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A NEW FORESTRY BACTERIAL ISOLATE *Brevundimonas* sp. NEGATIVELY AFFECTING ON *Caenorhabditis elegans*

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ABSTRACT

Bacteria are food sources for the *Caenorhabditis* nematodes. This interaction becomes a model to understand the effects of bacteria on the nematodes and other host organisms. In this research, we report the identification of the environmental *Brevundimonas* sp. CFBb114 in Cat Tien National Park with the 16S rDNA. CFBb114 inhabits within the microhabitats of the nematode genus *Caenorhabditis*. We fed *Caenorhabditis elegans* with CFBb114 to investigate the effects of CFBb114 on *C. elegans*, and found that the bacteria reduced longevity and reproduction and changed the behavior of *C. elegans*. This research will facilitate the study of how host organisms evolve mechanisms against the impacts of bacteria.

Keywords: bacteria, brood size, food preference, longevity, nematodes.

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INTRODUCTION

Caenorhabditis elegans, a nematode model for tracing numerous biological issues (Leung et al., 2008), eats bacteria over its entire lifetime. The development of *C. elegans*, therefore, depends on the nutrition of the bacteria. In the sufficiency of favorable foods, *C. elegans* has normal growth and reproduction (Alvarez et al., 2005; Mukhopadhyay & Tissenbaum, 2007). In stressful conditions, it ceases the development and reproduction (Golden & Riddle, 1984; Angelo & Van Gilst, 2009).

Although nutritional bacteria are beneficial, many others, so-called pathogenic bacteria, were found to be virulent factors affecting the fitness of *C. elegans*. They could repress nematodes to death. Thus, the *C. elegans*-bacteria communication is far more than a prey-predator intra-connection (Dirksen et al., 2020).

In previous studies, the *Brevundimonas* bacteria were found to be abundant in soils and lived with soil nematodes (Zheng et al., 2008; Baquiran et al., 2013; Topalovic et al., 2019). Each strain could affect nematodes differently depending on the nematode species (Hamana et al., 2001; Zheng et al., 2008; Duclairoir Poc et al., 2011; Baquiran et al., 2013; Topalovic et al., 2019; Li et al., 2022; Sun et al., 2023). To us, this may imply that the *Brevundimonas*-nematode interactions are specific.

In this research, we isolated a new isolate of forestry *Brevundimonas* sp. CFBb114 in Cat Tien National Park where many *Caenorhabditis* nematodes live.

Bacteria temporally develop in a habitat where the nematodes complete their entire life. In this scenario, the life of the nematodes is solely driven by the bacteria. Hence, the objectives of this study are 1) to isolate a new isolate of forestry *Brevundimonas* sp. CFBb114 in Cat Tien National Park, where many *Caenorhabditis* nematodes live; 2) to investigate the potential impacts of the bacteria on *C. elegans*.

MATERIALS AND METHODS

Nematode strains: *C. elegans* var Bristol (N2) (lab number: CFBN22023), *Escherichia coli* OP50, and a new bacterial *Brevundimonas* sp. CFBb114 in this research.

Nematode growth media (NGM): The NGM preparation followed the protocol (Stiernagle, 2006). 17 g of agar, 3 g NaCl, 01 mL of 1M $CaCl_2$, 01 mL of 1 M $MgSO_4$, 25 mL of 1 M KPO_4, 1 mL of 5 mg/mL cholesterol, and 5 g of peptone in 1 L of distilled water.

Luria-Bertani (LB) broth: 10 g of peptone, 5 g of yeast extract, 5 g of NaCl in 1 L of distilled water. The ingredient mixture was autoclaved at 121 ^{o}C for 25 min.

Lifespan assay: The P_0 worms were raised on bacteria-seeded NGM for over five generations at 20 ^{o}C and were transferred to a new plate for the F1 generation. Each group of sixty worms already acclimated to the bacteria (either OP50 or CFBb114) was raised on one bacteria-seeded plate. They were transferred to a new plate every two to three days until they died. One worm was dead if its body was silent on the agar surface of the plate when gently touched. Lifespan comparison was analyzed with the log-rank test in the coded functions of [Surv(stage,lifepan)] and [surdiff(sdt~bacteria)].

Preparation of bacteria: *Brevundimonas* sp. CFBb114 was isolated as formerly described (Le et al., 2022). CFBb114 was stored in the LB broth at 10 ^{o}C and subcultured in the fresh LB broth at room temperature (RT; nearly 25 ^{o}C) every six months. The bacteria were spread and incubated on a LB agar plate for three days at RT. One colony on the plate was picked and cultured in the LB broth. After, an amount of the culture was seeded on NGM plates. The same culture was sequenced for 16S rDNA. To identify the bacterial genus, we compare the 16S rDNA sequence with the DNA database of the National Center of Biotechnology Information (NCBI).

Reproduction assay: Generally, two types of reproduction tests were conducted. First,

the preliminary assays were to examine the effects of bacteria on the brood sizes of *C. elegans*. Three *C. elegans* worms at the L2 stage were fed bacteria onto a CFBb114- or control OP50-seeded plate. The worms developed populations of F1 and F2 progenies for 10 days. The count of progenies on the test bacteria-seeded plate was estimated and compared with the OP50-seeded plate. The conclusion of the preliminary tests should be "more, equal, or less reproduction". The populations with extremely low progeny counts, i.e., CFBb114 were selected for the next tests (brood size, lifespan, and bacterial preference).

In the second test for brood size, a group of three to four L2 worms laid by the parents that had already acclimated to the bacteria-seeded NGM for one to two generations was grown on one bacteria-seeded NGM plate. The tested worms were transferred to a new plate every day until reproduction stopped for two continuous days. The brood size of a single worm in one group was the total brood size divided by the grouped worms (i.e., three or four). The statistical analysis of multiple brood size comparisons was deployed with Dunnett's post-hoc test in the coded function of [summary(glht(aov(linfct=mcp(ind=" Dunnett"))))].

Bacterial preference: In preparation of test plates, on each NGM plate (5 cm), we marked two opposite spots that were kept 2 cm apart. Next, we seeded one spot with CFBb114, and the other with OP50. The plate was incubated for two days at room temperature (~ 25 ^{o}C). Thirty to 31 reproducible worms were placed in the middle of the two bacterial spots, and they were free to migrate anywhere on the surface of the test plate for 3 hours and 15 min. After, the worms were counted for four choices (on each bacterial lawn, the middle, and as lost) (Fig. 1). The food preference was quantified by the choice index (CI), which was the number of worms on each destination for the total tested worms. Statistical analyses for the effects of overall destination on CIs were deployed with the 2-way ANOVA test in the function [summary (aov(value~Source * Destination, data))] and of pairwise destinations with Dunnette post-hoc test in the function of [summary (glht(aov,linfct=mcp(ind="Dunnett")))].

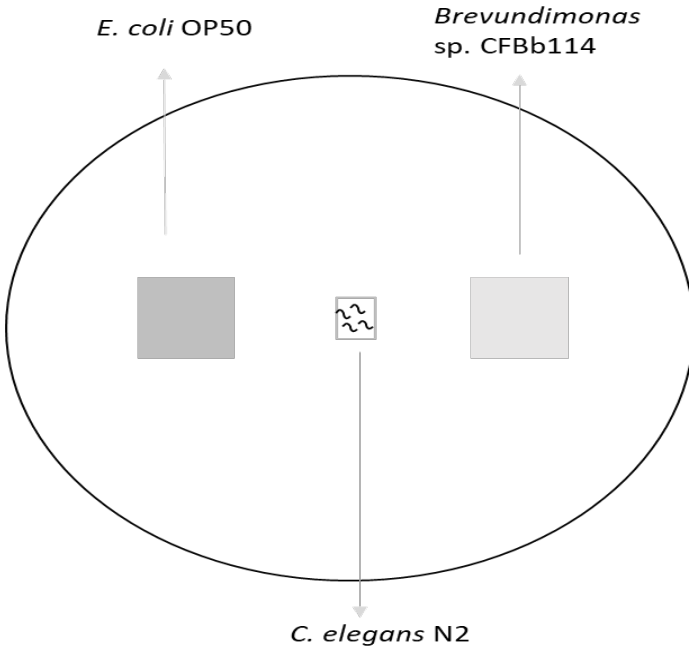

Figure 1. Diagram of bacterial preference. Two bacteria lawns were at a distance of 2 cm

RESULTS

Identification of bacteria

The bacteria CFBb114 associated with many *Caenorhabditis* nematodes (Le et al., 2022; Son et al., 2023a; Son et al., 2023b; Son et al., 2024) was isolated, cultured, and molecularly identified as sp. (GenBank accession number PQ460008) (Fig. 2). Because CFBb114 appeared to inhabit with the *Caenorhabditis* nematodes in Cat Tien National Park, the bacteria should have some effects on the nematodes. To address our curiosity, we fed *C. elegans* CFBb114 throughout the entire life to see any changes in survival, reproduction, and preference.

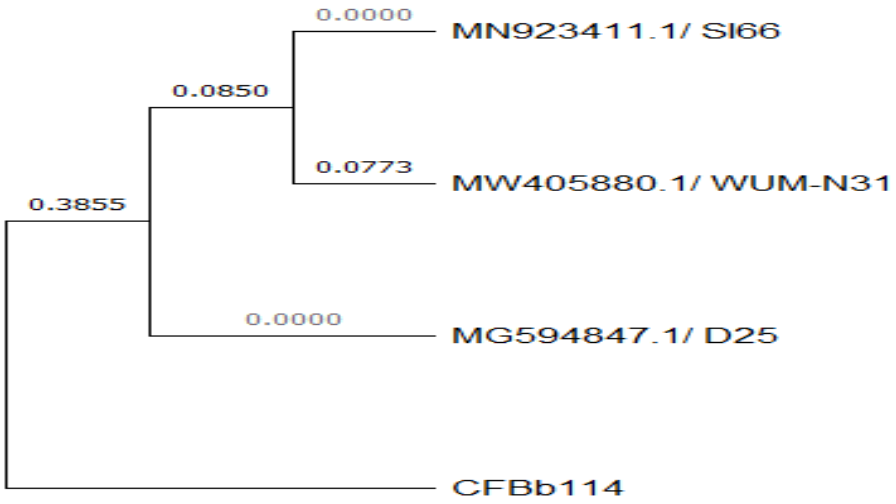

Figure 2. Phylogeny of CFBb114 with the 16S rDNA sequence. Phylogeny tree: Bootstrap replication 500. The top three hits, MN923411.1/*Brevundimonas diminuta* SI66 (16S rDNA sequence/ bacterial species strain), MW405880.1/*Brevundimonas vancanneytii* WUM-N31, and MG594847.1/ sp. D25 were cited from NCBI (Schoch et al., 2020) and close to CFBb114. The number on the branch - branch length.
The blast was conducted on Oct. 19, 2024

Lifespan

The worms raised on CFBb114 have a significantly shorter lifespan than OP50 (18.01 ± 2.38 (days) *versus* 19.18 ± 5.53 (days); $P = 0.002$; Fig. 3), suggesting that CFBb114 repressed the lifespan of *C. elegans*.

Brood size

The average gross brood size per worm on CFBb114 is smaller than that of OP50. Average daily brood sizes per worm are significantly different between the worms on CFBb114 and OP50 at four reproductive days (Day1/Day2/Day3/Day4: 11.33/20.62/20.18/ 0.27 *vesus* 14.00/113.47/11.28/0.07 larvae, respectively) (Fig. 3; $P < 0.001$; Pearson's Chi-squareed test). This indicates CFBb114 repressed the reproduction.

Regularly, the wild-type *C. elegans* N2 produces less progeny at Day1, most at Day2 and fewer at Day3 (Le et al., 2021; Le et al., 2022). We analyzed the pattern of daily reproduction and found that the worms on the CFBb114 have a significant difference from the control OP50 ($P < 0.001$) and no difference between Day2 and Day3 ($P = 0.90$) (Fig. 3). These results suggest that CFBb114 disrupted the reproductive physiology.

Bacterial preference

Worms on CFBb114 had negative impacts on longevity and reproduction. Thus, CFBb114 was likely virulent to *C. elegans*. To answer this in part, we assayed the impacts of CFBb114 on the food choice

ability of *C. elegans*. Gravid adult worms were grown on either CFBb114 or the control OP50, and after the worms were tested for food preference.

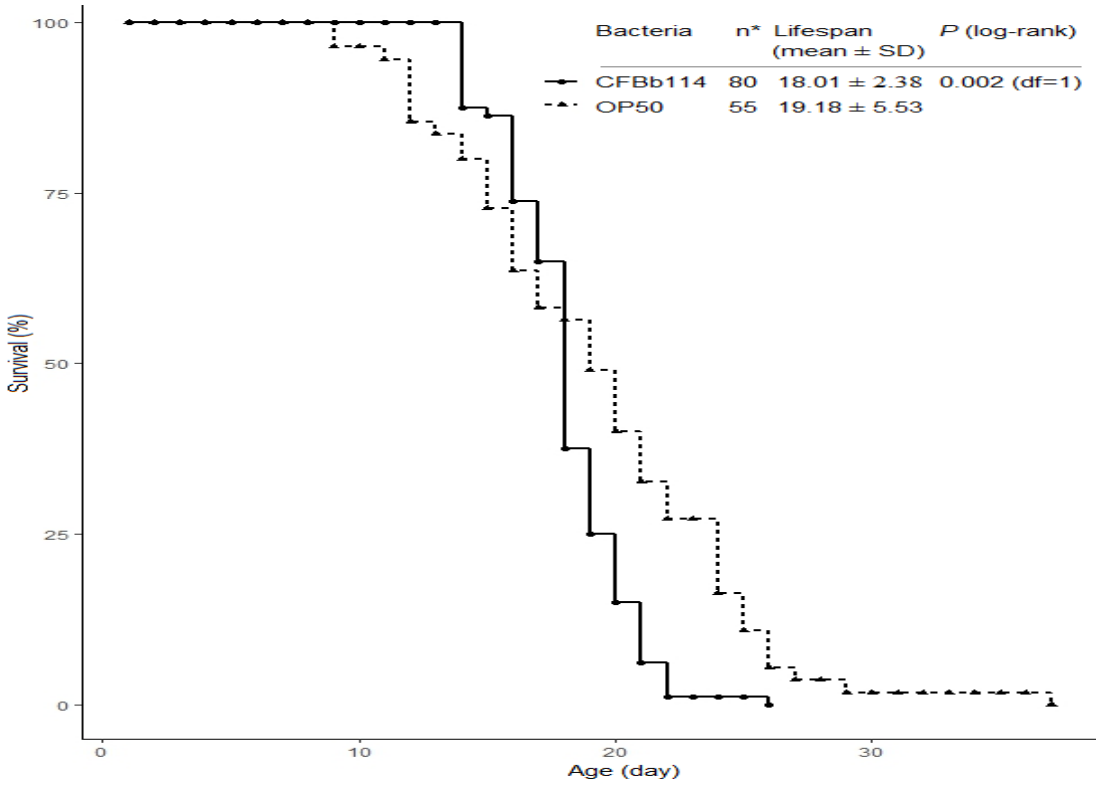

Figure 3. Survival rate of worms on bacteria. *The number of tested worms

Table 1. Brood size of *Caenorhabditis elegans*. *, N- the number of test plates, and n- the count of total test worms

Bacteria	N/n*	Brood size (larva) (mean ± SE)	*P* value (ANOVA test)
CFBb114	12/48	52.4 ± 1.35	< 2e-16
OP50	14/42	161.83 ± 4.05	

In general, the bacterial-destination preference of worms has significant biases ($P <$ 2e-16; Fig. 3). Next, we statistically compared pairwise CIs of every two destinations for each worm source. For the worms raised on CFBb114 (Fig. 4 (left)), pairwise comparisons of destinations showed non-significant CIs between paired destinations, i.e., Lost *versus* CFBb114 ($P = 0.06$) and Lost *versus* middle ($P = 0.09$), whereas the other pairwise destinations showed significance ($P \geq 0.03$), suggesting the worms did not have full ability to sense and reach destinations.

In contrast, the worms raised on the control OP50 have significantly different CIs, overall, higher for bacterial destinations and lower for non-bacterial areas ("Lost" and "Middle"). They have a similar CI between the OP50 and CFBb114 destinations (Fig. 5 (right)). The data suggest that the worms could sense and choose to move toward the destinations.

Given together, CFBb114 partially abolished the sensation and movement of worms.

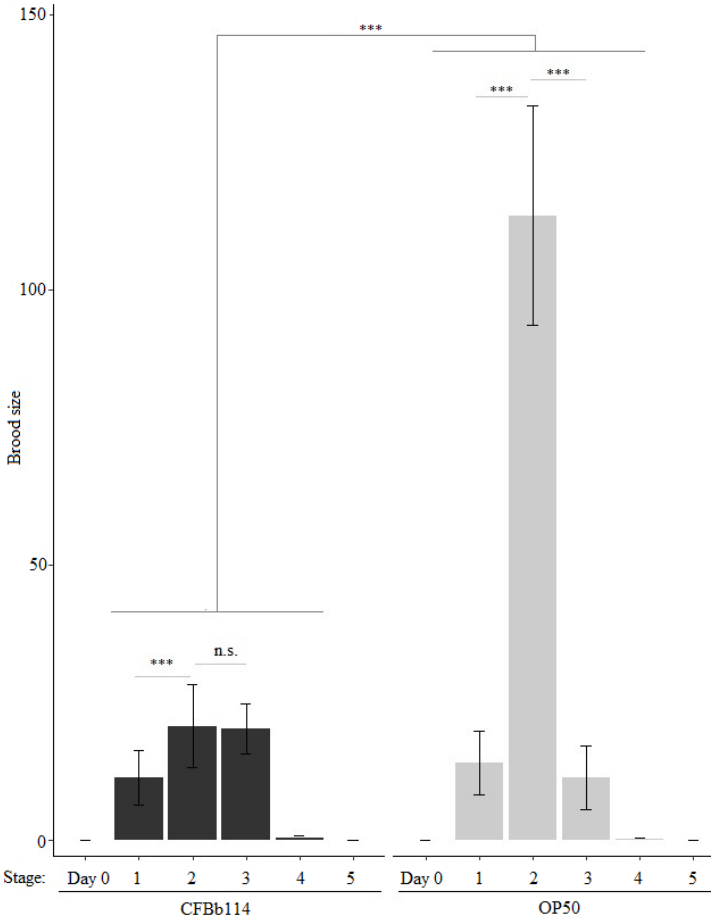

Figure 4. Daily reproductive brood sizes of worms on CFBb114 and the control OP50. "Brood size" is the count of larvae. Error bar - standard deviation. ****P* < 0.001. n.s. - no significant

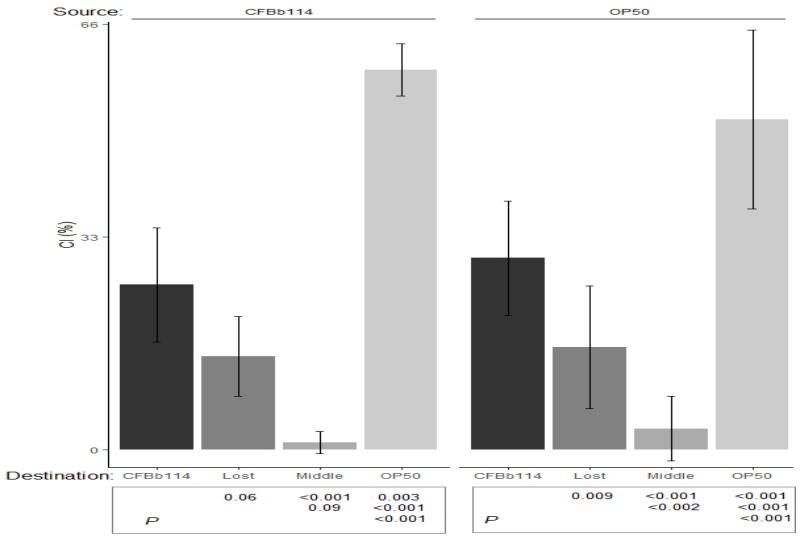

Figure 5. Choice index (CI) of *Caenorhabditis elegans*. Error bar - standard deviation

DISCUSSION

We isolated and identified a forestry *Brevundimonas* sp. CFBb114 is associated with the *Caenorhabditis* nematodes in Cat Tien National Park, Vietnam. CFBb114 is molecularly distinct from other strains within the bacterial genus globally distributed in different nations (Baquiran et al., 2013; Topalovic et al., 2019). To see any effects of CFBb114 on nematodes, we used the bacteria to feed the *C. elegans* nematode.

C. elegans could survive and produce offspring when feeding on the bacteria CFBb114, meaning that CFBb114 provides nutrients and growth factors for the nematodes. Of which, polyamines are profitable for the host in the wildness and for *C. elegans* in this report (Hamana et al., 2001; Duclairoir Poc et al., 2011; Tofalo et al., 2019; Michael, 2018; Xi et al., 2023).

However, *C. elegans* reduced 4.0% of longevity and 67.62% of reproduction in comparison with OP50, indicating that CFBb144 negatively affects *C. elegans*. In the tests of food preference, *C. elegans* chose OP50 over CFBb114, indicating that CFBb114 is not the best food choice. *C. elegans* raised on CFBb114 partially lost the ability to reach the bacterial food. This indicates that CFBb114 caused the malfunction in the food sensation of and/or the bacteria is not favorable for the starved *C. elegans*. As reported, wild-type *Brevundimonas* sp. strains can produce toxic compounds produced to antagonize nematodes (Zheng et al., 2008; Duclairoir Poc et al., 2011; Li et al., 2022; Sun et al., 2023). Therefore, CFBb114 may be repressed by *C. elegans* in the same theme.

CONCLUSION

We isolated a new forestry *Brevundimonas* sp. living with the *Caenorhabditis* nematodes in Cat Tien National Park. In comparison with the standard bacteria *E. coli* OP50, the bacterial isolate solely decreased longevity and reproduction, and it disrupted the food preference of *C. elegans*.

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