al.,* (1998). They reported that the EPS producing *Lactobacillus* possess a plasmid DNA of molecular weight greater than 20kb. The present study revealed that culture conditions have a clear impact on the growth and EPS production by *Lactobacillus acidophilus.* The carbon source has a remarkable influence on EPS production. Among the different sugar sources tested, glucose provides highest yield.

The biomass produced from the medium containing various carbon sources were analyzed for EPS production. Although many studies revealed the EPS production of *Lactobacillus* spp. is strongly linked to biomass levels (De Vuyst *et al.* 1998 and Torino *et al.* 2000). The present study is find out there is no correlation between the biomass levels and EPS yield. The quantity of carbohydrates in the medium affects EPS yield (Prasher *et al*. 1997) and high initial carbohydrate level tend to enhance the final EPS levels (De Vuyst *et al.* 1998). Our results are consistent with the previous reports of Cheirsilp *et al*. (2003) showing that the maximum EPS yield was obtained at the sugar concentration 5g/ml. The effect of temperature on EPS production was studied. Incubation at 45˚C clearly affected the biomass and yield. The biomass produced the temperatures 4 - 45˚C have a correlation with the EPS yield. The optimal growth temperature of 37˚C was also optimal for EPS production. Although the biomass is similar at the *p*H range from 5 to 6, it does not having any effect on EPS yield. However the biomass and EPS yield was high at *p*H.Figure 4 shows that supplementation of additional nitrogen source such as ammonium molyptate, beef, ammonium acetate, ammonium ferric citrate, urease, ammonium per sulphate, peptone, yeast and gelatin (1 - 5% (w/v) in the skim milk increased markedly the EPS production of *L. acidophilus*. However, no significant increase in EPS production was observed when the concentration of nitrogen sources was increased from 1% (w/v) to 5% (w/v), probably due to the fact that

supplementation of 1% (w/v) ammonium acetate provided enough nitrogen sources for the growth and EPS production by *L. acidophilus*. Zisu and Shah (2003) found that the EPS yield of *S. thermophilus* 1275 increased with supplementation of 0.5% (w/v) WPC in the skim milk, and the appearance of the EPS increased at higher WPC concentrations. A fivefold increase of EPS yield was obtained for *S. thermophilus* ST 111 when it was grown in milk medium supplemented with whey protein hytrolysate (Vaningelgem et al., 2004).

IV. Figures And Tables



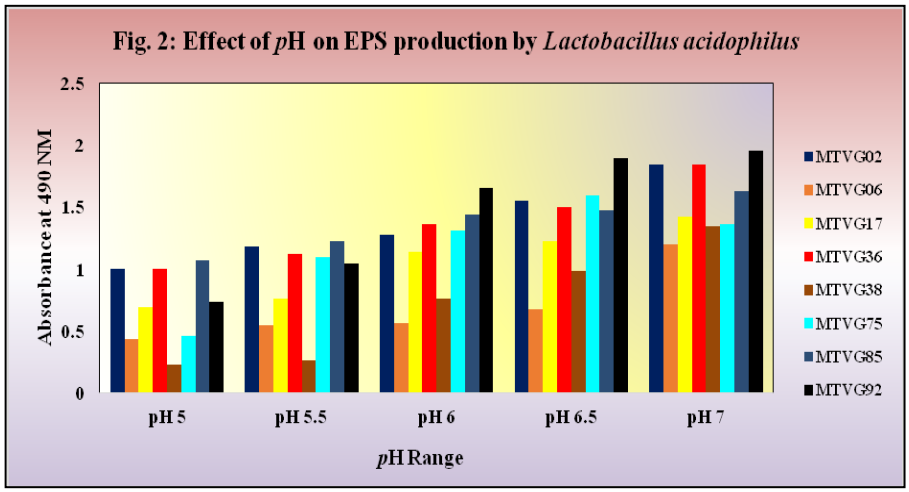
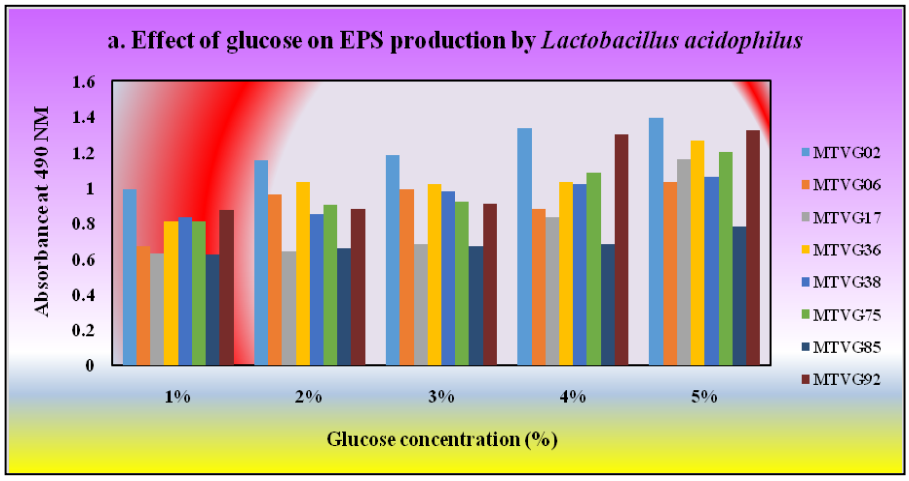


Fig.3. Effect of carbon source on EPS production by *Lactobacillus acidophilus*



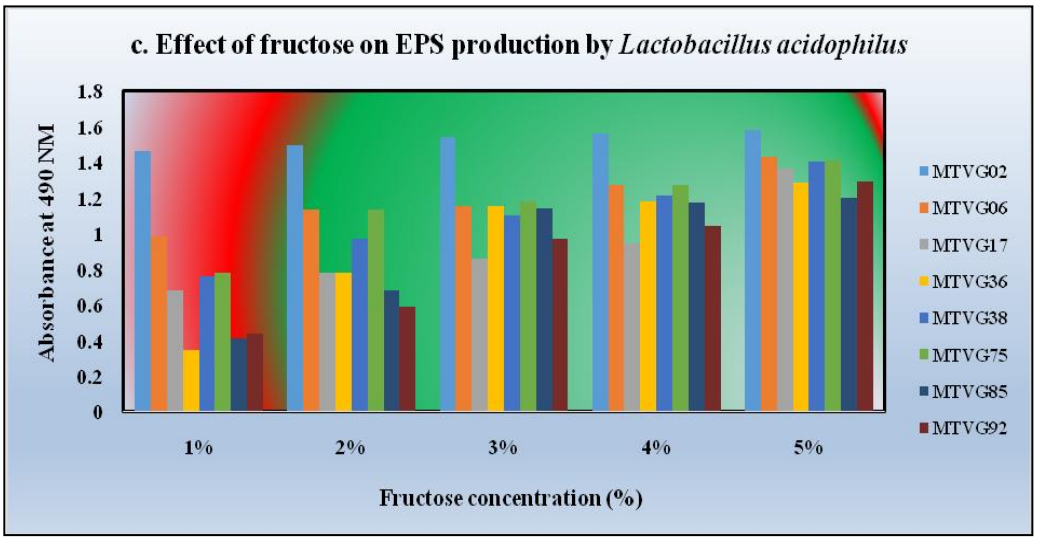
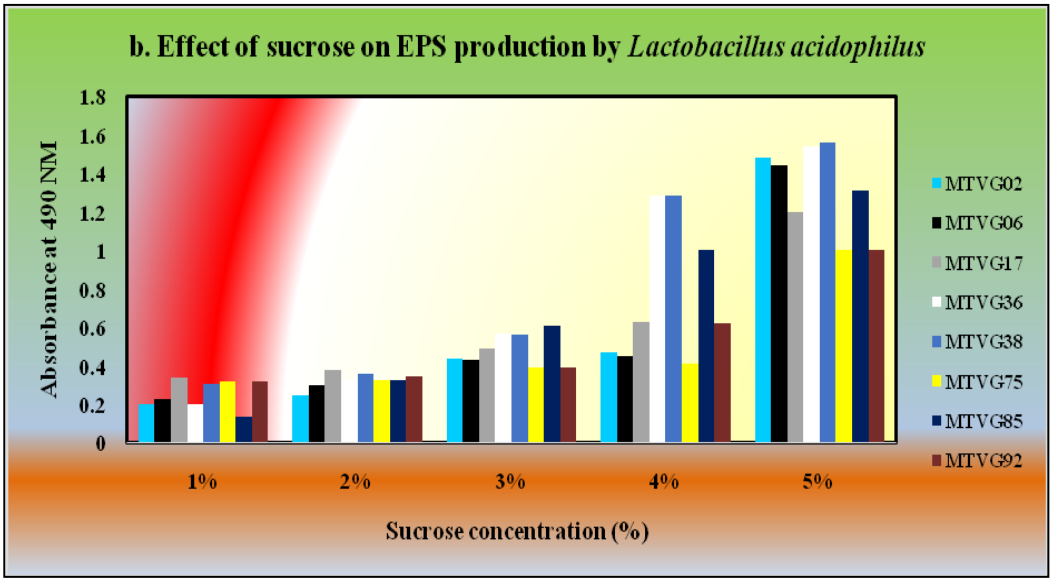
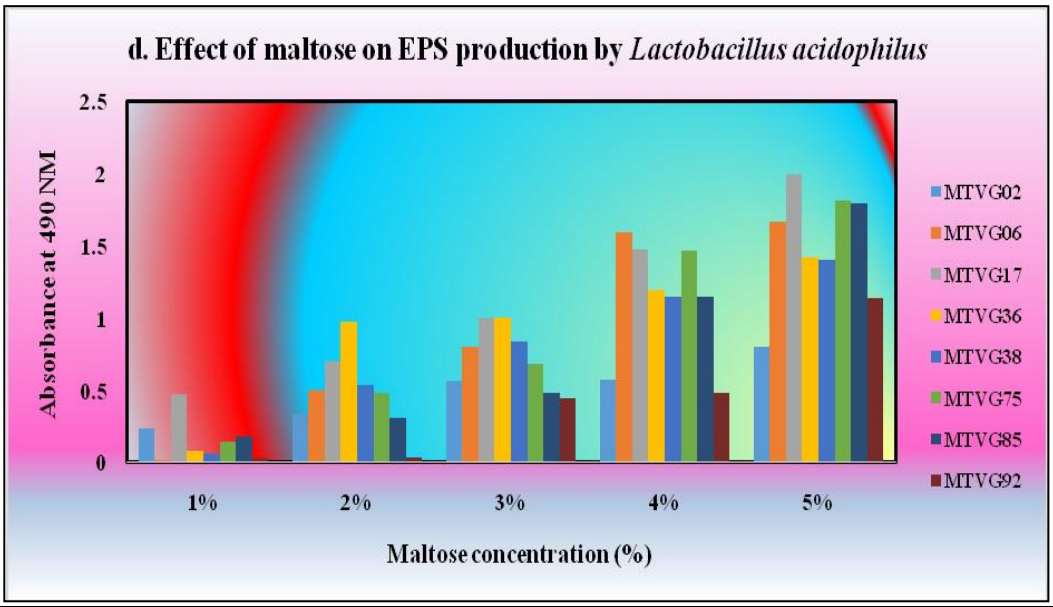
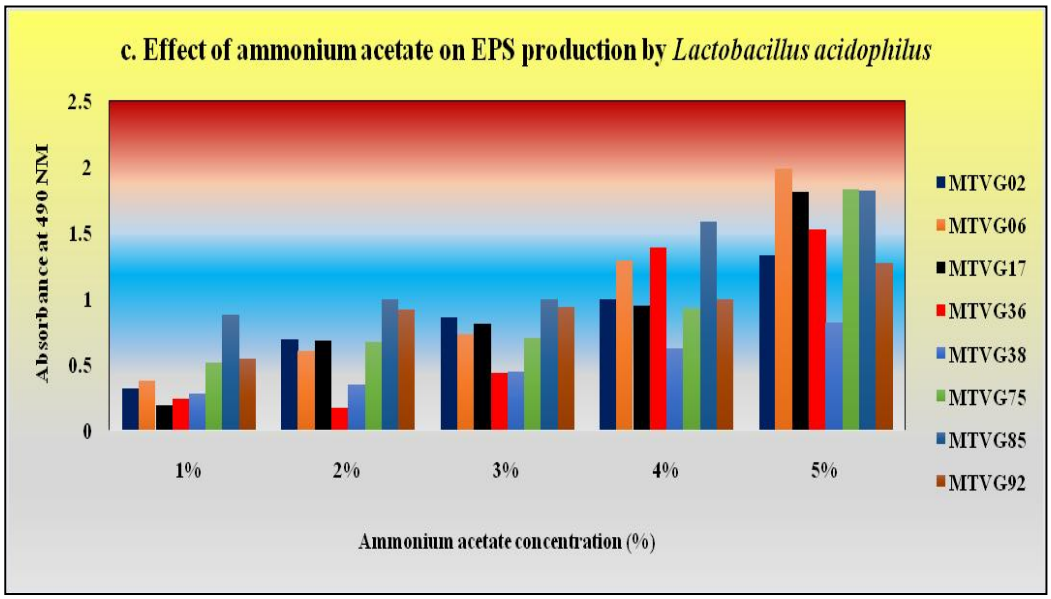
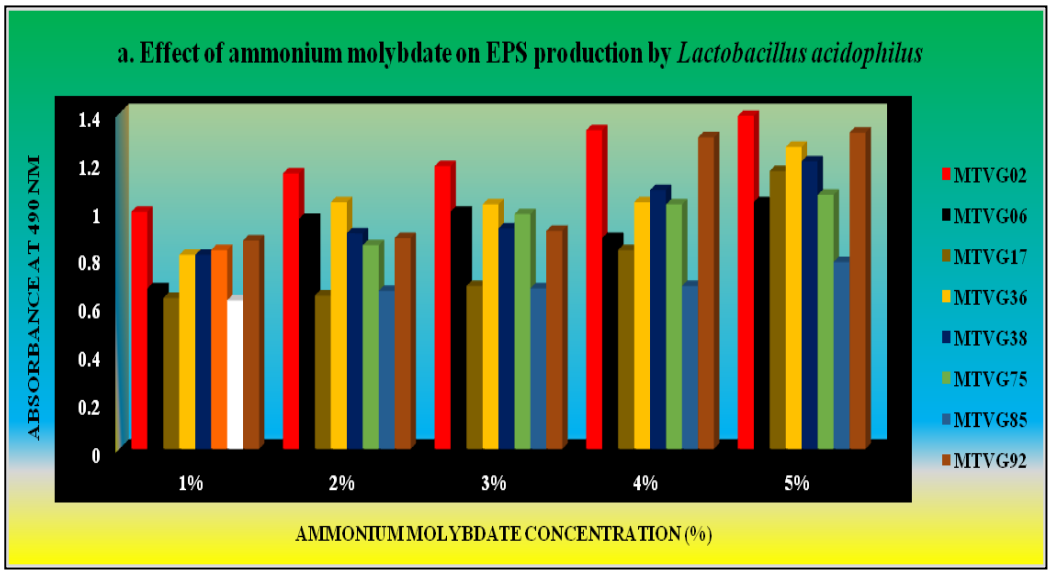


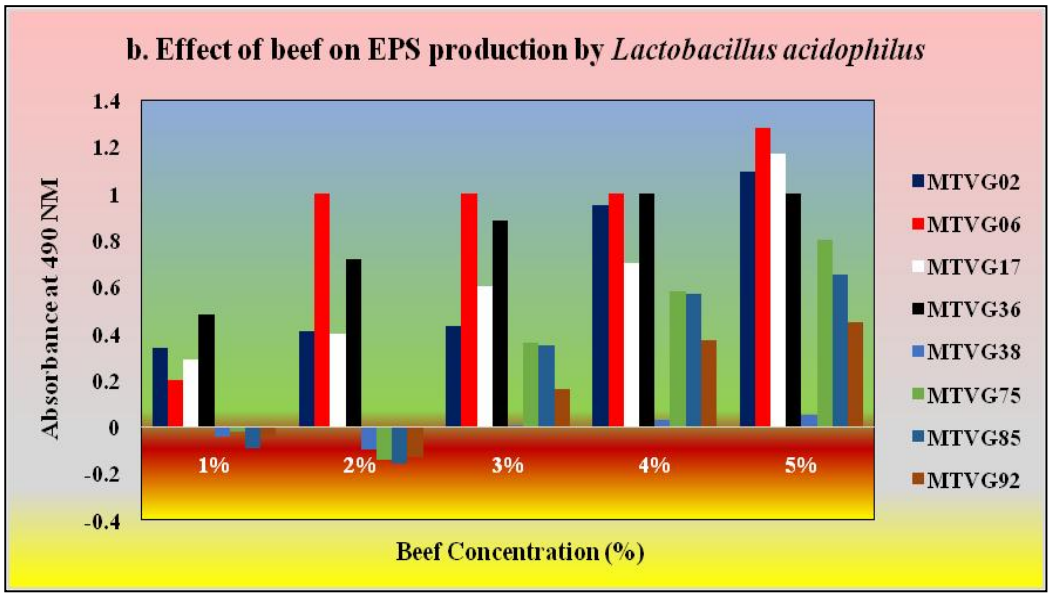
Fig.4. Effect of nitrogen source on EPS production by *Lactobacillus acidophilus*

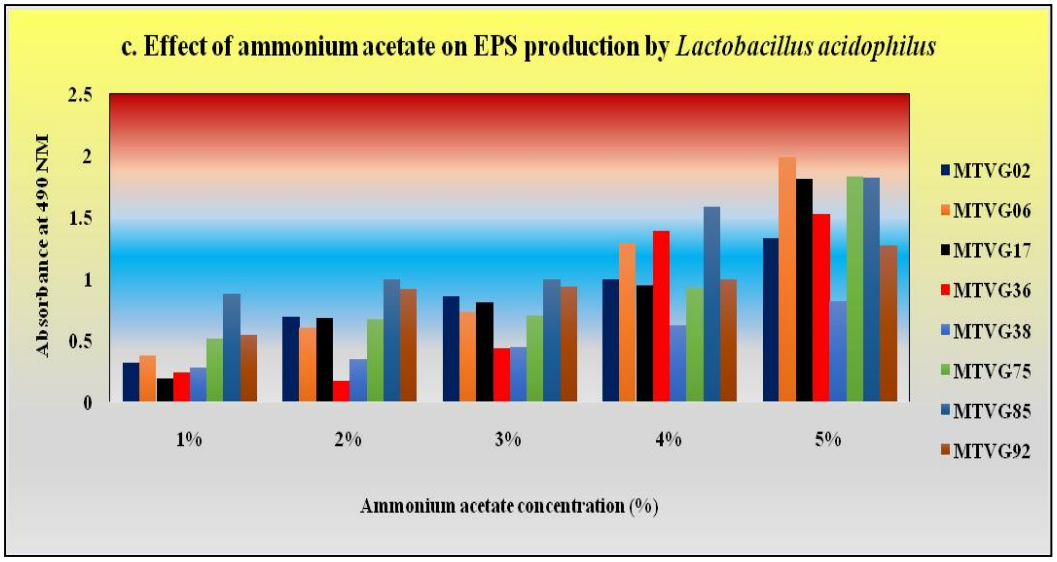





c. Effect of ammonium acetate on EPS production by Lactobacillus acidophilus
Absorbance at 490 NM
2.5
2
1.5
1
0.5
0
1%
2%
3%
4%
5%
Ammonium acetate concentration (%)
MTVG02
MTVG06
MTVG17
MTVG36
MTVG38
MTVG75
MTVG85
MTVG92


a. Effect of ammonium molybdate on EPS production by Lactobacillus acidophilus
ABSORBANCE AT 490 NM
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1%
2%
3%
4%
5%
AMMONIUM MOLYBDATE CONCENTRATION (%)
MTVG02
MTVG06
MTVG17
MTVG36
MTVG38
MTVG75
MTVG85
MTVG92


b. Effect of beef on EPS production by Lactobacillus acidophilus
Absorbance at 490 NM
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
1%
2%
3%
4%
5%
Beef Concentration (%)
MTVG02
MTVG06
MTVG17
MTVG36
MTVG38
MTVG75
MTVG85
MTVG92



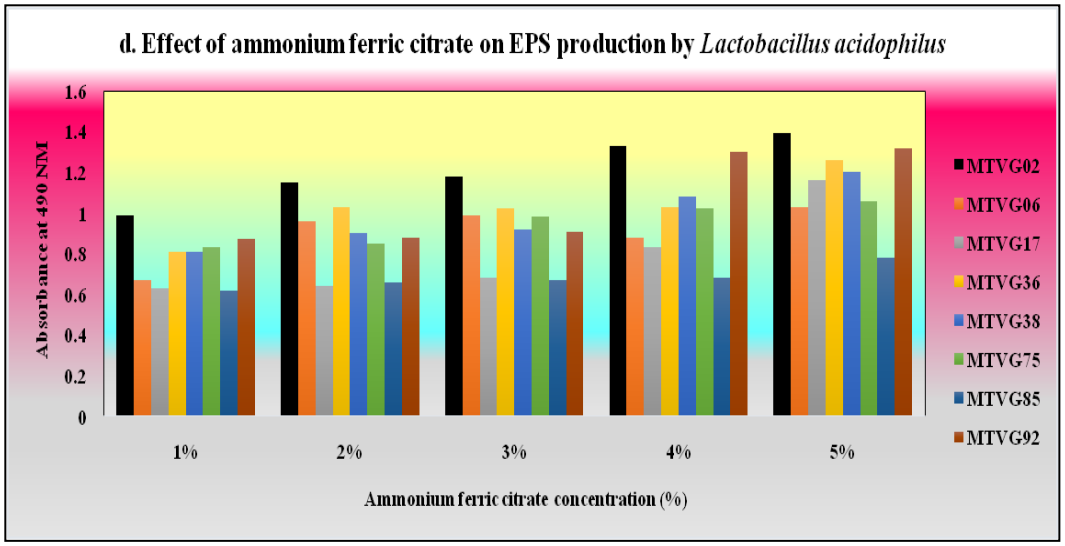
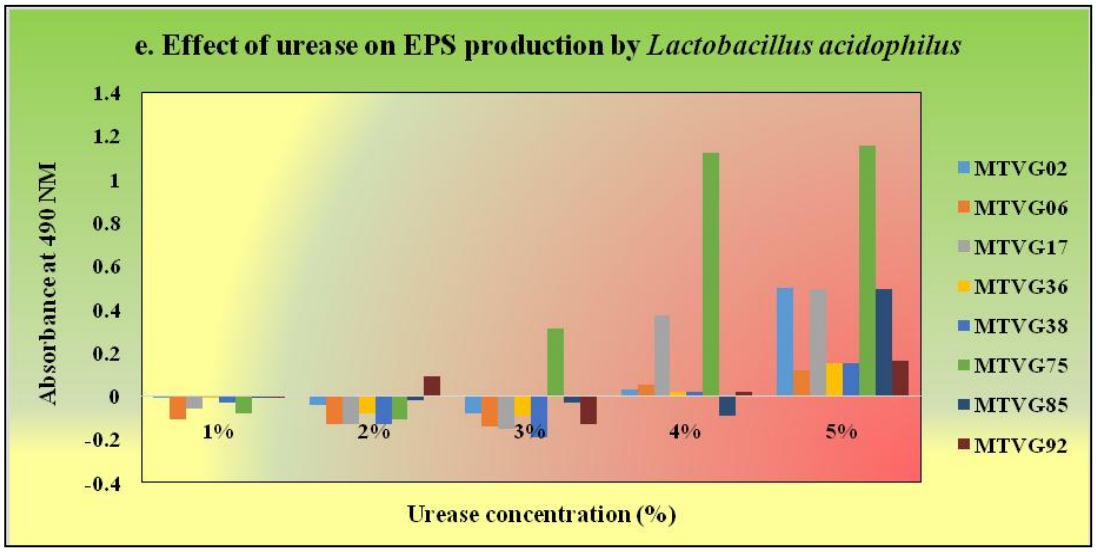
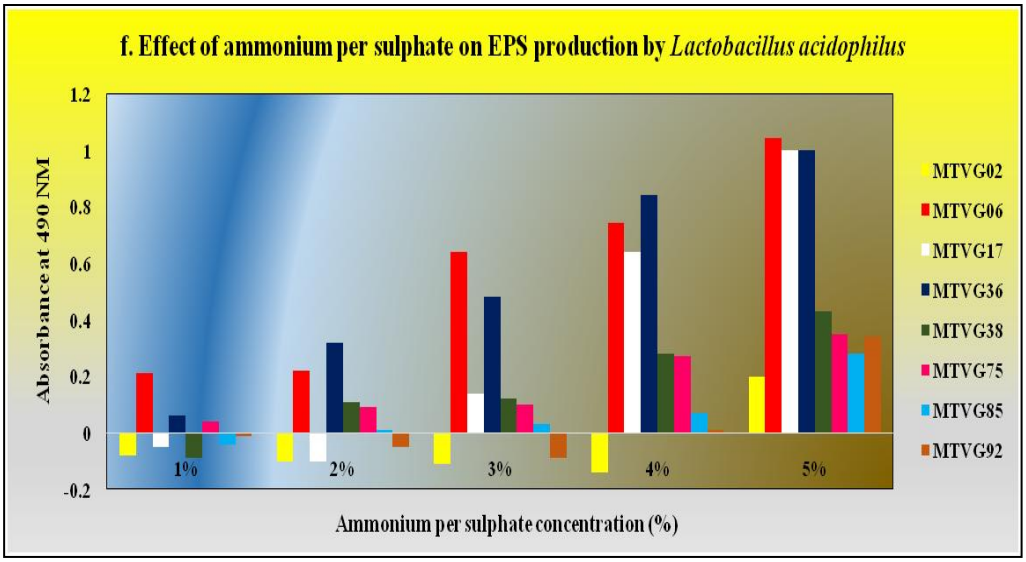
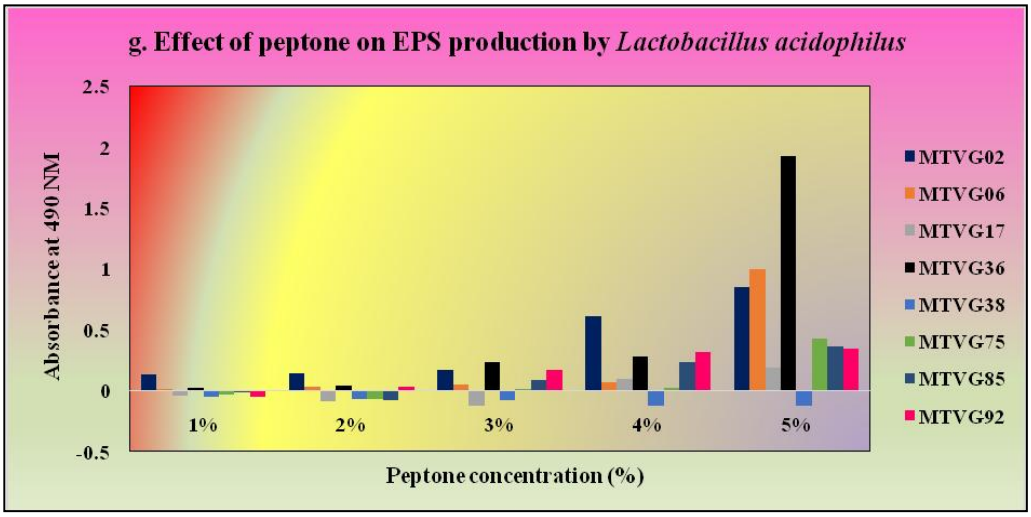


Fig.5. Effect of carbon source on total biomass of EPS producing *Lactobacillus acidophilus*




f. Effect of ammonium per sulphate on EPS production by Lactobacillus acidophilus
Absorbance at 490 NM
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
1%
2%
3%
4%
5%
Ammonium per sulphate concentration (%)
MTVG02
MTVG06
MTVG17
MTVG36
MTVG38
MTVG75
MTVG85
MTVG92


g. Effect of peptone on EPS production by Lactobacillus acidophilus
Absorbance at 490 NM
2.5
2
1.5
1
0.5
0
-0.5
1%
2%
3%
4%
5%
Peptone concentration (%)
MTVG02
MTVG06
MTVG17
MTVG36
MTVG38
MTVG75
MTVG85
MTVG92

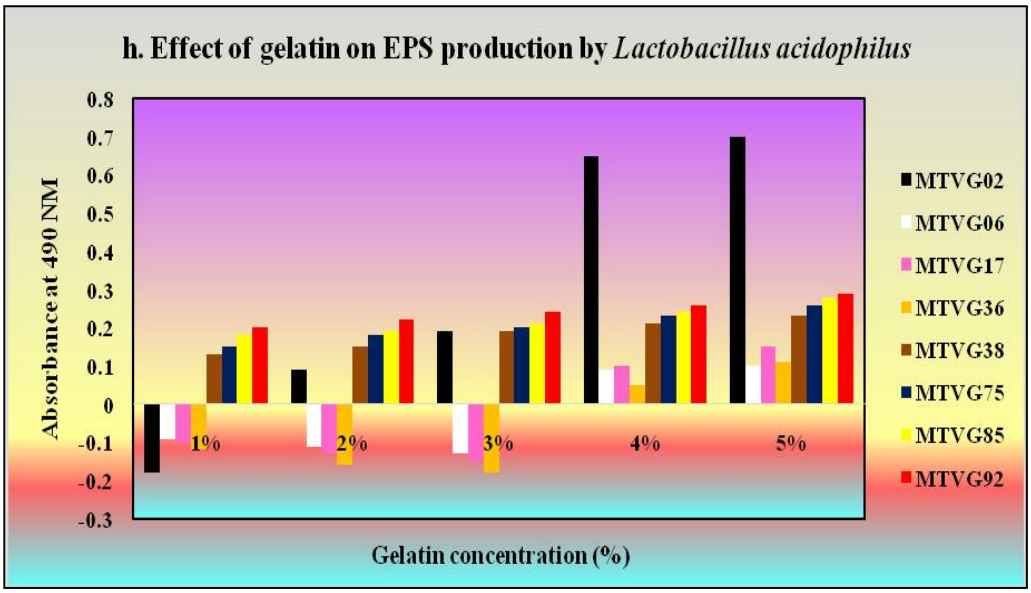
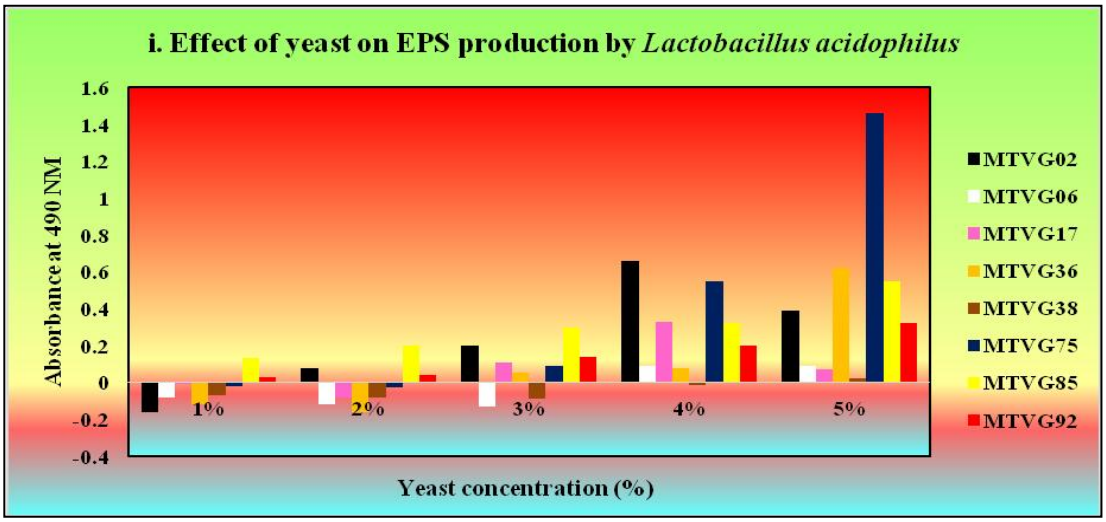
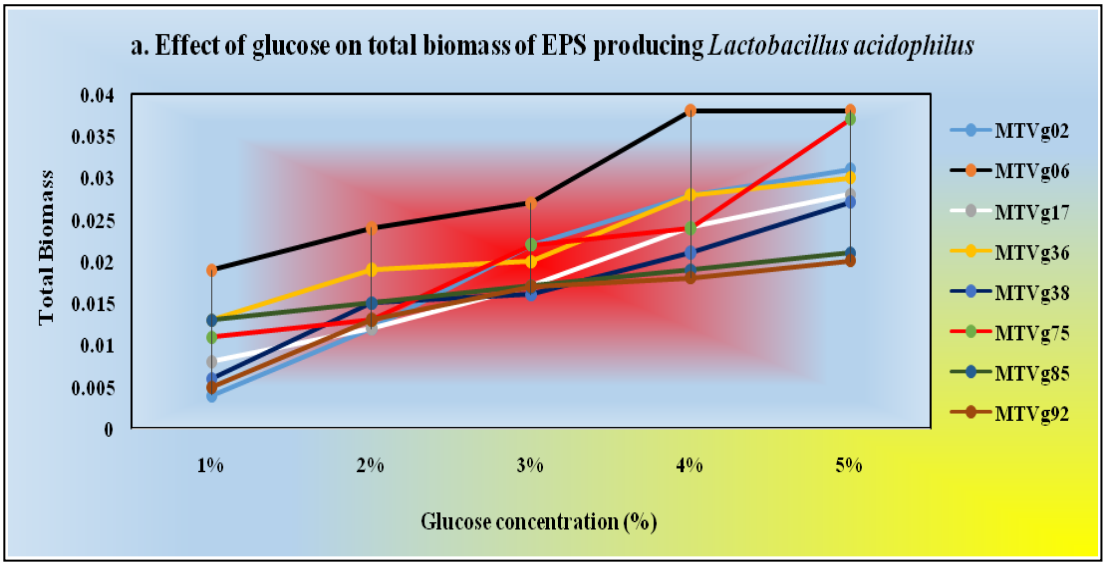
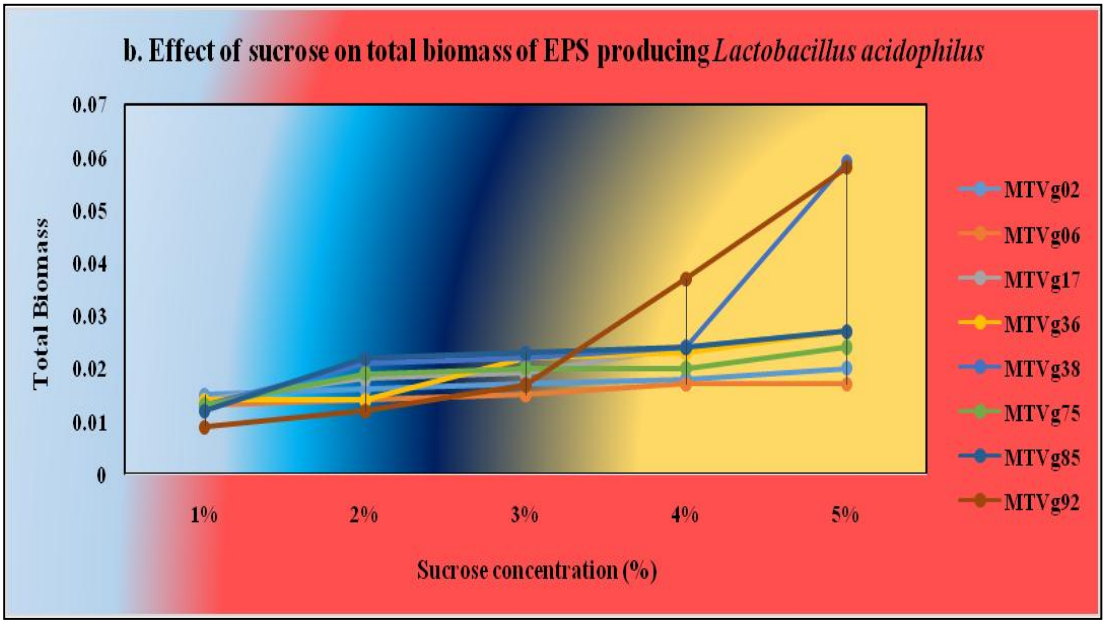
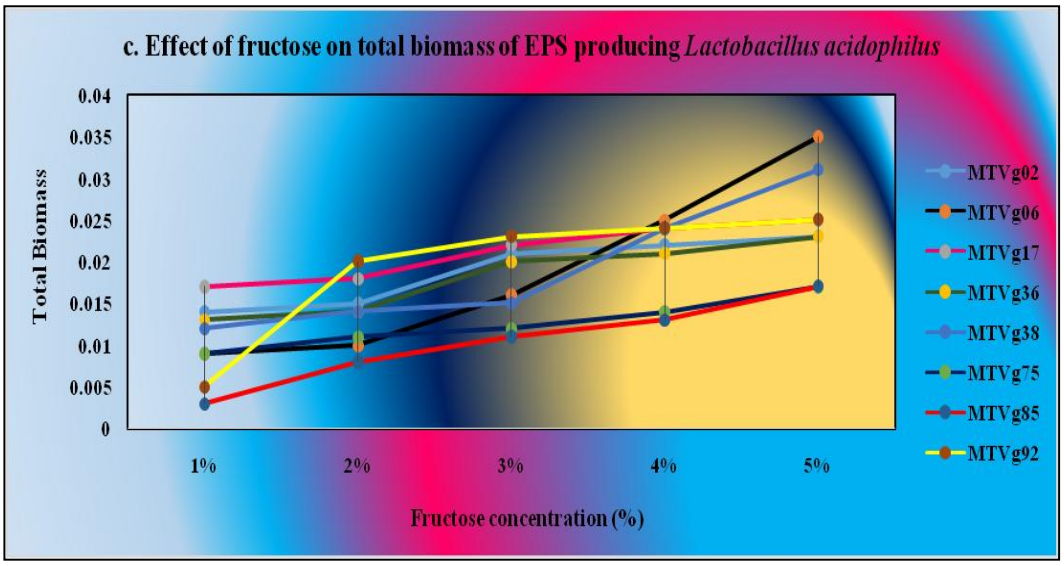

h. Effect of gelatin on EPS production by Lactobacillus acidophilus
Absorbance at 490 NM
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
1%
2%
3%
4%
5%
Gelatin concentration (%)
MTVG02
MTVG06
MTVG17
MTVG36
MTVG38
MTVG75
MTVG85
MTVG92

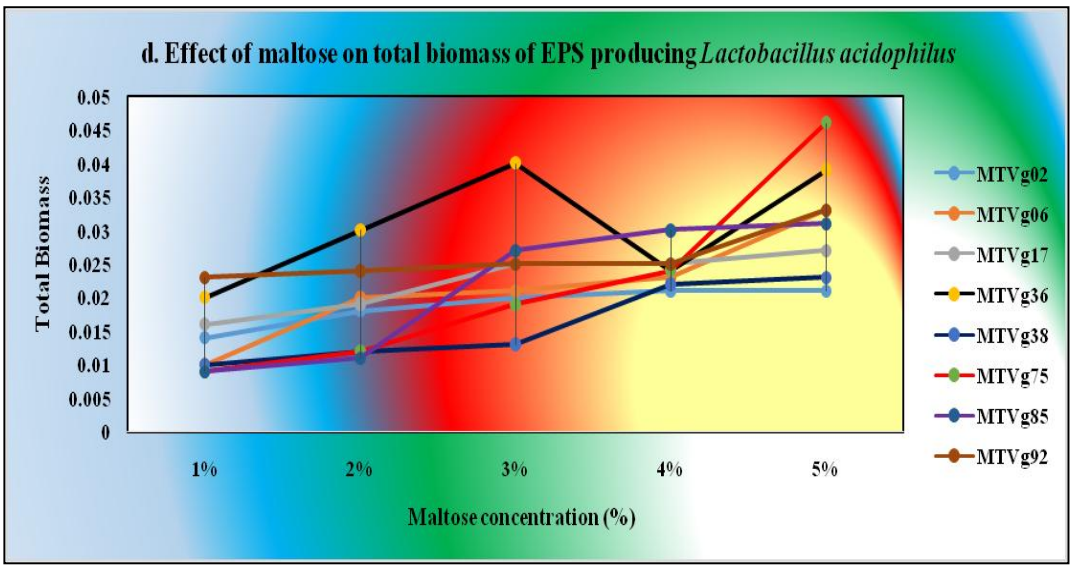
Fig. 6. Effect of nitrogen source on total biomass of EPS producing *Lactobacillus acidophilus*

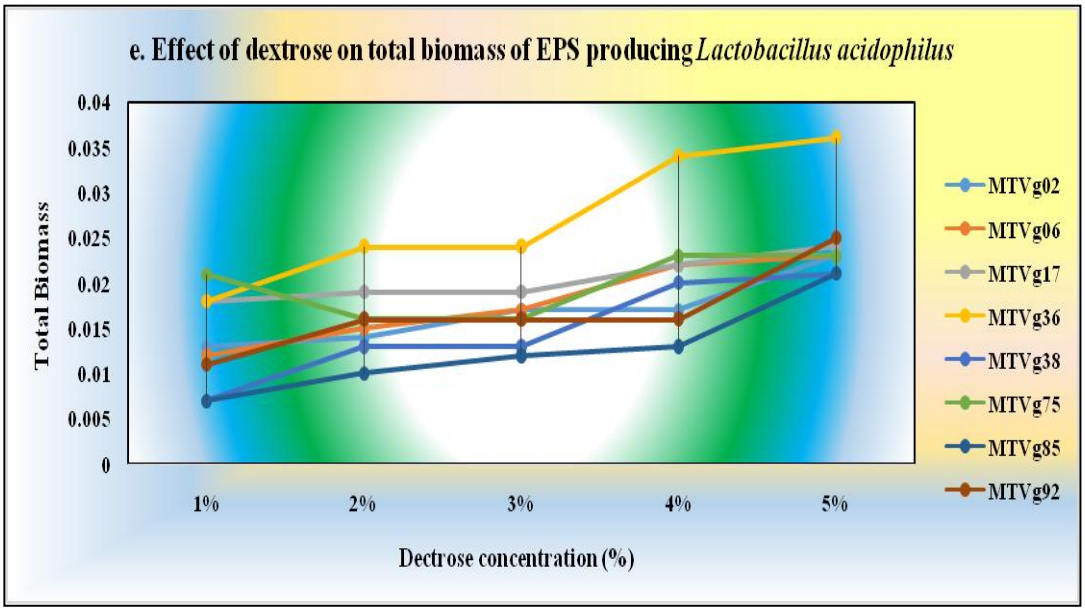


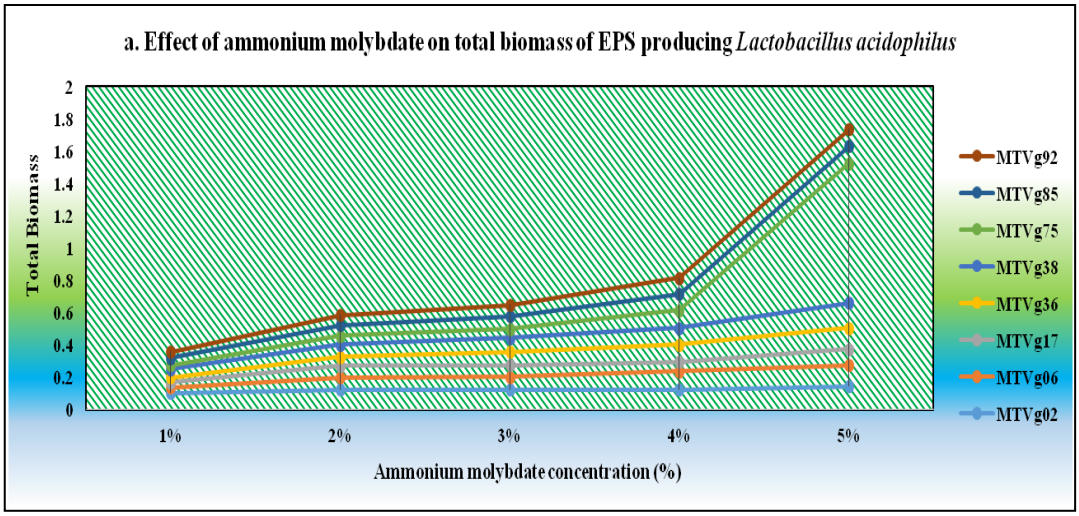


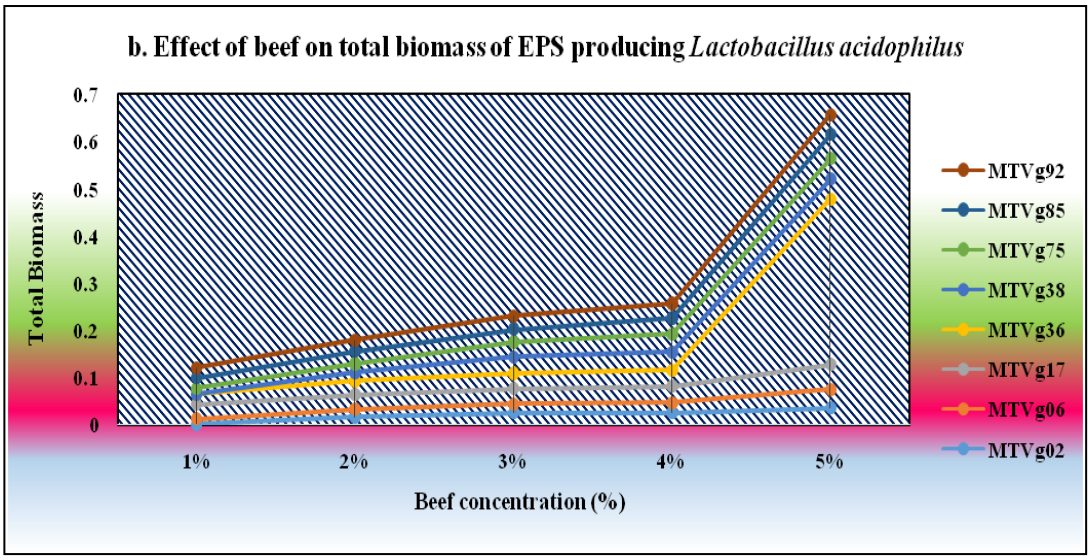


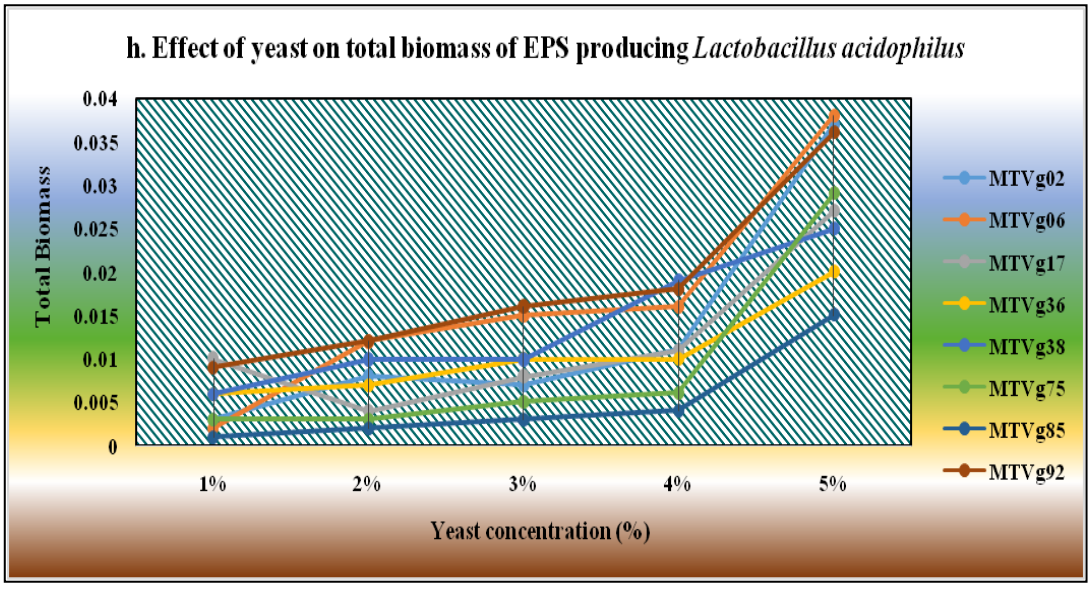

c. Effect of fructose on total biomass of EPS producing Lactobacillus acidophilus
Total Biomass
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
1%
2%
3%
4%
5%
Fructose concentration (%)
MTVg02
MTVg06
MTVg17
MTVg36
MTVg38
MTVg75
MTVg85
MTVg92

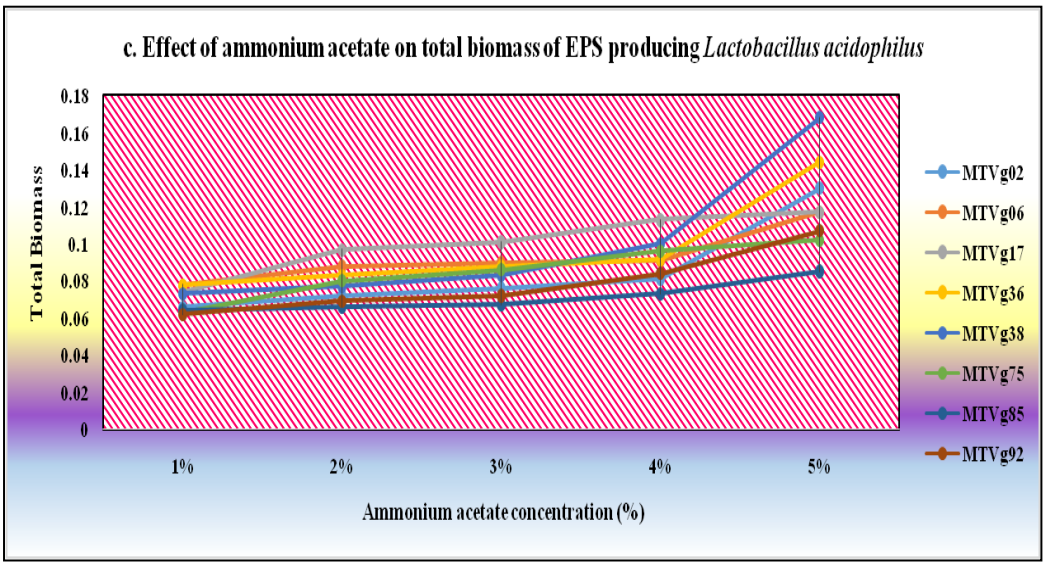

d. Effect of maltose on total biomass of EPS producing Lactobacillus acidophilus
Total Biomass
0.05
0.045
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
1%
2%
3%
4%
5%
Maltose concentration (%)
MTVg02
MTVg06
MTVg17
MTVg36
MTVg38
MTVg75
MTVg85
MTVg92

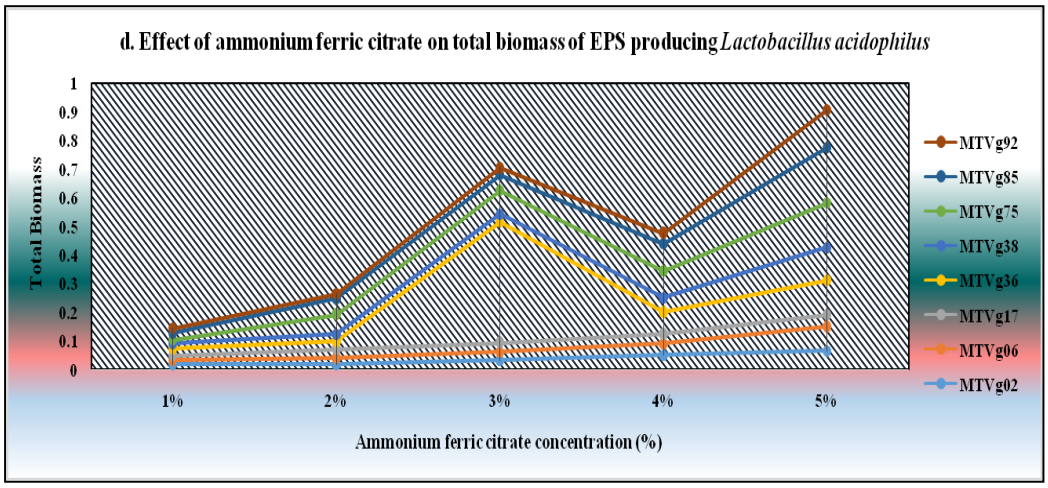

e. Effect of dextrose on total biomass of EPS producing Lactobacillus acidophilus
Total Biomass
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
1%
2%
3%
4%
5%
Dectrose concentration (%)
MTVg02
MTVg06
MTVg17
MTVg36
MTVg38
MTVg75
MTVg85
MTVg92

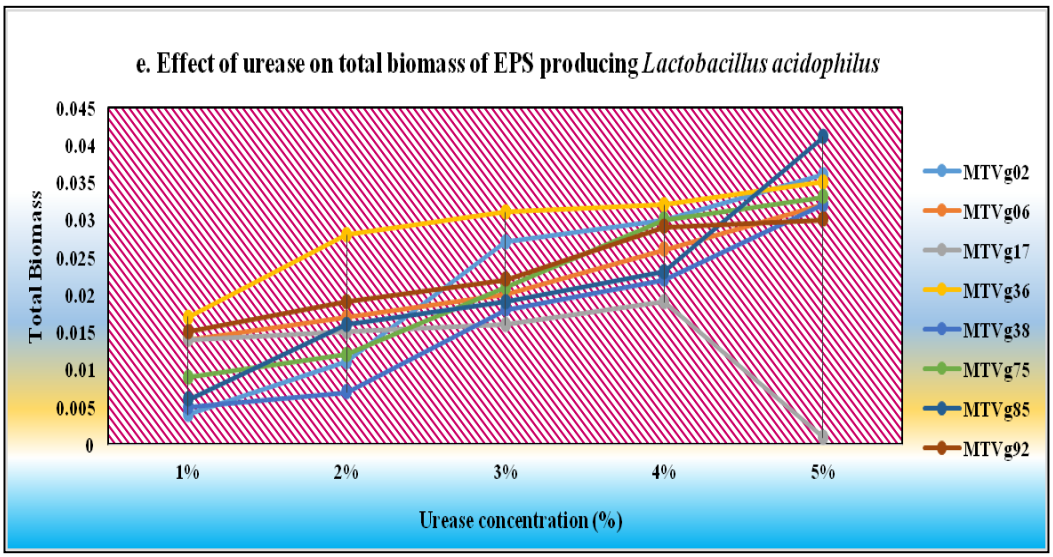

a. Effect of ammonium molybdate on total biomass of EPS producing Lactobacillus acidophilus
Total Biomass
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1%
2%
3%
4%
5%
Ammonium molybdate concentration (%)
MTVg92
MTVg85
MTVg75
MTVg38
MTVg36
MTVg17
MTVg06
MTVg02

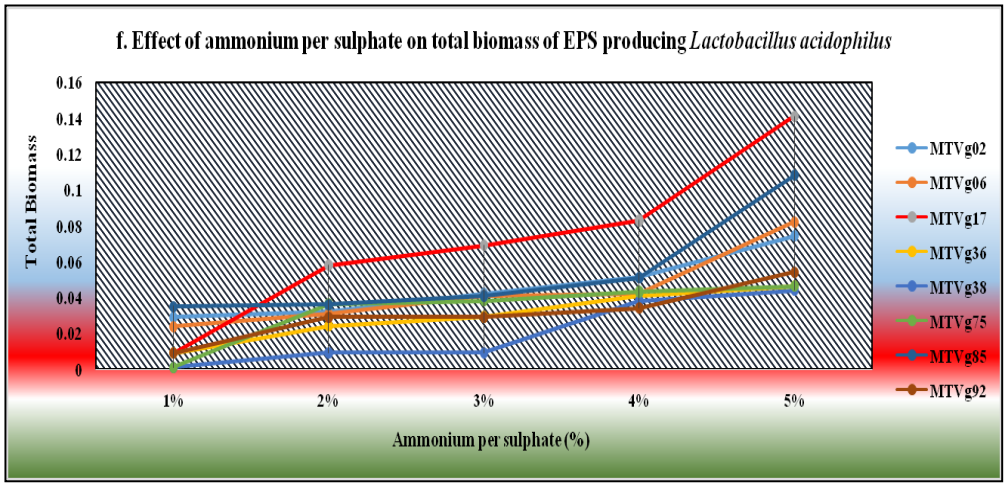

b. Effect of beef on total biomass of EPS producing Lactobacillus acidophilus
Total Biomass
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1%
2%
3%
4%
5%
Beef concentration (%)
MTVg92
MTVg85
MTVg75
MTVg38
MTVg36
MTVg17
MTVg06
MTVg02


h. Effect of yeast on total biomass of EPS producing Lactobacillus acidophilus
Total Biomass
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
1%
2%
3%
4%
5%
Yeast concentration (%)
MTVg02
MTVg06
MTVg17
MTVg36
MTVg38
MTVg75
MTVg85
MTVg92


c. Effect of ammonium acetate on total biomass of EPS producing Lactobacillus acidophilus
Total Biomass
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
1%
2%
3%
4%
5%
Ammonium acetate concentration (%)
MTVg02
MTVg06
MTVg17
MTVg36
MTVg38
MTVg75
MTVg85
MTVg92


d. Effect of ammonium ferric citrate on total biomass of EPS producing Lactobacillus acidophilus
Total Biomass
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1%
2%
3%
4%
5%
Ammonium ferric citrate concentration (%)
MTVg92
MTVg85
MTVg75
MTVg38
MTVg36
MTVg17
MTVg06
MTVg02


e. Effect of urease on total biomass of EPS producing Lactobacillus acidophilus
Total Biomass
0.045
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
1%
2%
3%
4%
5%
Urease concentration (%)
MTVg02
MTVg06
MTVg17
MTVg36
MTVg38
MTVg75
MTVg85
MTVg92



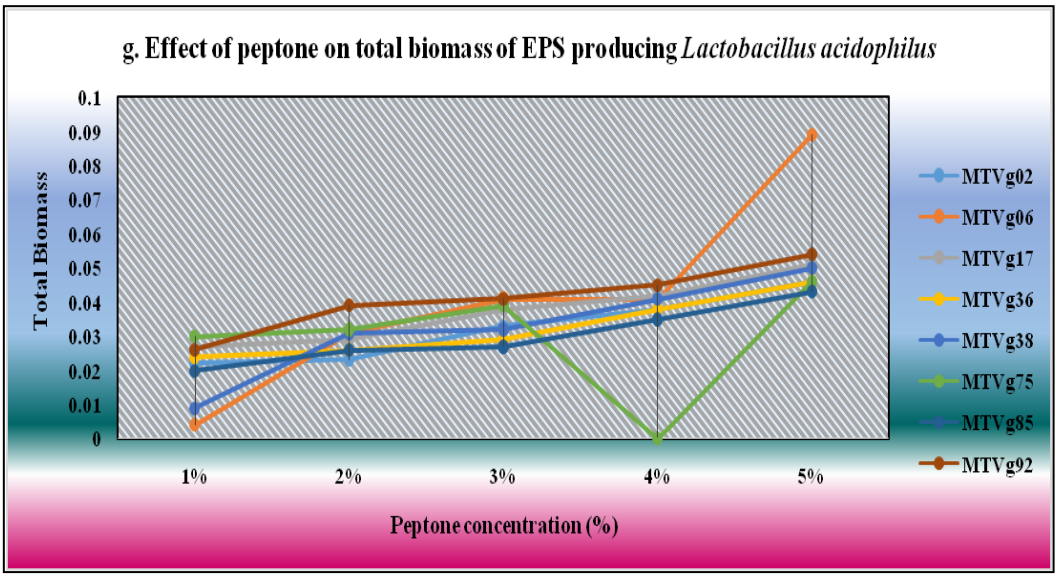


Table 1: Biochemical characterization of *Lactobacillus acidophilus*

S. No	Reaction	Result
01.	Indole	Negative
02.	Glucose	Positive
03.	Lactose	Positive
04.	Sucrose	Positive
05.	Motility	Negative
06.	Catalase	Negative
07.	Oxidase	Positive
08.	Methyl red	Positive
09.	Voges proskauer	Positive
10.	Citrate utilization	Positive
11.	Nitrate reduction	Positive
12.	Starch hydrolysis	Negative
13.	Urease	Positive
14.	Trible Sugar Iron	Positive

Table 2: Entrapment of *Lactobacillus acidophilus* isolated from childhood decay sample

S. No	Substrate used	Dilutions					
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
1.	Decay sample	Too numerous to count	Too numerous to count	>50 colonies	<50 colonies	<20 colonies	< 10 colonies

V. Plates

Plate 1: Confirmation of EPS production by mixture of tiny colonies in ethanol

Plate 1(a): Determination of biomass dry weight of EPS producing *Lactobacillus acidophilus*

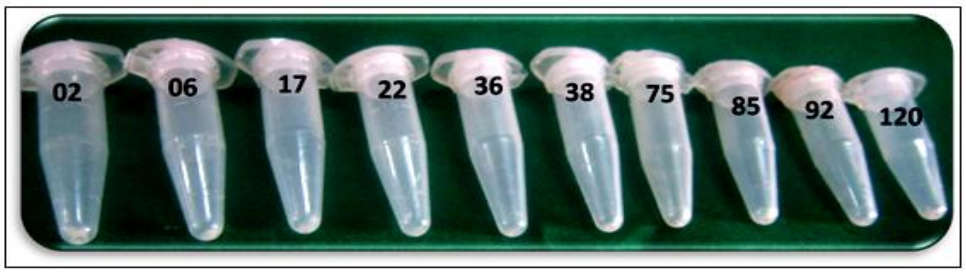


Plate 2: Thin-layer chromatographic analysis of the EPS isolated from cariogenic *Lactobacillus acidophilus*

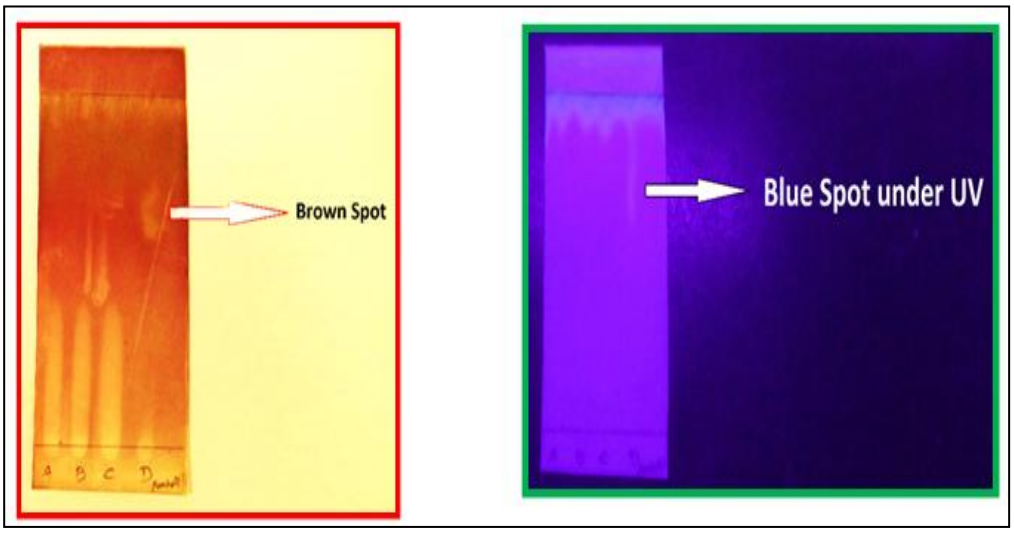


Plate 4: Effect of different Carbon source on EPS production by *Lactobacillus acidophilus*

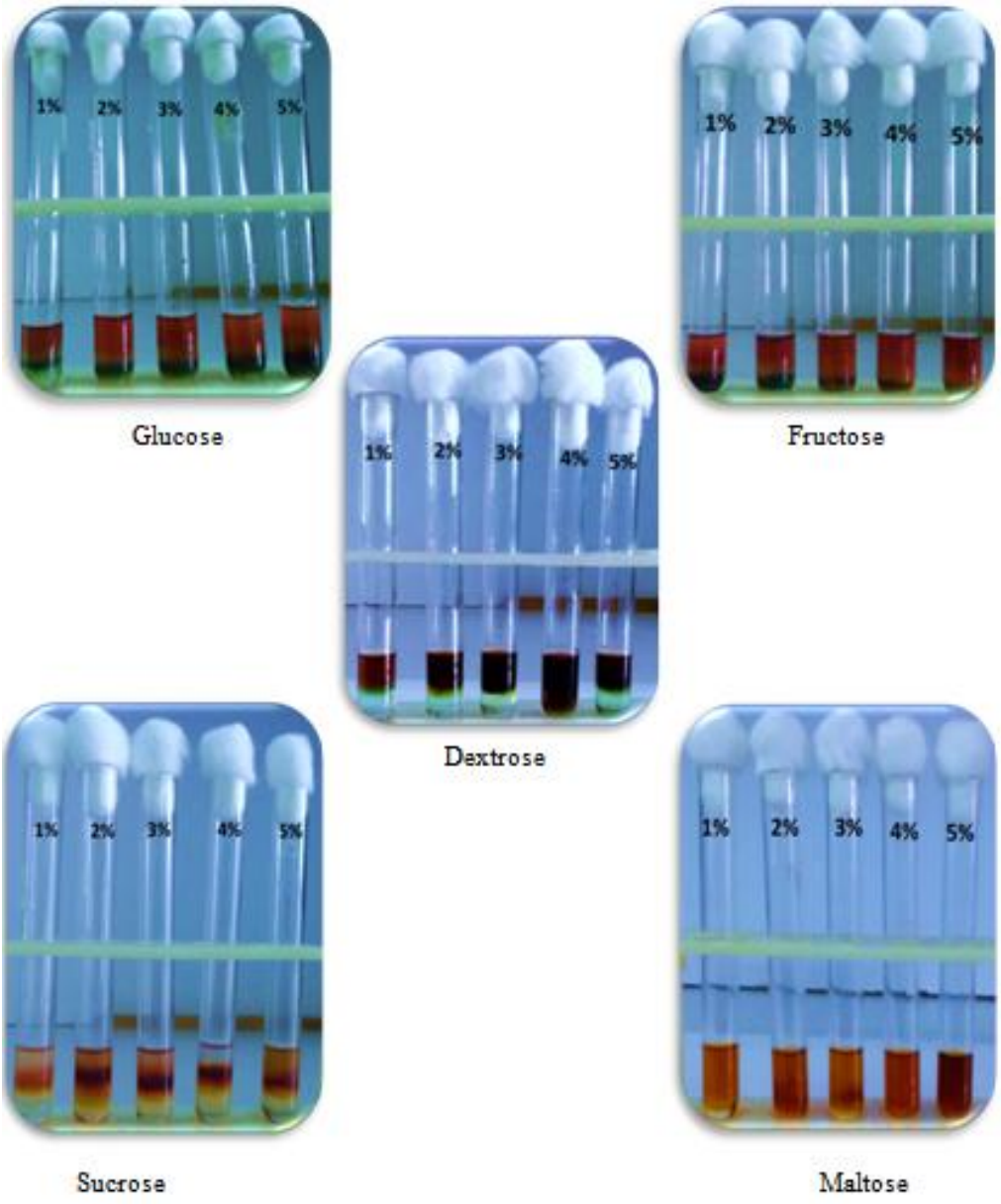
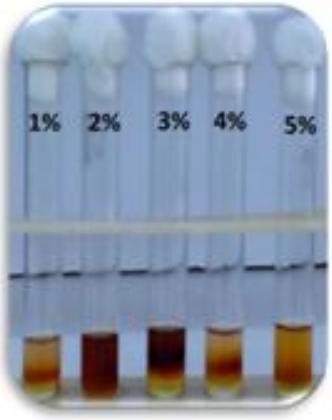
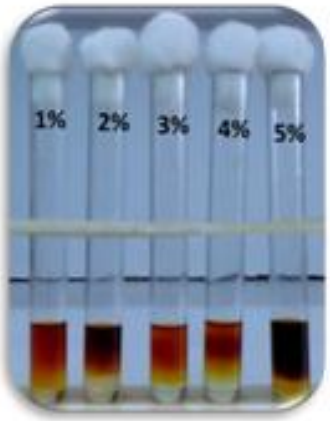


Plate 5: Effect of different Nitrogen source on EPS production by *Lactobacillus acidophilus*

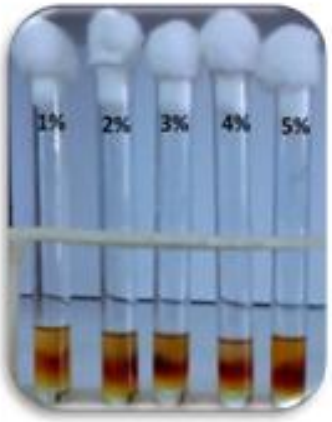


Peptone

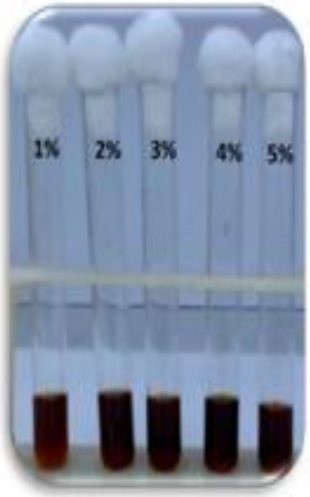
Urease



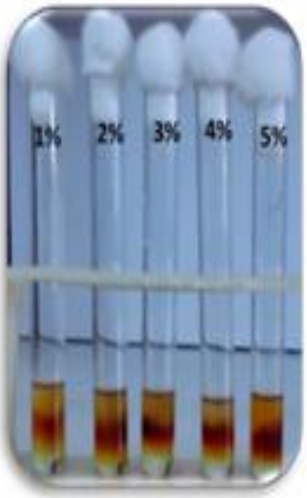
Yeast



Ammonium molybdate

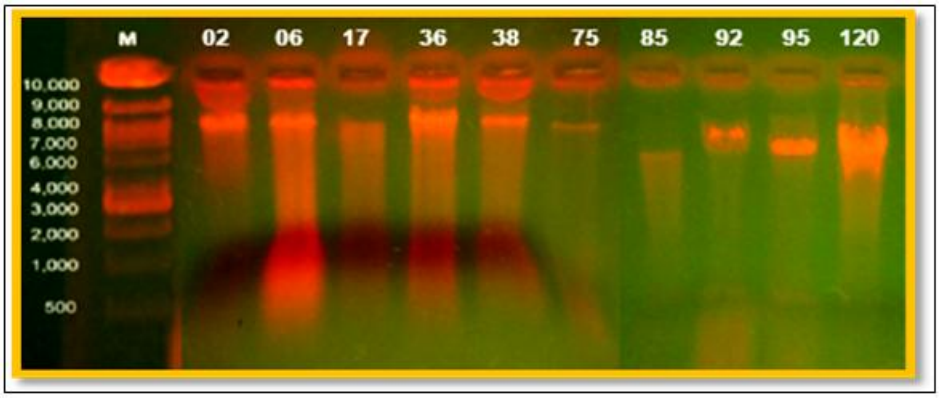


Ammonium Ferric Citrate



Beef

Plate 6: Isolation of plasmid from cariogenic *Lactobacillus acidophilus*



III. Conclusion

The present study concluded that the *Lactobacillus acidophilus* isolated from different decay caries were belongs to hetero fermentative and able to produce EPS in MRS medium. The production of EPS is partially growth associated and there is no correlation between the EPS yield. The cariogenic bacteria mainly it is preceded by accumulation of dental plaque. Dental plaque is a complex biofilm formed on teeth surface composed of self produced extracellular polysaccharides such as glucans. The fermentation of dietary sugars by acidogenic oral micro biota plays key role in the development of caries. The rapid emergence of drug resistant strains of microbial cariogenic pathogen especially those with multi drug resistance characteristics and the organism link with a plasmid have the ability to survive at low *p*H and low temperatures and had strongly to produce antimicrobial substance during fermentation process. These results also suggest that the consumption of carbonated drinks, eating of fast foods, chocolates and fruit juices from bakery shops. The products those enhances the food supply to growing plaque forming microorganisms and the condition favourable for development of pathogenic microorganisms. The main factor contributing the microbial resistance is the EPS production by microbes that allow them to with stand extreme environmental condition and antimicrobial agents due to lack of penetration of antimicrobial agent.

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