Discovery of beneficial bioactive compounds from fecal microbiota transplantation: Potential for therapies for intractable diseases using rare intestinal Actinobacteria

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Research objective

Diet and health are closely linked to the gut environment, with the gut microbiota playing a central role. We focused on *Clostridioides difficile* infection (CDI), which arises from disruptions in the gut microbiota, with the aim of developing treatments and preventive methods utilizing beneficial gut bacteria. CDI is refractory diarrhea and enteritis caused by *C. difficile*. Treatment is difficult because of the tendency for recurrence. Because *C. difficile* is a normal resident of the intestines in healthy individuals, there is a possibility of sudden onset of CDI, even in previously asymptomatic individuals. We found that when we performed fecal microbiota transplantation in patients with CDI, the proportion of diverse Actinobacteria increased as the patients recovered [1)]. Actinobacteria are typically known to inhabit aerobic environments, making their presence as commensals in the human gut a highly significant discovery. Therefore, building on these findings, this study isolated Actinobacteria from healthy human feces, evaluated their antimicrobial activity against pathogens, and investigated their inhibitory effects on *C. difficile* toxins.

Methods

1. Actinobacteria isolation and identification

Various culture conditions, including medium components, incubation temperature, incubation time, and anaerobic/aerobic conditions, were evaluated to isolate intestinal Actinobacteria from human feces. The selected isolates were subjected to 16S rRNA gene sequencing and basic local alignment search tool analysis. Additionally, molecular phylogenetic analysis using the nearest-neighbor method was performed using the probable bacterial identity and nucleotide sequences of the isolates to confirm species identification.

2. Evaluation of antibacterial activity of isolate metabolites

After culturing the isolates for 1 week in three media types (30, 60, and 301 media), metabolites were extracted using ethanol. The antimicrobial activities of the extracts against

the pathogens *C. difficile*, methicillin-susceptible *Staphylococcus aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA), *Bacillus subtilis*, and multidrug-resistant *Pseudomonas aeruginosa* were determined using the paper disc method.

3. Evaluation of inhibitory activity of isolate metabolites against *C. difficile* toxins

C. difficile toxins A and B were challenged with metabolites derived from intestinal Actinobacteria at 37 °C for 30 min, and then applied to cultured cells (MDCK-2 and Caco2). After 24 h, the cells were fixed and viability was assessed using crystal violet staining.

Results and Discussion

1. Isolation and identification of Actinobacteria from human feces

Based on human gut flora analysis data, we successfully isolated a total of 20 strains of gut Actinobacteria using our independently established culture techniques [2, 3] (Figure 1). For these isolates, 16S rRNA gene sequences were determined and similarity analysis using DNA databases was performed. The 20 strains were classified into 6 genera and 14 species. Among these are many rare Actinobacteria, including a new species. Generally, the isolation rate of rare Actinobacteria from natural environments (e.g., soil and vegetables) is extremely low; however, they were abundant in the gut. Furthermore, molecular phylogenetic analysis using the 16S rRNA gene sequence and neighbor-joining method revealed that intestinal Actinobacteria exhibited higher phylogenetic diversity than Actinobacteria derived from soil and vegetables (Figure 2). This suggests that intestinal Actinobacteria may have evolved and diversified within the intestinal environment. It is also conceivable that some Actinobacteria colonizing the intestine were introduced through dietary intake. Future isolation and comparison of intestinal Actinobacteria from human feces with different dietary habits are expected to clarify the relationship between diet and intestinal Actinobacteria.

Figure 1. Colonies of Actinobacteria isolated from human feces.

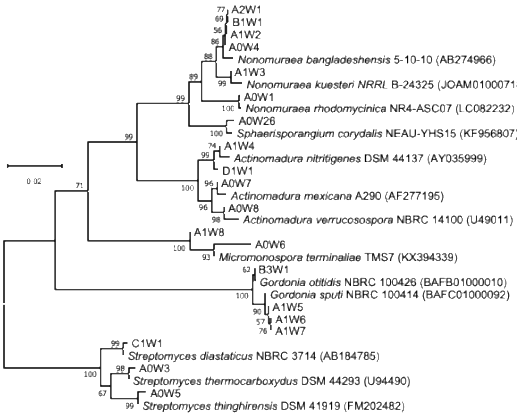

Figure 2. Phylogenetic tree of actinobacteria isolated from human feces (nearest-neighbor method).

2. Evaluation of antimicrobial activity of metabolites from isolates

Isolated intestinal Actinobacteria were cultured on various media, and their antimicrobial

activities against the five pathogens (*C. difficile*, etc.) were evaluated using the paper disc method. The results showed that *Nonomuraea bangladeshensis* exhibited antibacterial activity against MRSA; *Nonomuraea kuesteri* exhibited antibacterial activity against *P. aeruginosa*; *Nonomuraea rhodomycinica* exhibited antibacterial activity against MSSA, MRSA, and *B. subtilis*; and *Streptomyces thinghirensis* exhibited antibacterial activity against *C. difficile*. These findings demonstrated that metabolites from intestinal Actinobacteria possess selective antibacterial activity against pathogenic bacteria. Further elucidation of pathogen specificity of these metabolites at the molecular level may lead to the development of novel targeted therapies for infectious diseases.

3. Inhibitory effects of isolated metabolites on *C. difficile* toxins

Toxins produced by *C. difficile* (e.g., A and B) are known to exhibit potent cytotoxicity against cultured cells (MDCK). Therefore, the toxin-inhibitory activity of intestinal actinobacterial metabolites was evaluated. The results showed that *Gordonia sputi* and *Gordonia otitidis* exhibited suppressive effects against *C. difficile* toxins. Interestingly, these strains did not exhibit any antimicrobial activity against *C. difficile* itself. It is necessary to isolate the active compounds within the ethanolic metabolite pools, determine their mechanisms of action against toxins, and evaluate their biological activity and safety to explore their potential as novel therapeutic candidates.

Conclusion

To the best of our knowledge, this is the first study to successfully isolate intestinal Actinobacteria from the anaerobic environment of human feces. Some of the isolated strains exhibited antibacterial or inhibitory activity against *C. difficile* and its toxins. Revealing the selective mechanisms by which intestinal Actinobacteria metabolites exert their effects against pathogenic bacteria is expected to lead to the development of new intestinal disease treatments.

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