THE ROLE OF PROBIOTICS WITH LACTIC ACID-PRODUCING BACTERIA IN MODULATING THE BEHAVIOUR OF RODENT MODEL FOLLOWING CONTUSIVE SPINAL CORD INJURY: PRELIMINARY STUDY

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by

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PERANAN PROBIOTIK DENGAN BAKTERIA PENGHASIL ASID LAKTIK DALAM MEMODULASI TINGKAH LAKU MODEL MENCIT SUSULAN DARI KECEDERAAN KONTUSIF SARAF TUNJANG: KAJIAN AWAL

oleh

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LIST OF ABBREVIATIONS

BBB	Basso Beattie Brehnan
ANS	Autonomic nervous system
BLISs	Bacteriocin-like inhibitory substances
BT	Bacterial translocation
CF	Cystic fibrosis
CFTR	Cystic fibrosis transmembrane conductance regulator
CFU	Colony forming unit
CNS	Central nervous system
CSF	Cerebrospinal fluid
dpi	Day post-injury
EEG	Electroencephalogram
ENS	Enteric nervous system
GALT	Gut-associated lymphoid tissue
GI	Gastrointestinal
IBS	Irritable bowel syndrome
ILF	Isolated lymphoid follicles
LAB	Lactic acid bacteria
LAFAM	Laboratory Animal Facility and Management
LPS	Lipopolysaccharides
MALT	Mucosa-associated lymphoid tissue
MRI	Magnetic resonance imaging
PNS	Parasympathetic nervous system
PP	Peyer's patches
SCFAs	Short-chain fatty acids
SCI	Spinal cord injury
SEM	Standard error of mean
SNS	Sympathetic nervous system
UiTM	Universiti Teknologi MARA

LIST OF APPENDICES

PERANAN PROBIOTIK DENGAN BAKTERIA PENGHASIL ASID LAKTIK DALAM MEMODULASI TINGKAH LAKU MODEL MENCIT SUSULAN DARI KECEDERAAN KONTUSIF SARAF TUNJANG: KAJIAN AWAL

ABSTRAK

Gangguan dysbiosis usus selepas kecederaan saraf tunjang (SCI) adalah fenomena penting tetapi kurang difahami yang mungkin menjejaskan penyembuhan SCI. Kajian ini menangani keperluan untuk memeriksa pemulihan lokomotor selepas SCI dan membentangkan model haiwan yang boleh membantu memulihkan flora usus yang sihat dan meningkatkan terapi SCI. Objektif utama adalah untuk mencipta model haiwan SCI yang boleh dipercayai dengan dysbiosis usus untuk menguji rawatan probiotik. Dengan kadar keciciran 10% (1 tikus tambahan), kajian ini merangkumi 13 tikus. Tikus Wistar betina dewasa ditimbang dengan teliti dan diperuntukkan secara rawak kepada satu daripada empat kumpulan: Kumpulan 1 (kawalan, n = 3), Kumpulan 2 (kawalan dengan pengambilan antibiotik, n = 4), Kumpulan 3 (SCI dengan pengambilan antibiotik, n = 3), dan Kumpulan 4 (SCI dengan pengambilan antibiotik dan terapi probiotik, n = 3). Sebatang rod telah dijatuhkan daripada 25 mm untuk menyebabkan kecederaan lebam sederhana di kawasan toraks T9/T10 selepas laminektomi. Pembedahan yang berjaya telah ditubuhkan oleh kelumpuhan kaki belakang tikus. Lima hari sebelum SCI, tikus menerima campuran antibiotik untuk menghasilkan dysbiosis usus. Air minuman mereka termasuk 2 g/L streptomycin, 0.17 g/L gentamicin, 0.125 mg/L ciprofloxacin, dan 1 g/L bacitracin. Selepas pembedahan, tikus kembali kepada air minuman biasa mereka. Terapi probiotik pelbagai strain bermula pada hari ketiga belas selepas SCI dan berlangsung sehingga kelapan belas. Pemakanan paksa oral 3g 30 x 10^9 CFU/kg probiotik dalam air steril telah dilakukan.

Najis selepas kecederaan telah diuji untuk disbiosis usus pada hari 4, 11, 18, dan 25. Sebagai tambahan kepada analisis sampel najis, Skala Penilaian Lokomotor Basso Beattie Bresnahan digunakan untuk menilai pemulihan tingkah laku dan lokomotor tikus pada dpi ke-4, ke-11, ke-18 dan ke-25 selama 4 minit setiap tikus. Selepas enam hari terapi probiotik, morfologi sampel najis dan penilaian lokomotor BBB menunjukkan kesan ringan. Secara keseluruhannya, kajian ini memberi penerangan tentang hubungan yang signifikan antara SCI, dysbiosis usus, dan probiotik. Kajian mendapati bahawa probiotik boleh membantu pesakit SCI memulihkan pergerakan dan eubiosis usus. Lebih banyak kajian diperlukan untuk menentukan rangkaian penuh manfaat probiotik. Penyelidikan ini mendedahkan cara baharu untuk meningkatkan keputusan dan kualiti hidup pesakit SCI.

THE ROLE OF PROBIOTICS WITH LACTIC ACID-PRODUCING BACTERIA IN MODULATING THE BEHAVIOUR OF RODENT MODEL FOLLOWING CONTUSIVE SPINAL CORD INJURY: PRELIMINARY STUDY

ABSTRACT

The disruption of gut dysbiosis after spinal cord injury (SCI) is a crucial but poorly understood phenomenon that might affect SCI healing. This study addresses the need to examine locomotor recovery after SCI and presents an animal model that may help restore a healthy gut flora and improve SCI therapies. The primary objective is to create a reliable animal model of SCI with gut dysbiosis to test probiotic treatments. With a 10% dropout rate (1 additional rat), this study included 13 rats. Adult female Wistar rats were carefully weighed and randomly allocated to one of four groups: Group 1 (control, n = 3), Group 2 (control with antibiotic intake, n = 4), Group 3 (SCI with antibiotic intake, n = 3), and Group 4 (SCI with antibiotic intake and probiotic therapy, n = 3). A rod was dropped from 25 mm to cause a moderate contusion injury in the T9/T10 thoracic area after a laminectomy. Successful surgery was established by the rats' hindlimb paralysis. Five days before SCI, rats received antibiotic mixtures to produce intestinal dysbiosis. Their drinking water included 2 g/L streptomycin, 0.17 g/L gentamicin, 0.125 mg/L ciprofloxacin, and 1 g/L bacitracin. After surgery, the rats returned to their usual drinking water. Multi-strain probiotic therapy began on the thirteenth day post-SCI and lasted until the eighteenth. Oral forced feeding of 3g of 30 x 10^9 CFU/kg probiotics in sterile water was performed. Post-injury faeces were tested for gut dysbiosis on days 4, 11, 18, and 25. In addition

to faecal sample analysis, the Basso Beattie Bresnahan Locomotor Rating Scale was used to evaluate the rats' behavioural and locomotor recovery on the 4th, 11th, 18th, and 25th dpi for 4 minutes per rat. After six days of probiotic therapy, faecal sample morphology and BBB locomotor assessments showed a mild effect. Overall, this study sheds light on the significant connection between SCI, intestinal dysbiosis, and probiotics. The study found that probiotics may help SCI patients recover locomotion and gut eubiosis. More study is needed to determine the full range of probiotic benefits. This research reveals new ways to improve SCI patients' results and quality of life.

CHAPTER 1

INTRODUCTION

1.1 Background of The Study

Spinal Cord Injury (SCI) disrupts the critical brain-body communication system, changing life. It can cause lasting physical and functional issues that make even daily tasks difficult. SCI can impair independence and quality of life in everyday tasks like walking and dressing to more complicated ones like self-care and mobility. SCI generally requires major life and relationship changes, which can be emotionally and psychologically taxing. SCI-related immobility generally causes bedrest or wheelchair use, worsening gastrointestinal symptoms. Constipation is common in SCI patients due to decreased intestinal motility from inactivity. Chronic constipation affects quality of life and increases pressure ulcer risk, a prevalent condition in this demographic.

In 2013, Hospital Kuala Lumpur medical experts conducted detailed epidemiological research on Malaysian SCI patients' demographics. Most affected were individuals aged 16–30. Malaysians made up 59% of SCI cases, making them the most afflicted ethnic group. Over 75% were low-income. More over half of SCI patients were from the central area, with 14% each from the Northern, Southern, and East Coast regions. A lesser percentage of patients were from Sabah and Sarawak. Traumatic situations, including vehicle accidents, caused over 50% of SCI cases, mostly in men. Ibrahim et al. (2013) found that paraplegia was the most prevalent result in younger SCI patients and tetraplegia in those over 60.

This epidemiology data indicates the incidence of SCI in Malaysia, notably among young males and the poor. It emphasizes the critical need for effective treatments to improve SCI outcomes and quality of life. Understanding the unique issues SCI patients in Malaysia experience might help create customised healthcare strategies and support networks.

A growing concern about SCI is its potential to alter the gut microbiota, causing dysbiosis. In dysbiosis, pathogenic, pro-inflammatory bacteria outnumber benign, non-pathogenic strains in the gut microbiota. Neurological and psychological effects of traumatic SCI typically require further care and therapy (Valido et al., 2022). SCI patients may be more susceptible to gut dysbiosis due to these factors. Autonomic failure in SCI patients makes them more vulnerable to infections and requires frequent medications. Gut microbiome is essential to health. The gut has billions of bacteria, viruses, fungi, and other organisms. This complex microbial population aids digestion, food absorption, and immune system modulation. However, dysbiosis, an environmental imbalance, can harm health.

Stress, antibiotics, and digestive issues can cause gut dysbiosis (Kigerl et al., 2016). SCI can cause chronic stress due to psychological and emotional issues, including living with a life-changing injury. Stress affects the gut-brain axis, disrupting gut microbial balance. The vagus nerve and cortisol release are involved in gut-brain communication (Lalonde & Strazielle, 2021). Additionally, stress-induced gut microbiome changes might have serious effects. Changes in microbiota composition can affect central nervous system metabolites and signalling molecules. This may worsen SCI-related depression and anxiety.

SCI patients often get narcotic analgesics, steroids, muscle relaxants, and antibiotics. Surgery for SCI can induce respiratory, urinary tract, or surgical site infections. Antibiotics are needed to prevent or treat some life-threatening illnesses. Antibiotics reduce pneumonia and mucus but can promote gut dysbiosis. Disruption in the gut microbiota can harm metabolic capability and promote antibiotic-resistant bacteria (Afshari et al., 2021). Furthermore, numerous dysbiosis factors, including antibiotic usage, have been linked to bacterial translocation (BT). Immune cells in the gut-associated lymphoid tissue (GALT) activate to facilitate the migration of gut bacteria from the intestinal lumen to extraintestinal areas (Kigerl et al., 2016). Understanding BT's effects on SCI is crucial to improving patient outcomes and creating suitable treatments. Probiotics' ability to regulate gut microbiota and enhance immune function makes them a good option for BT mitigation and gut health restoration.

The mucosa-associated lymphoid tissue (MALT), part of the gut immune system, relies on the GALT. A thin yet permeable barrier between the body's interior and the outside world, the mucosal surface aids nutrition absorption. This makes the body vulnerable to infections, since many microorganisms use it for entry (Glaysher & Mabbott, 2007). The spleen, lymph nodes, and bone marrow have less plasma cells than GALT, which produces antibodies. Peyer's patches (PP) in the small intestine and several isolated lymphoid follicles (ILF) throughout the gastrointestinal tract are two types of GALT in humans and rodents. GALT structures vary in mammals, including appendiceal lymphoid tissue in humans and rodents, caecal and colonic patches in rabbits, and rectal lymphoid tissue in humans and rodents (Donaldson et al., 2015).

The gut microbiota, a large part of the MALT, shapes gastrointestinal immune responses. This complex link is called the gut-immune axis. A variety of gut bacteria

interact with immune cells, affecting immune system development and function. Immune homeostasis and avoiding allergies and autoimmune illnesses depend on these interactions. The gut-immune axis disturbance in SCI patients is complicated. Autonomic dysregulation in the sympathetic and parasympathetic nerve systems controls gastrointestinal motility and blood flow after trauma. This imbalance can reduce transit time and cause gastrointestinal problems.

In gastrointestinal health, probiotics are a promising area of study. Beneficial bacteria and yeast strains from the human body are grown. An abundance of beneficial and dangerous microorganisms lives in the human body. This community can become imbalanced after an infection. Protective strains of probiotics reduce harmful microorganisms to restore balance. Consuming probiotics improves gut microbial balance, improving health (Kim et al., 2019).

The use of lactic acid bacteria (LAB), Gram-positive microorganisms found in fermented foods, as probiotics is rising. Bioactive chemicals in LAB are abundant yet unknown (Garbacz, 2022). LAB-enriched medical-grade probiotics improve functional recovery and neuroprotection in mice after SCI (Kigerl et al., 2016). Rats are selected as the experimental subjects because they have a long history of being used in SCI research. Additionally, their anatomical and physiological characteristics bear similarities to those of humans, making them a suitable model for studying SCI (Pawlowski et al., 2023). Furthermore, their relatively small size makes them manageable for conducting experiments. The appropriateness of selecting the T9/T10 thoracic level for a contusion injury is based on its correlation with a crucial segment of the spinal cord that governs motor and sensory functions in the lower extremities. Injuries occurring at this particular level often led to notable functional impairments, rendering it a suitable option for investigating the effects of SCI.

1.2 Problem Statement

Due to its complexity, SCI is a major medical issue that can have devastating effects. Gut dysfunction and neurogenic bowel disorders are major issues for SCI patients, affecting their quality of life. This study investigates gut dysbiosis following SCI and possible treatment options utilizing probiotics supplemented with lactic acid-producing bacteria.

SCI patients require innovative treatments since a cure is still elusive after intensive study. SCI implications depend on gut dysbiosis, an imbalance in gut microbiota (Pawlowski et al., 2023). This dysbiosis increases intraspinal inflammation and slows neurological repair by activating Gut-Associated Lymphoid Tissue immune cells.

This study examines the impact of probiotics, especially lactic acid-producing bacteria, on immune response and neuroprotection in SCI to address current issues. This research aims to enhance SCI rehabilitation by understanding the complicated link between gut dysbiosis and SCI pathogenesis.

SCI-induced gut dysbiosis processes are unknown. This information gap limits focused treatment approaches. Probiotics supplemented with lactic acid-producing bacteria show promise, but the strains, doses, and treatment duration needed for optimum neuroprotection and gut eubiosis are still being studied internationally. SCI patients' probiotic effects in clinical studies are also limited and unclear. This study investigates how probiotics affect behaviour and locomotor recovery in SCI-induced gut dysbiosis to close this gap.

The key questions that steer this research are as follows; To what extent does antibiotic-induced gut dysbiosis occur in the context of SCI, how does the morphology

of faecal samples evolve following SCI and can probiotics mitigate the observed effects of gut dysbiosis, and what are the changes in locomotor recovery patterns in SCI patients and how do probiotics influence these patterns? By exploring these study questions, the study seeks to understand the complex link between gut dysbiosis and SCI, enabling more targeted and effective therapies. This study's findings should aid SCI management and rehabilitation clinical trials and research.

No permanent cure is available to treat a person with SCI. SCI patients frequently exhibit gut dysfunction and neurogenic bowel disorders. Additionally, dysbiosis worsens intraspinal inflammation and hinders the restoration of neurological function because of the significant modifications in GALT immune cell activation (Vidal Vera et al., 2022). The mechanism of gut dysbiosis after SCI and the improved restorative treatment for SCI needs to be understood. This study is essential to be carried out because the idea of SCI with induced gut dysbiosis and effects of probiotics can be understood and studied in depth. The exact number of doses and strains of probiotics that enriched with lactic acid-producing bacteria are still being studied worldwide. This study is expected to contribute to the ongoing study of the clinical trial.

1.3 Objectives of The Study

1.3.1 General Objective

The primary objective of this study is to create a robust and reproducible animal model of SCI-induced gut dysbiosis. The model will function as a fundamental framework for assessing potential therapeutic interventions that involve probiotics.

1.3.2 Specific Objectives

i. To validate contusion SCI with antibiotic intake as bacterial translocation (BT) and persistent gut dysbiosis, as this objective test contusion SCI and antibiotics to produce BT and sustain gut dysbiosis.

ii. To compare morphology of faecal sample. This objective compares the morphological characteristics of faeces from four groups: sham laminectomy, sham laminectomy + Antibiotics, SCI + Antibiotics, and SCI + Antibiotics + Probiotics. Sample will be taken on days 4, 11, 18, and 25 after injury to analyse probiotics and BT.

iii. To evaluate locomotor recovery. On days 4, 11, 18, and 25 post-injury, this objective compares locomotor recovery in the sham laminectomy, antibiotics, SCI, and probiotics groups. The Basso Beattie Breshnan Locomotor Rating Scale will test probiotics and BT.

1.4 Hypothesis

Ho: Contusion SCI, with the intake of antibiotics cocktails, does not result in gut dysbiosis.

H_1: Contusion SCI, with the intake of antibiotics cocktails, results in gut dysbiosis.

Ho: Collected faecal samples do not prove that probiotics create gut eubiosis.

H_2: Collected faecal samples proves that probiotics creates gut eubiosis.

Ho: Admission of probiotics does not enhance the behaviour and locomotor recovery of SCI rats.

H_3: Admission of probiotics enhances the behaviour and locomotor recovery of SCI rats.

CHAPTER 2

LITERATURE REVIEW

2.1 Classification of SCI

Spinal cord injuries (SCI) are classified by their features and processes. Complete and partial SCIs have different characteristics and recovery implications (S. Hu et al., 2003; B. Skinner & Fitzpatrick, 2008). A complete injury has no sacral sparing, no motor or sensory function below the damage, and reflexes (S. Hu et al., 2003). However, an incomplete injury preserves position sense, sacral sparing, and voluntary lower extremity movement (B. Skinner & Fitzpatrick, 2008).

SCI can also be categorised by mechanism, resulting in different spinal injuries:

Flexion Injury: The most frequent spinal injury is caused by landing on the heels or glutes or a strong shoulder blow. These injuries mainly affect the cervical spine, causing C5–C7 vertebral dislocations or compression fractures. Vertebral compression determines stability (B. Skinner & Fitzpatrick, 2008; J Maheshwari & A Mhaskar, 2015).

Flexion-Rotation Injury: One of the most serious spinal injuries, flexion-rotation injuries often cause a very unstable spine and neurological impairment. A powerful shoulder hit that flexes and rotates the trunk or a fall on the postero-lateral head cause these injuries. They can cause cervical vertebral fractures or facet joint dislocations (B. Skinner & Fitzpatrick, 2008; J Maheshwari & A Mhaskar, 2015).

Vertical Compression Injury: This occurs when an object falls straight on the head or when an upright fall hits the head's top. Vertical compression injuries cause burst fractures by crushing the vertebral body vertically. Dorso-lumbar spine injuries are rare due to its large canal, which protects against neurological impairments (B. Skinner & Fitzpatrick, 2008; J Maheshwari & A Mhaskar, 2015).

Extension Injury: Shallow water diving and motor vehicle accidents that cause the head to contact the ground, stretch the neck, or smash the windscreen cause extension injuries in the cervical spine. These injuries commonly cause vertebral anterior rim chip fractures and instability (J Maheshwari & A Mhaskar, 2015).

Flexion-Distraction Injury (Chance Fracture): Sudden braking while wearing a seatbelt allows the upper body to thrust forward while the lower body is held by the seat, causing flexion forces and distraction. Horizontal fractures commonly extend into the posterior spine (B. Skinner & Fitzpatrick, 2008; J Maheshwari & A Mhaskar, 2015).

In rare cases, direct injury and intense muscular contraction can fracture the cervical and lumbar vertebrae (J Maheshwari & A Mhaskar, 2015). To quantify spinal cord contusion severity, ventral nerve fibres that link the rostral and caudal spinal cord must be structurally intact (Research et al., 2014). Understanding SCI categorization and processes helps healthcare personnel diagnose, treat, and prognose the damage by identifying its magnitude and nature. Below in Table 2.1 shows the summary of SCI classification: -

Table 2.1 SCI Classification

Classification	Description
Completeness of Injury	Complete Injury: Reflexes, no sacral sparing, no motor or sensory function.
	Incomplete Injury: Lower extremity position sense, sacral sparing, and voluntary movement.
	Complete injuries improve less than incomplete ones.
Mechanism of Injury	Flexion Injury: Common, regularly injuring the cervical spine from falls or strong strikes.
	Flexion-Rotation Injury: Specific occurrences produce neurological injury and instability.
	Vertical Compression Injury: Burst fractures from objects falling on the head or upright falls cause vertical compression injury.
	Extension Injury: Common in the cervical spine from shallow water diving or accidents.
	Flexion-Distraction Injury (Chance Fracture): Sudden forward force and distraction, often in cars.
	Direct Injury: Bullets or direct impacts to the cervical vertebrae cause shattering or fractures.
	Violent Muscle Contraction: Sudden psoas contractions cause lumbar vertebral fractures.
Special Considerations	Spinal Cord Contusion: Ventral nerve fibres linking rostral and caudal regions remain unaltered.
	Residual nerve fibres indicate spinal cord contusion severity.

SCI is a complex illness with numerous implications that change the lives of those afflicted. Understanding these complications is essential in identifying effective treatments and enhancing SCI patients' quality of life.

Neurological issues are significant in SCI. These include paralysis, sensory loss, and reflexes. The SCI severity determines these neurological impairments' magnitude and location (Valido et al., 2022). Incomplete injuries may preserve function, whereas full injuries destroy motor and sensory functions below the damage site. Gut dysbiosis affects the immune system and inflammation, which may slow neurological healing.

The autonomic nervous system controls involuntary body activities. SCI often upsets this delicate balance, causing autonomic dysfunction. Orthostatic hypotension and gastrointestinal dysfunction might lower a patient's quality of life. Gut dysbiosis may affect autonomic function and the gut-brain axis, causing several difficulties. Understanding these linkages is crucial to creating solutions to reduce these issues.

Respiratory issues are critical in cervical and upper thoracic SCI patients. These injuries can affect respiratory muscles and lung capacity, making breathing difficult. Inflammation and immunological dysfunction from gut dysbiosis may affect respiratory health. Comprehensive SCI treatment requires studying the gut microbiome and respiratory issues.

Muscle atrophy, contractures, and osteoporosis are musculoskeletal complications. Muscle and bone density loss generally follows mobility and weight-bearing loss. Through inflammation and nutrition absorption, gut dysbiosis may worsen these disorders. Understanding how the gut microbiota affects musculoskeletal

health is crucial to designing therapies to preserve bone density and muscle mass in SCI patients.

Due to limited movement and feeling, SCI patients are at risk for pressure ulcers. Ulcers may be painful, difficult to cure, and cause serious infections. Gut dysbiosis may modulate the immune system and cause systemic inflammation, affecting skin and wound healing (Lalonde & Strazielle, 2021). SCI patients' gut-skin relationship must be studied to avoid and treat problems.

SCI patients often have constipation, diarrhoea, and bowel incontinence (Jing et al., 2021). Due to gut microbiome changes that affect digestion and motility, gut dysbiosis is closely linked to these issues. Understanding gut dysbiosis and gastrointestinal issues is essential for creating tailored therapies to enhance bowel function and digestive health.

Cardiovascular problems such deep vein thrombosis and orthostatic intolerance are more common in SCI patients. Gut dysbiosis may influence vascular function and inflammation, causing multiple issues. Understanding and treating cardiovascular complications requires studying the gut-cardiovascular axis. Depression, anxiety, and adjustment difficulties are also common among SCI patients (Valido et al., 2022). Neurological discomfort is another common and painful implication. Gut dysbiosis may affect these issues via the gut-brain axis, emphasizing the need of mental health and pain management in SCI treatment.

2.2 Current Treatment for SCI

The medical field continues to face ongoing challenges in regards to the regeneration of injured nerve cells or the enhancement of functionality in remaining neurons, particularly in cases of spinal cord damage (S. Hu et al., 2003). Although spinal cord injuries are irreversible, researchers are developing new treatments, such as drugs and prosthetic limbs, that prevent further damage and restore function (Scholtes et al., 2012; Shah et al., 2020). Pharmaceutical treatments with neuroprotective and neuroregenerative qualities are being studied (Shah et al., 2020). Well-known neuroprotective medication methylprednisolone reduces post-traumatic inflammation and membrane lipid peroxidation, improving neurological outcomes. Its therapeutic use is debated due to issues such increased gastrointestinal bleeding and wound infections (Wilson et al., 2013). Considering the hazards, current recommendations indicate providing methylprednisolone within eight hours of injury in certain cases (Hugenholtz et al., 2002; Shah et al., 2020).

Neuromodulation, which modifies neural circuitry through electrical stimulation, may cure spinal cord injuries (James et al., 2018; Shah et al., 2020). Studies have demonstrated that epidural or transcutaneous spinal cord stimulation improves motor function. These strategies have improved hand dexterity and lower limb voluntary movements in SCI patients (Angeli et al., 2014). However, neuromodulation therapy accessibility and cost-effectiveness remain challenges (James et al., 2018; Scholtes, 2012).

Robotic or motorized exoskeletons are popular SCI rehabilitation methods (Scholtes et al., 2012; Shah et al., 2020). Exoskeletons have been shown to reduce neuropathic pain, improve cardiovascular health, reduce energy expenditure, improve

body composition, gait, physical activity, and quality of life (Miller et al., 2016). These technologies evolve, and multidisciplinary collaboration is needed to make them more accessible. Further research and development of prosthetic devices for SCI patients is also possible (Gorgey, 2018).

2.3 Mechanism of Gut Dysbiosis

The gut microbiota acts as a crucial organ that supports host health. This complex microbial community of billions of bacteria affects many systemic and local activities, including nutrient synthesis, vitamin production, mucosal immune system development, bidirectional gut-to-brain communication, and oncogenesis. *Proteobacteria* is less common in the human microbiome than *Bacteroidetes*, *Firmicutes*, and *Actinobacteria*. Like other organs, the gut microbiota needs a steady microbial makeup to operate properly (Arumugam et al., 2011).

Dysbiosis, caused by large changes in these microbial phyla's ratios or the development of new bacteria, causes health issues (Walker et al., 2011). Dysbiosis reduces microbial diversity and increases *Proteobacteria*. This imbalance is associated to more problems and increases disease risk and severity (Walker et al., 2011). Dysbiosis is linked to inflammatory bowel illnesses, metabolic irregularities, immunological disorders, and neurological issues (Wlodarska et al., 2015; Gérard, 2016; Bibbò et al., 2016; Tremlett et al., 2017). Dysbiosis can cause or worsen illnesses at any age, from infancy, when necrotizing enterocolitis may develop, through adulthood, when colorectal cancer risk rises, and to old age, when *Clostridium* difficile-associated diarrhea more common (Seekatz & Young, 2014).

Even small changes in microbial diversity can make bacterial communities vulnerable to further disruption and dysbiosis (Carding et al., 2015). Bacteriocins, bacteriophages, and oxidative stress cause dysbiosis in microbiota. The threshold for dysbiosis depends on the bacterial groups involved. Overgrowth of peripheral groups can tilt the balance, although major alterations in *Bacteroidetes* and *Firmicutes* may not cause pathogenic consequences (Arumugam et al., 2011). The gut microbiome normally has a small *Enterobacteriaceae* population (Tenaillon et al., 2010). Inflammation can change the gut's oxidative environment, causing *Enterobacteriaceae* to multiply quickly. *Enterobacteriaceae* lipopolysaccharides are pyrogenic, hence their presence can worsen inflammation (Stecher et al., 2007; Walker et al., 2011).

Disruptions to gut eubiosis, a harmonious interspecies balance in the microbiota population, can cause infectious and non-infectious illnesses (Al-Rashidi, 2022). The host immune system and bacteria interact intricately to maintain intestinal homeostasis. Disruptions in this connection can cause gut microbiota-induced diseases. Microbiota influence host physiological activities, whereas the host offers microbial survival niches and resources (Al-Rashidi, 2022; Bajinka et al., 2020). The microbiota breaks down carbohydrates, synthesizes vitamins, develops gut-associated lymphoid tissue (GALT), modulates the gut immune system, and prevents pathogen colonization. Commensal bacteria trigger gut immune responses that modulate microbiota composition (Afzaal et al., 2022).

Understanding how SCI can cause gut dysbiosis is essential to understanding how neurological trauma affects the gut microbiota. The gut-brain axis connects the central nervous system (CNS) and gut for bidirectional communication (Wang et al., 2023). It controls intestinal homeostasis, immunological responses, and physiological functions. SCI alters this axis, altering gut-CNS signals. Injury to the spinal cord can

disrupt gastrointestinal motility, blood flow, and immune function signals. This disturbance can promote intestinal dysbiosis.

SCI typically causes immune system alterations. The acute phase involves a rise in injury-site inflammation followed by persistent immunological suppression. This immunological dysregulation can impair gut microbial balance (Jing et al., 2021). Gut immune cells like T and regulatory T cells shape the gut microbiota. SCI-induced immunological changes may upset this balance, allowing dangerous microorganisms to thrive.

SCI disrupts gastrointestinal motility, contributing to gut dysbiosis. Proper gut motility pushes food through the digestive system effectively, reducing dangerous bacteria development. SCI disrupts the autonomic nervous system's regulation over gastrointestinal motility, causing constipation and prolonged transit times (Wang et al., 2023). These disruptions allow some bacteria to grow, potentially causing dysbiosis.

Following a SCI, individuals often undergo alterations in their dietary habits and nutritional intake. Physical restrictions, difficulties with swallowing, and taste perception might cause these alterations. A change in diet can affect the gut microbiota (Abdullahi bet al., 2019). A low-fibre diet might deprive good gut bacteria of critical nutrients, favouring harmful species. Dietary components that cause inflammation or gut epithelial barrier disruption can also cause dysbiosis.

SCI patients are more likely to get urinary tract infections and other infections that need antibiotics (Jing et al., 2021). While antibiotics are necessary for illness treatment, they can harm the gut microbiota. Broad-spectrum antibiotics kill harmful

and beneficial microorganisms, disrupting the gut microbiota. This disturbance may allow harmful germs to proliferate.

Overall, SCI triggers a complicated chain of events that might cause gut dysbiosis. Disruptions in gut-brain connection, immune system dysfunction, altered gastrointestinal motility, food and nutrition modifications, and antibiotic usage contribute to dysbiosis in SCI patients. These pathways are essential to creating tailored treatments, such as probiotics, to restore gut health and minimize the negative effects of gut dysbiosis in SCI patients.

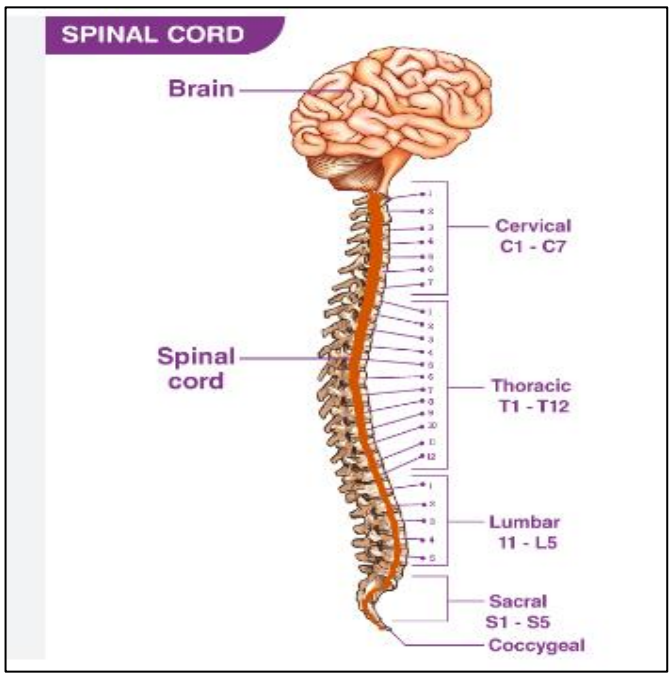

Figure 2.1 Human spinal cord (adapted from Al-Rashidi, 2022)

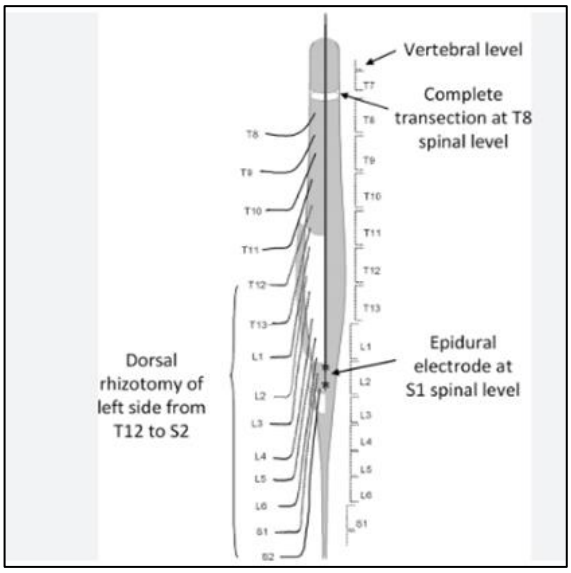

Figure 2.2 Rat spinal cord (adapted from Al-Rashidi, 2022)

Figure 2.1 above shows human spinal cord while Figure 2.2 shows rat spinal cord. These two images present some of the differences between a human and rat spinal cord thus demonstrating the influence of SCI on the two. Figure 2.1 shows the human spinal cord and Figure 2.2 shows the rat spinal cord, illustrating the essential structural differences between these two species. The depicted juxtaposition emphasizes SCI research and its impact on both humans and animals. While humans have a bigger and more complicated spinal cord, rats have anatomical similarities, making them useful for researching SCI. Rat model studies can enlighten and improve the knowledge of SCI in humans, as shown by this comparison. The figures also highlight the complicated neuronal networks in the spinal cord, stressing the need for treatments and interventions to restore function and mitigate SCI effects. The figures provide a striking visual narrative that emphasizes the importance of SCI research in bridging species and increasing spinal cord injury understanding.

2.4 History of Probiotics

The first intentional alteration of gut flora was the ancient Chinese use of excrement to cure disease and food poisoning. This microbiota-changing technique has a 500-year history. Only in the last 50 years has there been increasing interest in using particular bacterial strains to obtain targeted therapeutic results (Piqué et al., 2019). Lilly and Stillwell defined "probiotics" in 1965 as bacteria-produced compounds that promote bacterial growth. Early probiotics were mostly single bacteria from *Saccharomyces* or *Lactobacillus* species. Further meta-analyses showed that such probiotics can prevent viral and post-antibiotic diarrhoea, including *Clostridium* difficile-induced colitis (Goldenberg et al., 2017).

Many probiotic bacteria are developed to survive stomach pH. This caused several mutations with unknown physiological traits. The huge number of microbial combinations hampers comparisons, causing class-wide effects and probiotic prescriptions without specificity. Due to a lack of independent studies, each strain's physiological effects are unknown (de Simone, 2019; Ohkusa et al., 2019). The prebiotic activity of deceased probiotic bacteria's cellular components is another unexplored topic. The next generation of probiotics has better features and medicinal uses (Satokari, 2019). Human coevolution with specific bacterial species is now known to contribute to probiotic benefits (Piqué et al., 2019).

Due to the field's evolution and the discovery of new bacterial species each year, probiotic clinical research advances slowly. Lack of well-designed, impartial clinical studies also slows development. With many possible probiotic candidates, they are often presumed to be equally effective and not sufficiently scrutinized (Piqué et al., 2019).

2.4.1 Probiotics in The Gut Microbiota Ecosystem

The microbiota organises as a focussed ecosystem and differs from place to location, especially when contrasting bacteria living in mucus or sticking to the intestinal wall, known as the parietal microbiota, with germs dwelling in food in transit and faeces, known as the luminal microbiota (Caballero et al., 2015). According to the influence of nutrition, exposure to probiotic bacteria consumed, gut environment conditions, and other host-related factors, the microbiota composition is dynamic and personalised and may temporarily contain some new strains in the ecosystem (Derrien & van Hylckama Vlieg, 2015; Zhang et al., 2016).

The luminal microbiota changes with a probiotic therapy, suggesting survival throughout transit through the digestive system. Systemic metabolism, including insulin resistance, may be substantially more affected by probiotic changes to the parietal microbiota. Probiotic bacteria still make up a small portion of the local microbiota. Curiously, the only person who did not have the probiotic colonise the individual had ulcerative colitis (Alander et al., 1997; Piqué et al., 2019).

2.4.2 Probiotics and The Gut Barrier Function

Humans have a great symbiotic relationship with these bacteria despite the fact that gastrointestinal cells are constantly exposed to their antigens and metabolites. There are several components that help to make this arrangement possible (Piqué et al., 2019). The gut barrier functions extremely well under normal conditions because of complex, multifaceted mechanisms, including the presence of a mucus layer, tight junction proteins, antimicrobial factors, immunoglobulin A, and sentinels, including

intraepithelial lymphocytes and other adaptive immune cells (König et al., 2016; Wells et al., 2017).

The primary mechanism driving the onset of metabolic endotoxemia was directly connected to a change in the functioning of the gut barrier and the composition of the gut microbiota. In addition to the particular alterations in the composition of the gut microbiota, it is also proposed that T lymphocytes increase in the stomach of obese patients eating high-fat diets, a fact that corresponds with morbidity (Monteiro-Sepulveda et al., 2015). Furthermore, it has been proposed that individuals with type 2 diabetes and obesity have reduced amounts of certain immune cells, such as mucosa-associated invariant T cells, which release more Th1 and Th17 cytokines. (Magalhaes et al., 2015).

Numerous studies have confirmed that altering the gut microbiota using probiotics or faecal material transplantation may have an impact on host metabolism (Hiippala et al., 2018). The relevant literature primarily focuses on various strains, ranging from more contemporary contenders like *A. muciniphila* and *Faecalibacterium prausnitzii*, which are regarded as next-generation beneficial bacteria, to more established probiotics like the bacteria *Lactobacillus* and *Bifidobacterium* or the yeast *Saccharomyces boulardii* (O'Toole et al., 2017). All of these possibilities have encouraged gut barrier renewal, lowered inflammation, and eventually enhanced glucose homeostasis (Hiippala et al., 2018).

It's crucial to understand that not every strain affects the intestinal barrier, inflammation, body weight or fat mass, or glucose metabolism in the same way. The many modes of action mentioned before may help to explain this observation. As a result, it is essential to stress the fact that some bacteria's metabolic effects are strain-

specific and cannot be applied to all members of a particular genus. In other words, even while several pathways are seen when analysing a single strain, it is not expected that every strain will have the same effects (Piqué et al., 2019).

2.4.3 Probiotics with Lactic Acid Bacteria

LAB make up probiotics. They alter the human digestive system's microorganisms, particularly the intestinal microbiome. Catalase-negative, Gram-positive, non-sporulating bacteria make up the non-pathogenic heterogeneous probiotic LAB group (Zukiewicz-Sobczak et al., 2014).

From glucose, a number of growth inhibitors, including bacteriocins, bacteriocin-like inhibitory substances (BLISs), hydrogen peroxide, diacetyls, and carbon dioxide, they produce lactic acid as a primary product (Lalonde & Strazielle, 2021). For these bacteria to flourish, complex nutrients such amino acids, peptides, nucleotide bases, vitamins, fatty acids, and carbohydrates are essential. The cavities of both humans and animals contain them, as do dairy products, fermented meats, fish, drinks, pickled vegetables, and cereals (Lactic Acid Bacteria as Probiotics - PubMed, n.d.; Zukiewicz-Sobczak et al., 2014).

The metabolism of LAB is linked to the impact of probiotic bacteria on the intestinal flora. The major products of fermentation on carbohydrates are lactic acid and short-chain fatty acids like butyric acid, acetic acid, and propionic acid, which reduce the pH of the intestinal contents (Anjana & Tiwari, 2022; Zukiewicz-Sobczak et al., 2014). Additionally, some LAB secrete bacteriocins called lactocins, such as nisin, acidophilin, lactacin, and lactocidin, which have a bacteriostatic action and stop the growth of harmful microorganisms (Anjana & Tiwari, 2022).

Selecting bacterial strains is crucial to probiotic efficacy. Probiotics contain a variety of LAB with various health benefits. Table 2.1 briefly describes several common LAB in probiotic formulations. These LAB are crucial to gut health and have been intensively studied for medicinal purposes. Lactic acid-producing bacteria ferment substrates into lactic acid, making them the foundation of probiotics. *Lactobacillus acidophilus*, a well-known LAB, colonizes the small intestine, modulates immunological responses, and promotes gut health (Holzapfel et al., 2001). *Lactobacillus casei* may also relieve gastrointestinal discomfort and preserve gut microbial balance.

Bifidobacterium species like *B. adolescentis* and *B. bifidum* are also important LAB members. These bacteria are known for fermenting complex carbohydrates to produce short-chain fatty acids. SCFAs like butyrate nourish the intestinal epithelium and reduce inflammation (Holzapfel et al., 2001). SCI patients may have gut motility and transit time abnormalities, although *Bifidobacterium* strains in probiotics may improve gut function.

LAB species *Enterococcus faecalis* and *faecium* are tough and resilient. Probiotics must survive the stomach's acidic environment to reach the lower gastrointestinal system (Abdullahi bet al., 2019). These strains may boost gut defences and resistance against opportunistic infections.

Lactococcus lactis is another probiotic LAB. Although typically used in dairy fermentation, its potential to generate antimicrobial peptides and boost the immune system has made it a popular probiotic (Holzapfel et al., 2001). These qualities can prevent infections and boost the immune system in SCI problems. *Streptococcus thermophilus*, used in yogurt, is a probiotic. It helps digest lactose and produces