

# Report assessing the inhibitory effects of Lactobacillus Reuteri NCIMB 30351 on the growth of various Clostridium species

## Purpose:

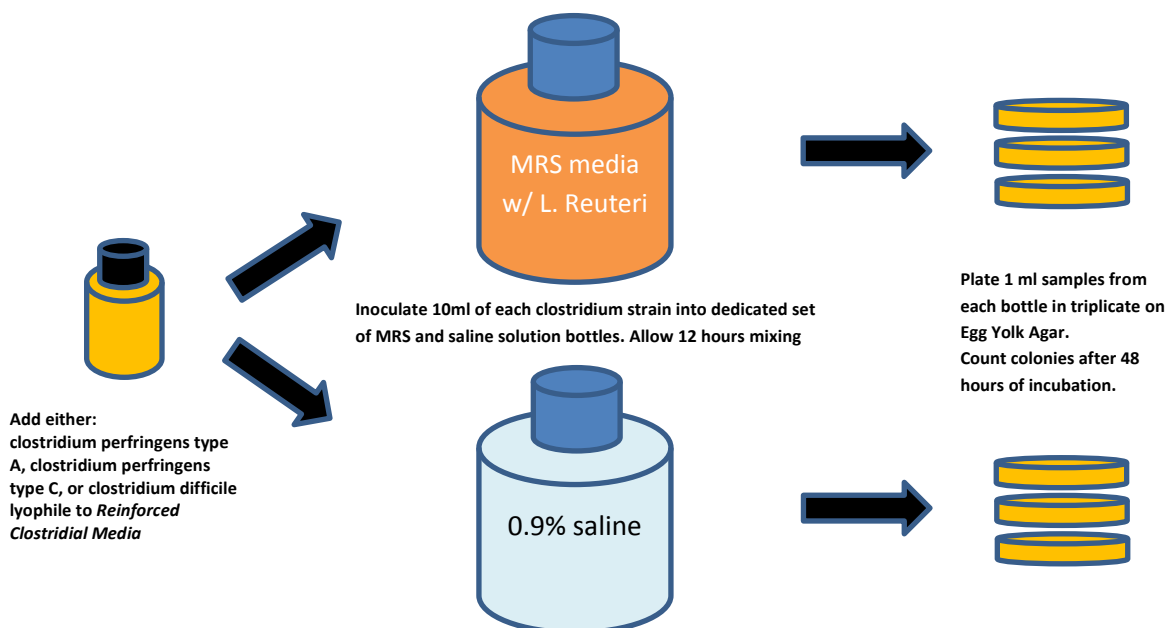
To test whether Lactobacillus Reuteri NCIMB 30351 is able to impair the growth of 3 different strains of Clostridium: Clostridium Perfringens Type A, Clostridium Perfringens Type C, and Clostridium Difficile.

## Materials:

- (3 x 100ml bottles) Reinforced Clostridial Medium, USP. Hardy Diagnostics cat no. U172
- (3x 1L bottles) DeMan, Rogosa, Sharpe Media (MRS). Hardy Diagnostics cat no. C5934.
- 0.9% saline solution.
- (18) Egg Yolk Agar plates. Hardy Diagnostics cat no. AG401.
- (3 x 10ml) Lactobacillus Reuteri NCIMB 30351 working wet seed (WWS)
- (1 lyophile vial) Clostridium Perfringens Type A ATCC®13124™
- (1 lyophile vial) Clostridium Perfringens Type C ATCC®3628™
- (1 lyophile vial) Clostridium Difficile ATCC®BAA-1870™

## Method:

- for each of the Clostridial strains being tested, take each lyophile vial (Clostridium Perfringens Type A ATCC®13124™, Clostridium Perfringens Type C ATCC®3628™, or Clostridium Difficile ATCC®BAA-1870™) and reanimate in dedicated Reinforced Clostridial Medium (100ml). Allow each strain to grow inside incubator anaerobically at 37°C for 48 hours.
- prepare three 1L bottles of autoclave sterilized 1L MRS media (Teflon magnetic stirrer deposited in bottle). When cooled down to room temperature, inoculate Lactobacillus Reuteri NCIMB 30351 WWS into each MRS bottle. Allow each bottle to grow inside incubator anaerobically at 37°C for 12 hours. [start fermentation at time that coincides with the end of fermentation in step (a)].
- prepare three 1L bottles of autoclave sterilized 0.9% saline solution (Teflon magnetic stirrer deposited in bottle).
- after clostridial strains have grown in their medium for 48 hours, pipet 10ml of each strain into their own dedicated Lactobacillus reuteri NCIMB 30351 fermentation and 0.9% saline solution 1L bottles.
- allow each 1L bottle inoculated with 10ml of each clostridial strain to mix while inside anaerobic incubator for further 12 hours.
- label 18 Egg Yolk Agar plates (This agar is selective for clostridial species):
  - 3 plates will be for testing Clostridium Perfringens Type A added to Lactobacillus Reuteri fermentation.
  - 3 plates will be for testing Clostridium Perfringens Type A added to 0.9% saline solution.
  - 3 plates will be for testing Clostridium Perfringens Type C added to Lactobacillus Reuteri fermentation.
  - 3 plates will be for testing Clostridium Perfringens Type C added to 0.9% saline solution.
  - 3 plates will be for testing Clostridium Difficile added to Lactobacillus Reuteri fermentation.
  - 3 plates will be for testing Clostridium Difficile added to 0.9% saline solution.
- pipet 1ml from each 1L bottle onto dedicated Egg Yolk Agar Plates. Streak sample uniformly across entire surface of the agar plates.
- place each agar plate into anaerobic incubator set at 37°C for 48 hours.
- after 48 hours, count number of colonies that form on each agar plate.



# Report assessing the inhibitory effects of Lactobacillus Reuteri NCIMB 30351 on the growth of various Clostridium species

## Results:

After anaerobic incubation for 48 hours at 37°C, each Egg Yolk agar plated sample was observed to check for colony formation. For each agar plate, the number of colonies observed were counted. Results are shown in Table 1 below.

**Table 1: Quantity of Colonies formed on Egg Yolk agar plate samples**

| Strain grown in Clostridial Media | Solution into which 10 ml Clostridial Sample was inoculated | Plate designation | Number of colonies formed on Egg Yolk Agar after 37°C/48 hour anaerobic incubation | Average number of colonies |
|-----------------------------------|-------------------------------------------------------------|-------------------|------------------------------------------------------------------------------------|----------------------------|
| Clostridium Perfringens Type A    | L. Reuteri NCIMB 30351 fermentation in MRS media            | 1                 | 28                                                                                 | 28.67                      |
|                                   |                                                             | 2                 | 32                                                                                 |                            |
|                                   |                                                             | 3                 | 26                                                                                 |                            |
|                                   | 0.9% Saline solution                                        | 1                 | 420                                                                                | 406.67                     |
|                                   |                                                             | 2                 | 412                                                                                |                            |
|                                   |                                                             | 3                 | 388                                                                                |                            |
| Clostridium Perfringens Type C    | L. Reuteri NCIMB 30351 fermentation in MRS media            | 1                 | 33                                                                                 | 32.33                      |
|                                   |                                                             | 2                 | 42                                                                                 |                            |
|                                   |                                                             | 3                 | 22                                                                                 |                            |
|                                   | 0.9% Saline solution                                        | 1                 | 368                                                                                | 386.33                     |
|                                   |                                                             | 2                 | 390                                                                                |                            |
|                                   |                                                             | 3                 | 401                                                                                |                            |
| Clostridium Difficile             | L. Reuteri NCIMB 30351 fermentation in MRS media            | 1                 | 21                                                                                 | 16.33                      |
|                                   |                                                             | 2                 | 16                                                                                 |                            |
|                                   |                                                             | 3                 | 12                                                                                 |                            |
|                                   | 0.9% Saline solution                                        | 1                 | 260                                                                                | 241                        |
|                                   |                                                             | 2                 | 223                                                                                |                            |
|                                   |                                                             | 3                 | 240                                                                                |                            |

## Discussion:

When comparing samples plated on Egg Yolk Agar that came from either the L. Reuteri fermentation or the 0.9% Saline solution, there was a marked difference in the number of colonies formed between both groups. (refer to Table 1: Quantity of Colonies formed on Egg Yolk agar plate samples)

Clostridium Perfringens Type A sample plates from L. Reuteri NCIMB 30351 fermentation in MRS media had an average 28.67 colonies. This is a drastic difference to the number of colonies observed to have grown after incubation in 0.9% Saline solution, with an average of 406.67 colonies. The samples from L. Reuteri NCIMB 30351 fermentation in MRS media in this grouping had only 7.05% of the colonies compared to samples from 0.9% saline solution.

Clostridium Perfringens Type C sample plates from L. Reuteri NCIMB 30351 fermentation in MRS media had an average 32.33 colonies. This is also a drastic difference to the number of colonies observed to have grown after incubation in 0.9% Saline solution, with an average of 386.33 colonies. The samples from L. Reuteri NCIMB 30351 fermentation in MRS media in this grouping had only 8.37% of the colonies compared to samples from 0.9% saline solution.

Clostridium Difficile sample plates from L. Reuteri NCIMB 30351 fermentation in MRS media had an average 16.33 colonies. This is also a drastic difference to the number of colonies observed to have grown after incubation in 0.9% Saline solution, with an average of 241 colonies. The samples from L. Reuteri NCIMB 30351 fermentation in MRS media in this grouping had only 6.78% of the colonies compared to samples from 0.9% saline solution.

## Conclusions:

The addition of each Clostridial species in this study to each L. Reuteri NCIMB 30351 fermentation shows that L. Reuteri is effective at inhibiting the presence of Clostridium Perfringens Type A, Clostridium Perfringens Type C, and Clostridium Difficile, compared to 0.9% saline solution alone.

There may be several reasons for this, as clostridial species may experience environmental competition for resources from L. Reuteri. Additionally, L. Reuteri is known to produce the antimicrobial REUTERIN, which may negatively affect clostridium survival. Lastly, L. Reuteri fermentation is highly acidic, with pH typically ranging between 2.0 to 3.0; with the clostridial species tested being unable to effectively survive in such a low pH environment.