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Probiotic-derived polyphosphate improves the intestinal barrier function through the caveolin-dependent endocytic pathway

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ABSTRACT

Probiotics exhibit beneficial functions for host homeostasis maintenance. We herein investigated the mechanism by which *Lactobacillus brevis*-derived poly P exhibited a beneficial function. Immunostaining indicated that poly P was captured in the plasma membrane via integrin β1 in Caco2/bbe cells. The uptake of poly P was reduced by the inhibition of integrin β1 as well as caveolin-1, a major component of lipid rafts. The function of poly P, including the induction of HSP27 and enhancement of the intestinal barrier function, was suppressed by the inhibition of caveolin-1, illustrating that the function of poly P was mediated by the endocytic pathway. High-throughput sequencing revealed that poly P induced tumor necrosis factor alpha-induced protein 3, which contributes to cytoprotection, including upregulation of the intestinal barrier function. The present study demonstrates a novel host–probiotic interaction through the uptake of bacterial substance into host cells, which is distinct from pattern recognition receptor pathways.

1. Introduction

It is suggested that over 1000 bacterial species live in the mammalian intestine and maintain host intestinal homeostasis. Previous reports have indicated that the perturbation of intestinal microflora is strongly associated with intestinal disorders [1–3]. It has been thought that the host–microbial interaction is mediated by pattern recognition receptors (PRRs), such as toll-like receptors (TLRs) and Nods, which recognize bacterial-derived molecules, including lipopolysaccharide (LPS) and lipoteichoic acid (LTA) [4,5]. TLR signaling induces the production of inflammatory cytokines and leads to the inflammation of several organs including the intestinal tract [6]. Nods activate the NF-kappaB pathway following intracellular stimulation by bacterial products [7,8]. Therefore, these PRRs are considered to recognize pathogenic bacteria-derived molecules and enhance inflammation [9,10]. However, several studies have shown that some TLRs contribute to the recognition of pathogenic as well as beneficial bacteria. Lee et al. proposed that TLR9 mediates the interaction between the intestinal epithelia and commensal bacteria through the recognition of bacteria-derived ISS-ODN (immunostimulatory sequence oligodeoxynucleotide) and contributed to the maintenance of intestinal homeostasis [11]. Podolsky et al. showed that TLR2 mediated the induction of host innate immune protection by bacteria-derived Pam3CysSK4 [12]. While these studies suggested the role of TLRs in the interaction between the beneficial bacteria and host intestine, it remains unclear why the activation of certain TLR pathways show anti- or pro-inflammation. Xiao et al. proposed that single immunoglobulin IL-1 receptor-related molecule (SIGIRR) inhibited the activation of TLRs, contributing to the prevention of excess inflammation by the stimulation of commensal bacteria [13]. This suggests that recognition systems for commensal or beneficial bacteria, other than TLRs, contribute to the regulation of intestinal inflammation.

Probiotics are defined as microbes which confer health benefits to the host when administered in adequate amounts. Our previous reports proposed that competence and sporulation factor (CSF) derived from *Bacillus subtilis* activates the cell survival Akt pathway

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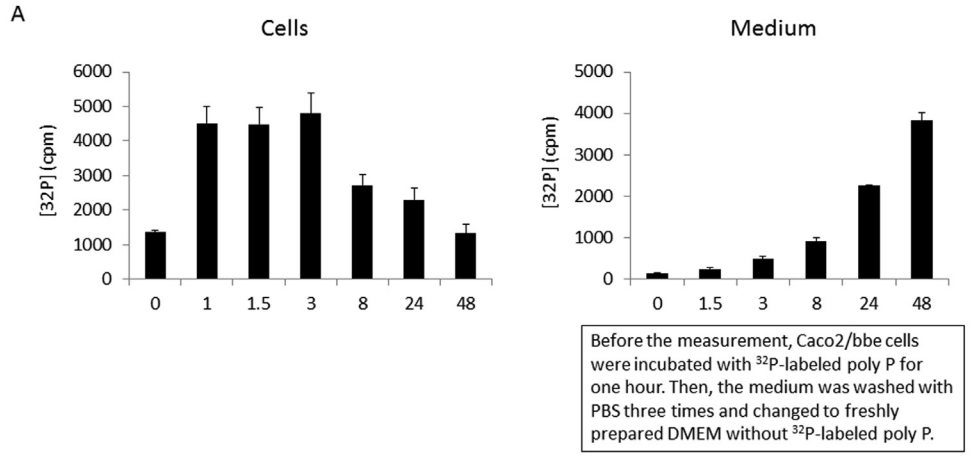
A
Cells
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[32P] (cpm)
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3000
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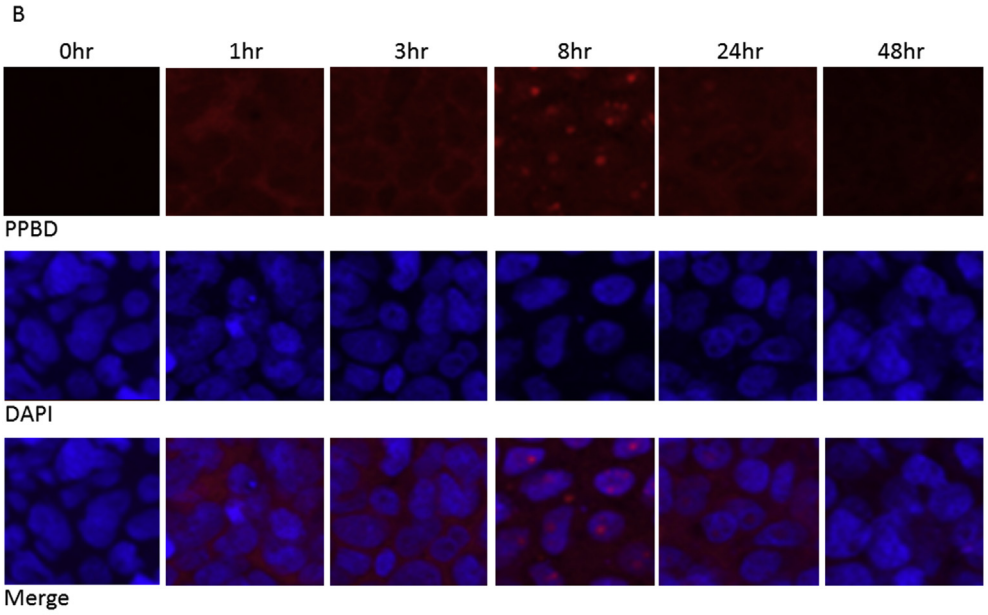
0 1.5 3 8 24 48
Before the measurement, Caco2/bbe cells were incubated with 32P-labeled poly P for one hour. Then, the medium was washed with PBS three times and changed to freshly prepared DMEM without 32P-labeled poly P.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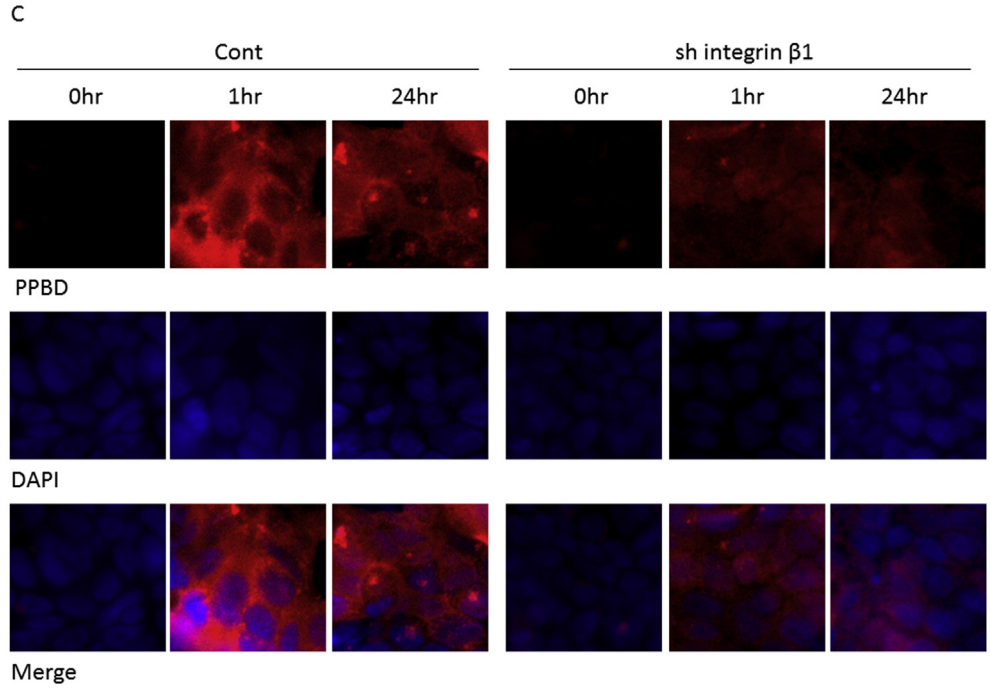
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