Isolation and banking of microbial isolates from premature infants.

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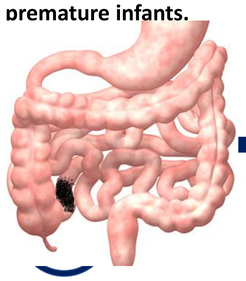
tiny lives SPECIAL CARE BABIES
Marie Skłodowska-Curie Actions

Introduction

- Necrotising enterocolitis (NEC) is a serious disease that occurs in premature babies, characterized by inflammation of the intestines. This potentially fatal disease is associated with a high requirement for surgery, as well as significant long term health complications in those surviving.
- The cause of NEC is unclear, however, an imbalance in gut microbes causing an inappropriate host immune response is considered to be a key factor.
- Previous work has shown *Bifidobacterium* to be present in healthy preterm infants, but absent in NEC infants[1].

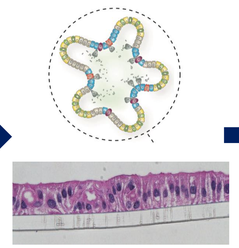
Overview

Collect pieces of tissue from premature infants.



Both healthy and necrotic tissue are collected.

Generate enteroid lines



Stem cells are used to create enteroid monolayers which are intestinal epithelium culture.

Anaerobic medium

The enteroids will be placed in anaerobic medium to simulate intestinal conditions and co-cultured with the microbial species isolated in this project.

Figure 1: Schematic overview showing the main experimental procedures.

Aims

1) Isolate a range of microbial species, including *Bifidobacterium,* from stool samples from premature infants for use in future experiments.

2) Count the number of goblet cells on enteroids which are histological intestinal epithelium cultures[2] to determine a link between NEC and healthy preterm infants.

Materials and methods

Aim 1 - Microbial species isolation:

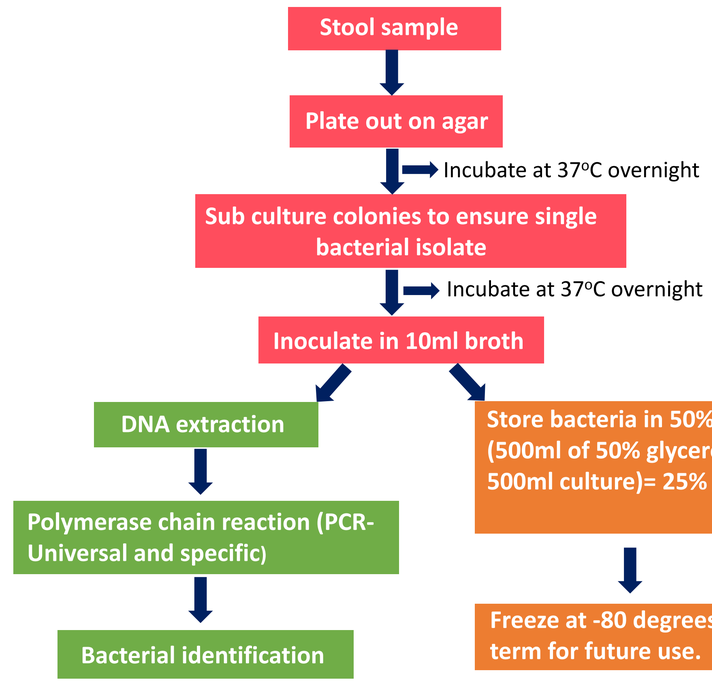
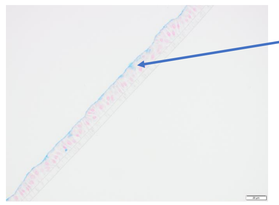


Figure 2: Flowchart depicting the steps from; isolating microbial species from both fresh and frozen stool samples through to identification and storage.

Aim 2 - Number of goblet cells in enteroid



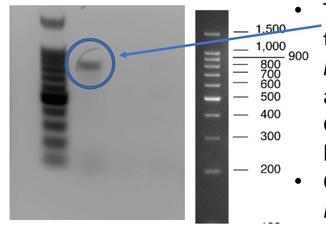
Arrow indicates goblet cell stained blue (by alcian blue).

Figure 3 : Image of an enteroid (intestinal epithelium culture grown from stem cells) from sample NEC2.1, stained with 154 for 8 hours at magnification x20.

References:
1. Stewart CJ, Embleton ND, Marrs ECL, Smith DP, Nelson A, Abdulkadir B, Skeath T, Petrosino JF, Perry JD, Berrington JE, Cummings SP, Temporal bacterial and metabolic development of the preterm gut reveals specific signatures in health and disease, Microbiome, 2016, 4:67.
2. Butt SE, Crawford SE, Ramani S, Zou WY, Estes MK, Engineered Human Gastrointestinal Cultures to Study the Microbiome and Infectious Diseases, Cellular and Molecular Gastroenterology and Hepatology, 2017, 5:3, 241-251

Results

Aim 1 – Isolated 24 species of bacteria including *Bifidobacterium*

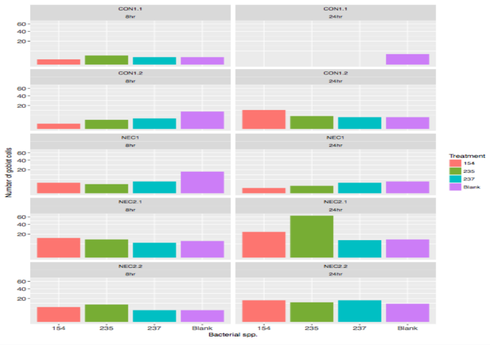


- The band present at 831kb indicates the presence of *Bifidobacterium longum* Bacterial identification achieved by sequencing by Eurofins Genomics, confirmed the sample to be *Bifidobacterium longum*.
- Other species of bacteria, e.g., *Lactobacilli* and *Enterococci,* were isolated.

Figure 4: DNA extracted from stool samples was amplified by PCR (using *B.longum* specific primers) and ran on Agarose Gel Electrophoresis image (left). 1kb DNA ladder (right) (New England Biolabs).

Aim 2 – Number of goblet cells differed between enteroid lines

Figure 5: Bar chart showing the number of goblet cells present on enteroids with variable treatments and durations from different patients.



Conclusions

- *Bifidobacterium longum* was successfully isolated from premature infant's stool samples.
- The number of goblet cells present in the NEC infants was higher, although whether this is significant is undetermined.
- The 23 frozen samples of known bacterial identity will be co-cultured with enteroids in future experiments to examine host microbial interactions.