Centanni, M., Bergmann, S., Turroni, S., Hammerschmidt, S., Chhatwal, G.S., Brigidi, P., Candela, M.
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(2012) Applied and Environmental Microbiology, 78 (7), pp. 2465-2469.

TNF-α modulates the dynamics of the plasminogen-mediated early interaction between *Bifidobacterium animalis* subsp. *lactis* and human enterocytes

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Abstract

The capacity to intervene with the host plasminogen system has been recently considered an important component in the interaction process between *B. animalis* subsp. *lactis* and the human host. However, its significance in the bifidobacterial microecology within the human gastrointestinal tract is still an open question. Here we demonstrated that human plasminogen favors the *B. animalis* subsp. *lactis* BI07 adhesion to HT29 cells. Prompting the HT29 cell capacity to activate plasminogen, TNF-α modulated the plasminogen-mediated bacteria-enterocyte interaction, reducing the bacterial adhesion to the enterocytes and enhancing the migration to the luminal compartment.

53 *Bifidobacterium* is an important, occasionally dominant, genus of the human intestinal
54 microbiota whose presence in the human gastrointestinal tract (GIT) has been associated with
55 several health benefits (12, 17). Merging health-promoting activities and technological properties,
56 *B. animalis* subsp. *lactis* (referred as *B. lactis* here) is one of the most common probiotic species in
57 North America and Europe (3). Because of its wide probiotic usage, different studies have been
58 recently carried out with the attempt to better comprehend the molecular mechanisms involved in
59 the bacteria-host interaction process (3, 11). In this scenario, the capacity of this microorganism to
60 intervene with the host plasmin(ogen) system has been recently regarded as a possible actor in the
61 interplay with human enterocytes (11, 28). However, the role of the human plasminogen (Plg)
62 system in the biology of the bifidobacteria-host interaction process is still to be determined. The 92-
63 kDa Plg is the monomeric pro-enzyme of the serine protease plasmin. Plg comprises an N-terminal
64 8-kDa preactivation peptide, five consecutive disulfide-bounded kringle domains (65 kDa) that
65 mediate the binding to lysine-containing protein receptors, and a serine protease domain (25 kDa).
66 Although it is produced mainly by hepatocytes, other tissue sources of Plg have been identified,
67 including the intestine (41). Plg is immobilized onto lysine-containing cell surface protein receptors
68 such as fibrin as well as components of the extracellular matrix. Plg activation is a process tightly
69 regulated by the balance between Plg activators (PA), such as urokinase (uPA) and tissue type (tPA)
70 activators, and their specific inhibitors PAI-1/2. Involved in fibrinolysis, enhancement of cell
71 migration and damages of tissue barriers, the Plg system has a key role in several human
72 physiological and pathological processes (27).

73 We previously reported the capability of *B. lactis* to bind human plasmin(ogen) (8). Plg binding
74 to the bifidobacterial cell surface is mediated by five cell wall protein receptors: DnaK, glutamine
75 synthetase, enolase, bile salt hydrolase and phosphoglycerate mutase. According to Candela *et al.*
76 (7), in the presence of Plg and host PAs, *B. lactis* acquires a surface-bound Plg-derived proteolytic
77 activity effective in the degradation of host physiological substrates. This modality of interaction
78 with the components of the host Plg system resembles the one characteristic of several

79 enteropathogens, such as *Salmonella enterica*, *Listeria monocytogenes* and *Escherichia coli* (16, 25,
80 29, 30, 32, 36). Differently from *Bifidobacterium*, the role of Plg in bacterial pathogenicity has been
81 determined and the bacterial capacity to intervene with the host Plg system has been traditionally
82 considered as a paradigm of pathogenicity (5, 16, 25, 29, 30, 32, 35). Facilitating the bacterial
83 transmigration through epithelial monolayers, for pathogens the acquisition of a Plg-dependent
84 surface-associated plasmin activity is necessary for dissemination in the host tissues. This process
85 has been defined as bacterial metastasis in analogy to the role of Plg in tumor cell invasion (10, 31).
86 Originally isolated from fermented milk (19) and commonly found in the gut of healthy adults and
87 infants (38), it is beyond any doubt that *B. lactis* possesses a mutualistic nature and its capacity to
88 intervene with the host Plg system must serve a different purpose than the bacterial metastasis (7).
89 Consequently, even if *B. lactis* and pathogens show the same modality of interaction with the
90 components of the host Plg system, the outcome of Plg-binding activity in the biology of interaction
91 with the host must be different and the comprehension of its significance in the bifidobacterial
92 ecology is needed. Here we studied the role of the human Plg system in the early interaction process
93 between *B. lactis* BI07 and the human enterocyte line HT29 (26). Moreover, since gastrointestinal
94 inflammatory processes involve unbalances of the components of the host Plg system (14, 20, 23),
95 we investigated the impact of the pro-inflammatory cytokine TNF-α on the dynamics of the Plg-
96 mediated *B. lactis* BI07-HT29 cell early interaction. TNF-α was selected since this cytokine has
97 been reported to play a central role in intestinal inflammation (18, 40).
98 **HT29 cell culture conditions**. HT29 cells were grown in Dulbecco's modified Eagle's minimal
99 essential medium with 4.5 g/l glucose (DMEM; PAA Laboratories) as reported by O'Hara *et al.*
100 (22). For adhesion and intracellular invasion assays, 2.5 x 10^5 HT29 cells were seeded per well in
101 24-well tissue culture plates (TPP) and 12 mm-diameter glass coverslips and grown to confluent
102 monolayers. For transmigration assay, 1 x 10^5 cells were layered on 3 μm-pore size transwell inserts
103 (Falcon – Becton Dickinson) in 24-well tissue culture plates and allowed to grow to confluent and
104 fully differentiated monolayers. The tightness of the cell layers was verified as described by Attali

105 *et al.* (2). Twenty-four h before each assay, the cell medium was changed with Interaction Medium
106 (IM) (DMEM, 25 mM HEPES, 1 g/l glucose (Gibco), 1% FCS); when necessary, 2 ng/ml human
107 recombinant TNF-α (Thermo Scientific) was added to induce a pro-inflammatory response (22).
108 **Role of human Plg in the interaction process between *B. lactis* BI07 and HT29 cells.** To
109 assess whether the HT29 cell endogenous Plg contributed to the early interaction between *B. lactis*
110 BI07 and HT29 cells, the impact of the enterocyte pre-treatment with polyclonal goat anti-human
111 Plg IgG (Kordia) (5 µg/ml) on the *B. lactis* BI07 adhesion was determined. *B. lactis* BI07 adhesion
112 to HT29 monolayers was measured by quantitative PCR (qPCR) as reported by Candela *et al.* (9).
113 For each experimental condition six independent replica experiments were performed. According to
114 our data, the anti-human Plg pre-treatment resulted in a slight, but not significant ($P > 0.05$),
115 decrease of the bifidobacterial adhesion to the enterocyte surface (-16%). These data indicated that
116 the endogenously produced Plg of HT29 cells exerted only a minimal contribution to the *B. lactis*
117 BI07-HT29 cell adhesion process. Consequently, in order to investigate the role of human Plg in the
118 *B. lactis* BI07-enterocyte interaction process, bacterial cells were pre-incubated with human Plg
119 (Sigma), as reported by Attali *et al.* (2), and then subjected to a conventional HT29 cell adhesion
120 assay (9). Thirty-min pre-incubation at 37°C with 100 µg/ml human Plg significantly enhanced *B.*
121 *lactis* BI07 adhesion to HT29 cells (+225%; $P < 0.001$) (Table 1). The incubation of Plg pre-treated
122 *B. lactis* BI07 cells with 5 µg/ml anti-human Plg IgG resulted in the complete abolition of the Plg
123 contribution to the bifidobacterial adhesion to the HT29 cell surface. These data demonstrated that
124 human Plg captured on the *B. lactis* BI07 cell surface significantly enhanced bacterial adhesion to
125 the host enterocytes. As suggested by Pancholi *et al.* (24), the bacterial bound Plg can act as a
126 molecular bridge between bacterial and enterocyte receptors, enhancing the bacterial adhesion to
127 the host epithelium. Incubation of Plg pre-treated *B. lactis* BI07 cells with 1 µg/ml uPA (Sigma)
128 decreased the HT29 cell adhesion of 50% with respect to the value obtained in the absence of uPA
129 ($P < 0.001$) (Table 1). The addition of 8 U/ml of the plasmin inhibitor aprotinin (Sigma) (2) was
130 effective in the complete recovery of the Plg-dependent enterocyte adhesion of *B. lactis* BI07 cells

pre-incubated with Plg + uPA (Table 1), proving that the acquisition of a cell surface plasmin activity in the presence of uPA was sufficient to dampen the contribution of Plg to the *B. lactis* BI07 adhesion to the enterocyte surface. Next, we investigated HT29 cell internalization and transmigration of untreated, pre-treated with Plg or with Plg and uPA *B. lactis* BI07 bacteria. Enterocyte internalization was analyzed by double immunofluorescence microscopy, which enables a differential staining of intracellular and adherent bacterial cells, as reported by Bergmann *et al.* (4). For each experimental condition three independent adhesion experiments were carried out. Antibodies against *B. lactis* BI07 were generated in Balb/C mice according to standard protocols. Microscopic analysis of the whole HT29 cell layer revealed no bacterial internalization by the HT29 enterocyte cell line in any of the tested conditions (Fig. 1A). For comparison and as a control, the internalization of *Salmonella enterica* serovar Typhimurium by HT29 cells was determined using a gentamicin protection assay (13). *S.* Typhimurium, provided by A. Essig (Dept. of Medical Microbiology, University of Ulm, Germany) was cultured as reported by Candela *et al.* (9). In accordance to data reported by Hess *et al.* (13), we determined a HT29 cell internalization value of $9.32 \pm 1.58 \times 10^4$ CFU of *S.* Typhimurium after 1 h of incubation with 1×10^8 CFU of this microorganism. In order to analyze the *B. lactis* BI07 transmigration through HT29 monolayers, a transwell system-based transmigration assay was performed as reported by Attali *et al.* (2). Neither untreated nor Plg pre-treated nor Plg and uPA pre-treated *B. lactis* BI07 cells showed transmigration through the HT29 monolayers (data not shown). In a control experiment, the transmigration of *S.* Typhimurium bacteria across HT29 monolayers was determined. In accordance with the data reported by Hess *et al.* (13), $7.84 \pm 1.03 \times 10^5$ bacteria penetrated from the apical to the basal compartment of HT29 cells after 1 h of co-incubation with 2×10^7 CFU. These last data proved that in *B. lactis* BI07 the acquisition of a surface-associated plasmin activity did not result in enterocyte internalization or bacterial transmigration through enterocyte monolayers, supporting the strict commensal nature of this health-promoting species.

Even if the modality of interaction between *B. lactis* and the components of the host Plg system resembles the one described for several pathogens (16, 25, 29, 30, 32, 36), our data demonstrated that the consequences of this interaction with respect to host colonization are different. In fact, for both *B. lactis* and pathogenic bacteria, cell-bound Plg enhances bacterial adhesion to the host epithelium (2, 24), but in the presence of host PAs the phenotype of interaction with the host becomes totally different. Differently from pathogens, in *B. lactis* the acquisition of a surface-associated plasmin activity does not result in pericellular invasion, but rather supports the bacterial migration to the luminal compartment. Thus, common to symbionts and pathogens (1, 15), the bacterial capacity to intervene with the host Plg system cannot be viewed as a determinant of pathogenicity by itself. Otherwise, it could be regarded as an ancestral mechanism of bacteria-host interaction evolved prior to the radiation of pathogens from commensals (6, 21). Acting in concert with other virulence attributes (2, 21), only in pathogens the capacity to intervene with the host Plg system becomes a fundamental prerequisite for host invasion (37).

Impact of TNF-α on the Plg-mediated early interaction between *B. lactis* BI07 and HT29 cells. To mimic an inflammatory status, HT29 cell layers were pre-incubated with the pro-inflammatory cytokine TNF-α and their early interaction with Plg or Plg + uPA pre-treated and untreated *B. lactis* BI07 cells was investigated by qPCR and immunomicroscopic analysis as reported above. Interestingly, in TNF-α stimulated HT29 monolayers we detected only a minor increase of *B. lactis* BI07 adhesion after Plg pre-treatment (Table 1). Moreover, after the TNF-α pre-incubation of HT29 layers, Plg pre-treated and Plg + uPA pre-treated *B. lactis* BI07 cells exhibited comparable adhesion values (Table 1). The addition of aprotinin effectively restored the Plg-dependent *B. lactis* BI07 adhesion to TNF-α stimulated HT29 layers for both Plg and Plg + uPA pre-treated *B. lactis* BI07 cells (Table 1). Analogously to the data obtained with non-inflamed HT29 cells, no bacterial internalization (Fig. 1B) and transmigration (data not shown) were detected when TNF-α stimulated HT29 cells were incubated with Plg, Plg + uPA pre-treated and untreated *B.*

181 *lactis* BI07 cells. Taken together these data indicated that, by enhancing the enterocyte capacity to
182 activate Plg, a TNF-α-mediated inflammatory response can modulate the dynamics of the Plg-
183 mediated early interaction between *B. lactis* and human enterocytes, lowering the bacterial adhesion
184 to the enterocyte surface and favoring the migration to the luminal compartment. Confirming this
185 hypothesis, in a conventional plasmin activity assay (2, 7) we demonstrated that the TNF-α pre-
186 treatment increased by two-fold the HT29 cell capacity to activate Plg ($P < 0.001$).

187 Our data suggest that *B. lactis* BI07 shows a different phenotype of interaction with the host Plg
188 system depending on the inflammatory status of the host GIT epithelium. While in a non-inflamed
189 GIT epithelium Plg enhances *B. lactis* adhesion, inflammation would result in the activation of
190 bacterial bound Plg and mediate the migration of the microorganisms to the luminal compartment.
191 We hypothesize that this particular dynamic of interaction between *B. lactis* and the components of
192 the host Plg system could be of some relevance in the bifidobacterial microecology in the human
193 GIT. In non-inflamed regions of the human GIT, which represent the suitable ecological niche for
194 *B. lactis*, the microorganism can take advantage of the host Plg for the adhesion to the mucosal
195 surface. On the other hand, in inflamed tissue sites *B. lactis* would acquire a surface-associated
196 plasmin activity that, facilitating the bacterial migration to the luminal compartment, could function
197 as a bacterial escape mechanism to circumvent the host inflammatory response. Unable to face
198 inflammation (34), *B. lactis* may thus utilize the host Plg system to sense and escape intestinal
199 inflammation, abandoning inflamed gastrointestinal sites in favor of non-inflamed ones. Our *in*
200 *vitro* model of interaction could explain, at least in part, the observed decrease of the relative
201 abundance of *Bifidobacterium* in IBD subjects (33, 39).

202 **Conclusions.** Investigating the role of Plg in the bifidobacterial biology in the human GIT, our
203 experimental work represents a step forward in the comprehension of the factors that play a role in
204 the dynamics of *Bifidobacterium*-host interaction in the human GIT. However, our *in vitro* data need
205 to be confirmed by *in vivo* studies specifically designed to prove the role of the host Plg system in
206 the inflammation-dependent bifidobacterial decrease in the human GIT. The achievement of this

goal will allow a better understanding of the biology of this health-promoting microbiota component in the human GIT.

Acknowledgements

This research was partially supported by a grant from the Italy–Germany Vigoni Program.

References

1. **Antikainen, J., V. Kuparinen, K. Lähteenmäki, and T. K. Korhonen.** 2007. Enolases from Gram-positive bacterial pathogens and commensal lactobacilli share functional similarity in virulence-associated traits. FEMS Immunol. Med. Microbiol. **51:**526-534.
2. **Attali, C., C. Durmort, T. Vernet, and A. M. Di Guilmi.** 2008. The interaction of *Streptococcus pneumoniae* with plasmin mediates transmigration across endothelial and epithelial monolayers by intercellular junction cleavage. Infect. Immun. **76:**5350-5356.
3. **Barrangou, R., E. P. Briczinski, L. L. Traeger, J. R. Loquasto, M. Richards, P. Horvath, A. C. Coûté-Monvoisin, G. Leyer, S. Rendulic, J. L. Steele, J. R. Broadbent, T. Oberg, E. G. Dudley, S. Schuster, D. A. Romero, and R. F. Roberts.** 2009. Comparison of the complete genome sequences of *Bifidobacterium animalis* subsp. *lactis* DSM 10140 and Bl-04. J. Bacteriol. **191:**4144-4151.
4. **Bergmann S., A. Lang, M. Rohde, V. Agarwal, C. Rennemeier, C. Grashoff, K. T. Preissner, and S. Hammerschmidt.** 2009. Integrin-linked kinase is required for vitronectin-mediated internalization of *Streptococcus pneumoniae* by host cells. J. Cell Sci. **122:**256-267.
5. **Bergmann, S., and S. Hammerschmidt.** 2007. Fibrinolysis and host response in bacterial infections. Thromb. Haemost. **98:**512-520.
6. **Blaser, M. J., and D. Kirschner.** 2007. The equilibria that allow bacterial persistence in human hosts. Nature **449:**843-849.

7. **Candela, M., G. Miccoli, S. Bergmann, S. Turroni, B. Vitali, S. Hammerschmidt, and P. Brigidi.** 2008. Plasminogen-dependent proteolytic activity in *Bifidobacterium lactis*. Microbiology **154:**2457-2462.
8. **Candela, M., S. Bergmann, M. Vici, B. Vitali, S. Turroni, B. J. Eikmanns, S. Hammerschmidt, and P. Brigidi.** 2007. Binding of human plasminogen to *Bifidobacterium*. J. Bacteriol. **189:**5929-5936.
9. **Candela, M., G. Seibold, B. Vitali, S. Lachenmaier, B. J. Eikmanns, and P. Brigidi.** 2005. Real-time PCR quantification of bacterial adhesion to Caco-2 cells: competition between bifidobacteria and enteropathogens. Res. Microbiol. **156:**887-895.
10. **Ertongur, S., S. Lang, B. Mack, K. Wosikowski, B. Muehlenweg, and O. Gires.** 2004. Inhibition of the invasion capacity of carcinoma cells by WX-UK1, a novel synthetic inhibitor of the urokinase-type plasminogen activator system. Int. J. Cancer **110:**815-824.
11. **Gilad, O., B. Svensson, A. H. Viborg, B. Stuer-Lauridsen, and S. Jacobsen.** 2011. The extracellular proteome of *Bifidobacterium animalis* subsp. *lactis* BB-12 reveals proteins with putative roles in probiotic effects. Proteomics **11:**2503-2514.
12. **Gill, S. R., M. Pop, R. T. Deboy, P. B. Eckburg, P. J. Turnbaugh, B. S. Samuel, J. I. Gordon, D. A. Relman, C. M. Fraser-Liggett, and K. E. Nelson.** 2006. Metagenomic analysis of the human distal gut microbiome. Science **312:**1355-1359.
13. **Hess, D. J., M. J. Henry-Stanley, E. A. Erickson, and C. L. Wells.** 2002. Effect of tumor necrosis factor alpha, interferon gamma, and interleukin-4 on bacteria-enterocyte interactions. J. Surg. Res. **104:**88-94.
14. **Killeen, S. D., J. H. Wang, E. J. Andrews, and H. P. Redmond.** 2009. Bacterial endotoxin enhances colorectal cancer cell adhesion and invasion through TLR-4 and NF-kappaB-dependent activation of the urokinase plasminogen activator system. Br. J. Cancer **100:**1589-1602.
15. **Kinnby, B., N. A. Booth, and G. Svensäter.** 2008. Plasminogen binding by oral streptococci from dental plaque and inflammatory lesions. Microbiology **154:**924-931.
16. **Lähteenmäki, K., S. Edelman, and T. K. Korhonen.** 2005. Bacterial metastasis: the host plasminogen system in bacterial invasion. Trends Microbiol. **13:**79-85.

17. **Ley, R. E., M. Hamady, C. Lozupone, P. J. Turnbaugh, R. R. Ramey, J. S. Bircher, M. L. Schlegel, T. A. Tucker, M. D. Schrenzel, R. Knight, and J. I. Gordon.** 2008. Evolution of mammals and their gut microbes. Science **320:**1647-1651.
18. **Ma, T. Y., G. K. Iwamoto, N. T. Hoa, V. Akotia, A. Pedram, M. A. Boivin, and H. M. Said.** 2004. TNF-alpha-induced increase in intestinal epithelial tight junction permeability requires NF-kappa B activation. Am. J. Physiol. Gastrointest. Liver Physiol. **286:**G367-376.
19. **Meile, L., W. Ludwig, U. Rueger, C. Gut, P. Kaufmann, G. Dasen, S. Wenger, and M. Teuber.** 1997. *Bifidobacterium lactis* sp. nov., a moderately oxygen tolerant species isolated from fermented milk. Syst. Appl. Microbiol. **20:**57-64.
20. **Mondino, A., and F. Blasi.** 2004. uPA and uPAR in fibrinolysis, immunity and pathology. Trends Immunol. **25:**450-455.
21. **Ochman, H., J. G. Lawrence, and E. A. Groisman.** 2000. Lateral gene transfer and the nature of bacterial innovation. Nature **405:**299-304.
22. **O'Hara, A. M., P. O'Regan, A. Fanning, C. O'Mahony, J. MacSharry, A. Lyons, J. Bienenstock, L. O'Mahony, and F. Shanahan.** 2006. Functional modulation of human intestinal epithelial cell responses by *Bifidobacterium infantis* and *Lactobacillus salivarius*. Immunology **118:**202-215.
23. **Okumura, S., K. Yuki, R. Kobayashi, S. Okamura, K. Ohmori, H. Saito, C. Ra, and Y. Okayama.** 2008. Hyperexpression of NOD2 in intestinal mast cells of Crohn's disease patients: preferential expression of inflammatory cell-recruiting molecules via NOD2 in mast cells. Clin. Immunol. **130:**175-185.
24. **Pancholi, V., P. Fontan, and H. Jin.** 2003. Plasminogen-mediated group A streptococcal adherence to and pericellular invasion of human pharyngeal cells. Microb. Pathog. **35:**293-303.
25. **Parkkinen, J., and T. K. Korhonen.** 1989. Binding of plasminogen to *Escherichia coli* adhesion proteins. FEBS Lett. **250:**437-440.
26. **Rousset, M.** 1986. The human colon carcinoma cell lines HT-29 and Caco-2: two in vitro models for the study of intestinal differentiation. Biochimie **68:**1035-1040.
27. **Saksela, O., and D. B. Rifkin.** 1988. Cell-associated plasminogen activation: regulation and physiological functions. Annu. Rev. Cell Biol. **4:**93-126.

28. **Sánchez, B., P. Bressollier, and M. C. Urdaci.** 2008. Exported proteins in probiotic bacteria: adhesion to intestinal surfaces, host immunomodulation and molecular cross-talking with the host. FEMS Immunol. Med. Microbiol. **54:**1-17.
29. **Schaumburg, J., O. Diekmann, P. Hagendorff, S. Bergmann, M. Rohde, S. Hammerschmidt, L. Jansch, J. Wehland, and U. Karst.** 2004. The cell wall subproteome of *Listeria monocytogenes*. Proteomics **4:**2991-3006.
30. **Sebbane, F., O. Jarrett, D. Gardner, D. Long, and B. J. Hinnebusch.** 2006. Role of the *Yersinia pestis* plasminogen activator in the incidence of distinct septicemic and bubonic forms of flea-borne plague. Proc. Natl. Acad. Sci. U S A **103:**5526-5530.
31. **Setyono-Han, B., J. Stürzebecher, W. A. Schmalix, B. Muehlenweg, A. M. Sieuwerts, M. Timmermans, V. Magdolen, M. Schmitt, J. G. Klijn, and J. A. Foekens.** 2005. Suppression of rat breast cancer metastasis and reduction of primary tumour growth by the small synthetic urokinase inhibitor WX-UK1. Thromb. Haemost. **93:**779-786.
32. **Sijbrandi, R., T. Den Blaauwen, J. R. H. Tame, B. Oudega, J. Luirink, and B. R. Otto.** 2005. Characterization of an iron-regulated alpha-enolase of *Bacteroides fragilis*. Microbes Infect. **7:**9-18.
33. **Sokol, H., P. Seksik, J. P. Furet, O. Firmesse, I. Nion-Larmurier, L. Beaugerie, J. Cosnes, G. Corthier, P. Marteau, and J. Doré.** 2009. Low counts of *Faecalibacterium prausnitzii* in colitis microbiota. Inflamm. Bowel Dis. **15:**1183-1189.
34. **Stecher, B. and W. D. Hardt.** 2008. The role of microbiota in infectious disease. Trends Microbiol. **16**:107-114.
35. **Steinert, M., U. Hentschel, and J. Hacker.** 2000. Symbiosis and pathogenesis: evolution of the microbe-host interaction. Naturwissenschaften **87:**1-11.
36. **Sun, H.** 2005. The interaction between pathogens and host coagulation system. Physiology **21:**281-288.
37. **Sun, H., U. Ringdahl, J. W. Homeister, W. P. Fay, N. C. Engleberg, A. Y. Yang, L. S. Rozek, X. Wang, U. Sjobring, and D. Ginsburg.** 2004. Plasminogen is a critical factor for group A streptococcal infection. Science **305:**1283-1286.
38. **Turroni, F., E. Foroni, P. Pizzetti, V. Giubellini, A. Ribbera, P. Merusi, P. Cagnasso, B. Bizzarri, G. L. de'Angelis, F. Shanahan, D. van Sinderen, and M. Ventura.** 2009. Exploring

the diversity of the bifidobacterial population in the human intestinal tract. Appl. Environ. Microbiol. **75:**1534-1545.

39. **Veiga, P., C. A. Gallini, C. Beal, M. Michaud, M. L. Delaney, A. DuBois, A. Khlebnikov, J. E. van Hylckama Vlieg, S. Punit, J. N. Glickman, A. Onderdonk, L. H. Glimcher, and W. S. Garrett.** 2010. *Bifidobacterium animalis* subsp. *lactis* fermented milk product reduces inflammation by altering a niche for colitogenic microbes. Proc. Natl. Acad. Sci. U S A **107:**18132-18137.

40. **Yan, Y., G. Dalmasso, H. T. Nguyen, T. S. Obertone, L. Charrier-Hisamuddin, S. V. Sitaraman, and D. Merlin.** 2008. Nuclear factor-kappaB is a critical mediator of Ste20-like proline-/alanine-rich kinase regulation in intestinal inflammation. Am. J. Pathol. **173:**1013-1028.

41. **Zhang, L., D. Seiffert, B. J. Fowler, G. R. Jenkins, T. C. Thinnes, D. J. Loskutoff, R. J. Parmer, and L. A. Miles.** 2002. Plasminogen has a broad extrahepatic distribution. Thromb. Haemost. **87:**493-501.

TABLE 1. qPCR quantification of the Plg-mediated adhesion of *B. lactis* BI07 to untreated and TNF-α pre-treated HT29 cells

Experimental conditions*	Bacteria / HT29 cell	
	TQ	**+ TNF-α**
BI07	18.87 ± 1.20	26.65 ± 2.02
BI07 + Plg	61.08 ± 2.85	41.09 ± 2.74
BI07 + Plg + anti-Plg	19.40 ± 1.24	-
BI07 + Plg + uPA	28.87 ± 1.93	36.57 ± 2.27
BI07 + Plg + uPA + Aprotinin	81.15 ± 3.58	79.81 ± 3.52
BI07 + Plg + Aprotinin	-	75.96 ± 5.67
BI07 + Aprotinin	20.23 ± 2.21	23.55 ± 1.55

*Untreated (TQ) and TNF-α pre-treated (+ TNF-α) confluent HT29 cell monolayers were incubated with 5 x 10^7 CFU of: untreated *B. lactis* BI07 cells (BI07); Plg pre-treated *B. lactis* BI07 cells (BI07 + Plg); Plg pre-treated *B. lactis* BI07 cells incubated with anti-Plg IgG (BI07 + Plg + anti-Plg); Plg and uPA pre-treated *B. lactis* BI07 cells (BI07 + Plg + uPA); Plg and uPA pre-treated *B. lactis* BI07 cells in the presence of aprotinin (BI07 + Plg + uPA + Aprotinin); Plg pre-treated *B.*

366 *lactis* BI07 cells in the presence of aprotinin (BI07 + Plg + Aprotinin); *B. lactis* BI07 cells in the
367 presence of aprotinin (BI07 + Aprotinin)
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375 **Figure legend**
376
377 **Fig. 1. Intracellular invasion assays of *B. lactis* BI07 into untreated (A) and TNF-α pre-treated**
378 **(B) HT29 cells.**
379 HT29 cell monolayers grown to confluence were incubated with 5 x 10^7 CFU of *B. lactis* BI07
380 untreated (BI07), pre-treated with Plg (+Plg) and pre-treated with Plg and uPA (+Plg+uPA).
381 Intracellular and adherent bacteria were differentiated by double immunofluorescence microscopy.
382 Adherent *B. lactis* BI07 bacteria (indicated by arrows) were stained with mouse anti-bifidobacterial
383 antiserum followed by a secondary Alexa-Fluor 488-conjugated anti-mouse antibody (green). After
384 HT29 cell permeabilization, internalized bacteria were stained with mouse anti-bifidobacterial
385 antiserum and a secondary Alexa-Fluor 568-conjugated anti-mouse antibody (red). HT29 cells are
386 visualized in the corresponding phase contrast images shown in the lower panel (magnification: X
387 100). For each experimental condition, the assay was repeated three times. A representative
388 experiment is shown.
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