ASSESSING THE SYNERGISTIC EFFECTS OF *Akkermansia muciniphila* AND *Bacillus subtilis* AGAINST ESBL-PRODUCING *Escherichia coli*: AN IN VITRO STUDY

Chris Diamante, Chris Calumpang, Kiara Denise Nodado, Karylle Dumanglas, Jeryn Portrias, Gwyneth Kadile, Elar Cataylo, Jansenn Dy, Samantha Bernadas, Kate Pacional, Hannah Tubio

Institute of Clinical Laboratory Sciences, Silliman University

Contact Info: chrispdiamante@su.edu.ph;hannahdtubio@su.edu.ph

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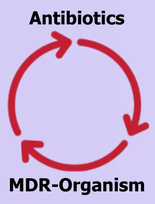
BACKGROUND OF THE STUDY

Multidrug-resistant (MDR) organisms

- particularly *Escherichia coli,* are recognized as one of the most serious global health threats of this century.

Resistance is worsened

- by the overuse of antibiotics such as fluoroquinolones, which has led to decreased efficacy in treatments for infections.

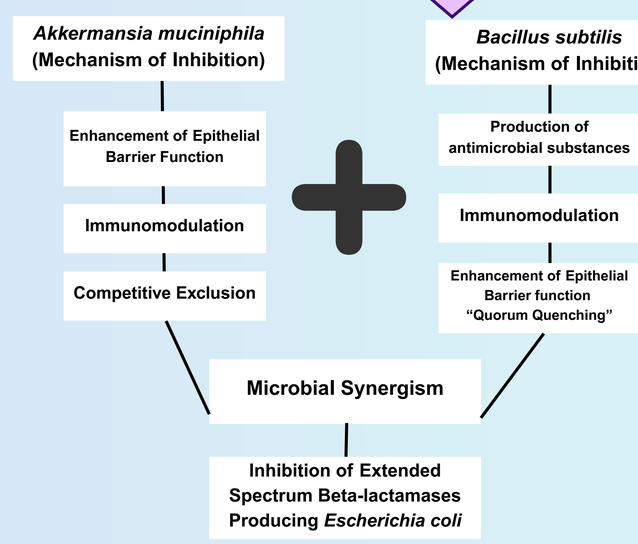


Potential of Probiotics

- This study assesses the potential of probiotics, specifically *A. muciniphila* and *B. subtilis*, as an alternative strategy against ESBL-producing *E. coli* through in vitro studies.

Alternative treatment approach

- The global increase in MDR organisms highlights the necessity for ongoing monitoring, responsible antibiotic use, and the creation of alternative treatment approaches.



Figrue 1. Conceptual Framework Between the Synergistic mechanisms of Both *A. muciniphila* and *B. subtilis*

METHODOLOGY

Ethics Approval
- Approved by Silliman University Research Ethics Committee

Procurement of Bacteria
- Probiotic Capsule *(A. muciniphila)*
- ATCC and CDC *(B. subtilis and E. coli)*

Bacterial Identity Confirmation
- Biochemical test
- Certificate of Analysis
- PCR using AM1 and AM2 primers

Preparation of Bacterial Suspension
- McFarland concentrations

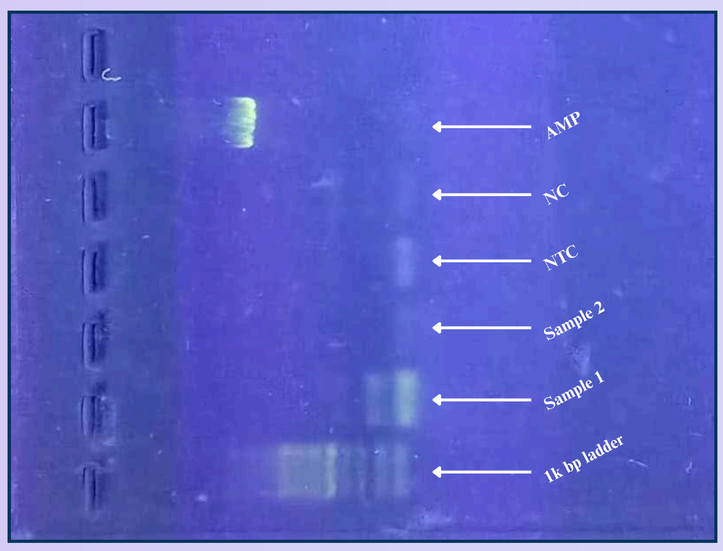
Antimicrobial Susceptibility Testing
- Broth Dilution (microdilution)

Viable Colony Count & Synergy Checkerboard Assay

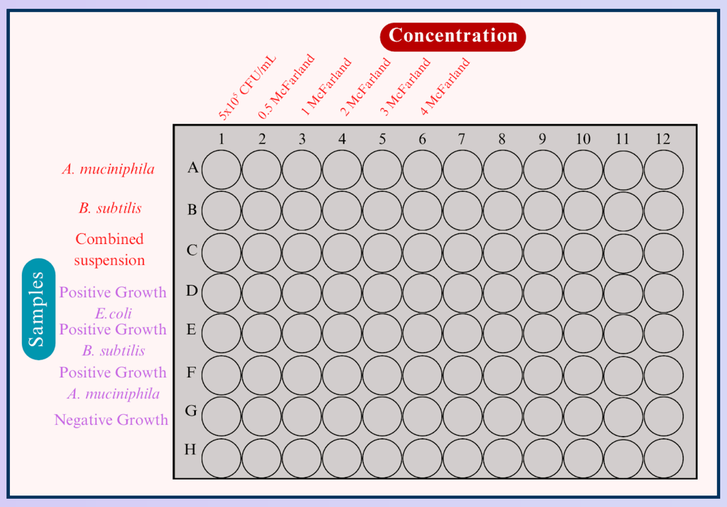
RESULTS & DISCUSSION

Bacterial Identity

Akkermansia muciniphila identity was confirmed through PCR using AM1 and AM2 primers, which are specific for *A. muciniphila. B. subtilis* and ESBL-producing *E. coli* identity were confirmed through the certificate of analysis from the supplier.



First Microdilution



In the first microdilution assay, conducted in a 96-well microplate, six concentrations of *A. muciniphila* and *B. subtilis* were tested against ESBL-producing *E. coli* at a working inoculum of 5×10^5 CFU/mL. The result shows no evidence of bactericidal activity.

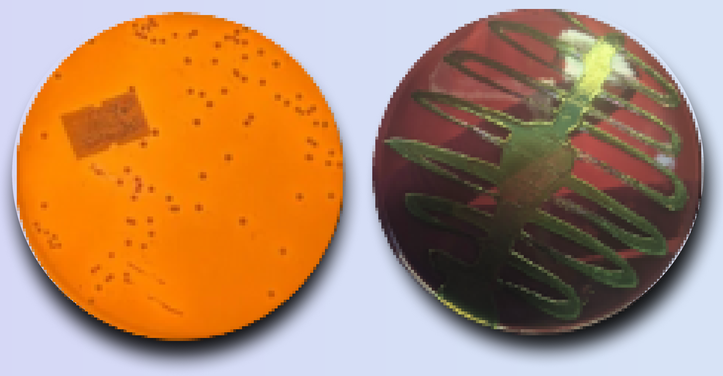
In response to this outcome, the researchers considered two possible explanations:

(1) the concentrations of *B. subtilis* and *A. muciniphila* may have been insufficient to kill the ESBL-producing *E. coli*; and

(2) the two organisms may not possess bactericidal properties against ESBL-producing *E. coli,* but rather exert only bacteriostatic effects, inhibiting replication without killing the bacteria.

Second Microdilution

The initial trial demonstrated no evidence of bactericidal activity. Therefore, a second trial was conducted to determine whether the organisms instead exhibited bacteriostatic effects. To facilitate this, the working ESBL-producing *E. coli* suspension was diluted from 5×10^5 CFU/mL to approximately 150 CFU/mL. A reference plate was also made to confirm that the concentration of ESBL-producing *E. coli* used in the assay is countable, In theory, if the organisms were bacteriostatic, colony counts should match the reference. If bactericidal, counts should be reduced. Second microdilution yielded to too numerous to count colonies in all wells.



CONCLUSIONS

- *A. muciniphila* (WB-STR-001), *B. subtilis* (ATCC 6051), and their combined suspension were found to exhibit neither bactericidal nor bacteriostatic effects against ESBL-producing *E. coli* (CDC-AR-0346).
- As a result of this outcome, the researchers did not proceed with the Synergy Checkerboard Assay.
- This study refines existing protocols and, for the first time, reports the isolation of *Akkermansia muciniphila* from a probiotic capsule verified through PCR, while encouraging exploration of other strains with variable antimicrobial activity.

RECOMMENDATIONS

- Future studies are encouraged to explore the use of alternative strains of *A. muciniphila* and *B. subtilis*, as antimicrobial activity can vary between strains.
- Employing other in vitro methods, such as agar well diffusion or time-kill assays, may serve as a pilot study before proceeding to microdilution as defined in this study.
- In vivo or ex vivo models may also offer more physiologically relevant data on the antagonistic potential of these probiotics against pathogenic bacteria.
- Although the study was halted due to the undesirable results and did not proceed to the Synergy Checkerboard Assay, the researchers chose to include the assay's methodology in the paper to serve as a reference for future researchers.

Read Full Paper and References