Submission ID #: 63707
Scriptwriter Name: Sweety Arora
Project Page Link: https://www.jove.com/account/file-uploader?src=19430683

Title: Investigating the Alleviating Effects of *Bacillus cereus* Administration on Colitis Through Gut Microbiota Modulation

Authors and Affiliations:

Kangliang Sheng[1,2,3], Yifan Xu[1,2,3], Jian Yang[1,2,3], Huijuan Ren[1,2,3], Ziwei Su[1,2,3], Yongzhong Wang[1,2,3,4]

[1]School of Life Sciences, Anhui University
[2]Key Laboratory of Human Microenvironment and Precision Medicine of Anhui Higher Education Institutes, Anhui University
[3]Anhui Key Laboratory of Modern Biomanufacturing
[4]Institute of Physical Science and Information Technology, Anhui University

Corresponding Author:
Yongzhong Wang yzwang@ahu.edu.cn

Co-authors:
Kangliang Sheng kangliang@ahu.edu.cn
Yifan Xu 18815615165@163.com
Jian Yang Y1997040637@163.com
Huijuan Ren rhuij224548@163.com
Ziwei Su suziwei731011@163.com

Author Questionnaire

1. Microscopy: Does your protocol demonstrate the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or similar? **YES**

2. Software: Does the part of your protocol being filmed demonstrate software usage? **NO**

3. Interview statements: Please select one.

☒ Interviewees self-record interview statements.

☐ Interview Statements are read by JoVE's voiceover talent.

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here:

When you are ready to submit your video files, please contact our China Location Producer, Yuan Yue.

Protocol Length
Number of Steps: 21
Number of Shots: 41

Introduction

NOTE: Timecodes are added as provided by authors. The writer has not reviewed the footage.

1. Introductory Interview Statements

1.1. **Yihan Zhang:** This protocol allows for evaluating the bidirectional regulating effect of probiotic supplementation on health and provides stable and reproducible results **[1].**

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. Author provided timecode: DSCF0249.mp4 00:00-00:11

1.2. **Ziwei Su**: An alternative DSS *(D-S-S)*-induced colitis model was established using pseudo-germ-free mice in order to avoid some laboratory limitations in using germ-free mice as receptors for probiotics **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. Author provided timecode: DSCF0250.mp4 00:00-00:16

1.3. **Huijuan Ren**: To explore the beneficial effects of probiotics in this technique is a promising approach for developing novel treatment strategies for alleviating the symptoms of chronic inflammatory-associated disorders **[1]**.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. Author provided timecode: DSCF0252.mp4 00:00-00:17

Ethics Title Card

1.4. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) of Anhui University, China

Protocol

NOTE: Timecodes are added as provided by authors. The writer has not reviewed the footage for regular (talent) shots.

2. Administration of Antibiotics

2.1. To begin, prepare a cocktail of antibiotics by dissolving ampicillin, neomycin sulfate, metronidazole, and vancomycin in drinking water **[1]**. After complete dissolution, store the solutions at 4 degrees Celsius **[2]**.

2.1.1. Talent preparing antibiotic solutions. Author provided timecode: DSCF0151.MOV 00:00-01:15

2.1.2. Talent storing the solutions. Author provided timecode: DSCF0151.MOV 00:00-01:15

2.2. Put the antibiotic cocktail solutions into a water bottle **[1]**. Ensure to use brown bottles or wrap the bottles with aluminum foil to protect the antibiotic solutions from light. Place the bottle on top of the mouse cage **[2]**.

2.2.1. Talent putting the antibiotic cocktail solutions into a water bottle. Author provided timecode: DSCF0158.MOV 00:02-00:40

2.2.2. Talent wrapping the bottles with aluminum foil. Author provided timecode: DSCF0158.MOV 00:02-00:40

3. Preparation of DSS Drinking Water and Induction of Colitis

3.1. To make a 2.5 percent dextran sulfate sodium solution, dissolve 2.5 g of DSS in 100 milliliters of distilled water. Replace the drinking water with the 2.5 percent DSS *(D-S-S)* solution in the colitis model mice group **[1]**.

3.1.1. Talent dissolving DSS in DI water. Author provided timecode: DSCF0140.MOV 00:00-00:14 and DSCF0141.MOV 00:00-00:53 for “To make a 2.5 percent…..of distilled water”

Author provided timecode: DSCF0157.MOV 00:00-00:47 for “Replace the drinking water…….the colitis model mice group”

3.2. To assess the positive effect of *B. cereus* on alleviating colitis, randomly assign the mice to the following three groups: the control group, the DSS-induced colitis model, and probiotic *B. cereus* supplementation for DSS-induced mice **[1]**.

3.2.1. Talent assigning mice to different groups. Author provided timecode: DSCF0155.MOV 00:02-00:14

3.3. To explore the role of gut microbiota in regulating the probiotic effects of *B. cereus* on DSS-induced colitis, randomly assign the mice into the following three groups: ABX *(A-B-X)*-control, ABX-DSS, and ABX-*B. cereus* plus DSS **[1]**.

3.3.1. Talent assigning mice to different groups. Author provided timecode: DSCF0154.MOV 00:03-00:11

3.4. Record the body weight of the mice as the baseline weight **[1].** After the administration of the antibiotic cocktail, immediately treat the male C57BL/6 *(C-Fifty-Seven-B-L-Six)* mice with 2.5 percent DSS solution in drinking water for 7 days **[2]**.

3.4.1. Talent recording mice weight. Author provided timecode: DSCF0173.MOV 00:00-00:14

3.4.2. Talent treating mice with DSS solution. Author provided timecode: DSCF0179.MOV 00:00-00:08

4. Quantification of *B. Cereus*

4.1. Prepare eight 1.5-milliliter microcentrifuge tubes filled with 900 microliters of sterile normal saline **[1]**. Pipette 100 microliters of logarithmic growth *B. cereus* **[2]** and dissolve in 900 microliters of sterile normal saline **[3]**.

4.1.1. Talent preparing microcentrifuge tubes with saline. Author provided timecode: DSCF0123.MOV 00:02-00:04

4.1.2. Talent pipetting B. cereus out of tubes. Author provided timecode: DSCF0123.MOV 02:09-02:11

4.1.3. Talent dissolving normal saline into tubes. Author provided timecode: DSCF0123.MOV 02:11-02:28

4.2. Serially dilute *B. cereus* using tubes **[1]**. Plate 40 microliters of each dilution onto the respective LB *(L-B)* agar plate labeled with the dilution ratio. Repeat 3 times for each dilution ratio **[2]**.

4.2.1. Talent diluting *B. cereus*. Author provided timecode: DSCF0123.MOV 02:34-03:22

4.2.2. Talent plating dilutions onto agar plates. Author provided timecode: DSCF0130.MOV 00:00-00:41 and DSCF0133.MOV 00:01-00:47

4.3. Culture the plates in a 37 degrees Celsius constant temperature incubator for 16 hours. Select the plates with approximately 20 to 70 colonies for counting **[1]**.

4.3.1. Talent culturing the plates. Author provided timecode: DSCF0134.MOV 00:01-00:08 and DSCF0255.mp4 00:02-00:28

5. Preparation of *B. cereus* for Gavage

5.1. After the quantification of *B. cereus*, collect 1 milliliter of *B. cereus* medium containing 1 x 10^9 CFU *(C-F-U)* **[1]** and centrifuge at 8,000 *g* for 10 minutes **[2]**.

5.1.1. Talent collecting medium containing CFU. Author provided timecode: DSCF0256.mp4 00:00-00:48

5.1.2. Talent centrifuging the solution. Author provided timecode: DSCF0257.mp4 00:00-00:18

5.2. Then, wash the bacterial cells 2 times with sterile water by centrifugation at 8,000 *g* for 10 minutes **[1]** and remove the supernatant **[2]**.

5.2.1. Talent centrifuging the bacterial cells. Author provided timecode: DSCF0259.mp4 00:00-01:15 and DSCF0260.mp4 00:00-00:08

5.2.2. Talent removing the supernatant. Author provided timecode: DSCF0261.mp4 00:00-00:07 and DSCF0262.mp4 00:00-00:28

5.3. Dilute the concentrated *B. cereus* with sterile normal saline to a final concentration of 2 x 10^8 CFU per 200 microliters **[1]**. Pipette the dilution up and down repeatedly to acquire a dispersed *B. cereus* suspension **[2]**

5.3.1. Talent diluting the solution with saline. Author provided timecode: DSCF0262.mp4 00:28-01:20

5.3.2. Talent dispersing the solution by pipetting up and down. Author provided timecode: DSCF0262.mp4 00:28-01:20

6. Application of *B. cereus* Supplements by Gavage

6.1. Wipe the outside of the gavage needle with 70 percent ethanol and maintain proper dose application **[1]**.

6.1.1. Talent wiping the needle with ethanol. Author provided timecode: DSCF0254.mp4 00:02-00:09

6.2. To restrain the mice, pick them up by their tail, and grasp the loose skin of the back firmly with the end of the tail fixed between the handler's last two fingers **[1]**.

6.2.1. Talent picking the mouse up. Author provided timecode: DSCF0175.MOV 00:01-00:10

6.3. Keep the mice in a vertical position. Then, insert the gavage needle carefully and gently through the mouth into the esophagus **[1-TXT]**.

6.3.1. Talent inserts the gavage needle through the mouse's mouth. **TXT: If no resistance, gently push the needle into the stomach** Author provided timecode: DSCF0179.MOV 00:00-00:05

6.4. Administer the *B. cereus* solution **[1]**, withdraw the needle gently **[2],** and return the mouse to its cage **[3]**.

6.4.1. Talent administering B. cereus solution. Author provided timecode: DSCF0179.MOV 00:05-00:09

6.4.2. Talent withdrawing the needle. Author provided timecode: DSCF0179.MOV 00:05-00:09

6.4.3. Talent returning the mouse to its cage. Author provided timecode: DSCF0179.MOV 00:05-00:09

7. Determination of the Disease Activity Index

7.1. Measure the body weight of the mice daily. Use a cotton swab to stimulate the contraction of the anus to promote defecation and collect the feces samples immediately in a sterilized centrifuge tube **[1]**.

7.1.1. Talent collecting fecal samples from the mice. Author provided timecode: DSCF0173.MOV 00:00-01:25 for "Measure the body weight of the mice daily"

Author provided timecode: DSCF0162.MOV 00:00-00:05 for "Use a cotton swab……. in a sterilized centrifuge tube"

7.2. Determine the fecal occult blood by stool occult blood test paper. Collect 10 milligrams of stool sample on an applicator stick **[1]**.

7.2.1. Talent collecting stool sample on the applicator stick. Author provided timecode: DSCF0162.MOV 00:05-00:18

7.3. Put the specimen on the test card **[1]** and apply a thin smear to the card **[2]**. Open the back flap **[3]** and apply the developer over the smear **[4]**.

7.3.1. Talent putting the specimen on the test card. Author provided timecode: DSCF0185.MOV 00:00-00:07

7.3.2. Talent applying a smear on the card. Author provided timecode: DSCF0185.MOV 00:07-00:22

7.3.3. Talent opening the back flap. Author provided timecode: DSCF0185.MOV 00:22-00:34

7.3.4. Talent applying developer on the smear. Author provided timecode: DSCF0185.MOV 00:34-01:05

8. Tissue Collection and Assessment of Historical Damage

8.1. Fix the mice supine on the operating table **[1]** and open the peritoneal cavity **[2]**. Separate the entire colon surgically **[3]** and measure the length with a ruler **[4]**.

8.1.1. Talent fixing the mice on the operating table. Author provided timecode: DSCF0182.MOV 00:00-00:08

8.1.2. Talent opening the peritoneal cavity. Author provided timecode: DSCF0182.MOV 00:08-01:11

8.1.3. Talent separating the colon surgically. Author provided timecode: DSCF0182.MOV 01:11-04:41

8.1.4. Talent measuring the length with a ruler. Author provided timecode: DSCF0183.MOV 00:00-00:09

8.2. Then, gently wash the colon with a 5-milliliter syringe filled with precooled PBS **[1]**. Dewax and dehydrate the colon sections **[2]**. Next, stain the colon sections with hematoxylin and eosin. Let two independent experimenters evaluate the histological scores of the sample under a light microscope **[3]**.

8.2.1. Talent washing the colon with PBS. Author provided timecode: DSCF0192.MOV 00:03-00:14

8.2.2. Talent dewaxing the colon sections. Author provided timecode: DSCF0199.MOV 00:01-00:14 for “Dewax”.

DSCF0205、DSCF0206、DSCF0208、DSCF0209、DSCF0210、DSCF0211、DSCF0212、DSCF0213、DSCF0214、DSCF0215、DSCF0216、DSCF0217、DSCF0218 for “dehydrate the colon sections”. **NOTE:** Use any one footage for “dehydrate the colon sections” from the above list of footage.

8.2.3. Talent staining the colon sections. DSCF0220 0:07-0:20, DSCF0221, DSCF0222, DSCF0223, DSCF0224, DSCF0225, DSCF0228 0:02-0:08 for “Next, stain the colon sections with hematoxylin and eosin.” **NOTE:** Use relevant footage from the above list of footage.

DSCF0231 for “Let two independent experimenters evaluate the histological scores of the sample under a light microscope.”

Results

9. Results: Effect of *B. cereus* on Colitis

9.1. Hematoxylin and Eosin staining **[1]** revealed that the colon tissue of the colitis model mice presented crypt loss, goblet cell damage, inflammatory cell infiltration, and the loss of epithelium. *B. cereus* treatment improved DSS *(D-S-S)*-induced histopathological damage **[2].**

9.1.1. LAB MEDIA: Figure 2 A-B

9.1.2. LAB MEDIA: Figure 2 A

9.2. The histopathological scores of colitis mice were significantly higher than in the control group. However, the histopathological scores in the *B. cereus* group were significantly lower than in the colitis mice **[1].**

9.2.1. LAB MEDIA: Figure 2 B

9.3. Mice were randomly assigned into three groups: the body weight loss, DAI *(D-A-I)* scores, and histopathology scores were higher, and the colon length was shorter in the ABX *(A-B-X)*-DSS group compared with mice in ABX-control group **[1]**.

9.3.1. LAB MEDIA: Figures 3 A-E

9.4. The body weight loss **[1]**, colon length **[2]**, DAI scores **[3]**, and histopathology scores **[4]** were not statistically different between the ABX-DSS and ABX-*B. cereus* plus DSS groups. The present results indirectly suggest that the gut microbiota plays a key role in the positive effects of *B. cereus* on colitis **[5]**

9.4.1. LAB MEDIA: Figures 3 B

9.4.2. LAB MEDIA: Figures 3 C

9.4.3. LAB MEDIA: Figures 3 D

9.4.4. LAB MEDIA: Figures 3 E

9.4.5. LAB MEDIA: Figures 3

Conclusion

NOTE: Timecodes are added as provided by authors. The writer has not reviewed the footage.

10. Conclusion Interview Statements

10.1. **Ziwei Su:** One of the most important steps is to use *Bacillus cereus* by gavage method to construct the colitis model in mice **[1].**

10.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. Author provided timecode: DSCF0251.mp4 00:00-00:11

10.2. **Huijuan Ren:** The treatment of *Bacillus cereus* ameliorated the DSS *(D-S-S)* induced colitis, which laid a foundation for the follow-up study of probiotics for the treatment of colitis **[1].**

10.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. Author provided timecode: DSCF0253.mp4 00:00-00:15