PROBIOTICS INNECROTIZING ENTEROCOLITIS- A PREVENTIVE APPROACH

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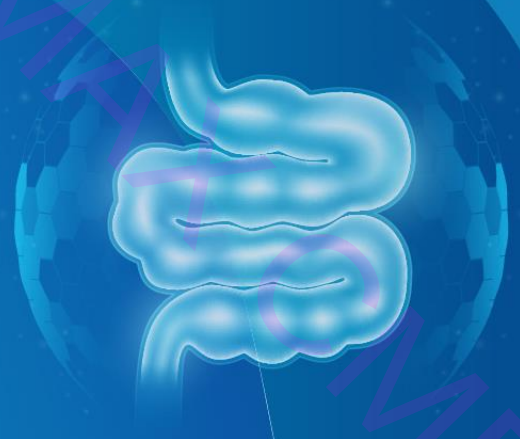
- DMA Award 2023

Probiotics in Necrotizing Enterocolitis:
A Preventive Approach



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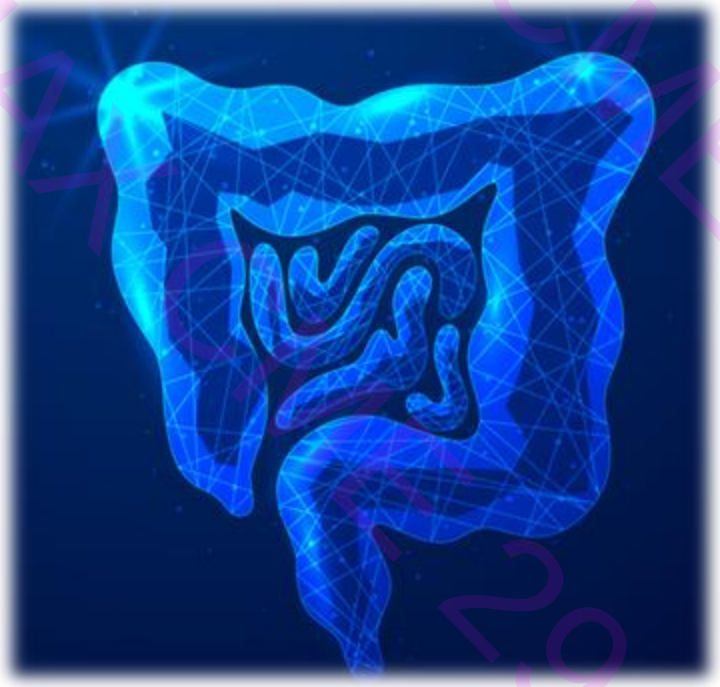

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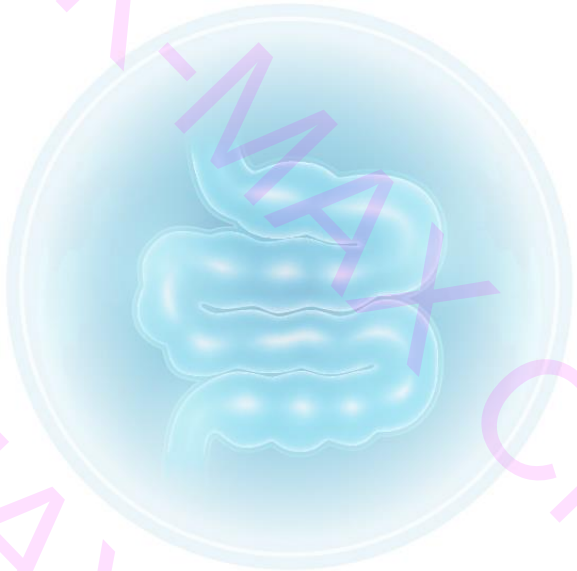
NEC: Necrotizing enterocolitis; SCFA: Short-chain fatty acid.



NEC Uncovered: **Understanding the Challenge**

NEC: Necrotizing enterocolitis.

NEC: A Neonatal Challenge

NEC is the most common serious gastrointestinal disease in preterm infants.[1–3]

It is also a leading cause of death in preterm infants between 2 weeks and 2 months of age.[2,3]

Healthcare providers classify NEC into different types based on the onset of symptoms and underlying causes as:[4]

01

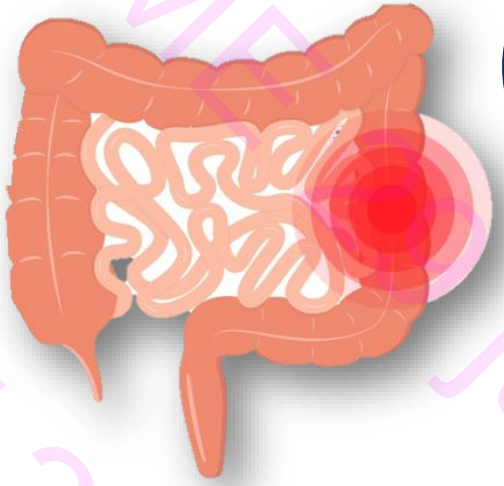
Classic

- Affects infants born **before 28 weeks**, typically occurring 3–6 weeks after birth.
- It often **appears suddenly** in otherwise stable infants.

02

Transfusion-associated

About **one in three** preterm infants develops NEC within 3 days of receiving a blood transfusion for anemia.



03

Atypical

Rarely occurs in the first week of life or before the first feeding.

04

Term infant

Full-term babies with NEC usually have **birth defects, such as congenital heart conditions, gastroschisis, or low oxygen levels at birth**.

1. Necrotizing enterocolitis. Available at: https://www.ncbi.nlm.nih.gov/books/NBK513357/. Accessed on: 23 September 2024.
2. Patel RM, *et al. N Engl J Med*. 2015;372(4):331–340.
3. Patel RM, *et al. Semin Pediatr Surg*. 2018;27(1):39–46.
4. Necrotizing enterocolitis (NEC). Available at: https://my.clevelandclinic.org/health/diseases/10026-necrotizing-enterocolitis. Accessed on: 23 September 2024.

NEC: Necrotizing enterocolitis.

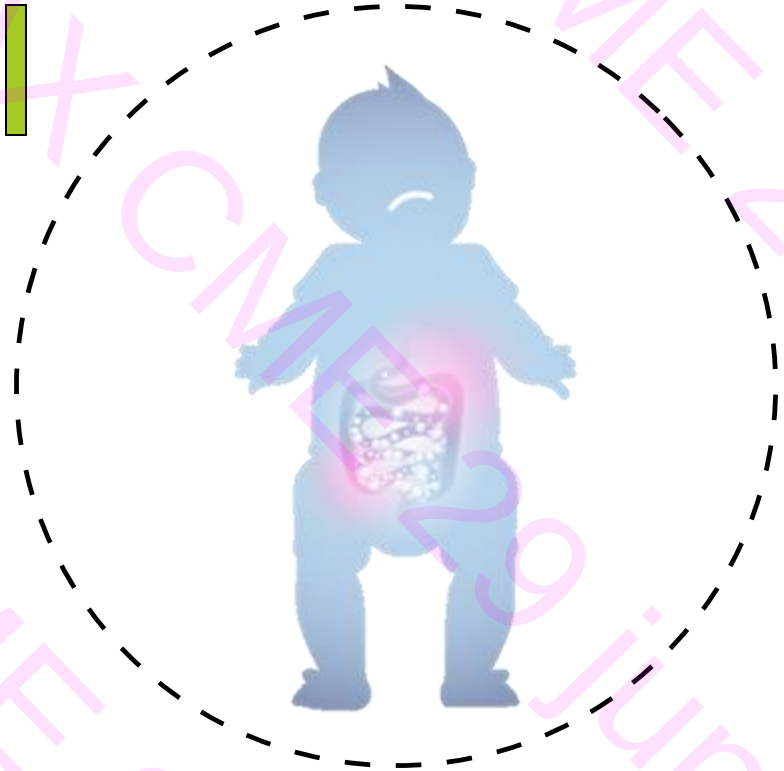
Prevalence of NEC: A Concern

NEC primarily affects infants **<32 weeks**, and the incidence decreases as the gestational age increases[1,2]

Gestational age breakdown
Approximately **70%** of NEC cases occur in infants born before 36 weeks of gestation[6]

Birth weight
NEC occurs in **6%–10%** of infants with a birth weight of <1500 g[5]

Global incidence
In high-income countries, the incidence of NEC in preterm and LBW infants is **7% and 22%,** respectively[3,4]



NICU admissions
NEC accounts for nearly **8%** of all NICU admissions[6]

VLBW infants
Evidence shows that **7 out of 100** VLBW infants in the NICU are likely to develop NEC[7]

Congenital heart disease
The incidence of NEC among neonates with congenital heart disease is **3.7%**[8]

NEC has an incidence rate of **1.26%** in India.[9]

1. Patel RM, *et al. Semin Pediatr Surg*. 2018;27(1):39–46.
2. Battersby C, *et al. Lancet Gastroenterol Hepatol*. 2017;2(1):43–51.
3. Battersby C, *et al. Arch Dis Child Fetal Neonatal Ed*. 2018;103(2):F182–F189.
4. Chekole Temere B, *et al. Pediatric Health Med Ther*. 2022;13:95–102.
5. Mekonnen SM, *et al. Glob Pediatr Health*. 2021;8:2333794X211019695.
6. Necrotizing enterocolitis. Available at: https://www.ncbi.nlm.nih.gov/books/NBK513357/. Accessed on: 23 September 2024.
7. Alsaied A, *et al. BMC Pediatr*. 2020;20(1):344.
8. Spinner JA, *et al. Pediatr Crit Care Med*. 2020;21(3):228–234.
9. Adhisivam B, *et al. J Matern Fetal Neonatal Med*. 2019;32(6):902–905.

LBW: Low birth weight; NEC: Necrotizing enterocolitis; NICU: Neonatal intensive care unit; VLBW: Very low birth weight.

Impact of NEC: The Consequences in Infants

The higher occurrence and fatality of NEC in premature infants are linked to their immature immune systems and less diverse gut microbiomes.[1,2]

An infant with NEC is at risk for other problems, such as:[3]

01

Abdominal infection
NEC can cause a **perforation in the intestinal wall**, leading to peritonitis and a heightened risk of sepsis.

02

Intestinal stricture
About **one in three** infants develops intestinal strictures, which narrow the intestines and obstruct food passage. Surgery may be needed.

03

SBS
Damage from NEC can result in **SBS, impairing nutrient absorption**. Lifelong nutritional support, including enteral feedings, may be necessary.

04

Growth failure and developmental delays
Long-term complications include growth failure and neurodevelopmental delays, especially in infants who had surgery, requiring ongoing monitoring.

Mortality rates for NEC in premature infants in the NICU are reported to be between **20% and 30%.**[4]

1. Singh DK, *et al. Front Pediatr*. 2023;10:1107404.
2. Claud EC, *et al. Proc Natl Acad Sci USA*. 2004;101(19):7404–7408.
3. Necrotizing Enterocolitis (NEC). Available at: https://my.clevelandclinic.org/health/diseases/10026-necrotizing-enterocolitis. Accessed on: 23 September 2024.
4. Battersby C, *et al. Arch Dis Child Fetal Neonatal Ed*. 2018;103(2):F182–F189.

NEC: Necrotizing enterocolitis; NICU: Neonatal intensive care unit; SBS: Short bowel syndrome.

Impact of NEC: The Consequences on Survivors and Parents

NEC survivors and their parents face long-term complications affecting their physical and mental health, social experiences, and quality of life.

Long-term outcomes and life-impacts of NEC survivors and parents

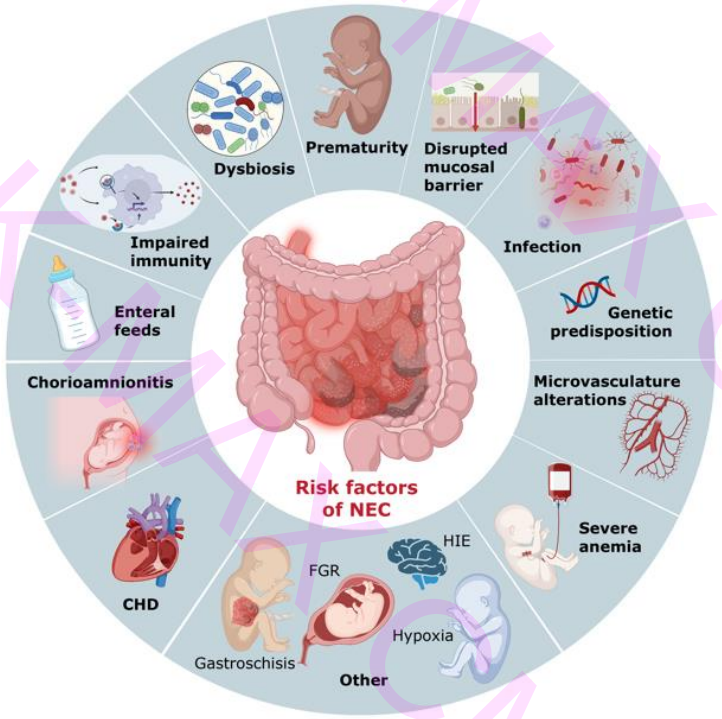
GI/SBS symptoms	Scars/self image	Social concerns	Anxiety/ depression	Access to care
Diarrhea/pain/IBS	Insecure	Bullied	Lonely	Lack of information
Bowel obstructions	Poor body image	Embarrassed	Worried/anxious	Inexperienced staff
Malabsorption/malnourished	Painful scars	Limited activities	Helpless/hopeless	Financial concerns
"Twice my son needed surgery for bowel obstructions from severe adhesive scarring throughout his intestines"	*"People make fun of his scar"*	*"People visit less and we travel less"*	*"The emotional issues I've faced from NEC and SBS have left me feeling depressed and lonely"*	*"NICU bankrupted us. And most doctors are over 100 miles away"*
"GI related symptoms have dictated my life..both career-wise and in my personal life"	*"I've always felt deformed due to the surgical scars on my abdomen from NEC"*	*"I'm embarrassed to go to someone's house for dinner"*	*"I get anxious going places that do not have toilet facilities"*	*"I have never been asked about it by any healthcare provider, nor informed there could be any complications"*

Canvasser J, *et al. Semin Perinatol*. 2023;47(1):151696.

GI: Gastrointestinal; IBS: Irritable bowel syndrome; NEC: Necrotizing enterocolitis; NICU: Neonatal intensive care unit; SBS: Short bowel syndrome.

Identifying Risk Factors for NEC

Prematurity is the most significant risk factor for NEC.[1,2]



Risk factors differentiated[1,3]

Maternal factors

- Microbiome
- Nutrition
- BMI
- Stress
- Hypertension
- Drugs
- Smoking
- Chorioamnionitis

Neonatal factors

- Prematurity
- LBW
- Dysbiosis
- Premature immune system
- Premature gut
- Transfusion
- Genetic/ congenital

Dysbiosis factors

- Mode of delivery
- Formula feeding
- NICU environment
- Antibiotics
- Acid-suppressing drugs
- Acute hypoxia

NEC factors

- Increased TLR4 expression
- Increased ER stress
- Increased cytokine levels
- PMN infiltration
- Apoptosis
- Bowel necrosis/gut barrier disruption
- Sepsis/organ disfunction

1. Bautista GM, *et al. Front Pediatr*. 2023;11:1161342.
2. Fitzgibbons SC, *et al. J Pediatr Surg*. 2009;44(6):1072–1076.
3. Aziz M, *et al. Cell Host Microbe*. 2022;30(5):612–616.

BMI: Body mass index; CHD: Congenital heart disease; ER: Endoplasmic reticulum; FGR: Fetal growth restriction; HIE: Hypoxic-ischemic encephalopathy; LBW: Low birth weight; NEC: Necrotizing enterocolitis; NICU: Neonatal intensive care unit; PMN: Polymorphonuclear neutrophil; TLR4: Toll-like receptor 4.

Diagnosing NEC: Keys to Early Intervention

The diagnosis of NEC is primarily based on clinical features and imaging findings[1–3]

However, early stages are often missed, and once typical features appear, the condition progresses rapidly.[3]

Key imaging test

- **Abdominal plain film series**
  - Anterior-posterior and left lateral decubitus views
- **Diagnostic findings**
  - •Dilated loops of bowel
  - •Pneumatosis intestinalis
  - •Portal venous air
- **X-rays**

Laboratory tests

- **Leukopenia**
- **Fecal test**
- **Comprehensive Metabolic panel**
- **Blood cultures**
- **Breath hydrogen test**

Monitoring
Serial abdominal radiographs every 6 hours to track disease progression until treatment

1. Necrotizing enterocolitis (NEC). Available at: https://my.clevelandclinic.org/health/diseases/10026-necrotizing-enterocolitis. Accessed on: 26 September 2024.
2. Necrotizing enterocolitis. Available at: https://www.ncbi.nlm.nih.gov/books/NBK513357/. Accessed on: 26 September 2024.
3. Liu XC, *et al. Front Microbiol*. 2022;13:969656.

NEC: Necrotizing enterocolitis.

Predicting NEC: The Role of Biomarkers in Early Detection

For over 20 years, gut tissue injury biomarkers have been investigated as reliable, noninvasive predictors of NEC onset, facilitating earlier interventions and improving outcomes[1,2]

Noninvasive gut biomarkers and their origins (sample) of detection[1]

Measurement of tissue oxygenation or blood flow

NIRS and USS doppler

Clinical features of NEC

Clinical biomarkers

HRV shown to predate NEC

Directly from bowel lumen

Stool biomarkers

VOCs
Microbiota
Calprotectin

Loss of bowel wall integrity so can cross into blood vessels

Filtered through kidneys and passed into urine

Serum blood biomarkers

ATP, NADH, HIF, VEGF, IL, TNFα, PAF, TLR4, CRP, PCT, SAA, platelets, FABPs, TFF3, Gene polymorphisms and metabolomics

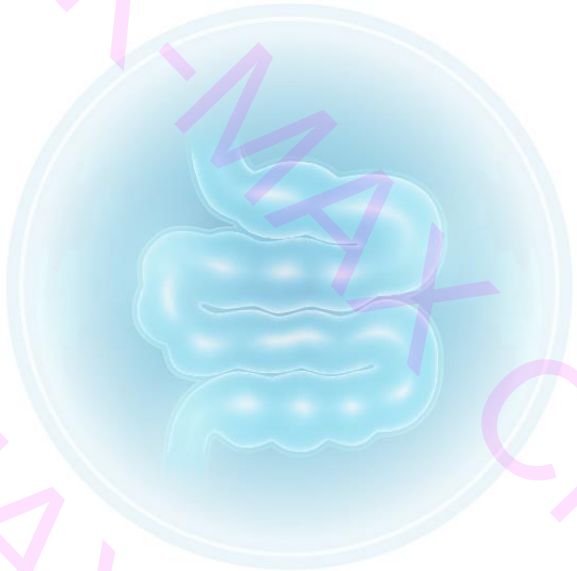
Urine biomarkers

FABPs, fibrinogen peptides, PGE-MUM, urine proteins, metabolomics

SCFAs such as **acetic, propionic, and butyric acid** are being investigated as biomarkers for the early detection of NEC[2]

1. Howarth C, *et al. Front Pediatr*. 2022;10:1048322.
2. Liu XC, *et al. Front Microbiol*. 2022;13:969656.

ATP: Adenosine triphosphate; CRP: C-reactive protein; FABP: Fatty acid binding protein; HIF: Hypoxia-inducible factor; HRV: Heart rate variability; IL: Interleukin; NADH: Nicotinamide adenine dinucleotide; NEC: Necrotizing enterocolitis; NIRS: Near-infrared spectroscopy; PAF: Platelet-activating factor; PCT: Procalcitonin; PGE-MUM: Prostaglandin E2 major urinary metabolite; SAA: Serum amyloid A; SCFA: Short-chain fatty acid; TFF3: Trefoil factor 3; TLR-4: Toll like receptor 4; TNFα: Tumor necrosis factor alpha; USS: Ultrasound scan; VEGF: Vascular endothelial growth factor; VOC: Volatile organic compound.



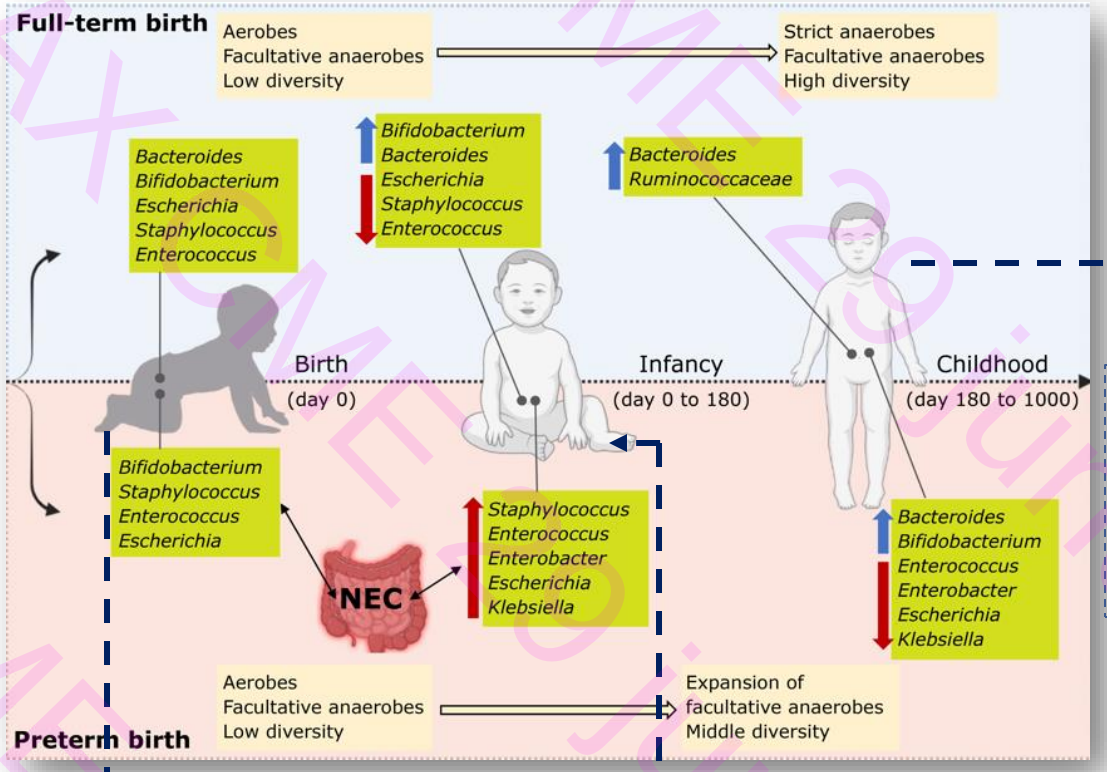
Dysbiosis:
A Key Contributor to NEC

NEC: Necrotizing enterocolitis.

Microbial Dysbiosis in Preterm Infants: The First 1000 Days

In the **first 1000 days**, preterm infants experience more gut dysbiosis compared with full-term infants.

Microbiota varies significantly between individuals, influenced by gestational age at birth



Increased diversity and decreased variability with age
Microbial diversity increases and interindividual variability decreases

Elevated pathogens in preterm infants
Higher levels of facultative anaerobes and opportunistic pathogens.

Lower microbiota diversity in growing preterm infants
Gut microbiota diversity increases but remains lower than that of full-term infants

The gut microbiota-immune system relationship is vital for infant growth, and its disruption can lead to diseases like **NEC.**

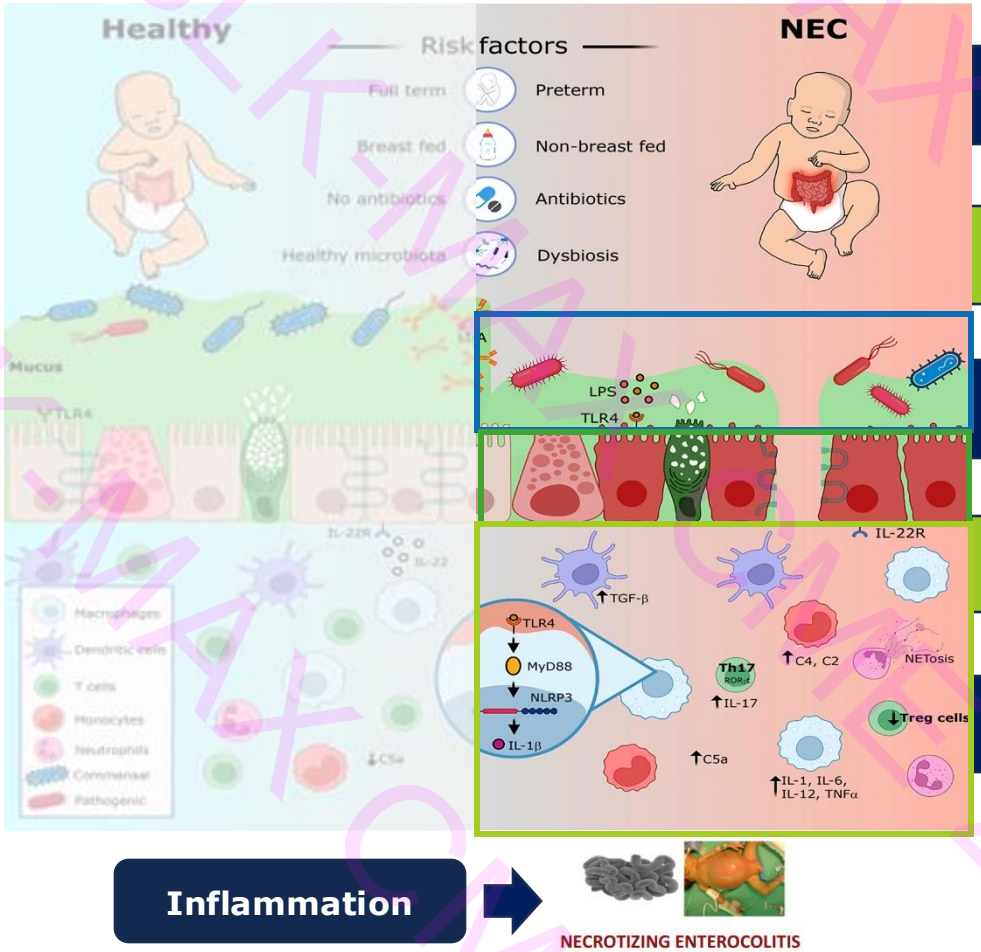
Zeng S, *et al. Front Microbiol*. 2022;13:905380.

NEC: Necrotizing enterocolitis.

Dysbiosis: Breaking the Gut Barrier in NEC

Dysbiosis in preterm infants is mainly caused by **physiological immaturity and elevated oxygen levels** in the gut.[1]

Elevated oxygen levels inhibit strict anaerobes, causing dysbiosis that increases the risk of chronic diseases later in life[1]



1. **Bacterial invasion:** NEC is caused by bacterial invasion into the intestinal wall.[2]
2. **Inflammation and destruction:** This invasion leads to inflammation and cellular destruction of the intestinal wall.[2]
3. **Immune response alterations:** Altered innate and adaptive immune responses impair the intestinal barrier.[3]
4. **Increased inflammation:** The impaired barrier results in increased inflammation.[3,4]
5. **Immature immune defense:** Immature intestinal immune defense is a key factor contributing to the high morbidity and mortality rates due to NEC.[3]

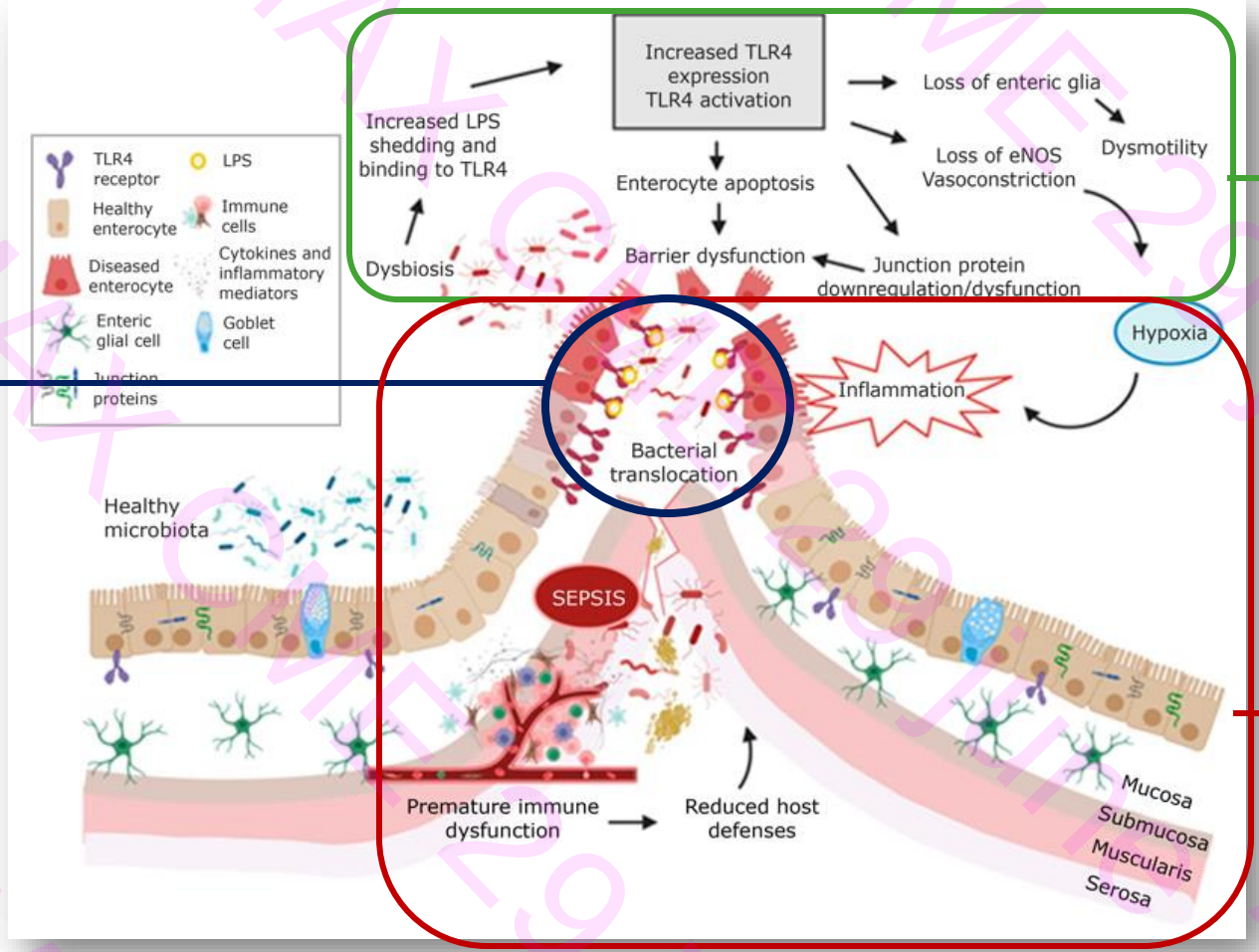
1. Zeng S, *et al. Front Microbiol.* 2022;13:905380.
2. Necrotizing enterocolitis. Available at: https://www.ncbi.nlm.nih.gov/books/NBK513357/. Accessed on: 26 September 2024.
3. Singh DK, *et al. Front Pediatr.* 2023;10:1107404.
4. Neu J, *et al. Semin Perinatol.* 2017;41(1):29–35.

C2: Complement component 2; C4: Complement component 4; C5a: Complement component 5a; IL: Interleukin; LPS: Lipopolysaccharide; MyD88: Myeloid differentiation primary response 88; NEC: Necrotizing enterocolitis; NETosis: Neutrophil extracellular trap formation; NLRP3: NOD-like receptor family pyrin domain containing 3; RoRγt: Retinoic acid-related orphan receptor gamma t; SIgA: Secretory immunoglobulin A; TGF-β: Transforming growth factor β; Th17: T helper 17; TLR4: Toll-like receptor 4; Treg: Regulatory T cell.

Unpacking NEC: Pathophysiology and Dysbiosis

Premature and VLBW infants have underdeveloped immune systems, increasing their susceptibility to pathogens and leading to immune dysregulation and dysbiosis.[1–3]

Dysbiosis and TLR4 activation:
An imbalance in gut microbiota activates TLR4 on the intestinal epithelium, leading to excessive signaling in response to LPS, which impairs immune function.[1–3]

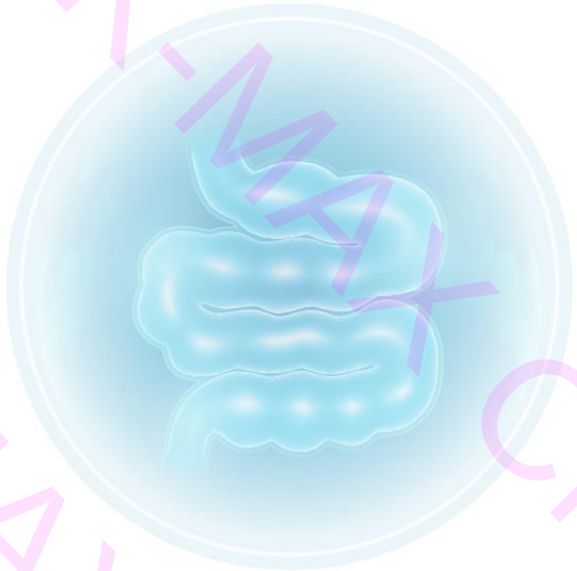


Inflammatory response and cellular damage:
TLR4 activation triggers proinflammatory signaling pathways, recruiting neutrophils and causing epithelial cell death through apoptosis, autophagy, and necroptosis, impairing mucosal repair.[1–3]

Gut barrier injury and consequences:
This damage results in irreversible gut barrier impairment, allowing bacterial translocation, which ultimately leads to intestinal ischemia and the clinical manifestations of NEC.[1–3]

1. Duess JW, *et al. Gut Microbes*. 2023;15(1):2221470.
2. Wu H, *et al. Front Pediatr.* 2022;10:954735.
3. Neu J, *et al. Semin Perinatol.* 2017;41(1):29–35.

eNOS: Endothelial nitric oxide synthase; LPS: Lipopolysaccharide; NEC: Necrotizing enterocolitis; TLR4: Toll-like receptor 4; VLBW: Very low birth weight.



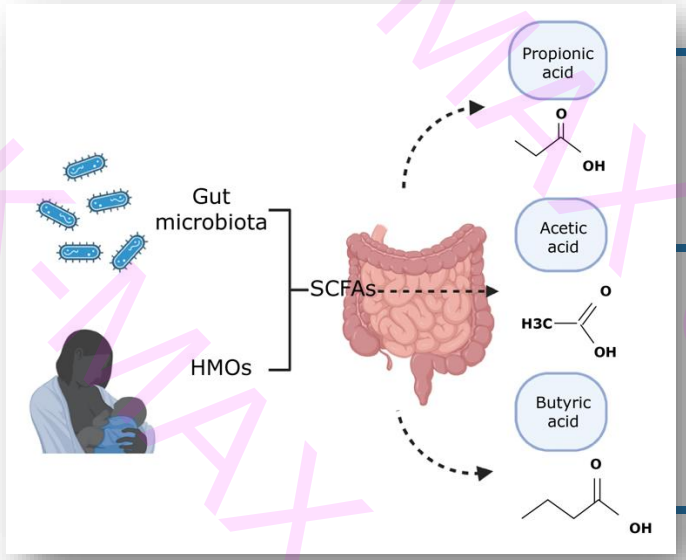
SCFAs:
The Unsung Heroes in NEC Defense

NEC: Necrotizing enterocolitis; SCFA: Short-chain fatty acid.

SCFAs: Powerhouses for Gut Health and Inflammation Control

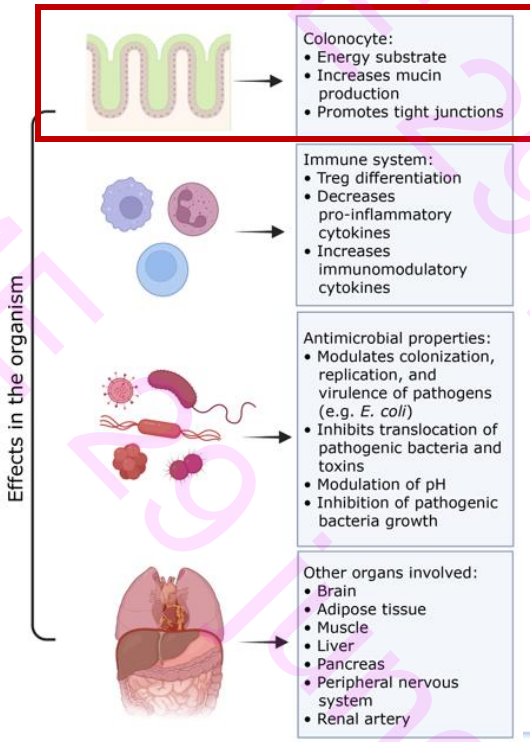
Evidence indicates that SCFAs, the end products of intestinal bacterial metabolism, play a pivotal role in mediating immune development during early life.[1]

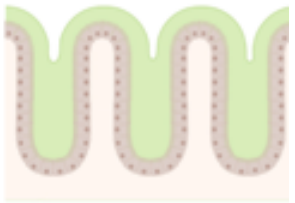
Advances in metagenomics and metabolomics have established SCFAs as essential for gut health.[2]



Acetic, butyric, and propionic acids account for 90%–95% of SCFAs in the human colon.[3]

They **support colonocytes and reduce** inflammation, benefiting conditions like NEC.[2,4]





Colonocyte:[2]
- Energy substrate
- Increase in mucin production
- Promotes tight junction

Butyrate, acetate, and propionate are key SCFAs that support gut health, enhance barrier integrity, and reduce inflammation via epigenetic modifications.[4]

1. Chun J, *et al. Nutrients*. 2022;14(18):3670.
2. Cifuentes MP, *et al. Curr Res Microb Sci*. 2024;6:100219.
3. Xiong J, *et al. Front Cell Infect Microbiol*. 2022;12:1030588.
4. Alsharairi NA. *Life (Basel).* 2023;13(2):561.

E.coli: Escherichia coli; HMO: Human milk oligosaccharides; NEC: Necrotizing enterocolitis; SCFA: Short-chain fatty acid; Treg: Regulatory T cell.

SCFAs and Beneficial Bacteria: Combatting NEC

In a recent study, infants with NEC showed lower levels of acetic acid, propionic acid, butyric acid, and total SCFAs.[1]

SCFAs may reduce inflammation in neonatal NEC by protecting intestinal cells from proinflammatory cytokines and chemokines through the inhibition of cellular signaling pathways.[2]

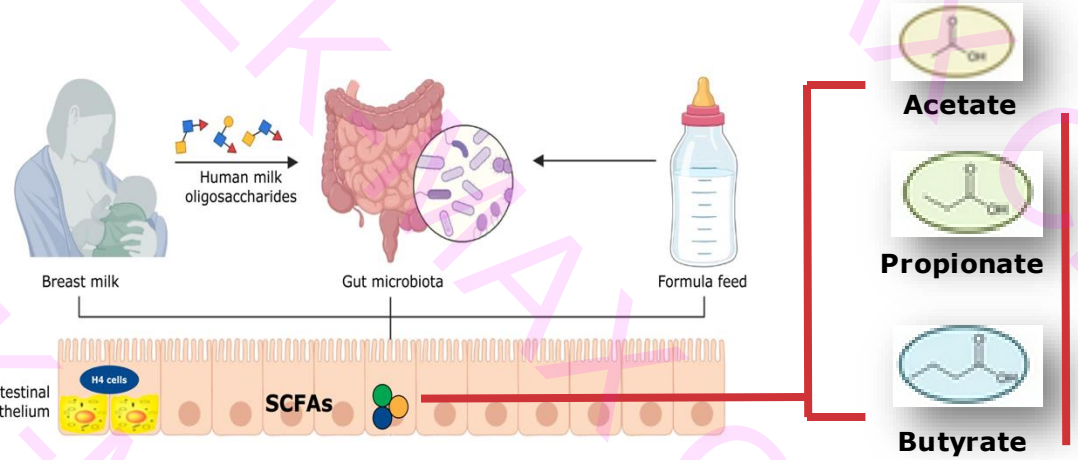
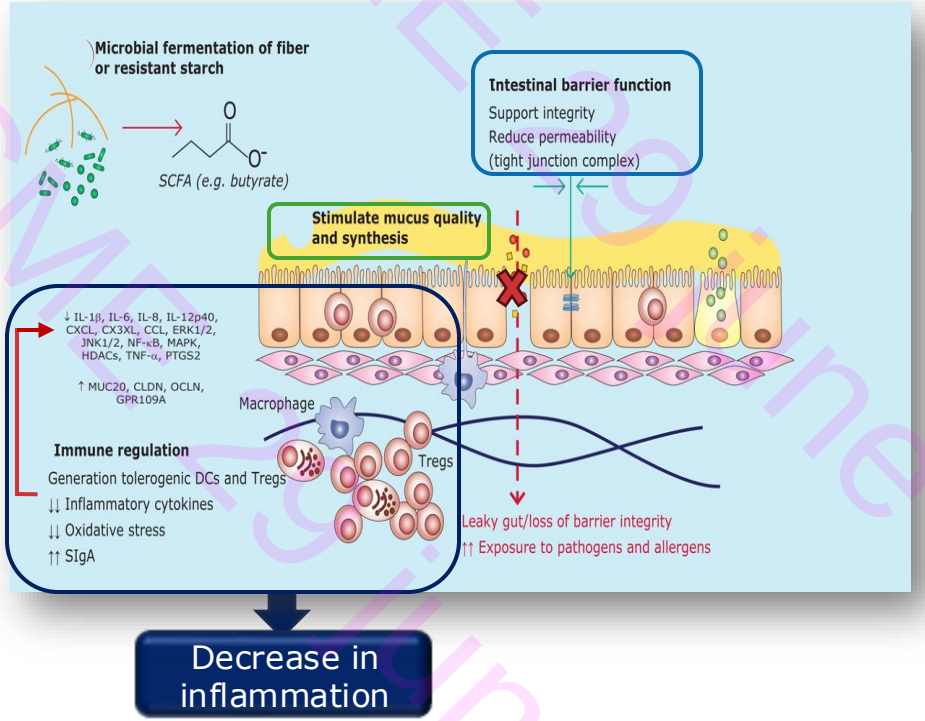


Image adapted from: Chun J, *et al. Nutrients*. 2022;14(18):3670.



SCFAs & barrier integrity
SCFAs improve intestinal barrier integrity, which is crucial in preventing NEC.[2–4]

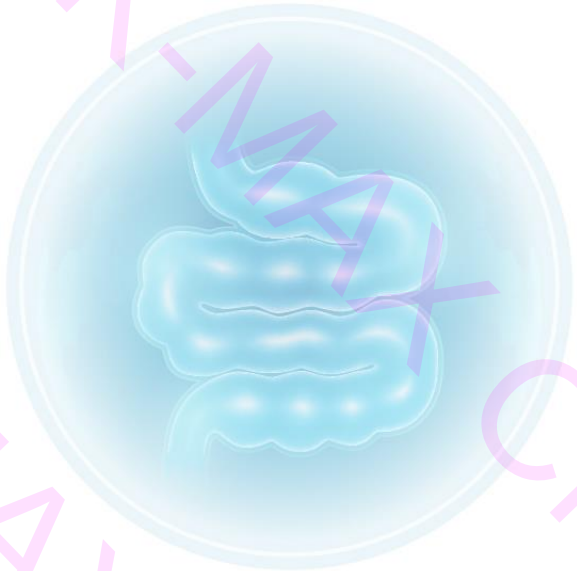
Mucus layer restoration
SCFAs, like butyric acid, aid in restoring the mucus layer.[3,4]

Gene expression
SCFAs boost mucin production, strengthen tight junctions, and reduce proinflammatory cytokines[2–4]

SCFAs mediate microbe–host immune interactions and may help treat NEC-induced inflammation.[2]

1. Xiong J, *et al. Front Cell Infect Microbiol*. 2022;12:1030588.
2. Alsharairi NA. *Life (Basel)*. 2023;13(2):561.
3. Cifuentes MP, *et al. Curr Res Microb Sci*. 2024;6:100219.
4. Blaak EE, *et al. Benef Microbes*. 2020;11(5):411–455.

Ca: Calcium; CCL: C-C motif chemokine ligand; CLDN: Claudin; CX3CL: C-X3-C motif chemokine ligand; CXCL: C-X-C motif chemokine ligand; DC: Dendritic cells; ERK: Extracellular signal-regulated kinase; GPR109A: G protein-coupled receptor 109A; HDAC: Histone deacetylase; IL: Interleukin; JNK: c-jun N-terminal kinase; MAPK: Mitogen-activated protein kinase; MUC20: Mucin 20; NEC: Necrotizing enterocolitis; NF-αB: Nuclear factor kappa-light-chain-enhancer of activated B cells; PTGS2: Prostaglandin-endoperoxide synthase 2; SCFA: Short-chain fatty acid; SIgA: Secretory immunoglobulin A; TNF-α: Tumor necrosis factor-alpha; Treg: Regulatory T cells.



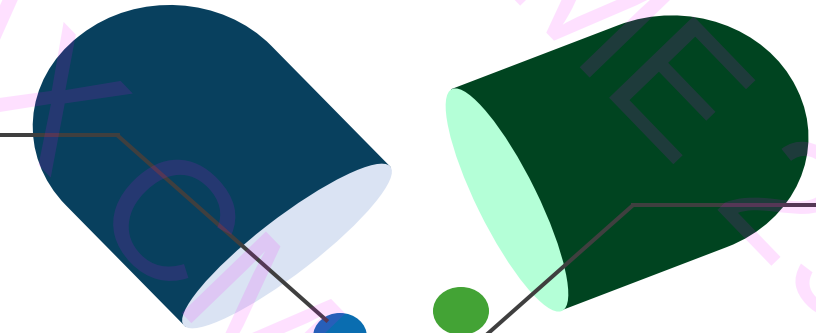
Probiotics:
Powerful Allies Against NEC

NEC: Necrotizing enterocolitis.

Probiotics: Strengthening Gut Health

Probiotics treat dysbiosis by restoring microbial diversity, regulating gut microbiota, enhancing immunity, increasing beneficial bacteria, and alleviating symptoms of various diseases.[1,2]

Characteristics of an ideal probiotic strain[3,4]



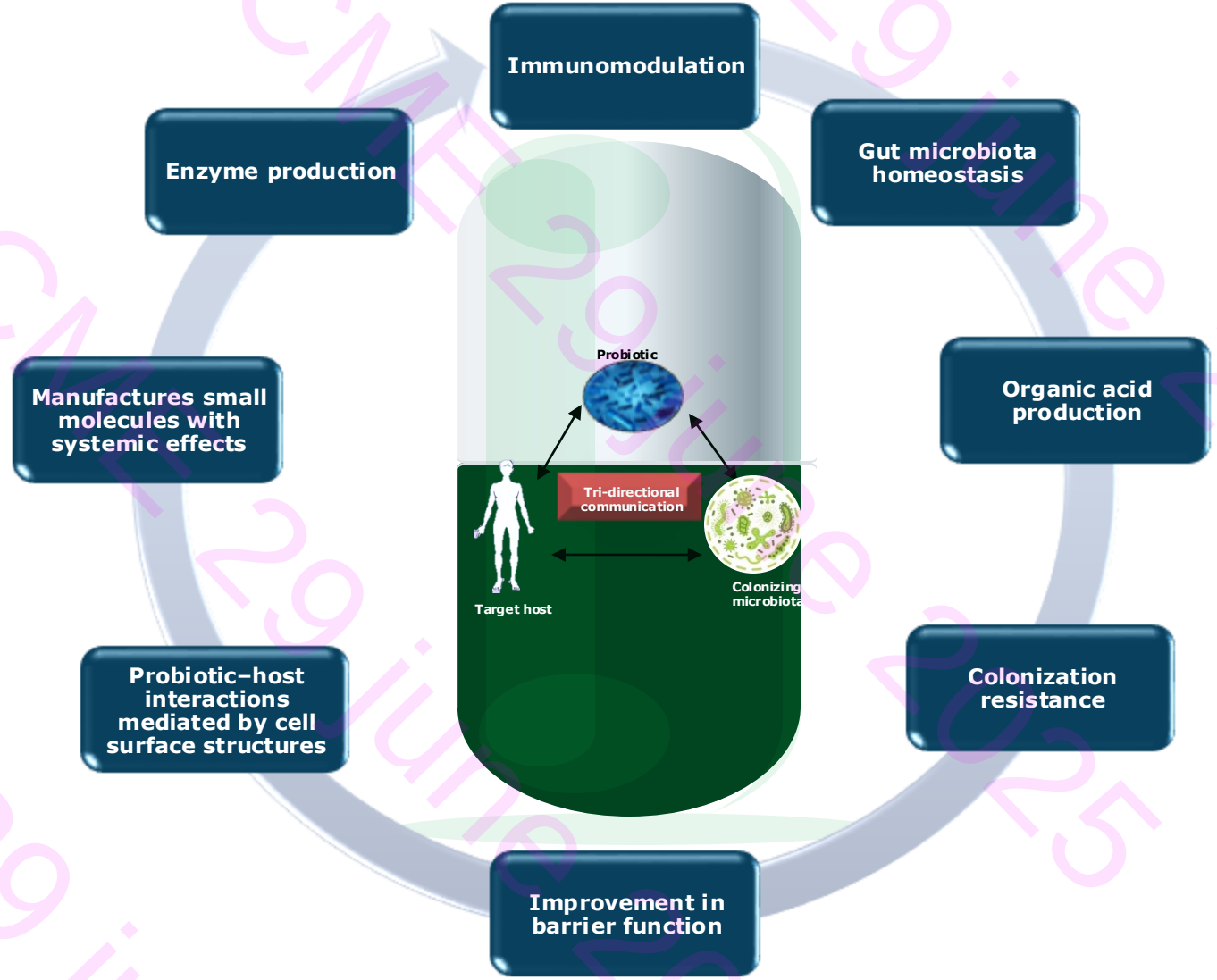
- Nonpathogenic (GRAS)
- Acid- and bile-tolerant
- Effective adhesion to gut lining
- Robust and surviving processing conditions
- Antigenotoxic property
- Short generation time
- Genetically stable
- Lactic acid producer
- Sensitive to antibiotics
- Resistant to digestive enzymes

1. Plaza-Diaz J, *et al. Adv Nutr*. 2019;10(suppl_1):S49–S66.
2. Chandrasekaran P, *et al. Int J Mol Sci*. 2024;25(11):6022.
3. Choudhary R, *et al*. Probiotics and human health. In: molecular biology its approaches and developments in the recent era. 2023. p. 87–91.
4. Pandey KR, *et al. J Food Sci Technol*. 2015;52(12):7577–7587.

GRAS: Generally recognized as safe.

Probiotic Benefits Explained: Mechanisms of Interaction and Impact

Probiotic microorganisms act through various mechanisms, including

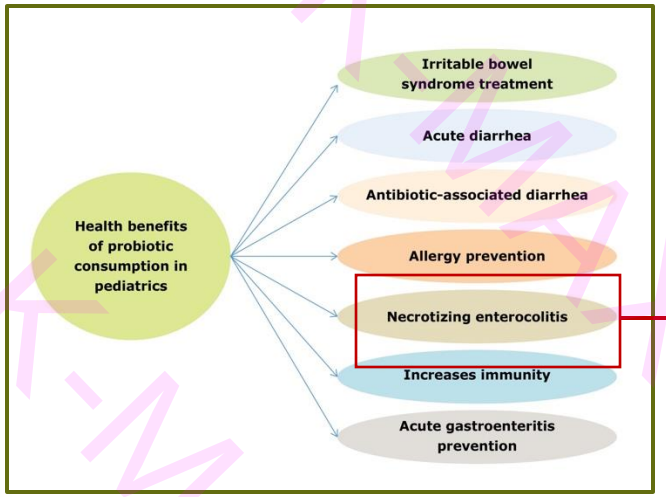


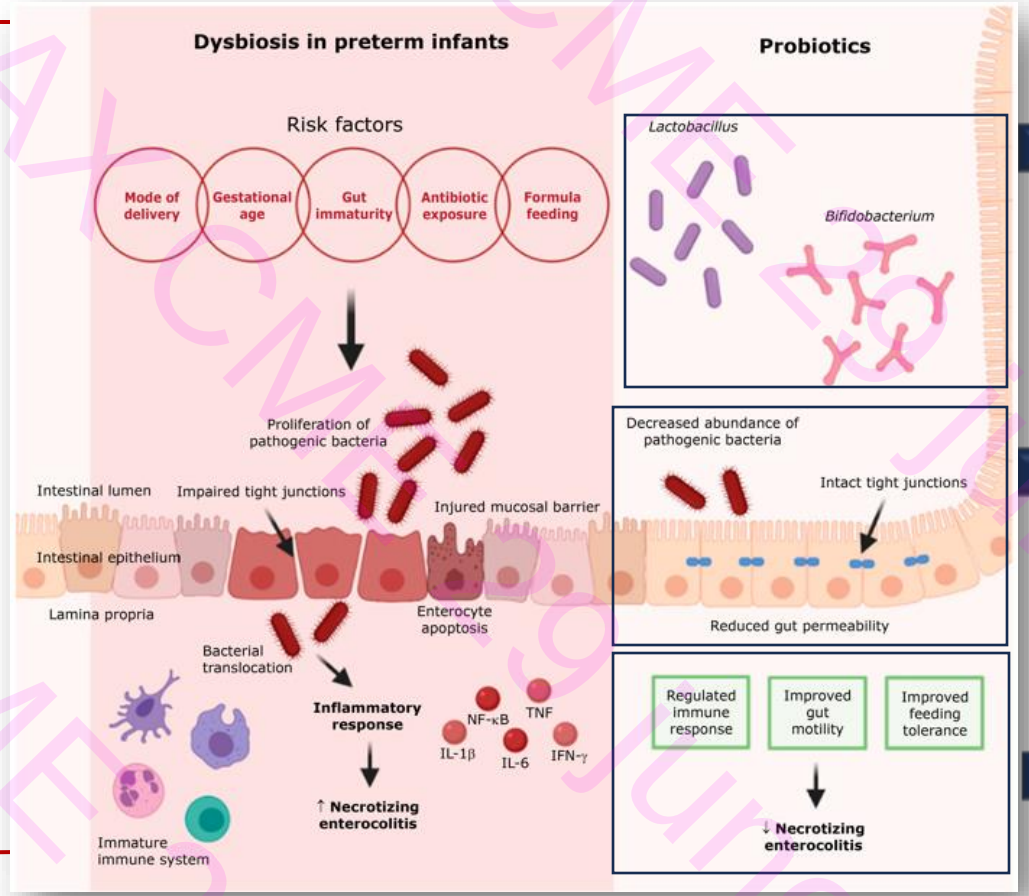
Sanders ME, *et al. Nat Rev Gastroenterol Hepatol*. 2019;16(10):642.

Probiotic Benefits in Very Preterm and Low Birth Weight Infants

Probiotics have been studied as single-strain or multistrain supplements in both preterm and term neonates.[1]

Health benefits in pediatric population[2]





Microbiome diversity:
Probiotics increase beneficial bacteria like *Bifidobacterium* and decrease pathogenic bacteria such as *Enterobacteriaceae* and *Clostridium*[1]

Intestinal barrier protection:
Probiotic supplementation in preterm infants can enhance intestinal barrier function, reducing intestinal permeability, and improving tight junction expression[1]

NEC protection:
Probiotics may reduce the risk of NEC by regulating immune response, enhancing gut motility, and improving feeding tolerance[1]

Several studies underscore the critical role of probiotics in regulating the microbiome and reducing neonatal morbidities, such as NEC.[1]

1. Nolan LS, *et al. Nutrients.* 2020;12(10):3052.
2. Vijayaraghavan S, *et al. IJEB.* 2021;59(10):653–661.

IFN-γ: Interferon-gamma; IL: Interleukin; NEC: Necrotizing enterocolitis; NF-αB: Nuclear factor kappa-light-chain-enhancer of activated B cells; TNF: Tumor necrosis factor.

Cochrane Review on Preterm Infants for the prevention of NEC

- 56 Trials
- 10,812 neonates
- **The probiotics can significantly reduce the risk of necrotizing enterocolitis.**

IAP Consensus Guidelines 2022

The IAP recommends probiotic supplementation for prevention of necrotizing enterocolitis in preterm infants.

- Most of the studies have used multi-strain probiotics and a few used single-strain probiotics.
- Probiotic supplementation was continued till discharge, till reaching full feeds, or for a prespecified duration between 7 and 21 days.
- The meta-analysis showed reduced risk of NEC≥ stage II , late-onset sepsis and mortality in probiotic group.
- Probiotics also reduced the time to full feeds and duration of hospital stay.
- Since there is low to moderate level of certainty about the effects of probiotic supplements on the risk of NEC and associated morbidity and mortality, large, high-quality studies are needed.

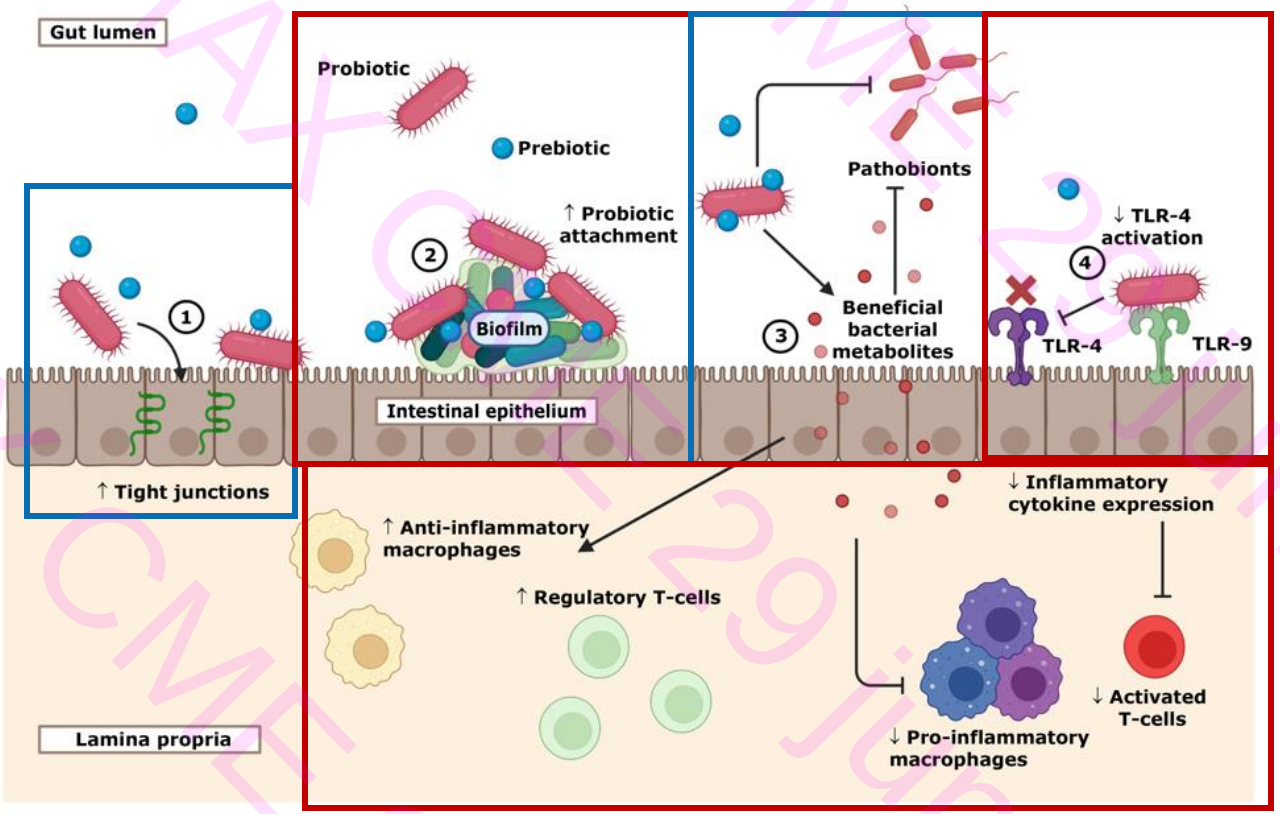
Probiotic Impact on Gut Development in NEC

Administering probiotics to **very preterm, very LBW infants may lower the risk of NEC** and probably reduce mortality compared with placebo or no treatment.[1]

Gut barrier enhancement & cytoprotection
Probiotics enhance gut barrier integrity by preserving tight junction proteins (e.g., claudin 4, occludin), supporting barrier maturation, and providing an antiapoptotic, cytoprotective effect[2,3]

Probiotic actions
Probiotics compete with pathobionts and produce antimicrobial compounds while metabolizing substrates like tryptophan into beneficial SCFAs[2,3]

TLR pathway inhibition
Some probiotics inhibit the TLR-4 pathway via interaction with TLR-9, reducing inflammatory cytokines and increasing regulatory T cells to mitigate inflammation[2,3]



Adhesion and biofilm formation
Highly adhesive probiotics form complex biofilms that improve attachment and overall efficacy in the gut[2,3]

Effects of SCFAs
SCFAs promote anti-inflammatory responses, nourish colonocytes, and lower pH to suppress pathogenic growth[2,3]

Immune modulatio
Probiotics regulate cellular immunity by balancing the Th1/Th2 response, competing with other microbes, and downregulating proinflammatory gene expression to support immune balance[2,3]

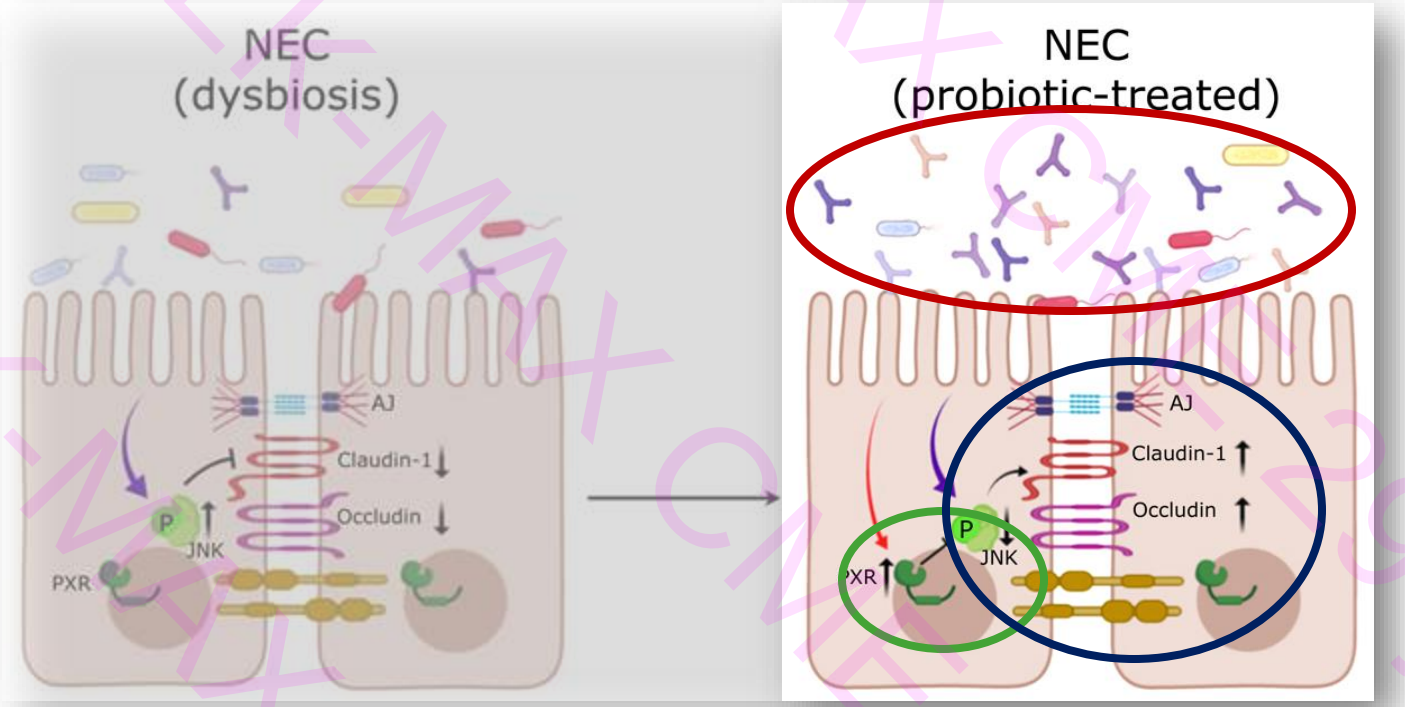
With careful strain selection and optimized dosing, probiotics can effectively prevent NEC.[2]

1. Sharif S, *et al. Cochrane Database Syst Rev*. 2023;7(7):CD005496.
2. Sajankila N, *et al. Front Pediatr.* 2023;11:1120459.
3. Patel RM, *et al. Semin Pediatr Surg*. 2018;27(1):39–46.

LBW: Low birth weight; NEC: Necrotizing enterocolitis; SCFA: Short-chain fatty acid; Th1: T-helper 1 cells; Th2: T-helper 2 cells; TLR4: Toll-like receptor 4; TLR: Toll-like receptor.

Probiotics: Enhancing Gut Microbiota and Barrier Function

Impaired intestinal barrier function, marked by reduced expression of tight junction components, has been observed in the pathogenesis of NEC.



Microbiota remodeling
Probiotics remodel the community of the intestinal microbiota.

PXR activation
Probiotics induce the activation of PXR, which may subsequently inhibit the phosphorylation of JNK.

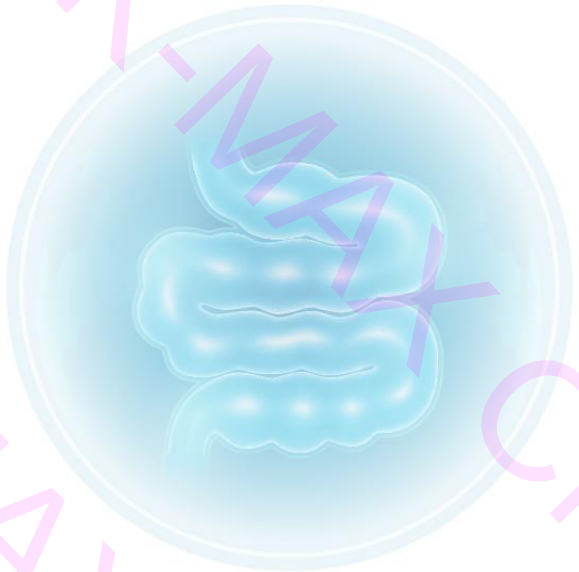
Tight junction enhancement
Inhibition of JNK phosphorylation elevates the expression of tight junction components, strengthening the epithelial barrier.

Improved barrier function in NEC
This process leads to improved intestinal barrier function, potentially preventing NEC.

Probiotics can help alleviate intestinal lesions by strengthening mucosal barrier function.

Zhao X, *et al. Cell Biosci.* 2021;11(1):20.

AJ: Adherent junctions; JNK: c-Jun N-terminal kinase; NEC: Necrotizing enterocolitis; PXR: Pregnane X receptor.



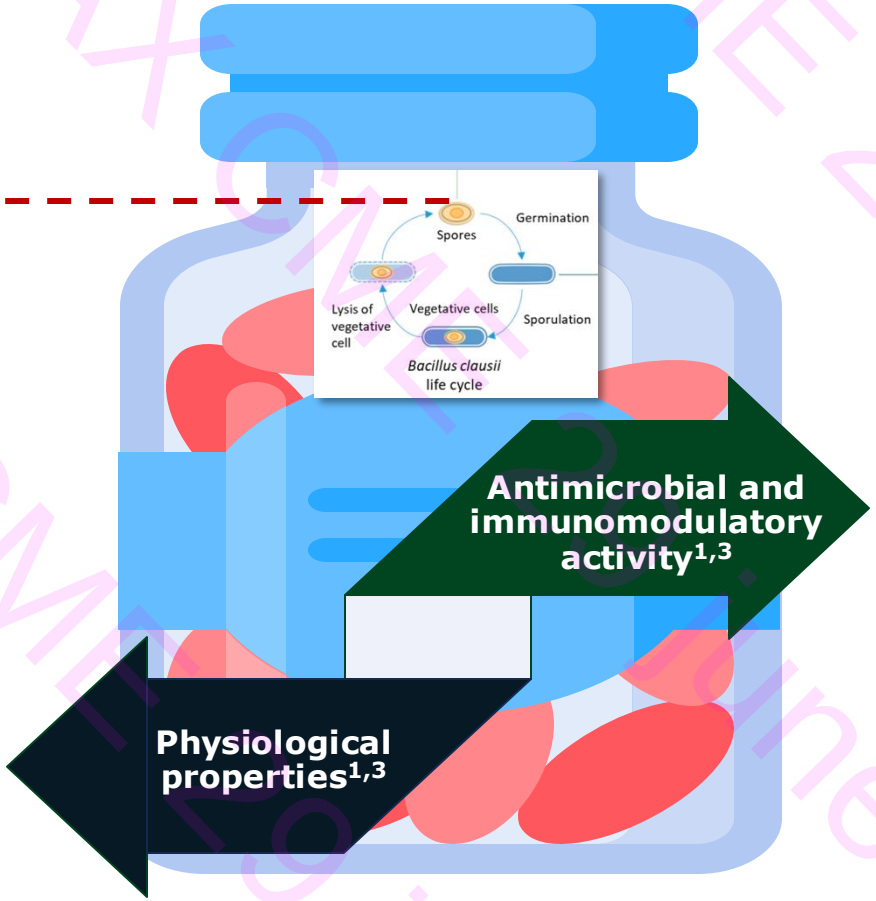
Bacillus clausii: **Strong Defense for Tiny Tummies**

B. clausii: Strong Probiotic for Pediatric Health

B. clausii is an extremely stable probiotic that survives stomach acid, reaches the intestine intact, and remains viable in a desiccated state without refrigeration.[1]

It has been safely used for decades, notably improving outcomes in **NEC** and various other conditions.[2]

- Tolerance to heat, acid, and salt
- Enhances gut barrier function
- Vitamin synthesis
- Antibiotic resistance
- Other biochemical and metabolic properties



- ↑ Mucin secretion
- ↓ Gut permeability
- Production of antibiotics
- Altered levels of pro and anti-inflammatory cytokines
- Serine protease activity on enterotoxins
- Proliferation of CD4+T cells
- Inhibition of enteropathogens

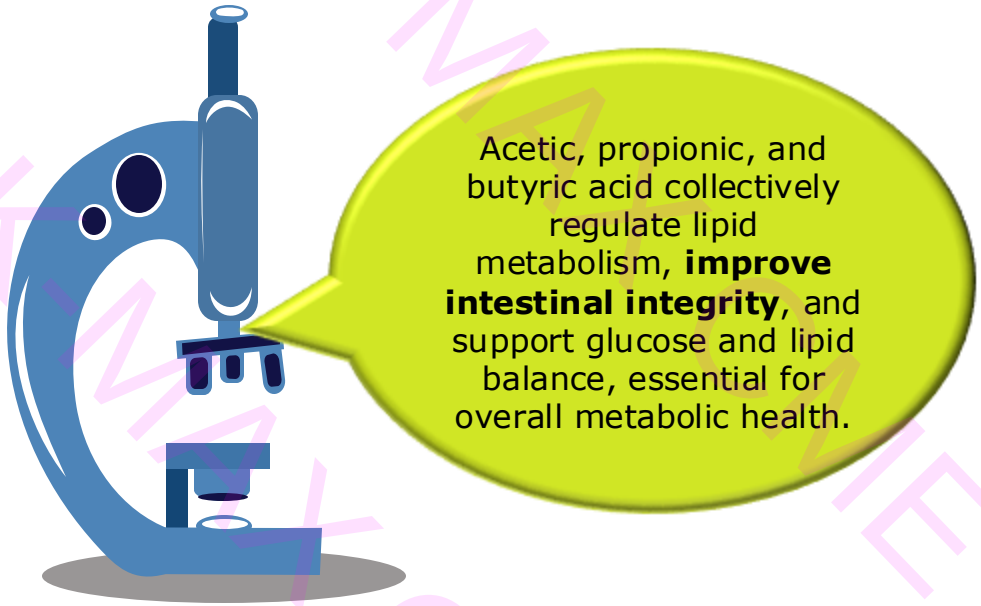
1. Ghelardi E, *et al*. *Microorganisms*. 2022;10(6):1246.
2. Wong-Chew RM, *et al*. *Expert Rev Clin Immunol*. 2022;18(7):717–729.
3. Chelliah R, *et al*. *LWT*. 2024;203:116291.

B. clausii: Bacillus clausii; CD4+T:Cluster of differentiation 4-positive T cell; NEC: Necrotizing enterocolitis.

B. clausii: Balancing Physiology With SCFAs-Clinical Evidence 1

Microbial fermentation of complex carbohydrates takes place in the human intestine → Generating SCFA → Deficient SCFA levels are linked to various diseases → **Probiotic microbacteria**
- Counteract SCFA deficiency
- Restore gut health

Study: *In vitro* tests allow a better understanding of the therapeutic effects of commercialized probiotic strains.



Study results
Production of SCFA by *B. clausii* strain

Bacterial strain	Acetic acid	Propionic acid	Butyric acid
Bacillus clausii NR	+	+[#]	+
Bacillus clausii OC	+	+[#]	+
Bacillus clausii SIN	+	+[#]	+
Bacillus clausii T	++*	++[#]	+

**Bacillus clausii* T was one of the strongest producers of acetic acid.
[#]*Bacillus clausii* T produced the highest concentrations of propionic acid, which differed significantly from *Bacillus clausii* NR (p=0.0374) and *Bacillus clausii* SIN (p=0.0112).

***B. clausii* (O/C, SIN, N/R, T) was found to secrete acetic acid, propionic acid, and butyric acid.**

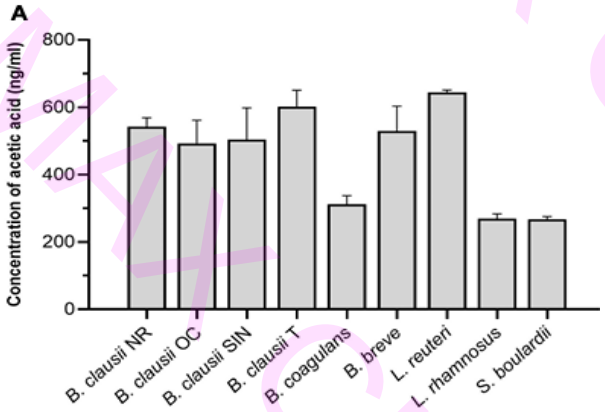
Pellegrino P, *et al*. *Evid Self Med*. 2024;4:240013.

B. clausii: Bacillus clausii; N/R: Novobiocin and rifampicin; O/C: Chloramphenicol; SCFA: Short-chain fatty acid; SIN: Neomycin and streptomycin; T: Tetracycline.

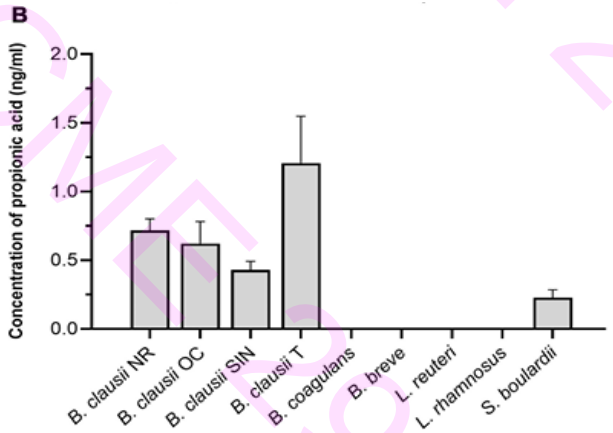
B. clausii: Balancing Physiology With SCFAs-Clinical Evidence 2

Study: HPLC-MS-MS quantification of SCFAs actively secreted by probiotic strains[1]

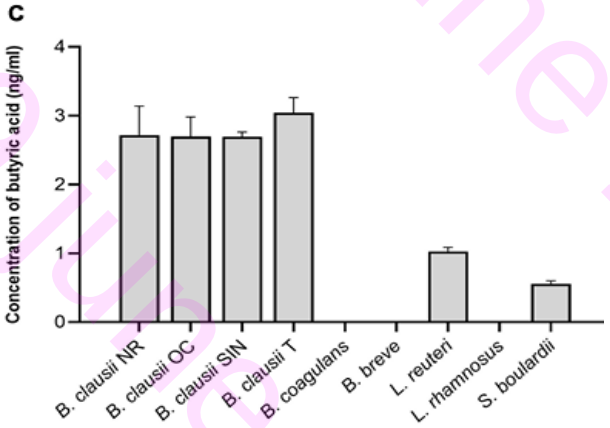
Concentration of (A) acetic acid; (B) propionic acid; and (C) butyric acid in the culture supernatants of bacterial strains tested.[1]



***B. clausii* T** was the **second-highest producer** among tested strains to secrete acetic acid (602.00±54.15 ng/mL).



***B. clausii* T** produced the **highest levels** of propionic acid among the tested strains (1.21±0.38ng/mL).



All *B. clausii* strains showed comparable secretion of butyric acid and were higher compared with *L. reuteri* and *S. boulardii*.

B. clausii produces SCFAs, which play a key role in mediating microbe–host immune interactions.[2]
This suggests that *B. clausii* may help alleviate NEC-induced inflammation and its related symptoms.

1. Calvigioni M, *et al. Front Microbiol*. 2023;14:1124144.
2. Alsharairi NA. *Life (Basel).* 2023;13(2):561.

B. breve: Bifidobacterium breve; *B. clausii: Bacillus clausii; B. coagulans: Bacillus coagulans;* HPLC-MS-MS: High-performance liquid chromatography coupled to tandem mass spectrometry*; L. reuteri: Limosilactobacillus reuteri; L. rhamnosus: Lacticaseibacillus rhamnosus;* NEC: Necrotizing enterocolitis; *S. boulardii: Saccharomyces boulardii*; SCFA: Short-chain fatty acids.

Comparative Analysis of Original *Bacillus clausii* Strains (O/C, N/R, SIN, T) vs. Generic Strains: Evaluating Label Claims

Parameters	Enterogermina®	Tufpro®	Ecogro™	Ospor®	Entromax®
Label claim for bacterial/spore count	***Bacillus clausii* 2×10⁹ spores/5 mL and 2×10⁹ CFU/g**				
Label claim test results	Matched claim	Lower than claim	Minor deviations	Lower than claim	Matched claim
Species identification	100% *B. clausii*	91% *B. cereus*	33% *B. clausii*	100% *B. clausii*	100% *B. subtilis*
Identification of viable/nonviable bacteria	Homogenous *B. clausii* population	Mixed bacterial population (*B. cereus*)	Mixed bacterial population (*B. subtilis*)	*B. clausii* population	Mixed bacterial population (*B. amyloliquefaciens*)

Among the commercial probiotics analyzed, only Enterogermina® (*B. clausii* [O/C, N/R, SIN, T]) adhered to its label claims.

Patrone V, *et al. Int J Food Microbiol*. 2016;237:92–97.

B. amyloliquefaciens: Bacillus amyloliquefaciens; B. cereus: Bacillus cereus; B. clausii: Bacillus clausii; B. subtilis: Bacillus subtilis; CFU: Colony-forming unit; N/R: Novobiocin and rifampicin; O/C: Chloramphenicol; SIN: Neomycin and streptomycin; T: Tetracycline.

Comparison of Original Strains (O/C, N/R, SIN, T) and Generic Strains of *Bacillus clausii*-Quality Analysis

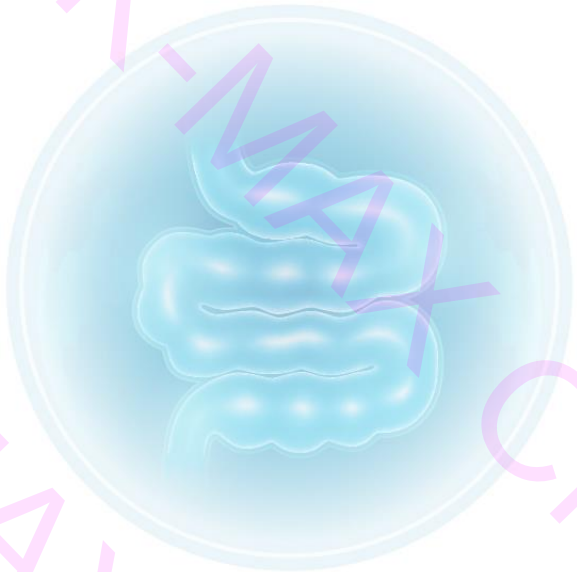
Product	Antibiotic resistance to all four strains	Contamination	Titer
Enterogermina (Reference)	✓	✗	**2×10^9 /5 mL**
BIFILAC Clausii	✓	✓	8E+8/5 mL
ENTROMAX	✗	✓	3.8E+7/5 mL
PROGERMINA	✗	✓	2.2E+9/5 mL
ENTROFLORA	✗	✓	3.5E+8/5 mL
NOVOGERMINA	✗	✓	3.7E+8/5 mL
TUFPRO	✗	✓	2.8E+8/5 mL
GUTGERMINA	✗	✓	1.1E+8/5 mL
ECO-ALL	✗	✓	2.1E+9/5 mL
ENTERO CLAUSI	✗	✓	6.7E+8/5 mL
MEDOGERMINA	✗	✗	3.0E+9/5 mL

- **Resistance to all four tested** antibiotics (tetracycline hydrochloride, chloramphenicol, streptomycin sulfate, and rifampicin)
- **Lack of contamination**.
- **High bacterial count** of 2×10^9 CFU/5 mL.

***Enterogermina*® (*B. clausii* [O/C, N/R, SIN, T]) stands out with its inherent antibiotic resistance and superior compositional quality, unlike its competitors that face varying antibiotic profiles, bacterial counts, and contamination concerns.**

Sanofi Internal Data Sheet.

B. clausii: *Bacillus clausii*; CFU: Colony-forming unit; N/R: Novobiocin and rifampicin; O/C: Chloramphenicol; SIN: Neomycin and streptomycin; T: Tetracycline.



Key Insights

NEC Prevalence
NEC is the leading serious gastrointestinal disease in preterm infants and a major cause of death between 2 weeks to 2 months of age.

Vulnerability in Preterm Infants
Premature and VLBW infants are highly susceptible to pathogens due to underdeveloped immune systems, leading to immune dysregulation and dysbiosis.

SCFAs Role
SCFAs play a key role in microbe–host immune interactions, helping mediate inflammation, particularly in conditions like NEC.

Probiotics Impact
Probiotics, including *B. clausii*, are critical in regulating the gut microbiome, potentially reducing neonatal morbidities like NEC.

***B. clausii* Potential**
B. clausii produces SCFAs, suggesting its potential role in reducing NEC-induced inflammation and related symptoms.

B. clausii: Bacillus clausii; NEC: Necrotizing enterocolitis; SCFA: Short-chain fatty acid; VLBW: Very low birth weight.

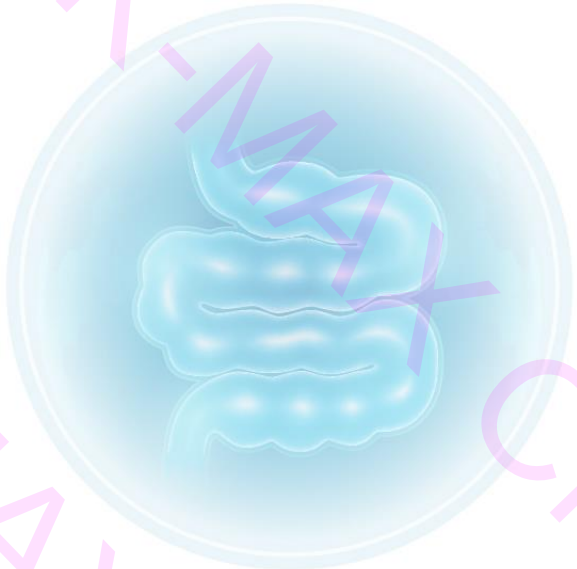
Preventive Strategies : NEC

Maternal Health Optimization:
Reducing preterm birth & Antenatal corticosteroids

Feeding Practices:
Human milk feeding
Standardized feeding protocols

Probiotics: While studies on probiotics are promising, there is ongoing research to determine optimal strains, dosages, and treatment durations for preventing NEC.

Other Considerations:
- Minimizing infections
- Fluid restriction
- Limiting unnecessary antibiotics
- Avoiding gastric acid suppression
- Reducing exposure to blood transfusions



THANK YOU