Title: Characterization of gut and liver microbiome alterations in a streptozotocin induced rat model of liver damage

Abstract

Liver cirrhosis or damages in liver cells are well reported in many clinical conditions. However, the participation of microbiota in these conditions are being relatively less explored. The present study aimed to evaluate the changes in microbiome in both the liver and the gut in a rat model of liver damage by Streptozotocin (STZ). The experiment was performed on adult male Wistar rats, divided in control and experimental groups. The experimental rats were treated with STZ (65mg/kg b.w.) dissolved in citrate buffer, while the control subjects were treated by injecting 0.5 ml normal saline. The liver and gut samples were collected after the 14^{th} week of drug/normal saline treatment. The experimental results demonstrated large increase in microbes' colonies in liver with respect to the gut. The results showed appearance of grooves, flagella-like structures, and undulated surfaces was observed in the three bacterial isolates *i.e.*, control liver (CSBEBT37_1C_CL); STZ-treated liver (CSBEBT37_1E_SL) and STZ-treated colon (CSBEBT37_2E_C), respectively. The cocci bacterial cells exhibited smooth surfaces or undulated surfaces with few projections. The bacterial isolates of the STZ-treated colon were found to be rod in shape but had different surface features in CSBEBT37_1E_C, CSBEBT37_4E_C, and CSBEBT37_5E_C, the surface displayed undulations without any projections. The findings indicate increase in bacterial colonies by 9.06 folds in the liver damage and 2.4-fold in gut community in the STZ treated rat model compared to the control and hence confirms the role of microbiota in STZ induced experimental liver damages.

Keywords: Bacterial isolates; Liver damage; Liver microbiome; Streptozotocin

Introduction

Streptozotocin (STZ) is derived from a soil bacterium called *Streptomyces achromogenes* var. *streptozoticus*. Its chemical name is 2-deoxy-2-(3-methyl-3-nitrosourea)-1-D glucopyranose, and morphologically more or less similar to a glucose molecule. Various organs and living systems frequently consume and use glucose for energy. There are glucose membrane transporters located in the plasma membrane of the cells of the visceral organs like the pancreas, liver, kidneys, and small intestine, which transfer STZ. A specific glucose transporter known as Glucose transporter 2 (GLUT2) aids in the movement of glucose or other carbohydrates across the cell membranes. GLUT2 is reportedly found in organisms like rats, humans, and monkeys but is present in different numbers, more in rats and less in humans. However, STZ causes injury to the functional cells of these organs when it gains access to them through GLUT2 and targets DNA, resulting in its breakdown [1-3]. Rashmi *et al*. (2018)[4] have mentioned that the hepatic cells are impaired by the existence of the reactive oxygen species (hydrogen peroxide, superoxide, and hydroxyl radicals) formed due to STZ. The chemical can penetrate the bacterial cells via the phosphoenolpyruvate-carbohydrate phosphotransferase channel and exists inside the cell in its phosphorylated form. The hydrolyzed STZ releases diazomethane, which causes damage to the bacterial DNA [3].

The hepatic portal vein fulfils nearly seventy percent of the liver blood requirement. Subsequently, it encounters the bacterial cells and their products; thereby, the antimicrobial cells like Kupffer cells and B lymphocytes help in protecting the organ [5]. The gastrointestinal tract comprises about a hundred trillion germs and two thousand distinct bacterial species. The microbes usually start to grow in the gut in the early stages of life and assist in breaking down food, regulating antimicrobial agents, synthesizing vitamins and producing cholic acid and chenodeoxycholic acid [6]. Anatomically, the gut and liver are interconnected by the hepatic portal vein. The portal vein carries blood from the digestive tract, gall bladder, pancreas, and spleen to the liver [7]. Moreover, the association between the organs is mutual; the bile secreted by the liver is transported to the digestive tract, which is utilized by the microbes living there, and the microbes or their byproducts migrate into the liver [8]. However, some microbial byproducts, like short-chain fatty acids, minor breakdown products of cholic acid act as the causative molecules for non-alcoholic fatty liver disease [9].

STZ is directly involved with cytotoxic effects, oxidative stress, and inflammatory properties that cause profound alterations to the composition of the gut microbiome and produce liver damage. In the overall picture of STZ-induced diabetes models, the gut-liver

axis plays a crucial role, as alterations in the gut microbiome worsen liver toxicity caused by the drug [10]. Several authors have also reported the possible link between liver damage and gut microbial populations. This link can also be labelled as the liver-gut axis. The gastric mucosal barrier forms the leading interacting site between the gut and microbes, comprising the intestinal epithelial cells. The barrier contains goblet cells that produce immunoglobulin, a rich mucous that only allows contactless interaction between the gut lining and microbes. Bile also hinders microbial growth, inactivates microbial toxins [11-13]. The physical damage in this barrier by STZ is responsible for an impaired defence mechanism, and an alteration in the bile flow, can lead to complications in the liver. This damage leads to the loss of permeability of the barrier, which is followed by the presence of antibiotics, a high-fat diet and sepsis [6]. Consequently, the microbes residing in the gut effortlessly travel via the portal vein to the liver, where they cause notable damage to the liver or aggravate the damaged condition.

The present study, tried to explore the effect of STZ on the preexisting bacterial colonies found in the liver and gut of the control and STZ-induced liver damage of rat models, which ultimately provides a microbial basis for diagnosing and analysing the relationship between gut and STZ-induced liver damage.

Materials and Methods

Subjects and Drug Induction

Six male Wistar rats of 10-11 weeks, weighing 100 ± 10 grams were acquired from the animal house of Birla Institute of Technology, Mesra, Ranchi (India) for performing the present work, and the study was approved by the Institutional Animal Ethics Committee (1972/PH/BIT/81/24/IEAC). All the rats were maintained at room temperature (24 ± 1 ºC) inside polypropylene cages with adequate food and water supply. These were acclimatized for one week before the experiment. The subjects were divided into two groups: (i) Control and (ii) Experimental or drug treated. Streptozotocin (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 100mM citrate buffer (pH 4.5), and the prepared solution (65mg/kg b.w.) was administered to all the experimental rats through the intraperitoneal (*i.p.*) route [14]. The control rats were treated on the same way as the experimental subjects, however by injecting 0.5 ml normal saline to them. The liver and gut samples were collected after the 14^{th} week of drug/normal saline treatment.

Isolation, Staining and Characterization

After 14 weeks of drug induction, the liver and colon were extracted and isolated after sacrificing the rats and washed thrice with 0.1M (pH 7.4) Phosphate Buffer Saline (PBS). Smaller portions of the liver were stored in sterile water (0.9% NaCl) for further processing followed by dissolution of waste material from the colon region in 0.9% NaCl. 100µL of organ and gut samples were spread plated on nutrient agar plates and incubated for 24 hours at 37 ºC to observe the growth of different types of bacteria. Several streaking was done to obtain the purified isolates. Different purified isolates underwent Gram's staining using the standard protocol [15] using Gram-staining kit. The stained bacterial isolates were examined at 40X and 100X under a microscope (Leica microsystems, Wetzlar, Germany). Subsequently, 16S rRNA sequencing was also conducted to identify the bacterial isolates.

Field Emission Scanning Electron Microscope (FESEM) analysis

FESEM analysis was carried out for all the isolates to study the surface morphology. Smears of purified isolates were prepared and air-dried before the conductive coating of the sample. The samples were loaded into the FESEM equipment (Carl Zeiss, Gemini, Model-Sigma 300, Germany), and a high-magnification examination was performed [16].

Results

Isolation of bacteria and staining analysis

The STZ-treated rat liver showed more bacterial colonies than the control rat liver as shown in figure 1(a-d). Five different bacterial isolates were obtained in both the conditions, control and STZ-treated rat liver. The colony count of different isolates in both groups is tabulated in table 1. After computing the ratio, it has been concluded that the colonies that appeared in the experimental liver were approximately nine times more as compared to the control liver.

In liver samples, only gram-positive bacteria survived in both groups. However, according to the gram's staining results, gram-positive and gram-negative bacterial isolates were observed in gut samples of different groups. Comparatively, more gram-positive isolates were observed in the experimental group of gut samples. All the isolates of the liver in both conditions were shown in figure 2(A); purified bacterial colonies were obtained through the T-streaking method in the control rat liver and (B) STZ-treated rat liver. Similarly, the gut isolates of different conditions were shown in figure 3(A) Control rat gut and (B) STZ-treated rat gut.

The phenotypic characteristics of the bacterial isolates of different groups of liver are shown in tables 2 and table 3. The characteristics of purified cultures of the gut at different

conditions are shown in table 4 and table 5 along with the staining property. The gut isolates of different groups showed significant differences. The types of isolates obtained were four, but with a huge number of colonies in control gut sample and four isolates were seen under experimental conditions with fewer colonies.

Analysis of FESEM micrographs

The purified colonies of the liver (control rat and STZ-treated rat) and colon (control rat and STZ-treated rat) were examined under FESEM, and their surface morphology was closely studied. The appearance of grooves, flagella-like structures, and undulated surfaces was observed in the three bacterial isolates *i.e.*, control liver (CSBEBT37_1C_CL); STZ-treated liver (CSBEBT37_1E_SL) and STZ-treated colon (CSBEBT37_2E_C), respectively. The cocci bacterial cells exhibited smooth surfaces or undulated surfaces with few projections. The bacterial isolates of the STZ-treated colon were found to be rod in shape but had different surface features in CSBEBT37_1E_C, CSBEBT37_4E_C, and CSBEBT37_5E_C, the surface displayed undulations without any projections. All the FESEM images are illustrated in figures 4 (A & B) of the control rat liver and figures 5 (A & B) of the STZ-induced rat liver.

Analysis of 16S rRNA gene sequencing

16S rRNA gene sequencing was performed to identify the species of the isolates obtained, which is illustrated in table 6.

Discussion

In the present study, the effect of streptozotocin on the diversity of the bacterial population of the liver and the gut was observed using an STZ-induced rat model of liver damage at the end of 14 weeks. The drug STZ was used in many of the studies for the induction of diabetic conditions [17]. After five weeks of drug injection, the effect of STZ on diabetic conditions started falling, and finally, after six weeks, the diabetes was found to be normalized.

The gut comprises an abundance of bacteria forming the gut microbiome and the movement of bacterial colonies or their byproducts from the intestinal mucosa to the liver happens, and the mechanism is known as bacterial translocation [11]. A fair number of bacteria have been isolated from patients with liver diseases. Some of them showed decreased growth, including Enterococcaceae, Staphylococaceae in the case of non-alcoholic fatty liver disease while increased growth of *Enterococcus faecalis* and *Candida albicans* in the case of alcoholic fatty liver disease and steatosis. Also increased growth of Enterococcaceae,

Staphylococaceae in the case of cirrhosis can also be observed [8, 9]. In normal conditions, there is an unequal distribution of good and bad bacteria in the digestive system [18].

In this study, the association between the gastrointestinal tract and liver was also examined using a rat model after 14 weeks. STZ has also been documented to degrade liver cells [14, 19, 20]. According to literature, the hepatic portal vein connects the liver to the digestive tract and the tract contains diverse forms of bacteria. It should also be highlighted that it supplies nutrients rich blood but devoid of oxygen. Hence, the bacteria travelling through this vein must be either a facultative anaerobe or an obligate anaerobe. Interestingly, the former category of bacteria (phylum *Bacillota*) is more capable of migrating towards the liver from the tract.

The investigation showed that the bacterial species belonging to the phylum Bacillota showed greater migration efficiency. Almost all the bacteria isolated from the control and experimental liver falls under this phylum. These bacterial species have been identified by the *16S rRNA* sequencing, which resulted different genera like, *Enterococcus faecalis, Mammaliicoccus sciuri and Bacillus cereus*. Literatures revealed that these are considered mostly as facultative anaerobes. Exceptionally, one of the bacterial strains CSBEBT37_5C_CL, identified as *Microbacterium trichothecenolyticum* isolated from the control rat was found to be of Actinobacteria phylum.

The intestinal barrier is a portal through which the bacteria can pass and reach the liver under certain physiological conditions, including sepsis, antibiotics or a high-fat diet. Few studies have reported that some bacteria increase their colony population in the cirrhotic liver such as Enterococcaceae and Staphylococaceae family microbes [8, 9]. Two strains, one from control rat liver (CSBEBT37_3C_CL) and other obtained from the experimental rat liver (CSBEBT37_3E_SL) identified as *Enterococcus faecalis* belonged to the Enterococcaceae family while CSBEBT37_4C_CL, obtained from the control rat liver and CSBEBT37_4E_SL, obtained from the liver damage model of rat are identified as *Mammaliicoccus sciuri* belonged to Staphylococaceae family. Furthermore, *Enterococcus faecalis* has also been known to form biofilms [21]. The increase in the abundance of both bacteria in the experimental group indicated the liver cirrhotic state of the rat. *Bacillus cereus* is not directly linked with liver cirrhosis but acute liver failure [22]. A subspecies of it has also shown healing effects in damaged liver [23]. Its increased colonial growth in the STZ-treated rat liver may be linked with liver damage. The *Microbacterium trichothecenolyticum* is reportedly extracted from soil [24], human blood culture [25, 26], and gut of mole cricket

[27] but was never reported in the healthy rat liver. Moreover, its abundance became low in the experimental rat liver spread plate.

Thus, it can be concluded that the STZ-induced liver damage can be a vulnerable condition for the growth of different bacterial species. The gut harbours an enormous number of bacteria, likely more than any region of the digestive tract, as it has optimum pH, lacks bile and lacks many antibacterial agents [28]. However, the microbial population of the STZ-treated colon was determined to be lower when compared to the control colon plate. This decrease in colonies may suggest the influence of antibacterial activity of STZ as it has been documented that different antibiotics may affect the microbial population [29].

The lower abundance of bacterial colonies in the colon may also suggest the transitioning of native bacteria of the colon to other organs of the living system. Furthermore, a bacterium isolated from the colon was identified as *Bacillus marisflavi* (a facultative anaerobe), which was formerly reported from the mud flat of the Yellow Sea in Korea [30] and disease-free marine fish's gut. It was also experimentally found harmless to trout fishes [31]; this highlights the present study, as it has appeared in the STZ-treated rat gut.

Conclusion

Significant information about liver and colon microbiota in rodent subjects submitted to STZ treatment was studied and explained. In the case of the liver microbiome, our finding indicated that the abundance of bacteria increased by 9.06 folds in the liver damage of the rat model as compared to the control rat. Similarly, the abundance of the gut community also gets altered in the treated rat model as the control gut as the intestinal barrier is disrupted and bacterial translocation is observed. The abundance of colonizing isolates was 2.4 folds more in the treated model compared to the control model of rats. Nevertheless, the origin of bacterial colonies thriving in the healthy liver and the migration of colon-based bacteria to other visceral organs apart from the digestive tract can be extensively explored for future research work.

Acknowledgements

Authors are grateful for the help and support received from Ms. Kahakashan Parween, Research Scholar, Department of Bioengineering and Biotechnology, Birla Institute of Technology, Mesra (India) in conducting the experiment.

References

1. Kahraman S, Aydin C, Elpek GO, Dirice E, Sanlioglu AD, Diabetes-resistant NOR mice are more severely affected by streptozotocin compared to the diabetes-prone NOD Mice: correlations with liver and kidney GLUT2 Expressions. Journal of Diabetes Research, 2015: 450128.
2. Rerup C, Tarding F, Streptozotocin-and alloxan-diabetes in mice. European Journal of Pharmacology 1969; 7: 89-96.
3. Zhang J, Yakovlieva L, de Haan BJ, de Vos P, Minnaard AJ, Witte MD, Walvoort MT, Selective modification of streptozotocin at the C3 position to improve its bioactivity as antibiotic and reduce its cytotoxicity towards insulin-producing β cells. Antibiotics 2020; 9: 182.
4. Rashmi R, Magesh SB, Ramkumar KM, Suryanarayanan S, SubbaRao MV, Antioxidant potential of naringenin helps to protect liver tissue from streptozotocin-induced damage. Reports of Biochemistry & Molecular Biology 2018; 7: 76-84.
5. Son G, Kremer M, Hines IN, Contribution of gut bacteria to liver pathobiology. Gastroenterology Research and Practice 2010: 453563.
6. Wang L, Wan YJY, The role of gut microbiota in liver disease development and treatment. Liver Research 2019; 3: 3-18.
7. Carneiro C, Brito J, Bilreiro C, Barros M, Bahia C, Santiago I, Caseiro-Alves F, All about portal vein: a pictorial display to anatomy, variants and physiopathology. Insights into Imaging 2019; 10: 38.
8. Xu M, Luo K, Li J, Li Y, Zhang Y, Yuan Z, Wu X, Role of intestinal microbes in chronic liver diseases. International Journal of Molecular Sciences 2022; 23: 12661.
9. Albillos A, De Gottardi A, Rescigno M, The gut-liver axis in liver disease: Pathophysiological basis for therapy. Journal of Hepatology 2020; 72: 558-577.
10. Lefever DE, Xu J, Chen Y, Huang G, Tamas N, Guo TL, TCDD modulation of gut microbiome correlated with liver and immune toxicity in streptozotocin (STZ)-induced hyperglycemic mice. Toxicology and Applied Pharmacology 2016; 304: 48-58.
11. Alexopoulou A, Agiasotelli D, Vasilieva LE, Dourakis SP, Bacterial translocation markers in liver cirrhosis. Annals of Gastroenterology 2017; 30: 486-497.
12. Muñoz L, Borrero MJ, Úbeda M, Conde E, Del Campo R, Rodríguez-Serrano M, Albillos A, Intestinal immune dysregulation driven by dysbiosis promotes barrier disruption and bacterial translocation in rats with cirrhosis. Hepatology 2019; 70: 925-938.

13. Wiest R, Garcia-Tsao G, Bacterial translocation (BT) in cirrhosis. Hepatology 2005; 41: 422-433.
14. Zafar M, Naqvi N, Ahmed M, Kaimkhani Z, Altered liver morphology and enzymes in streptozotocin induced diabetic rats. International Journal Morphololology 2009; 27: 719-725.
15. Thairu Y, Nasir IA, Usman Y, Laboratory perspective of gram staining and its significance in investigations of infectious diseases. Sub-Saharan African Journal of Medicine 2014; 1: 168-174.
16. Arunachalam K, Davoodbasha M, Imaging bacteria and biofilm by field emission scanning electron microscopy. Analytical Methodologies for Biofilm Research 2021: 205-222.
17. Ghasemi A, Jeddi S, Streptozotocin as a tool for induction of rat models of diabetes: a practical guide. EXCLI Journal 2023; 22: 274-294.
18. Maslennikov R, Poluektova E, Zolnikova O, Sedova A, Kurbatova A, Shulpekova Y, Ivashkin V, Gut Microbiota and Bacterial Translocation in the Pathogenesis of Liver Fibrosis. International Journal of Molecular Sciences 2023; 24: 16502.
19. Aldahmash BA, El-Nagar DM, Ibrahim KE, Attenuation of hepatotoxicity and oxidative stress in diabetes STZ-induced type 1 by biotin in Swiss albino mice. Saudi Journal of Biological Sciences 2016; 23: 311-317.
20. Rodríguez Valeria, Plavnik Luis and Talamoni NL, Naringin attenuates liver damage in streptozotocin-induced diabetic rats. Biomedicine & Pharmacotherapy 2018; 105: 95-102.
21. Kim MA, Rosa V, Min KS, Characterization of Enterococcus faecalis in different culture conditions. Scientific Reports 2020; 10: 21867.
22. Schreiber N, Hackl G, Reisinger AC, Zollner-Schwetz I, Eller K, Schlagenhaufen C, Ehling-Schulz M, Acute liver failure after ingestion of fried rice balls: A case series of Bacillus cereus food poisonings. Toxins 2021; 14: 12.
23. Li YT, Ye JZ, Lv LX, Xu H, Yang LY, Jiang XW, Li LJ, Pretreatment with Bacillus cereus preserves against D-galactosamine-induced liver injury in a rat model. Frontiers in Microbiology 2019; 10: 1751.
24. Mondani L, Piette L, Christen R, Bachar D, Berthomieu C, Chapon V, Microbacterium lemovicicum sp. nov., a bacterium isolated from a natural uranium-rich soil. International Journal of Systematic and Evolutionary Microbiology 2013; 63: 2600-2606.

25. Gneiding K, Frodl R, Funke G, Identities of Microbacterium spp. encountered in human clinical specimens. Journal of Clinical Microbiology 2008; 46: 3646-3652.
26. Lau SK, Woo PC, Woo GK, Yuen KY, Catheter-related Microbacterium bacteremia identified by 16S rRNA gene sequencing. Journal of Clinical Microbiology 2020; 40: 2681-2685.
27. Kim DY, Shin DH, Jung S, Kim H, Lee JS, Cho HY, Park HY, Novel Alkali-Tolerant GH10 Endo-β-1,4-Xylanase with Broad Substrate Specificity from Microbacterium trichothecenolyticum HY-17, a Gut Bacterium of the Mole Cricket Gryllotalpa orientalis. Journal of Microbiology and Biotechnology 2014; 24: 943-953.
28. Kohl KD, Dearing MD, Bordenstein SR, Microbial communities exhibit host species distinguishability and phylosymbiosis along the length of the gastrointestinal tract. Molecular Ecology 2018; 27: 1874-1883.
29. Yoon JH, Kim IG, Kang KH, Oh TK, Park YH, Bacillus marisflavi sp. nov. and Bacillus aquimaris sp. nov., isolated from sea water of a tidal flat of the Yellow Sea in Korea. International Journal of Systematic and Evolutionary Microbiology 2003; 53: 1297-1303.
30. Yoon MY, Yoon SS, Disruption of the gut ecosystem by antibiotics. Yonsei Medical Journal 2018; 59: 4-12.
31. Akayli T, Albayrak G, Ürkü Ç, Canak Ö, Yörük E, Characterization of Micrococcus luteus and Bacillus marisflavi recovered from common dentex (Dentex dentex) larviculture system. Mediterranean Marine Science 2016; 17: 163-169.

316 **Table 1:** Comparison of the number of colonies in (a) liver and (b) gut of the STZ treated
317 subjects with respect to the control.

Liver			Gut		
Control	**STZ Treated**	**Ratio**	**Control**	**STZ Treated**	**Ratio**
160	1450	1450/160 = 9.06	205	485	485/205 = 2.4

318

319

Table 2: Morphological characteristics of bacterial colonies observed after spread plating for control liver (C- control group; CL- control liver).

S. No.	Phenotype	CSBEBT_1C_CL	CSBEBT_2C_CL	CSBEBT_3C_CL	CSBEBT_4C_CL	CSBEBT_5C_CL
1.	Colony colour	White	Yellow	Grayish white	White	Pale orange
2.	Colony shape	Irregular	Irregular	Small	Circular	Small
3.	Colony margin	Undulate	Smooth	Smooth	Smooth	Smooth
4.	Colony elevation	Flat	Raised	Flat	Convex	Convex
5.	Colony opacity	Opaque	Opaque	Transparent	Opaque	Opaque
6.	Gram's stain	+ve	+ve	+ve	+ve	+ve
7.	Shapes	Rod	Cocci	Cocci	Cocci	Rod

Table 3: Morphological characteristics of bacterial colonies observed after spread plating for STZ-treated liver (E- Experimental group; SL- STZ treated liver).

S. No.	Phenotype	CSBEBT_1E_SL	CSBEBT_2E_SL	CSBEBT_3E_SL	CSBEBT_4E_SL	CSBEBT_5E_SL
1.	Colony colour	White	Yellow	Grayish white	White	Pale orange
2.	Colony shape	Irregular	Irregular	Small	Circular	Small
3.	Colony margin	Undulate	Smooth	Smooth	Smooth	Smooth
4.	Colony elevation	Flat	Raised	Flat	Convex	Convex
5.	Colony opacity	Opaque	Opaque	Transparent	Opaque	Opaque
6.	Gram's stain	+ve	+ve	+ve	+ve	+ve
7.	Shapes	Rod	Cocci	Cocci	Cocci	Rod

Table 4: Characteristics of different cultures of the gut at control condition (C- control group; G- control gut).

S. No.	Phenotype	CSBEBT37_1C_G	CSBEBT37_2C_G	CSBEBT37_3C_G	CSBEBT37_4C_G
1.	Colony colour	White	Creamish white	Greyish-white	Yellow
2.	Colony shape	Small	Round	Small	Small
3.	Colony margin	Irregular	Smooth	Smooth	Smooth
4.	Colony elevation	Flat	flat	Convex	Umbonate
5.	Colony opacity	Opaque	Opaque	Translucent	Translucent
6.	Gram's stain	+ve	+ve	-ve	-ve
7.	Shapes	Rod	Cocci	Rod	Rod

Table 5: Characteristics of different cultures of the gut at treated condition (E- Experimental group; G- Gut).

S. No.	Phenotype	CSBEBT37_1E_G	CSBEBT37_2E_G	CSBEBT37_3E_G	CSBEBT37_4E_G
1.	Colony colour	Creamish white	Off-white	White	White
2.	Colony shape	Irregular	Filamentous	Circular	Round with radiating margins
3.	Colony margin	Irregular (Undulate)	Irregular	Smooth	Irregular
4.	Colony elevation	Crateriform	Flat	Convex	Umbonate
5.	Colony opacity	Opaque	Opaque	Opaque	Opaque
6.	Gram's stain	-ve	+ve	+ve	+ve
7.	Shapes	Rod	Rod	Rod	Rod

Table 6: Results of 16S rRNA sequencing along with their accession numbers.

S. No	**Bacterial Isolates**	**Similarity percentage (%)**	**Identification of bacteria**
1.	CSBEBT37_1C_CL	100%	*Bacillus cereus*
2.	CSBEBT37_3C_CL	99.29%	*Enterococcus faecalis*
3.	CSBEBT37_5C_CL	99.93%	*Microbacterium trichothecenolyticum*
4.	CSBEBT37_4E_SL	100%	*Mammaliicoccus sciuri*
5.	CSBEBT37_4E_G	100%	*Escherichia coli*
6.	CSBEBT37_3E_G	99.46%	*Bacillus marisflavi*

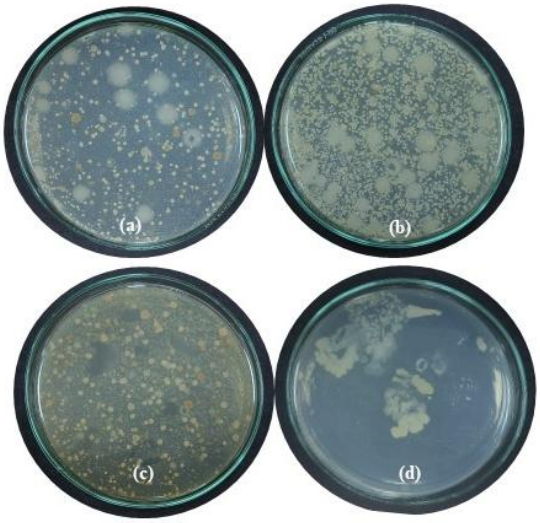
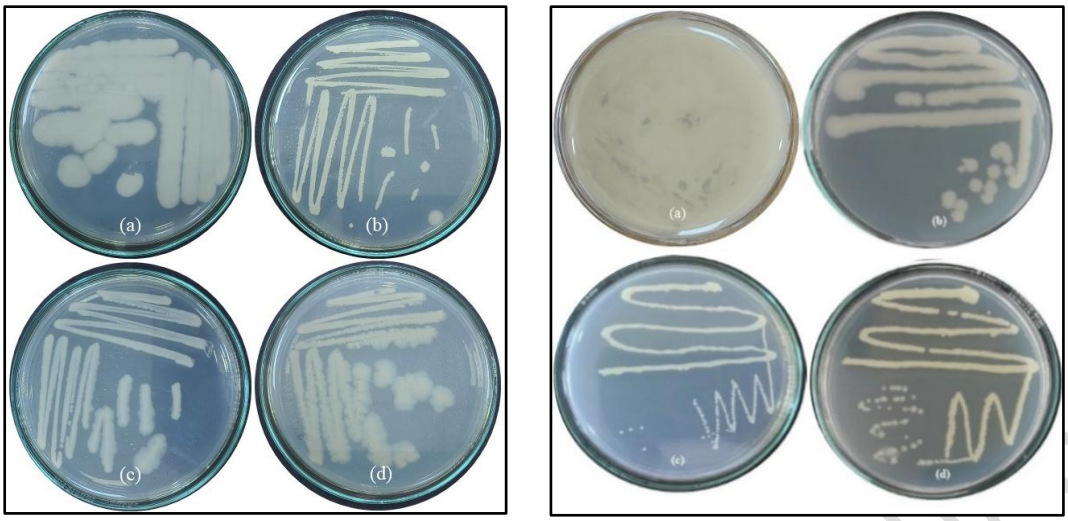


Figure 1: Colonies obtained from (a) the liver of a control rat, (b) the liver of an STZ-treated rat, (c) the gut of a control rat, and (d) the gut of an STZ-treated rat.

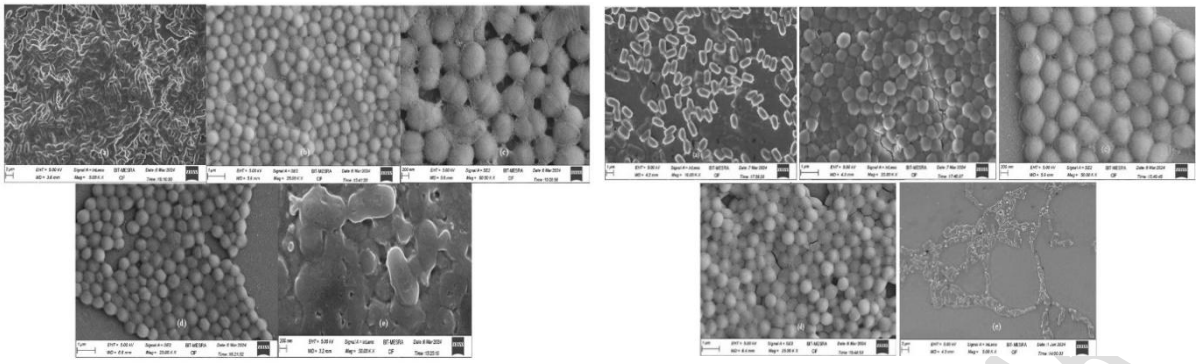
(A) (B)

Figure 2: (A) Purified bacterial colonies obtained through the T-streaking method in the control rat liver: (a) CSBEBT37_1C_CL, (b) CSBEBT37_2C_CL, (c) CSBEBT37_3C_CL, (d) CSBEBT37_4C_CL, and (e) CSBEBT37_5C_CL. **(B)** STZ-treated rat liver: (a) CSBEBT37_1E_SL, (b) CSBEBT37_2E_SL, (c) CSBEBT37_3E_SL, (d) CSBEBT37_4E_SL, and (e) CSBEBT37_5E_SL.



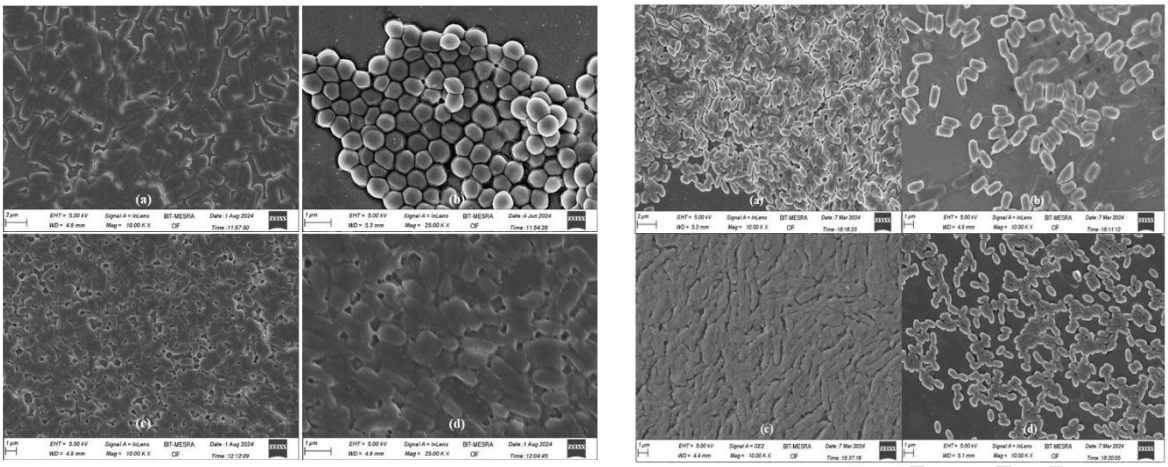
(A) (B)

Figure 3: Pure bacterial colonies obtained in **(A)** control rat gut: (a) CSBEBT37_1C_G, (b) CSBEBT37_2C_G, (c) CSBEBT37_3C_G and (d) CSBEBT37_4C_G and **(B)** STZ-treated rat gut: (a) CSBEBT37_1E_G, (b) CSBEBT37_2E_G, (c) CSBEBT37_3E_G, (d) CSBEBT37_4E_G.



(A) (B)

Figure 4: FESEM images: **(A)** isolated from a control rat liver: (a) CSBEBT37_1C_CL, (b) CSBEBT37_2C_CL, (A) CSBEBT37_3C_CL, (d) CSBEI (B) 4C_CL, and (e) CSBEBT37_5C_CL. **(B)** STZ-treated rat liver: (a) CSBEBT37_1E_SL, (b) CSBEBT37_2E_SL, (c) CSBEBT37_3E_SL, and (d) CSBEBT37_4E_SL and (e) CSBEBT37_5E_SL.



(A) (B)

Figure 5: FESEM images: **(A)** Control rat gut: (a) CSBEBT37_1C_G, (b) CSBEBT37_2C_G, (c) CSBEBT37_3C_G, (d) CSBEBT37_4C_G and (**B)** STZ-treated rat colon: (a) CSBEBT37_1E_G, (b) CSBEBT37_2E_G, (c) CSBEBT37_3E_G, (d) CSBEBT37_4E_G.