Biochemical and Biophysical Research Communications 734 (2024) 150448

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Establishment of the improved colonization of *Escherichia coli* laboratory strain in the intestine mediated by single gene deletion

Atsushi Minami [a,1], Takehiro Asai [b,1], Tsuyoshi Tachibana [b], Yu Tanaka [b], Mitsuyuki Nakajima [b], Shiori Tamura [c], Morihiro Nakazawa [c], Yoshiharu Tsuru [d], Yoichi Fujiyama [e], Yoh-ichi Tagawa [c], Tomohisa Kuzuyama [a,f], Shigeru Kakuta [b,f,g,*], Tetsuhiro Ogawa [a,f,**]

[a] Department of Biotechnology, The University of Tokyo, Yayoi, Bunkyo-ku, Tokyo, 113-8657, Japan
[b] Department of Veterinary Medical Sciences, The University of Tokyo, Yayoi, Bunkyo-ku, Tokyo, 113-8657, Japan
[c] School of Life Science and Technology, Tokyo Institute of Technology, Nagatsuta-cho, Midori-ku, Yokohama, 226-8501, Japan
[d] Life Science Laboratory, Primetech Corporation, Yayoi, Bunkyo-ku, Tokyo, 113-8657, Japan
[e] Technology Research Laboratory, Shimadzu Corporation, Hikaridai, Seika-cho, Soraku-gun, Kyoto, 619-0237, Japan
[f] Collaborative Research Institute of Innovative Microbiology, The University of Tokyo, Japan
[g] Research Center for Food Safety, The University of Tokyo, Yayoi, Bunkyo-ku, Tokyo, 113-8657, Japan

ARTICLE INFO

Keywords:
Gut microbiome
Biofilm
Probiotics
Bacterial colonization
On-chip colonization assay
Oral administration

ABSTRACT

In light of the emerging importance of the gut microbiome in human health, there is a need to improve the colonization efficiency of therapeutic bacteria called probiotics. Despite their recognized potential, artificially administered bacteria exhibit poor colonization in the intestine, limiting their therapeutic efficacy. Addressing this challenge requires innovative strategies; however, reported examples are limited. In nature, including in the intestinal tract, bacteria live via biofilm formation. Recently, it has been reported that RNase I, a member of the RNase T2 family conserved among almost all species, including bacteria, inhibits biofilm formation in *Escherichia coli*. In this study, we focus on these results and investigate the relationship between high biofilm formation and intestinal attachment using a non-settling *E. coli* laboratory strain as a probiotic model. The intestinal colonization abilities were evaluated through a microfluidic device mimicking the intestinal tract and through oral administration to mice. The *in vitro* and *in vivo* experiments showed that the *E. coli* strain lacking RNase I exhibited remarkable stability in intestinal colonization. We investigated the observation of colonization using fluorescence *in situ* hybridization, and inoculated *E. coli* cells were aggregated with the gut microbiome in the cecum and colon. This study proposes a technique to improve the intestinal colonization of bacteria by simply manipulating a single gene disruption, and it is expected to contribute to future research on the colonization of useful bacteria.

1. Introduction

Higher organisms, like animals and plants, live in symbiosis with various microorganisms. Approximately 90 % of the bacteria colonized in humans are found in the gastrointestinal tract, called the gut microbiome [1]. In recent years, the influence of the gut microbiome on systemic diseases and health, such as cancer prevention and immune system development, has been recognized [2,3]. Moreover, the brain and gut are closely related via the autonomic nervous system and humoral factors known as the gut–brain axis, which has recently been reported to be influenced by the gut microbiome [4]. Many studies have focused on maintaining health and prevention of disease using beneficial living bacteria called probiotics, such as *Lactobacillus* and *Bifidobacterium* [5]. Probiotics are defined as those that affect the maintenance of the gut microbiome, suppress pathogen propagation, and regulate the immune system [6]. These bacteria can maximally benefit from

Abbreviations: CFU, colony-forming unit; FISH, fluorescence *in situ* hybridization; OD, optical density.

* Corresponding author. Department of Veterinary Medical Sciences, The University of Tokyo, Yayoi, Bunkyo-ku, Tokyo, 113-8657, Japan.
** Corresponding author. Department of Biotechnology, The University of Tokyo, Yayoi, Bunkyo-ku, Tokyo, 113-8657, Japan.
E-mail addresses: a-skakuta@g.ecc.u-tokyo.ac.jp (S. Kakuta), atetsu@g.ecc.u-tokyo.ac.jp (T. Ogawa).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.bbrc.2024.150448
Received 12 July 2024; Accepted 24 July 2024
Available online 25 July 2024

colonizing the intestine; however, artificially administered bacteria typically cannot establish themselves in the intestinal tract for a long period. Such low colony-forming capacity is due to the elimination pressure caused by the endogenous bacteria in the intestine, intestinal immunity, and intestinal movement, often resulting in their excretion shortly after administration [7]. Therefore, attempts have been made to improve the colonization of probiotics in the intestine [8–11]. One of the keys to solve the issue lies in bacterial aggregate structures called biofilms. Most environmental bacteria, including the gut microbiome, live by forming biofilms [12,13]. Therefore, if the probiotics can improve their ability to form a biofilm, their performance will be maximized. Joshi's group reports one successful example [10]. They expressed curli fibers involved in biofilm formation from plasmids to induce cell–cell aggregation in Nissle 1917, the only *Escherichia coli* strain recognized as probiotics. In addition, they succeeded in promoting intestinal epithelial repair by fusing the curli fibers with a therapeutic factor. However, this method is restricted to bacteria that can stably retain the plasmid; therefore, its use seems limited. Recently, Weinert and co-workers demonstrated that deleting the gene for RNase T2 (it is called RNase I in *E. coli*) significantly improves the biofilm formation ability of laboratory strain *E. coli* BW25113 [14]. They showed that host RNA degradation intermediates, 2′,3′-cyclic nucleotide monophosphates, produced by RNase I, act as second messengers to suppress biofilm formation. Therefore, we expected that only a single gene deletion confers a high colonization ability in the intestinal tract via biofilm formation. This study shows that increasing biofilm-forming capacity improves the stable settlement of bacteria in the intestinal tract using the RNase I-deficient *E. coli* as a model bacterium. These results are expected to break new ground by establishing methodologies to improve useful bacterial functions.

2. Materials and methods

2.1. Bacterial strains and plasmids

Strains and plasmids used in this study are listed in Supplementary Table S1. The RNase I-deficient strain (Δ*rna*) and pseudogene-deleted strain (Δ*intG*), used as a negative control, were obtained from the Keio Collection, provided by the National BioResource Project-E. coli (National Institute of Genetics, Japan) [15]. The kanamycin-resistance cassette of Δ*rna* was eliminated by homologous recombination using the plasmid 707-FLPe (Gene Bridges, Heidelberg, Germany).

The plasmid placlux8 encoding *Photorhabdus luminescens luxCDABE* genes was kindly gifted by Dr. Takeshi Shimizu (Chiba University, Japan) [16]. The pAcGFP1 and ptdTomato plasmids were purchased from TaKaRa Bio (Shiga, Japan). The replacement of the ampicillin-resistance cassette with that of kanamycin in placlux8, pAcGFP1, and ptdTomato was carried out by the seamless cloning method (In-Fusion HD Cloning Kit, TaKaRa Bio, Shiga, Japan). *E. coli* cells were cultivated in L-broth (1 % tryptone, 0.5 % yeast extract, and 0.5 % NaCl) with ampicillin (100 μg/mL) or kanamycin (30 μg/mL) added as required.

2.2. Mice

Slc:ICR strain female mice aged 4–10 weeks were obtained from Japan SLC (Shizuoka, Japan). All mice were fed a pellet diet (CRF-1, Oriental Yeast) and were allowed to drink tap water or tap water-solvent antibiotic solution *ad libitum*. Mice were housed in negative pressure racks in a controlled laboratory environment with a temperature of 23 ± 2 °C, a humidity of 55 ± 5 %, and a 12-h light/dark cycle. Mice were acclimated to the facility environment for at least one week before experiments. All experiments complied with The University of Tokyo Manual for the Conduct of Animal Experiments and were approved by the Institutional Animal Care and Use Committee of the Graduate School of Agricultural and Life Sciences, The University of Tokyo, with Approval Nos. P18–144H07 and P23–034H02.

2.3. On-chip colonization assay

The porous membrane of the microfluidic device was coated with 2 % atelocollagen (KOKEN, Tokyo, Japan), and 2×10^4 cells of C2BBe1 (ATCC, VA, USA), a subline of Caco-2 cells, were seeded on this porous membrane in the apical channel. The device was cultured statically, and the medium was changed daily. After 3 days, the plateau was reached in the trans Epithelial Electrical Resistance (TEER) of C2BBe1 cells. An overnight culture of *E. coli* in Dulbecco's Modified Eagle Medium (DMEM, high glucose) containing 100 μg/mL ampicillin, 10 % fetal bovine serum (FBS), and 1 % transferrin was diluted to an optical density at 600 nm (OD_{600}) of 0.01, and 100 μL of that was added to the upper channel of the device. After standing for 30 min, incubation was carried out for 30 h at a flow rate of 500 μL/h.

2.4. Observation of colonization in vivo

The colonization of inoculated *E. coli* in the mouse intestinal tract was observed based on a previous report [10]. Tap water containing 0.25 g/L carbenicillin was given *ad libitum* one day before the bacteria inoculation. For the inoculation of *E. coli* carrying a plasmid coding a kanamycin-resistant gene, 3 g/L kanamycin was used for 3 d instead of carbenicillin. Mice were fasted on the morning of the inoculation day for *E. coli* culture. *E. coli* cells were cultured until OD_{660} reached 0.8. After 4–6 h of fasting, mice were inoculated orally with 200 μL of *E. coli* culture from each strain. Feces were collected at the indicated time, crushed, and suspended in 0.9 % saline using a BioMasher II (Nippi, Tokyo, Japan). To measure the bioluminescence of cells expressing bacterial luciferase, 100 μL of fecal suspension was applied to a luminometer AB-2200 (ATTO, Tokyo, Japan). The viable bacterial cell number was determined as follows: Cells in fecal suspension were serially diluted with saline and spread to L-broth medium plates containing 100 μg/mL ampicillin or 30 μg/mL kanamycin. After incubation at 37 °C overnight, the plates were photographed with an ImageQuant LAS 500 (GE Healthcare, IL, USA). Then, the colony-forming unit (CFU) was calculated by counting the number of colonies with bioluminescence. *In vivo* imaging using Ami HTX (Spectral Instruments Imaging, AZ, USA) was performed to visualize the colonization of *E. coli* cells in the mouse intestine.

2.5. Competitive colonization assay

Wild-type and Δ*rna E. coli* strains were cultivated in L-broth. When OD_{660} reached 0.8, the 200 μL of each cell culture was mixed, and the resultant 400 μL of the mixture was administered to mice 3 d after the pretreatment with antibiotics. Cell suspension was prepared from feces and spread on L-broth plates, as described in Section 2.4. Then, the plates were photographed with an iBright FL1500 imaging system (Thermo Fisher Scientific, MA, USA). The CFUs of wild-type and Δ*rna* strains were calculated by measuring the number of colonies exhibiting the fluorescence derived from GFP and tdTomato, respectively.

2.6. Fluorescence in situ hybridization (FISH) analysis

FISH assays were performed as previously described [17] with slight modifications. Mice were euthanized by cervical dislocation, and the cecum and colon were sampled. The tissue samples were immersed and fixed in methanol-Carnoy's fixative solution (60 % methanol, 30 % chloroform, and 10 % glacial acetic acid) for 4 h at room temperature. Subsequently, the tissue pieces were transferred to methanol and stored. After xylene displacement treatment, the fixed tissues were then embedded in paraffin. Tissues were sectioned at 10 μm thickness, dried, and deparaffinized by preheating at 50 °C, followed by bathing in xylene for 10 min, 100 % ethanol for 10 min, 90 % ethanol for 5 min, and 70 %

ethanol for 5 min, and treated with 0.1 M hydrochloric acid.

The hybridization step was performed at 50 °C overnight in the dark with a 3′ Alexa Fluor 488-labeled EUB338 probe and a 3′ Alexa Fluor 647-labeled ECO636 probe [18] diluted to a final concentration of 1 % in hybridization buffer (0.9 M NaCl, 20 mM Tris-HCl [pH 7.6], and 0.01 % SDS). The EUB338 and ECO636 probes were used for pan bacteria and *E. coli*, respectively. After washing in FISH washing buffer (0.45 M NaCl, 20 mM Tris-HCl [pH 7.6], and 0.01 % SDS) at 50 °C, slides were immersed in PBS for 10 min at room temperature, followed by staining with DAPI (Vector Laboratories, CA, USA) and covering with a coverslip. The slides were observed with a confocal laser scanning microscopy LSM700 (Carl Zeiss, Oberkochen, Germany). The sequences of the two probes used in this study are shown in Supplementary Table S2. These were synthesized by Integrated DNA Technologies, Inc. (IA, USA).

3. Results and discussion

3.1. Visualization of *E. coli* colonization in vitro using a microfluidic intestinal device

First, we evaluated the *in vitro* colonization ability of the Δ*rna* strain, showing a high biofilm-forming ability (Supplementary Fig. 1), using a microfluidic device that mimics the intestine environment. The device has a two-layered structure, with C2BBe1 cells adhering to one of the layers and bacteria flowing in the channel above it; as a result, its colonization ability can be evaluated (Fig. 1A). The culture of *E. coli* wild-type or Δ*rna* strain expressing GFP from a plasmid was added to the flow path of the device. Their colonization on the cells was visualized over time for 30 h. At the end of 21 h, biofilms of the Δ*rna* strain began to form, and clear structures were observed in the next 7 h. In contrast, no biofilm formation was observed in the wild-type strain during the observation period (Fig. 1B, Supplementary Movies S1 and S2). These results suggested that the Δ*rna* strain was retained in the intestinal tract for a more extended period than wild-type one (Fig. 1B).

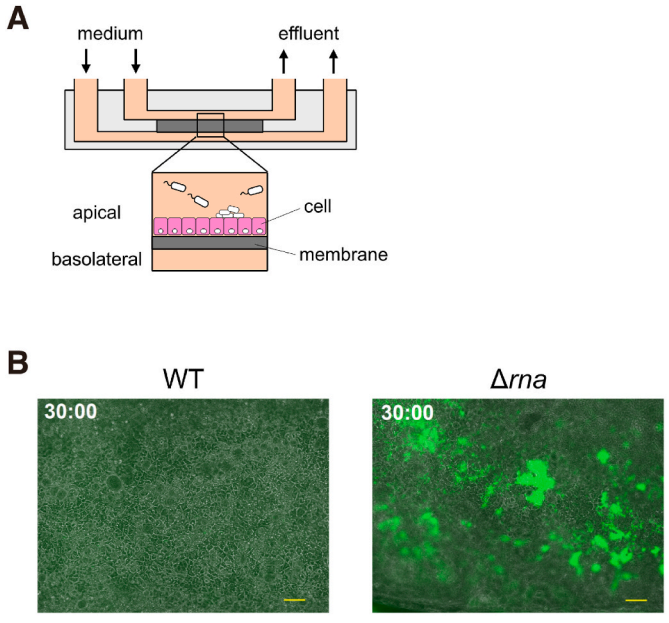

Fig. 1. Visualization of the colonization ability of *E. coli* strains in the microfluidic devices
(A) Schematic diagram of the microfluidic device structure mimicking the intestine. (B) Observation of colonization on the microfluidic devices using time-lapse fluorescence microscopy. Images at 30 h, when biofilms in the Δ*rna* strain were considerably observed, were used as representative. The scale bars represent 100 μm.

3.2. Utilization of a method for evaluation of the colonization of *E. coli* inoculated to mice in the intestine

To determine whether the Δ*rna* strain stably colonizes in the intestinal tract, we orally inoculated the strain into mice and attempted to assess its retention. The evaluation of intestinal bacterial colonization ability in mice relies on measuring the number of viable bacteria in fecal suspensions [19]. This method depends on the measurement of CFUs, which can be laborious in the case of large numbers of samples. To improve the efficiency of the experiment process, we used *E. coli* cells carrying the plasmid placlux8. This plasmid encodes a *Photorhabdus luminescens luxCDABE* gene cluster encoding luciferase and its substrate-producing enzymes, enabling observation of autoluminescence without needing an external substrate supply [16]. Therefore, cell number can be estimated based on the intensity of bioluminescence in the fecal suspension. We confirmed that the intensity of bioluminescence in the fecal suspension correlated with CFU (Supplementary Fig. S2). Here, carbenicillin was included in the drinking water before and after inoculation with *E. coli* to standardize between individuals by eliminating endogenous bacteria in the mouse intestinal tract and maintaining placlux8 (carrying the ampicillin resistance gene, see Section 2.1). We assessed the colonization ability of wild-type and Δ*rna* strains in the intestinal tract of 5-, 7-, 9-, and 11-week-old mice. The Δ*rna* strain was shed in feces for over two weeks in mice of all ages used in the experiments. In contrast, the wild-type strain either ceased to be shed or shed significantly less throughout the experiment (Fig. 2A).

To further evaluate the colonization ability of the inoculated *E. coli* in mice, non-invasive *in vivo* imaging was performed on mice 14 d after inoculation (Fig. 2B). Strong intensity of bioluminescence was observed near the intestinal tract of mice inoculated with the Δ*rna* strain. In contrast, such bioluminescence was not observed in mice inoculated with the wild-type strain. Next, to identify the colonization site of *E. coli* in the mouse intestinal tract in more detail, the mouse intestine was dissected after euthanasia, and the intensity of bioluminescence was measured. Bioluminescence of the Δ*rna* cells was observed from the cecum to the large intestine, and it was most prominent in the cecum (Fig. 2C). The cecum plays the role of a “food fermentation tank” to maintain the balance of the gut microbiome in mice. Consequently, Δ*rna* cells may mainly accumulate in the cecum.

3.3. Confirmation of enhanced colonization ability by competitive assay

Our results show that Δ*rna* strains are stably retained in the mouse intestinal tract. However, after repeated experiments, a few individuals occasionally showed extremely low retention of inoculated bacteria. (Supplementary Fig. S3). Considering that insufficient elimination of endogenous bacteria was the cause of this problem, we changed the antibiotic used from carbenicillin to kanamycin, and the conditions for the concentration used in drinking water were examined. As a result, using kanamycin at 3 g/L improved the reproducibility of the results to a certain extent; however, it sometimes provided individual differences. Therefore, we performed the competitive assay in which two strains were administered simultaneously, and the ratio of the colonized cell numbers was calculated. To distinguish between the two bacterial strains, we introduced plasmids expressing AcGFP1 and tdTomato to the wild-type and Δ*rna* strains, respectively, and the ratio of the CFU with the different fluorescence was determined. As a result, the proportion of colonies derived from wild-type strains declined significantly within 1 d of inoculation, and by day 4 post-inoculation, almost all the colonies were Δ*rna*-derived (Fig. 3). Therefore, this result indicates that the Δ*rna* strain can be dominant against *E. coli* with low colonization ability.

3.4. Micro-level observation of colonization of inoculated *E. coli* in the intestinal tract

In vivo imaging (Fig. 2B and C) shows that administered *E. coli* cells

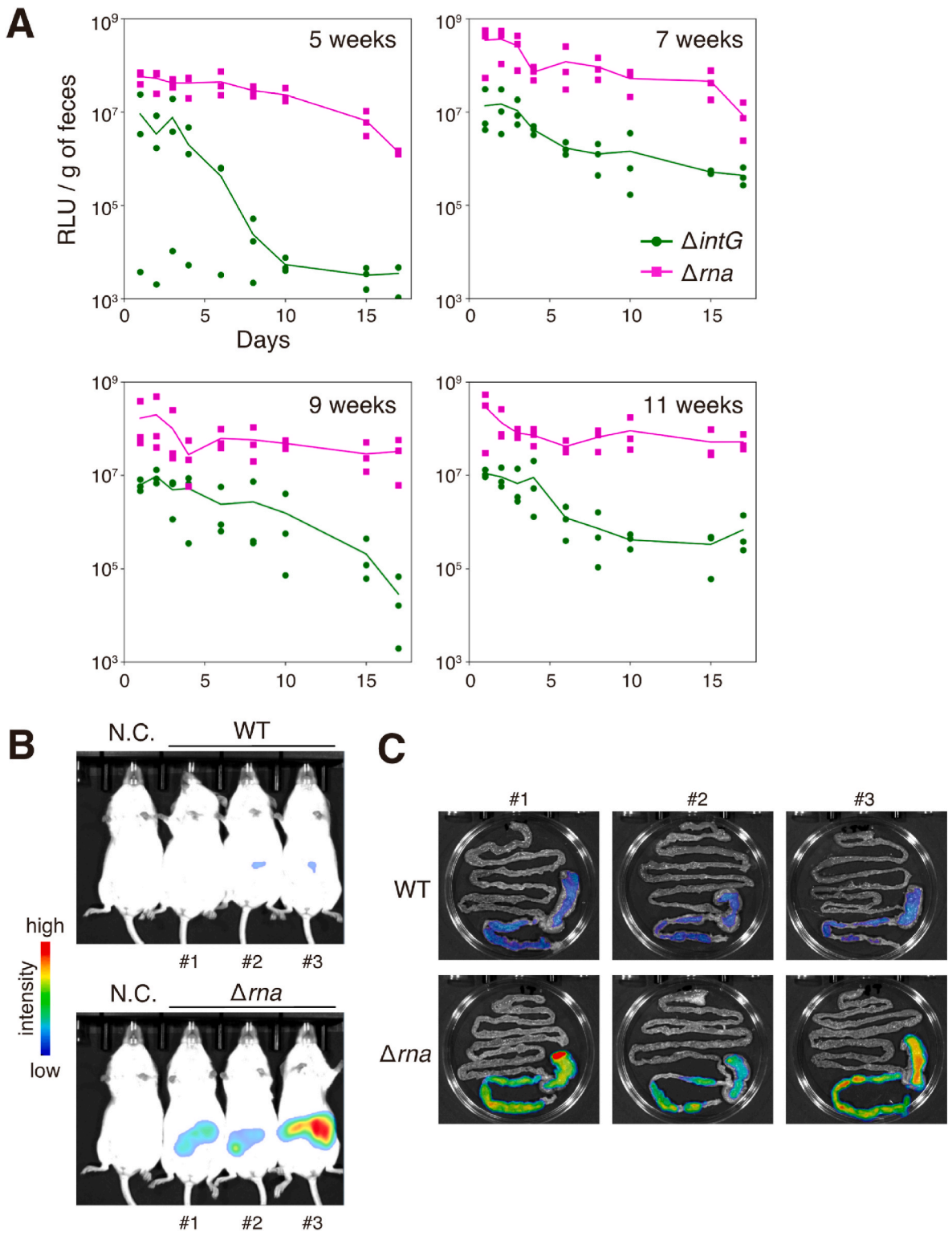

Fig. 2. Observation of colonization of the inoculated *E. coli* strains in the intestine
(A) 5-, 7-, 9-, and 11-week-old mice shown in the figure were inoculated with the Δ*rna* strain (magenta, square mark) or control strain (Δ*intG*, green, circle mark). Each strain harbors the bacterial luciferase expression plasmid placlux8, and the bioluminescence of the fecal suspension was measured at the indicated days. "RLU" indicates relative light units. (B) Non-invasive *in vivo* imaging of 9-week-old mice 14 d after *E. coli* inoculation was visualized using Ami HTX. "N.C." indicates the non-inoculated mice used as a negative control. (C) Incised intestines from mice shown in (B) were imaged using Ami HTX. Representative individual images are shown. The numbers above panels in (C) correspond to the number of mice from which the extractions were made, shown at the bottom of (B). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

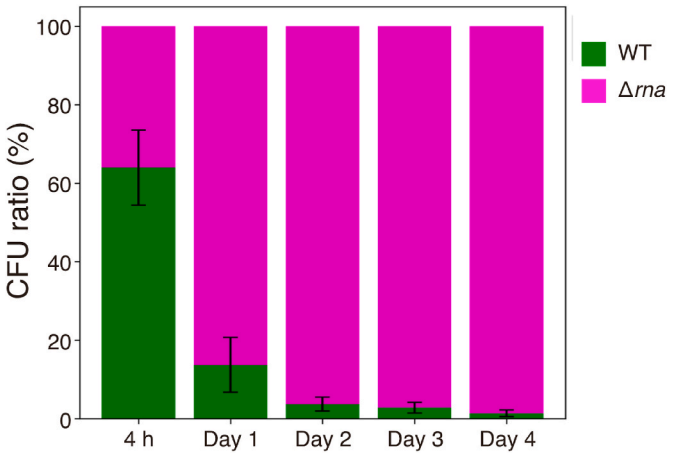

Fig. 3. Quantification of the colonization ability of the Δ*rna* strain using a competitive assay
The CFU ratio between the wild-type strain (green) and the Δ*rna* strain (magenta) in mouse feces is shown. Wild-type and Δ*rna* strains carrying the plasmid AcGFP1-Km or tdTomato-Km, respectively, were inoculated into 9-week-old mice. After visible colonies were obtained from mouse feces by plating on L-broth, fluorescent images of the plates were visualized, and the number of colonies with each type of fluorescence emission was counted. Data are expressed as the means ± standard error of eight individual mouse feces. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

were colonized mainly in the cecum and the colon. Then, the FISH assay was carried out after 14 d of inoculation to visualize a detailed view of the colonization of the inoculated *E. coli* Δ*rna* cells on the intestinal epithelium. Consistent with the bioluminescence imaging of the extracted intestine (Fig. 2C), administered Δ*rna* cells were detected in the cecum and colon when an *E. coli*-specific probe was used (Fig. 4). The colony formation of inoculated *E. coli* cells appears to be a co-aggregation with endogenous cells in the gut microbiome, indicating that *E. coli* cells do not form colonies alone but that multiple species of bacteria aggregate to form colonies near mucus layer of colon. Due to the limited detection sensitivity of FISH, more *E. coli* cells may have aggregated into a so-called biofilm shape, as in the result of the on-chip assay.

In summary, we demonstrated that *E. coli* with high biofilm-forming ability (Δ*rna* strain) stably attaches to the mouse intestine; the retention lasts at least 2 weeks in this study. Our findings are expected to apply to technologies that enable the long-term establishment of valuable bacteria in the gut [20]. Recent advances in synthetic biology have made it possible to incorporate a variety of valuable functions into model bacteria, such as *E. coli* [21,22]. Although the RNase T2 family is widely present, it has not yet been found in gram-positive bacteria such as lactic acid bacteria. Finding a ribonuclease that exhibits a function equivalent to RNase T2 in gram-positive bacteria is expected to expand the availability of valuable bacteria significantly.

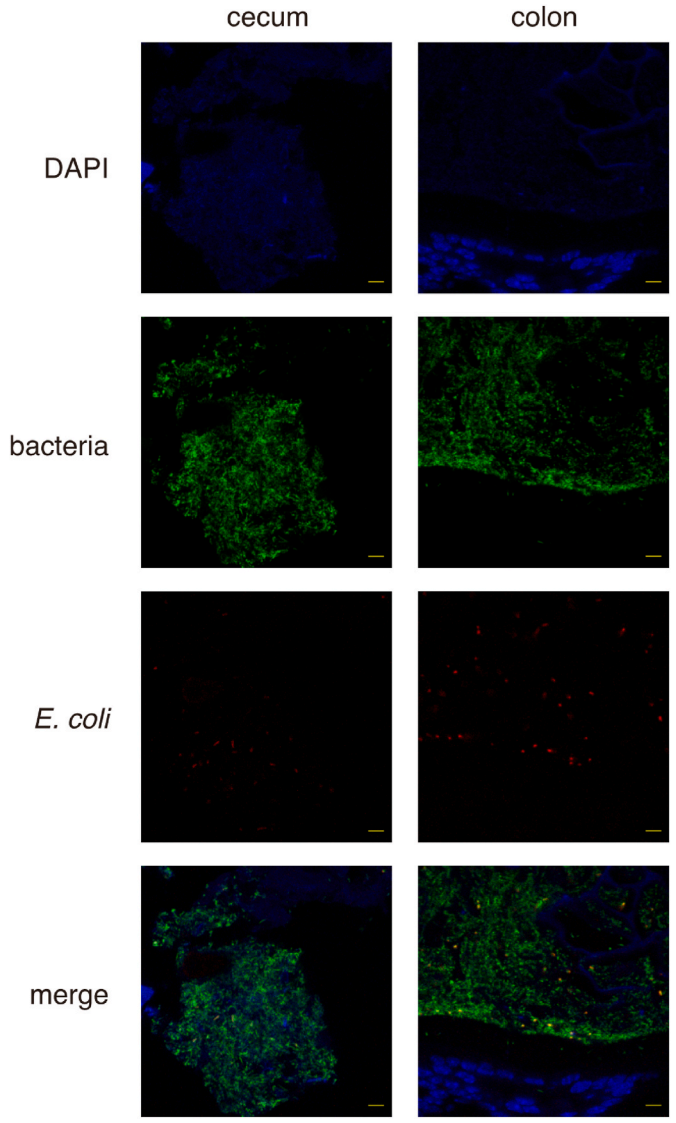

Fig. 4. Visualization of administered *E. coli* strain colonization in the intestine using FISH
The FISH assay was applied to the cecum and colon tissue fixed with methanol-Carnoy's fixative solution. The EUB338 (green) and the ECO636 (red) probes were used to detect pan bacteria or *E. coli*, respectively. The nucleolus was stained with DAPI (blue). Representative images of the cecum and colon 14 d after inoculation with Δ*rna* cells were shown. The scale bars indicate 10 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Funding

This work was supported by the JSPS KAKENHI (grant numbers JP21K18229 to S.K. and JP20K21266 and JP23H02122 to T.O.), by funding from the Institute for Fermentation, Osaka (IFO), Japan to T.O., and by JST SPRING (grant number JPMJSP2108) to A.M.

CRediT authorship contribution statement

Atsushi Minami: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Resources, Visualization, Writing – original draft. **Takehiro Asai:** Investigation, Visualization, Writing – review & editing. **Tsuyoshi Tachibana:** Investigation, Visualization. **Yu Tanaka:** Investigation, Visualization. **Mitsuyuki Nakajima:** Investigation. **Shiori Tamura:** Investigation, Visualization. **Morihiro Nakazawa:** Investigation. **Yoshiharu Tsuru:** Resources. **Yoichi Fujiyama:** Resources. **Yoh-ichi Tagawa:** Supervision, Writing – review & editing. **Tomohisa Kuzuyama:** Supervision, Writing – review & editing. **Shigeru Kakuta:** Funding acquisition, Resources, Supervision, Writing – review & editing. **Tetsuhiro Ogawa:** Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing finantial interests or personal relationships that could have appeared to influence

the work reported in this paper.

Acknowledgments

We are grateful to Dr. Takeshi Shimizu for providing the plasmid and Dr. Keita Nishiyama for technical assistance with the experiments.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrc.2024.150448.

References

[1] R. Sender, S. Fuchs, R. Milo, Revised estimates for the number of human and bacteria cells in the body, PLoS Biol. 14 (2016) e1002533, https://doi.org/10.1371/journal.pbio.1002533.
[2] J.L. Round, S.K. Mazmanian, The gut microbiota shapes intestinal immune responses during health and disease, Nat. Rev. Immunol. 9 (2009) 313–323, https://doi.org/10.1038/nri2515.
[3] A.W. Walker, T.D. Lawley, Therapeutic modulation of intestinal dysbiosis, Pharmacol. Res. 69 (2013) 75–86, https://doi.org/10.1016/j.phrs.2012.09.008.
[4] G. Agirman, K.B. Yu, E.Y. Hsiao, Signaling inflammation across the gut-brain axis, Science 374 (2021) 1087–1092, https://doi.org/10.1126/science.abi6087.
[5] P. Hemarajata, J. Versalovic, Effects of probiotics on gut microbiota: mechanisms of intestinal immunomodulation and neuromodulation, Therap. Adv. Gastroenterol. 6 (2013) 39–51, https://doi.org/10.1177/1756283X12459294.
[6] C.M. Thomas, J. Versalovic, Probiotics-host communication: modulation of signaling pathways in the intestine, Gut Microb. 1 (2010) 148–163, https://doi.org/10.4161/gmic.1.3.11712.
[7] S. Han, Y. Lu, J. Xie, Y. Fei, G. Zheng, Z. Wang, J. Liu, L. Lv, Z. Ling, B. Berglund, M. Yao, L. Li, Probiotic gastrointestinal transit and colonization after oral administration: a long journey, Front. Cell. Infect. Microbiol. 11 (2021) 609722, https://doi.org/10.3389/fcimb.2021.609722.
[8] S. Etzold, D.A. MacKenzie, F. Jeffers, J. Walshaw, S. Roos, A.M. Hemmings, N. Juge, Structural and molecular insights into novel surface-exposed mucus adhesins from Lactobacillus reuteri human strains, Mol. Microbiol. 92 (2014) 543–556, https://doi.org/10.1111/mmi.12574.
[9] M.X. Maldonado-Gómez, I. Martínez, F. Bottacini, A. O'Callaghan, M. Ventura, D. van Sinderen, B. Hillmann, P. Vangay, D. Knights, R.W. Hutkins, J. Walter, Stable engraftment of Bifidobacterium longum AH1206 in the human gut depends on individualized features of the resident microbiome, Cell Host Microbe 20 (2016) 515–526, https://doi.org/10.1016/j.chom.2016.09.001.
[10] P. Praveschotinunt, A.M. Duraj-Thatte, I. Gelfat, F. Bahl, D.B. Chou, N.S. Joshi, Engineered E. coli Nissle 1917 for the delivery of matrix-tethered therapeutic domains to the gut, Nat. Commun. 10 (2019) 1–14, https://doi.org/10.1038/s41467-019-13336-6.
[11] A.N. Samuels, M. Roggiani, J. Zhu, M. Goulian, R.M. Kohli, The SOS response mediates sustained colonization of the mammalian gut, Infect. Immun. 87 (2019), https://doi.org/10.1128/IAI.00711-18.
[12] H.-C. Flemming, S. Wuertz, Bacteria and archaea on Earth and their abundance in biofilms, Nat. Rev. Microbiol. 17 (2019) 247–260, https://doi.org/10.1038/s41579-019-0158-9.
[13] J.-P. Motta, J.L. Wallace, A.G. Buret, C. Deraison, N. Vergnolle, Gastrointestinal biofilms in health and disease, Nat. Rev. Gastroenterol. Hepatol. 18 (2021) 314–334, https://doi.org/10.1038/s41575-020-00397-y.
[14] B.M. Fontaine, K.S. Martin, J.M. Garcia-Rodriguez, C. Jung, L. Briggs, J. E. Southwell, X. Jia, E.E. Weinert, RNase I regulates Escherichia coli 2',3'-cyclic nucleotide monophosphate levels and biofilm formation, Biochem. J. 475 (2018) 1491–1506, https://doi.org/10.1042/BCJ20170906.
[15] T. Baba, T. Ara, M. Hasegawa, Y. Takai, Y. Okumura, M. Baba, K.A. Datsenko, M. Tomita, B.L. Wanner, H. Mori, Construction of Escherichia coli K-12 in-frame, single-gene knockout mutants: the Keio collection, Mol. Syst. Biol. 2 (2006) 2006.0008, https://doi.org/10.1038/msb4100050.
[16] T. Shimizu, Y. Ohta, H. Tsutsuki, M. Noda, Construction of a novel bioluminescent reporter system for investigating Shiga toxin expression of enterohemorrhagic Escherichia coli, Gene 478 (2011) 1–10, https://doi.org/10.1016/j.gene.2011.01.006.
[17] M.E.V. Johansson, G.C. Hansson, Preservation of mucus in histological sections, immunostaining of mucins in fixed tissue, and localization of bacteria with FISH, Methods Mol. Biol. 842 (2012) 229–235, https://doi.org/10.1007/978-1-61779-513-8_13.
[18] R. Aoi, S. Shimizu, K. Yamazaki, T. Sawabe, Y. Kawai, Rapid quantification of Escherichia coli as an indicator of food contamination using fluorescence in situ hybridization with filter cultivation (FISHFC), Nippon Shokuhin Kagaku Kogaku Kaishi 58 (2011) 483–489, https://doi.org/10.3136/nskkk.58.483.
[19] G.W. Tannock, Analysis of the intestinal microflora: a renaissance, Antonie Leeuwenhoek 76 (1999) 265–278, https://doi.org/10.1023/A:1002038308506.
[20] M. Irie, Structure-function relationships of acid ribonucleases: lysosomal, vacuolar, and periplasmic enzymes, Pharmacol. Ther. 81 (1999) 77–89, https://doi.org/10.1016/s0163-7258(98)00035-7.
[21] B.J. Russell, S.D. Brown, N. Siguenza, I. Mai, A.R. Saran, A. Lingaraju, E.S. Maissy, A.C. Dantas Machado, A.F.M. Pinto, C. Sanchez, L.-A. Rossitto, Y. Miyamoto, R. A. Richter, S.B. Ho, L. Eckmann, J. Hasty, D.J. Gonzalez, A. Saghatelian, R. Knight, A. Zarrinpar, Intestinal transgene delivery with native E. coli chassis allows persistent physiological changes, Cell 185 (2022) 3263–3277.e15, https://doi.org/10.1016/j.cell.2022.06.050.
[22] J.P. Lynch, C. González-Prieto, A.Z. Reeves, S. Bae, U. Powale, N.P. Godbole, J. M. Tremblay, F.I. Schmidt, H.L. Ploegh, V. Kansra, J.N. Glickman, J.M. Leong, C. B. Shoemaker, W.S. Garrett, C.F. Lesser, Engineered Escherichia coli for the in situ secretion of therapeutic nanobodies in the gut, Cell Host Microbe 31 (2023) 634–649.e8, https://doi.org/10.1016/j.chom.2023.03.007.