Potential Effects of Indole-3-Lactic Acid, a Metabolite of Human-Residential Bifidobacteria (HRB) on Neuronal Differentiation

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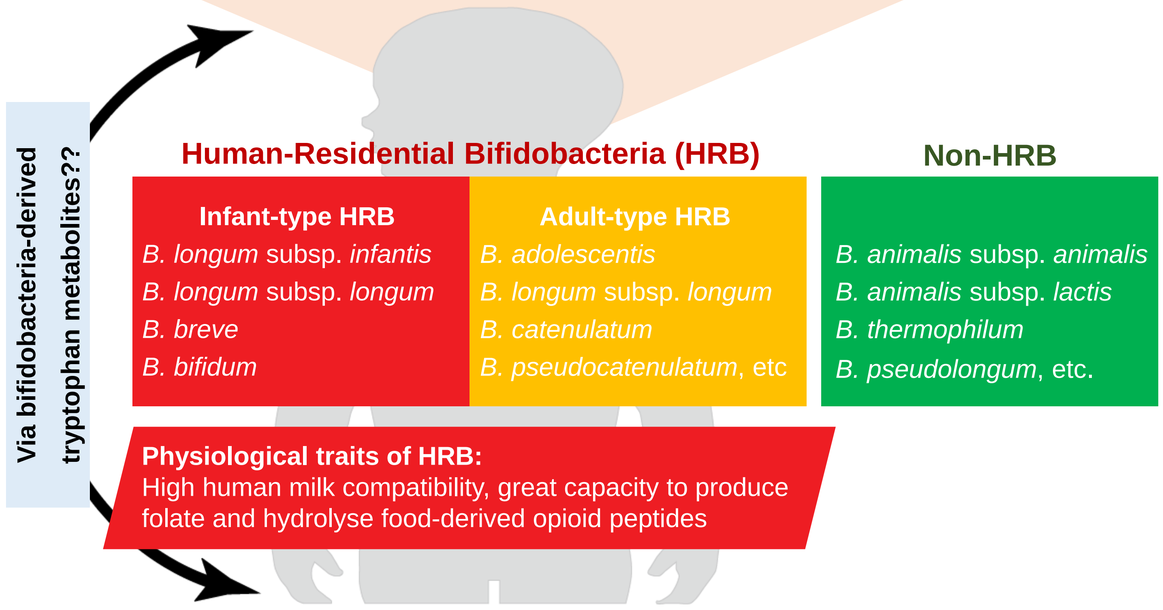
Abstract

Accumulating evidence suggests that *Bifidobacterium* strains that are naturally occurred in the human intestines, known as Human-Residential Bifidobacteria (HRB) possess unique physiological characteristics including high compatibility with human milk, adapted metabolic affinities, capabilities to produce folate and degrade food-derived opioid peptides, but how and why HRB strains are more beneficial to the human host, especially infants, remain obscure. It is suggested that the postbiotic metabolites produced by bifidobacteria could mediate the host-microbial interactions and may contribute to their adaptability and functionality in human infant gut. In this study, we examined the production of tryptophan metabolites by bifidobacterial strains of different residential origins and sought to explore the potential role of the metabolite in neuronal differentiation.

Tryptophan metabolites produced in bifidobacterial culture supernatants were quantified using LC-MS/MS. Indole-3-lactic acid (ILA) was the only tryptophan metabolite produced in bifidobacteria culture supernatants. No others, including indole-3-propionic acid (IPA), indole-3-acetic acid (IAA), and indole-3-aldehyde (IAld), were produced. Interestingly, infant-type HRB strains, such as *Bifidobacterium longum* subsp. *longum*, *B. longum* subsp. *infantis*, *B. breve*, and *B. bifidum*, produced higher levels of ILA than other strains, suggesting infant-type HRB might play a specific role in microbiota-host crosstalk by producing ILA in human infants. Next, we examined the neurite outgrowth of PC12 cells following exposure to ILA and NGF induction via fluorescence immunostaining of βIII-tubulin and elucidate the role of aryl hydrocarbon receptor (AhR) in ILA-enhanced neurite outgrowth. We found that ILA substantially enhanced NGF-induced neurite outgrowth of PC12 cells, in a dose-dependent manner, and had the most prominent effect at 100 nM. ILA was found to act as the aryl hydrocarbon receptor (AhR) agonist and evoked neurite outgrowth in an AhR-mediated manner. These new findings provide clues into the potential involvement of ILA as the mediator in bifidobacterial host-microbiota crosstalk and the neuronal developmental processes.



Methodology

We tested the ability of 19 bifidobacterial strains obtained from public culture collection to produce tryptophan-derived indole compounds. The concentration of the four tryptophan metabolites (IAA, IAld, IPA, and ILA) in the culture supernatants was quantified using LC-MS/MS.

Next, we sought to explore the potential role of ILA in neuronal differentiation. We examined the neurite outgrowth of PC12 cells following exposure to ILA (100 nM) and nerve growth factor (NGF; 25 ng/mL) induction. Following treatment for five days, cell body and processes were immuno-stained with anti-βIII-tubulin antibody. The nuclear was labelled with DAPI. Cells displaying projections of at least 1.5 times longer than the length of the cell body were considered positive and counted as neurite bearing cells. The % of neurite bearing cells was calculated as the % of the number of neurites divided by the total number of cells. The potential role of AhR in ILA-induced neurite outgrowth was evaluated. The protein expression of AhR in PC12 cells treated with or without the AhR antagonist, α-napthaflavone, were quantified via Western blot analysis.

Conclusion

This study suggests that infant-type HRB might play a specific role in microbiota-host crosstalk by producing ILA in human infants. The findings obtained provide clues into the potential involvement of bifidobacteria-derived postbiotics metabolites in infant health.

Conflict of Interest

The authors declare no conflict of interest.

Results and Discussion

1. ILA production by HRB strains

ILA was the only tryptophan metabolite produced in bifidobacterial culture supernatants. No others, including IPA, IAA, and IAld, were produced. Interestingly, infant-type HRB strains, such as *B. longum*, *B. infantis*, *B. breve*, and *B. bifidum*, produced higher levels of ILA than other strains

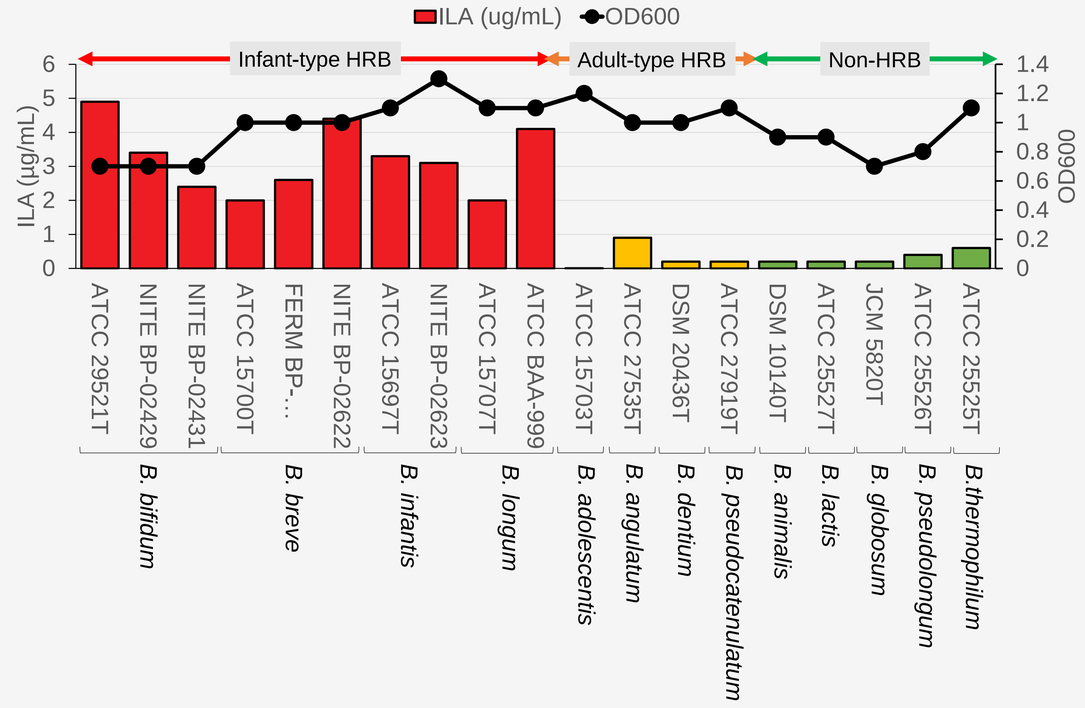


Figure 1. Production of ILA by bifidobacterial strains in public culture collection.

2. Effects of ILA on neurite outgrowth of PC12 cells

ILA promoted NGF-induced neurite outgrowth in an AhR-dependent way.

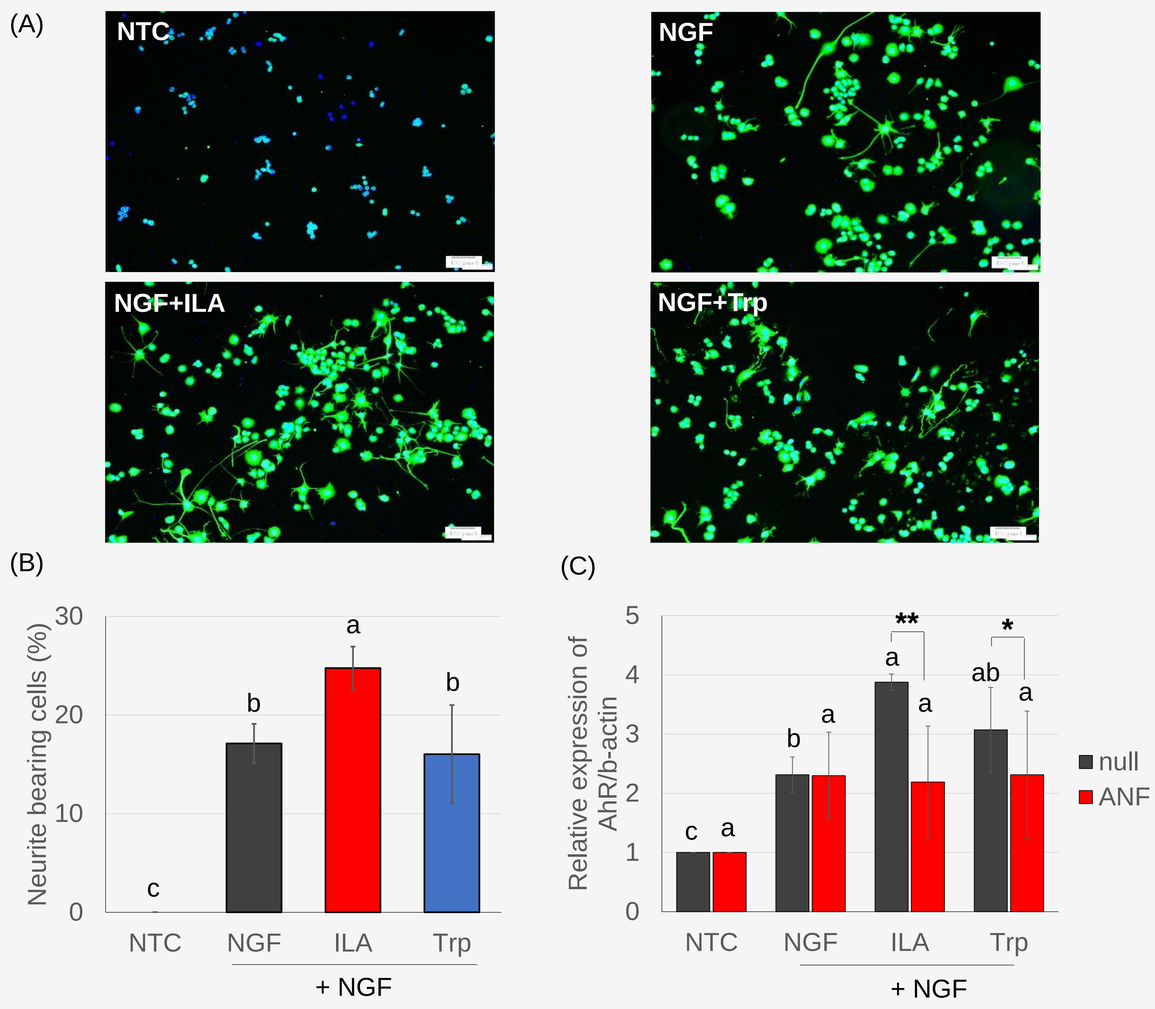


Figure 2. (A) Immunostaining images of βIII-tubulin (green) of PC12 cells at a x100 magnification. (B) Percentage of neurite-bearing cells in PC12 cells. (C) Effects of ILA on the AhR in PC12 cells treated with or without the AhR antagonist, α-napthaflavone (ANF). [abc]P<0.05 intergroup differences by one-way ANOVA with Tukey's post hoc tests. *P<0.05, *P<0.01 for intragroup differences compared by an independent Student's t-test.

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