Pathogen Virulence Impedes Mutualist-Mediated Enhancement of Host Juvenile Growth via Inhibition of Protein Digestion

Graphical Abstract

Highlights

- *Lactobacillus plantarum*<sub>WJL</sub> sustains gut peptidase expression in *Drosophila*
- *Lp*<sub>WJL</sub> association enhances dietary protein digestion and host amino acid levels
- Foodborne infection impedes *Lp*<sub>WJL</sub>-mediated intestinal peptidase activity
- Pathogen virulence impedes *Lp*<sub>WJL</sub>-mediated promotion of juvenile growth

In Brief

The microbial environment impacts animal physiology. Erkosar et al. reveal that commensal *Lactobacillus plantarum*<sup>WJL</sup> enhances *Drosophila* growth by promoting intestinal peptidase expression, thereby increasing protein digestion and amino acid levels. However, acute infection impedes this physiological benefit, suggesting that the host activates immune defense at the expense of mutualist-mediated growth promotion.
Pathogen Virulence Impedes Mutualist-Mediated Enhancement of Host Juvenile Growth via Inhibition of Protein Digestion

Berra Erkosar,1,2,4 Gilles Storelli,1,4 Mélanie Mitchell,1 Loan Bozonnet,1 Noémie Bozonnet,1,3 and François Leulier1,4

1Institut de Génomique Fonctionnelle de Lyon, Ecole Normale Supérieure de Lyon, Centre National de la Recherche Scientifique, Université Claude Bernard Lyon 1, Unité Mixte de Recherche S242, 69364 Lyon, Cedex 07, France
2Present address: Department of Ecology and Evolution, University of Lausanne, CH 1015 Lausanne, Switzerland
3Present address: University of Massachusetts Medical School, 373 Plantation Street, Suite 319, Worcester, MA 01605, USA
4Co-first author
*Correspondence: francois.leulier@ens-lyon.fr
http://dx.doi.org/10.1016/j.chom.2015.09.001
This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

SUMMARY

The microbial environment impacts many aspects of metazoan physiology through largely undefined molecular mechanisms. The commensal strain Lactobacillus plantarumWJL (LpWJL) sustains Drosophila hormonal signals that coordinate systemic growth and maturation of the fly. Here we examine the underlying mechanisms driving these processes and show that LpWJL promotes intestinal peptidase expression, leading to increased intestinal proteolytic activity, enhanced dietary protein digestion, and increased host amino acid levels. LpWJL-mediated peptidase upregulation is partly driven by the peptidoglycan recognition and signaling cascade PGRP-LE/Imd/Relish. Additionally, this mutualist-mediated physiological benefit is antagonized upon pathogen infection. Pathogen virulence selectively impedes LpWJL-mediated intestinal peptidase activity enhancement and juvenile growth promotion but does not alter growth of germ-free animals. Our study reveals the adaptability of host physiology to the microbial environment, whereby upon acute infection the host switches to pathogen-mediated host immune defense at the expense of mutualist-mediated growth promotion.

INTRODUCTION

Metazoans establish diverse forms of functional interactions with their microbial environment, and such interactions contribute to many aspects of animal development, physiology, and evolution (McFall-Ngai et al., 2013). Mutualistic interactions are a type of symbiosis where both partners bring reciprocal functional benefits to each other. In contrast, in pathogenic or parasitic interactions, the association is beneficial for one partner and deleterious for the other (Douglas, 2010). Despite recent progress, the molecular mechanisms through which the microbial environment exerts its beneficial influences on animal biology are still largely undefined.

Drosophila melanogaster has recently emerged as a powerful model organism to study beneficial host-bacteria interactions (Erkosar et al., 2013; Lee and Brey, 2013). Drosophila is associated with bacterial communities of low complexity composed of a handful of dominant species (mostly of the Acetobacteraceae and Lactobacillaceae families). The powerful genetic tools in Drosophila, coupled to the simplicity to cultivate germ-free (GF) animals and to manipulate its commensal bacterial species makes Drosophila an ideal host model to study the molecular mechanisms underlying bacteria-mediated physiological benefits. Commensal bacteria affect Drosophila biology throughout its life cycle (Buchon et al., 2013; Erkosar et al., 2013; Lee and Brey, 2013). In adults, they influence lifespan (Brummel et al., 2004; Guo et al., 2014; Ren et al., 2007), dictate host nutrition and metabolic responses (Wong et al., 2014; Yamada et al., 2015), shape mating preference (Sharon et al., 2010), mediate social attraction (Venu et al., 2014), increase host resistance to several intestinal pathogens (Blum et al., 2013), modulate intestinal immune homeostasis (Bosco-Drayon et al., 2012; Lhocine et al., 2008; Paredes et al., 2011), and promote intestinal epithelium renewal (Buchon et al., 2009a; Shin et al., 2011). During the juvenile phase (i.e., from the end of embryogenesis until metamorphosis), commensal microbes sustain larval growth and maturation rate, a feature even more pronounced when the host is facing undernutrition (Shin et al., 2011; Storelli et al., 2011). We previously showed that a selected strain of Lactobacillus plantarum isolated from Drosophila intestine, Lactobacillus plantarumWJL (LpWJL) (Ryu et al., 2008), is sufficient on its own to recapitulate the beneficial effect of more complex Drosophila-associated bacterial communities on host juvenile growth, especially upon undernutrition (Storelli et al., 2011). Using this monoxenic animal model (one microbe-one host), we revealed that LpWJL exerts its beneficial activity by acting upstream of hormonal signals known to coordinate Drosophila systemic growth and maturation rates. Indeed, we demonstrated that LpWJL association requires optimal mTOR signaling activity in the fat-body (Buchon et al., 2013; McFall-Ngai et al., 2013)."
acids (AAs), which are the main activators of TOR kinase activity. We therefore proposed that \(Lp^{WJL}\) may exert its growth-promoting effect by enhancing protein assimilation in the host to promote optimal AAs availability that triggers mTOR signaling and subsequently sustains systemic growth and maturation (Storelli et al., 2011).

In this manuscript, we directly address this hypothesis and show that \(Lp^{WJL}\) association sustains intestinal peptidase expression, partly via the PGRP-LE/Imd/Relish signaling cascade, which translates into increased intestinal proteolytic activity, enhanced protein digestion, and improved AA levels in the host. In addition, we reveal that this mutualist-mediated physiological benefit is antagonized upon foodborne pathogen infection.

**RESULTS**

**Lactobacillus plantarum Association Sustains Intestinal Peptidase Gene Expression during Juvenile Growth**

Previously, we studied changes in host transcriptome in adult flies associated with a cocktail of commensal bacterial strains and detected several peptidase genes whose expressions are significantly upregulated as compared to their GF siblings (Eroksar et al., 2014). Using the FlyAtlas tool, we identified that most of these peptidase genes have a basal expression level enriched in both the adult and larval midguts of the conventional individuals (CONV; i.e., carrying commensal microbes), a signature confirmed in adult midguts by RT-qPCR (Eroksar et al., 2014). Since we hypothesized that \(Lp^{WJL}\) may exert its growth-promoting activity during juvenile development by enhancing protein assimilation in the host (Storelli et al., 2011), this observation prompted us to investigate the expression of these peptidase genes by time course RT-qPCR in larval midguts of GF or \(Lp^{WJL}\)-associated individuals on a low-nutrition diet (see Figure 1A for detailed experimental scheme). We found the expression profile of 7 peptidases (Jon66Cii, Jon66Ci, Jon44E, Jon65Ai, Jon99Ci, CG18179, and CG18180) presents a robust and detectable transcriptional signature during larval development (Figures 1B–II). Principal-component analysis (PCA) on the whole dataset (expression levels of the seven genes at all time points in both conditions) reveals that PC1, which explains 80.5% of the observed variance, is enough to separate the GF and \(Lp^{WJL}\) groups. Such separation indicates that peptidase expression levels in the midguts of \(Lp^{WJL}\)-associated larvae remarkably differ from the GF condition (Figure 1B). This difference is also strongly significant when the whole dataset is analyzed by multivariate analysis of variance (MANOVA-Bacteria: \(p < 0.001\); Figure 1B; Table S1); furthermore, we compared each of the seven peptidases in GF and \(Lp^{WJL}\) larvae during the same defined period of growth and found that the expression of any given peptidase is significantly elevated in \(Lp^{WJL}\)-associated midguts (Figures 1C–II and ANOVA-Bacteria; Table S1). Notably, the peptidase gene expression tends to increase during larval development even in GF condition (MANOVA-Time and ANOVA-Time and GF Group; Table S1), but the trend of increase is only marginally affected by the bacterial association (MANOVA-Bacteria \(\times\) Time and ANOVA-Bacteria \(\times\) Time; Table S1). Taken together, these results demonstrate that \(Lp^{WJL}\) association triggers an overall increase in the expression levels of several intestinal peptidases but does not alter their expression dynamics during juvenile growth.

**Lactobacillus plantarum Association Enhances Intestinal Proteolytic Activity, which is Necessary and Sufficient for Juvenile Growth Promotion**

Next, we measured intestinal proteolytic activity upon \(Lp^{WJL}\) association. To this end, we dissected midguts from either GF or \(Lp^{WJL}\)-associated larvae grown on low-nutrition diet, and assayed intestinal proteolytic activity over time. Similarly to the expression profile of the peptidase genes delineated in Figure 1, the intestinal protease activities in GF larval midguts increase steadily during the period tested, and upon \(Lp^{WJL}\) association, the intestinal proteolytic activities are consistently higher than that in the GF animals (Figure 2A). To rule out the possibility that such observed increase of proteolytic activities are the result of a mere addition of the bacterial protease activity to the system, we monitored the proteolytic activities of live \(Lp^{WJL}\) cells and in the supernatant harvest from an over-night culture. With \(10^9\) \(Lp^{WJL}\) cells, a quantity several logs above the bacterial load detected in \(Lp^{WJL}\)-associated midguts (see Figure 6D) or in the supernatant, we detected marginal proteolytic activities using the same biochemical assay as compared to the activity detected from either GF or \(Lp^{WJL}\)-associated midguts (Figure S1).

We then questioned the functional importance of such intestinal proteolytic activity for Drosophila juvenile growth. To this end, we grew Drosophila GF larvae on the low-nutrition diet mixed with increasing quantities of either a complete Protease Inhibitor Cocktail (PIC) or a specific irreversible serine-protease inhibitor (AEBSF) and quantified the length of all individual larvae fed on such diet 7 days after egg deposition (AED). The growth rate of GF larvae was reduced, resulting in smaller larvae at day 7 AED as the quantities of protease inhibitors in their diet increase (Figures 2B–2D). Interestingly, \(Lp^{WJL}\) association, which enhances peptidase expression and activity, buffers the deleterious effect of protease inhibitors on juvenile growth, as exemplified by a reduced sensitivity to protease inhibitors of \(Lp^{WJL}\)-associated larvae (Figures 2B and 2C), yet at higher protease inhibitors concentration, \(Lp^{WJL}\)-mediated growth promotion was also diminished (Figures 2B and 2D). Our results therefore demonstrate that \(Lp^{WJL}\) association sustains intestinal protease activity and that intestinal peptidase activity is required for juvenile growth in general and \(Lp^{WJL}\)-mediated growth promotion in particular.

Next, we tested if the induced expression of an intestinal protease is sufficient to trigger larval growth. To this end, we directed the ectopic expression of Jon66Cii (the protease most differentially expressed upon \(Lp^{WJL}\) association) in the midgut of young GF larvae and assayed their longitudinal growth. We observed a marked growth promotion upon Jon66Cii induction in the enterocytes of both the entire midgut (mex-GAL4; Figure 2E) (Phillips and Thomas, 2006) and of the acidic region of the middle midgut (labial-GAL4; Figure 2F) (Hoppler and Bienz, 1994). These results therefore demonstrate that intestinal peptidase expression is sufficient to trigger juvenile growth. Collectively, our results demonstrate that \(Lp^{WJL}\) association enhances intestinal protease expression and activity, which is necessary and sufficient for juvenile growth promotion upon undernutrition.
Lactobacillus plantarum Association Promotes Protein Digestion and Sustains AA Levels during Juvenile Growth

We next questioned the physiological consequences of LpWJL-mediated enhanced intestinal proteolytic activity and wondered if that phenomenon increases protein digestion and free AAs levels in the host. To this end, we analyzed the relevant data from a global metabolomic study of GF and LpWJL-associated animals; the emergence of the first white pupae is used as an anchor for relative timings in each condition (1 = day 10 AED for LpWJL condition and D12 for GF condition). AED: after egg deposition.

AAs and 50 dipeptides were detected and quantified in the lysates of GF and LpWJL larvae (Table S1). A PCA analysis of this dataset tends to group the biological replicates generated from the same condition and separates the samples according to the bacterial association for either the free AAs (Figure 3A) or the dipeptides datasets (Figure 3B). Interestingly, the levels of most free AAs, including essential AAs, are increased in LpWJL larvae (Table S1; Figure 3D).

Figure 1. L. plantarum Association Sustains Intestinal Peptidase Gene Expression during Juvenile Growth

(A) Experimental setup for the RT-qPCR analysis. Actual and relative developmental timings and developmental stages are indicated for GF and LpWJL-associated animals; the emergence of the first white pupae is used as an anchor for relative timings in each condition (1 = day 10 AED for LpWJL condition and D12 for GF condition). AED: after egg deposition.

(B) Projection of the RT-qPCR dataset into the space of the first and second PCs. d: size of the background grid.

(C–I) Mean ± SEM of ΔCt\text{mem}/ΔCt\text{rp49} ratios for (C) Jon66Cii, (D) Jon66Ci, (E) Jon44E, (F) Jon65Ai, (G) Jon99Ci, (H) CG18179, and (I) CG18180 detected in midguts of GF (red) and LpWJL-associated (blue) larvae along larval development. p values of the MANOVA analysis from all variables (B) and gene-specific two-way ANOVA (C–I) are given (“Bacteria” effect only). See also Tables S1 and S2.
These observations strongly suggest that \( L.\ p.\ ^{W.U.} \) association increases protein digestion to increase host’s AAs levels.

**PGRP-LE/Imd/Relish Pathway Partly Regulates *Lactobacillus plantarum*-Mediated Intestinal Peptidase Gene Expression during Juvenile Growth**

We next wondered how \( L.\ p.\ ^{W.U.} \) association triggers the upregulation of the host intestinal peptidases. To tackle this question, we analyzed the expression dynamics of the peptidases genes influenced by \( L.\ p.\ ^{W.U.} \) association in a mutant background where the host transcriptomic response to the microbial environment is impaired, namely in *Dredd* mutants. Loss of *Dredd* results in complete loss of function of the Imd/Relish signaling pathway (Leulier et al., 2000). This signaling cascade, which is triggered by the direct sensing of peptidoglycan fragments of bacterial origin, is the prerequisite to induce the expression of hundreds of immune-regulated genes (De Gregorio et al., 2002), mount efficient immune responses to infectious bacteria (Myllymäki et al., 2014), and trigger immune tolerance to commensal microbes (Bosco-Drayon et al., 2012; Lhocine et al., 2008; Paredes et al., 2011).

Importantly, we and others have recently demonstrated the central role of the Imd/Relish cascade activity to promote the expression of many microbiota-regulated genes in the adult midgut (Broderick et al., 2014; Erkosar et al., 2014). We therefore analyzed the expression levels of the midguts in the midguts of wild-type and *Dredd* mutant larvae during juvenile growth on the low-nutrition diet in either GF or \( L.\ p.\ ^{W.U.} \)-associated conditions (Figures 4A–4H). As a control, we quantified the expression of PGRP-SC1a/b, a known microbiota-regulated gene whose induction in the larval midgut upon \( L.\ p.\ ^{W.U.} \) association is dependent on the Imd/Relish cascade activity (Figure 4I) (Bosco-Drayon et al., 2012). We first analyzed the entire expression dataset (for all time points, genotypes, and conditions) by projecting the expression results on two principal components (PCs) (Figure 4A). This PCA clearly singled out the \( L.\ p.\ ^{W.U.} \)-mediated enhancement of expression of these peptidases in wild-type animals (as seen in Figure 1C). Of note, \( L.\ p.\ ^{W.U.} \)-associated peptidase expression data points grouped in two distant pools separated by genotype (WT versus *Dredd*), while the GF individuals data grouped closer. This signature indicates that *Dredd* governs peptidase expression in the larval midgut, with a marked effect upon \( L.\ p.\ ^{W.U.} \) association. The statistical analysis of the whole dataset confirms this observation and reveals a very strong statistical interaction between bacterial association and genotype (Figure 4A and MANOVA-Bacteria × Genotype; Table S1). In addition, we further analyzed the time course expression profiles of individual peptidase genes with or without \( L.\ p.\ ^{W.U.} \) in different genetic background (Figures 4B–4H). First, the expression levels of Jon66Ci, CG18179, and PGRP-SC1a/b in both GF and \( L.\ p.\ ^{W.U.} \)-associated animals are *Dredd* dependent, and loss of *Dredd* markedly dampens the \( L.\ p.\ ^{W.U.} \)-mediated enhancement of expression of these peptidases to a level similar to that of the wild-type GF animals (Figures 4B, 4C, and 4I). The basal expression level (i.e., in GF) and \( L.\ p.\ ^{W.U.} \)-mediated induction of Jon66Ci and Jon65Ai only exhibit a partial *Dredd* dependency (Figures 4D and 4E). In contrast, Jon44E, Jon99Ci, and CG18180 induction upon \( L.\ p.\ ^{W.U.} \) association is moderately *Dredd* dependent, despite statistical significance being reached (Jon44E and Jon99Ci; Figures 4F and 4G) or even *Dredd* independent (Figure 4H). Finally, the basal expression level of Jon44E is reduced in *Dredd* mutant, while basal expression levels of Jon99Ci and CG18180 are increased in the same context (Figures 4G and 4H). Taken together, these gene specific signatures illustrate the complexity of the intestinal peptidase gene regulation. Yet our results clearly demonstrate that the activity of the Imd/Relish cascade is necessary to mediate the expression of several peptidases in the larval midgut upon \( L.\ p.\ ^{W.U.} \) association.

We next focused on Jon66Ci and CG18179, whose induction is strongly upon \( L.\ p.\ ^{W.U.} \) association and markedly *Dredd*...
dependent, and tested if PGRP-LE, one of the pattern recognition receptor acting upstream of the Imd/Relish signaling cascade (Kaneko et al., 2006; Takehana et al., 2004) is necessary for the induction of these two peptidases upon LpWJL association. Similarly to PGRP-SC1a/b (Bosco-Drayon et al., 2012) (Figure 4L), Jon66Cii and CG18179 induction upon LpWJL association was strongly impaired in PGRP-LE mutant larvae (Figures 4J and 4K). PGRP-LE is enriched in both adult and larval midgut (Neyen et al., 2012) and has been proposed to function as a direct peptidoglycan sensor in the adult and larval enterocytes (Bosco-Drayon et al., 2012; Neyen et al., 2012). We therefore wanted to test if PGRP-SC1a/b, Jon66Cii, and CG18179 induction upon LpWJL association requires the PGRP-LE/Imd/Relish pathway activity specifically in the enterocytes. Pirk is an inhibitor of this signaling cascade that alters Imd activation by PGRPs (Aggarwal et al., 2008; Kleino et al., 2008; Lhocine et al., 2008). We thus selectively overexpressed Pirk in enterocytes with mex-GAL4 and observed that LpWJL-mediated induction of Jon66Cii, CG18179, and PGRP-SC1a/b is markedly reduced (Figures 4M–4O). Therefore, the PGRP-LE/Imd/Relish pathway activity is required in enterocytes to mediate the induction of selected intestinal peptidase gene upon LpWJL association.

Having demonstrated that the LpWJL-mediated peptidase expression enhancement is at least partly Dredd dependent and requires the PGRP-LE/Imd/Relish pathway activity in the enterocytes, we detected no alteration of the intestinal proteolytic activity of Dredd mutant larval midguts in either GF or LpWJL-associated condition (Figures S2 A–S2C) or any marked impact of Dredd loss of function on LpWJL-mediated juvenile growth promotion (Figure S2 D). These results indicate that the Dredd dependency is partial and not rate limiting for LpWJL-mediated intestinal peptidase activity and juvenile growth promotion and suggest a more complex regulation of peptidase expression.

**Infection by Foodborne Pathogen Antagonizes Lactobacillus plantarum-Mediated Intestinal Proteolytic Activity during Juvenile Growth**

We recently reported the existence of a transcriptional switch upon foodborne infection in adults, where the induction of infection-mediated midgut genes occurs while several microbiota-mediated midgut genes are silenced (Erkosar et al., 2014). We therefore wondered if that phenomenon applies to LpWJL-mediated gene regulation in the larval midguts. To test this hypothesis, we studied the expression of the peptidases in LpWJL-associated larvae after an acute oral infection by the intestinal pathogen Pectobacterium carotovorum spp. carotovorum strain 15 (referred to as Ecc15) (Basset et al., 2003). As a control of the effective infection, we analyzed the expression of the antimicrobial peptide gene Attacin-D (AttD), which is a marker of the host intestinal immune response to this infection (Bosco-Drayon et al., 2012; Tzou et al., 2000). As expected, AttD is potently induced in the LpWJL-associated larval midgut upon Ecc15 infection (Figure 5A). In contrast, the expression of all seven LpWJL-regulated peptidases was dramatically
reduced upon infection (Figures 5B–5H). Accordingly, when we analyzed the intestinal proteolytic activity of LpWJL-associated midguts after infection, we detected an almost complete inhibition of the LpWJL association effect, with proteolytic activity levels decreasing upon infection to levels similar to those seen on GF midguts (Figure 5I). Collectively, these results demonstrate that acute pathogen infection antagonizes the LpWJL-mediated promotion of peptidase activity during juvenile growth.

Pathogen Virulence Impedes LpWJL-Mediated Juvenile Growth Promotion by Antagonizing LpWJL-Mediated Enhancement of Host Protein Digestion Capacities

We next addressed the physiological consequence of an acute oral infection on LpWJL-mediated juvenile growth promotion. To this end, we acutely infected a subset of GF and LpWJL-associated larvae at day 3 AED with increasing quantities of virulent Ecc15 or the avirulent mutant Ecc15Evf (Basset et al., 2003) and measured the size of GF and LpWJL-associated larvae on day 7 AED on the low-nutrition diet (Figures 6A, 6B, and 6E). Upon acute challenge with Ecc15, the enhanced longitudinal growth of LpWJL-associated larvae is significantly altered in an Ecc15 dose-dependent manner (Figure 6A). However, when LpWJL-associated larvae were acutely infected with increasing quantities of the avirulent mutant Ecc15Evf, the LpWJL-mediated benefit on larval growth persisted in all infection conditions tested (Figure 6B). Importantly, the alteration of LpWJL-mediated benefit on larval growth by the acute Ecc15 infection is not the result of a competition between LpWJL and Ecc15, since we detected similar amounts of LpWJL in the larvae pre- and post-infection (Figure 6D). Interestingly, GF larvae that were acutely infected with increasing quantities of Ecc15 at day 3 AED (Figure 6C) or at day 5 AED (GF larvae on day 5 AED match day 3 AED LpWJL-associated larvae in size; Figure S3) grew to their usual average size. Hence, our
results demonstrate that the virulence of Ecc15 specifically antagonizes the beneficial activity of LpWJL on juvenile growth but does not alter basal growth rate (i.e., as seen in GF). These results reveal that a selective physiological switch occurs in LpWJL-associated juveniles when facing Ecc15 foodborne infection whereby longitudinal growth is stunted. Next, we tested if Ecc15 infection would alter LpWJL-mediated benefit on larval growth in Dredd mutant larvae and observed that Ecc15 infection also antagonizes LpWJL-mediated benefit on the systemic growth in Dredd larvae. Therefore, the Imd/Relish signaling is not rate limiting for this physiological switch to occur (Figure S4).

Taken collectively, our results suggest that Ecc15 infection impedes LpWJL-mediated juvenile growth promotion by antagonizing LpWJL-mediated enhancement of host protein digestion capacities. To formally validate this model, we forced the expression of Jon66Cii in the midgut of Ecc15-infected animals and tested if it would rescue LpWJL-mediated juvenile growth promotion as expected under this hypothesis. To this end, we directed the expression of Jon66Cii either in all enterocytes of the larval midgut (Figure 6F) or just in the enterocytes of the acidic region of the middle midgut (Figure 6G) and assayed larval longitudinal growth of LpWJL-associated animals at day 7 AED after an Ecc15 infection at day 3 AED (Figure 6E). In both cases, we observed a marked rescue of LpWJL-mediated longitudinal growth when Jon66Cii was expressed under the control of the UAS/GAL4 system upon Ecc15 infection (Figures 6F and 6G). These results therefore demonstrate that Ecc15 infection alters LpWJL-mediated growth by antagonizing intestinal protease expression.

**Figure 5. Foodborne Pathogen Infection Antagonizes L. plantarum-Mediated Intestinal Peptidase Expression and Activity during Juvenile Growth**

(A–H) Mean ± SEM of ΔCt/DCT ratios for (A) Attacin-D, (B) Jon66Cii, (C) Jon66Ci, (D) Jon44E, (E) Jon65Ai, (F) Jon99Ci, (G) CG18179, and (H) CG18180 detected in midguts of wild-type larvae associated with LpWJL for 8 days AED. 8 hr prior midgut dissection, animals were infected with Ecc15 (OD 100) or sham treated. Corrected p values obtained from comparison of group means by Student’s t test with Welch correction are indicated.

(i) Proteolytic activity (mean ± SEM) in midguts dissected from size-matched GF (12 days AED) and LpWJL-associated animals (8 days AED), sham-treated or 8 hr post-Ecc15 infection. Proteolytic activity is expressed as a.u. p values obtained by one-way ANOVA are indicated. Results of Tukey’s post hoc pairwise comparisons confirm the statistical significance observed. See also Figure S2 and Table S1.
Our results support the notion that the regulation of intestinal protease expression by commensal microbes plays an important role in the context of mutualist-mediated juvenile growth promotion upon undernutrition. We show that association of GF Drosophila juveniles with the mutualistic bacterial strain \( Lp^{Wt} \) promotes intestinal peptidase expression and activity, and such gut functionality is necessary and sufficient to sustain host systemic growth. In addition, we reveal that \( Lp^{Wt} \) association enhances dietary protein digestion—a feature characterized by increased levels of dipeptides and free AAs in \( Lp^{Wt} \)-associated animals. This observation, together with our previous results positing that \( Lp^{Wt} \)-mediated juvenile growth promotion genetically requires the AA transporter Slimfast and the kinase mTOR, indicates that \( Lp^{Wt} \) promotes juvenile growth via enhanced intestinal peptidase expression and increased AAs assimilation, which optimizes the activity of the mTOR kinase in endocrine tissues producing dILPs and Ec dyscone, the two main drivers of systemic growth and maturation (Figure 7).

Recently, Yamada et al. (2015) showed that in the context of Drosophila aging, Issatchenka orientalis, a Drosophila commensal fungus, extracts AAs directly from nutrient-poor diets and increases AAs flux to the host by being a direct food source for Drosophila. Hence, \( I. \) orientalis association increases the lifespan of undernourished flies. This work indicates that, upon undernutrition, commensal fungi can become a food source for their host and may impact host physiology by fortifying the diet (Yamada et al., 2015). Whether this concept applies to commensal bacteria in general and \( Lp^{Wt} \)-mediated juvenile growth promotion in particular is an intriguing question. At this point, we cannot entirely rule out this possibility. However, we have previously demonstrated that other strains of \( L. \) plantarum \((L^{BDM1}\) and other strains), which can colonize efficiently the larval gut and the nutritional environment, do fail to mediate juvenile growth promotion (Storelli et al., 2011) (G.S. and F.L., unpublished data). Furthermore, in this study, we show that \( Lp^{Wt} \) association triggers a host transcriptional response in the Drosophila larval intestine to support essential gut functionalities necessary for systemic growth. These results support a model where \( Lp^{Wt} \) sustains Drosophila systemic growth via the promotion of specific host biological activities, rather than by being a mere food source for its host. In this light, our work now reveals that enhanced host intestinal peptidase expression should be considered as a host biological activity required for physiological benefit mediated by a mutualist association.

While we still lack a complete understanding of how \( Lp^{Wt} \) mediates the induction of intestinal peptidase gene expression, we found that some peptidase gene induction occurs in larval enterocytes and are partly regulated by the PGRP-LE/Imd/Relish cascade, a signaling pathway devoted to bacterial sensing by the host and previously associated with regulation of host immunity in the intestinal epithelium (Bosco-Drayon et al., 2012; Neyen et al., 2012). These results therefore demonstrate that in addition to regulating immune responses, the PGRP-LE/Imd/Relish cascade also influences dietary protein digestion in the midgut, albeit without being rate limiting to this biological process. Interestingly, we and others have previously shown that in adult midguts the Imd/Relish cascade is required for microbiota-induced expression of other digestive enzymes such as lipases, glycosyl-hydrolases, and alkaline phosphatase, thus raising the possibility that this signaling cascade may influence other digestive processes beyond dietary protein breakdown (Broderick...
is promoted in enterocytes while intestinal proteases expression is silenced. Virulence causes a transcriptional switch whereby immune genes expression

(L. plantarum) and promotes increases production of dILPs and Ecdysone, which together

increases the digestion and uptake of dietary proteins into dipeptides and AAs.

Drosophila's

PGP-LE/Imd/Relish cascade only contributes partly to their regulation (Figure 7). Therefore, our work paves the way to further dissections of the regulatory networks underlying intestinal peptidase gene induction, which may shed light on regulatory cross talks between immunity and digestion.

The hypothesis of regulatory cross-talks between immunity and digestion is also supported by the observation that in adult midguts a transcriptional switch occurs upon foodborne bacterial infection, whereby several digestive enzymes expression is silenced while immune-related gene expression is induced (Buchon et al., 2009b). Interestingly, such transcriptional switch favors the induction of infection-mediated midgut genes at the expense of microbiota-mediated midgut genes (Erkosar et al., 2014). Here we discovered the same transcriptional switch in LpWJL-associated juveniles upon infection with the pathogenic strain Ecc15 by showing that LpWJL-mediated intestinal protease induction is silenced upon Ecc15 infection. Consequently, LpWJL-mediated intestinal protease activity was suppressed. This striking observation allowed us to investigate the consequences of such transcriptional switch on the host physiology. LpWJL association to GF juveniles has a profound effect on host physiology by promoting juvenile longitudinal growth (Storelli et al., 2011). We now reveal that in LpWJL-associated juveniles, Ecc15 foodborne infection triggers a physiological switch stunting longitudinal growth. This switch is triggered by pathogen virulence, since infection with an Ecc15 avirulent mutant (Ecc15Avr) did not trigger stunting of LpWJL-associated juveniles. Surprisingly, age-matched or size-matched GF juveniles were not stunted by Ecc15 foodborne infection, suggesting that Ecc15 virulence selectively antagonized LpWJL-mediated benefit to host longitudinal growth. These results therefore illustrate how the host adapts its physiology when facing either mutualists or pathogens by manipulating its digestive activity to influence its growth patterns during the juvenile phase of its life cycle.

Infection-associated host physiological switches were previously reported in Drosophila; Listeria monocytogenes or Salmonella typhimurium infections trigger anorexia (Ayres and Schneider, 2009), Streptococcus pneumoniae infection triggers the loss of circadian locomotor activity (Shirasu-Hiza et al., 2007), and Mycobacterium marinum and Listeria monocytogenes infections trigger metabolic switches and wasting (Chambers et al., 2012; Clark et al., 2013; Dionne et al., 2006), yet these studies were performed by injecting into the body cavity of adult flies a lethal dose of pathogens isolated from humans or other heterologous animals. These lethal infection models have furthered the understanding of the physiopathology of such lethal infection models but do not recapitulate the interaction between Drosophila and its natural microbial environment. In our experimental system, we used a natural pathogen and a natural mutualist of Drosophila so that we can reveal the physiological adaptability of Drosophila to its natural microbial environment, at least during the juvenile phase of its life cycle. With this setting, we find that pathogen virulence antagonizes a mutualist-mediated physiological benefit. Interestingly, it was previously established that, in the larval midgut, mutualists (including LpWJL) promote intestinal immune tolerance while pathogens (including

et al., 2014; Erkosar et al., 2014). Taken together with the finding in this study, we propose that there are shared regulatory modules between the digestive and immune processes and that the PGRP-LE/Imd/Relish is at the cornerstone of this regulation. This observation echoes the theory proposing a common evolutionary origin for immunity and digestion in primitive guts based on the observation that many enzymes used as antimicrobial effectors during intestinal immune responses also play a role in digestion or share molecular ancestry with digestive enzymes (Broderick, 2015; Lemaitre and Miguel-Aliaga, 2013). Our study bolsters the theory by identifying some of the regulatory modules of these processes. Yet our results show that the regulation of intestinal protease expression is complex and that the PGRP-LE/Imd/Relish cascade only contributes partly to their regulation (Figure 7). Therefore, our work paves the way to further dissections of the regulatory networks underlying intestinal peptidase gene induction, which may shed light on regulatory cross talks between immunity and digestion.
Ecc15) induce potent intestinal immune and tissue repair responses (Bosco-Drayon et al., 2012; Buchon et al., 2013). We therefore propose a model whereby, upon association with a mutualist microbe (such as LpWJL), the host optimizes its juvenile growth and immune tolerance to more quickly reach the reproductive stage of its life cycle, while upon acute pathogen infection (such as Ecc15 infection), the juvenile stunts its growth, allowing the triggered intestinal immune and tissue repair mechanisms to efficiently resolve the infectious episode that could be detrimental to its reproductive success at the adult stage. In this model, the PGRP-LE/Imd/Relish cascade activation and the regulation of intestinal peptidase expression stand as molecular cornerstones in these events (Figure 7).

Given the importance of the microbial environment in the ecological success and evolution of host species (McFall-Ngai et al., 2013) and the importance of immunity and juvenile growth in this context, our work, which illustrates the profound impact of the microbial environment on both traits, paves the way for future studies focusing on the adaptive value of the physiological switch triggered by pathogen virulence in the host.

**EXPERIMENTAL PROCEDURES**

**Drosophila Diets, Stocks, and Breeding**

*Drosophila* stocks were cultured at 25°C with 12/12 hr dark/light cycles (light switch at 1:00 PM) on a yeast/cornmeal medium containing 50 g/l inactivated yeast as described in Erkosar et al. (2014). The low-nutrition diet was obtained by reducing the amount of inactivated yeast to 6 g/l. AEBSF (Sigma, ref. #A8456) and PIC (Sigma, ref. #P2714) were included in the diets at indicated concentration. GF stocks were established as described in Erkosar et al. (2014). *Drosophila* y,w flies were used as the reference strain in this work.

**Bacterial Strains**

*Lactobacillus plantarum* (referred as LpWJL) (Ryu et al., 2008), *Pectobacterium carotovorum* spp. *carotovorum* (referred as Ecc) and *Pectobacterium carotovorum* spp. *carotovorum* (referred as Ecc) (Basset et al., 2003) were used in this study.

**Colonization and Infection of Larvae**

40 embryos collected from GF females were transferred to a fresh low-nutrition medium poured in small petri dishes (ø = 5 cm). Colonization and Infection of Larvae were used in this study. 40 embryos collected from GF females were transferred to a fresh low-nutrition medium poured in small petri dishes (ø = 5 cm). Colonization and Infection of Larvae were used in this study.

**Larval Size Measurements**

*Drosophila* larvae were collected 7 days AED, washed in distilled water, transferred on a microscopy slide, killed with a short heat treatment (5 s at 90°C), mounted in 80% glycerol/PBS, and pictured under a Leica stereomicroscope M205FA. Larval longitudinal size (length) was quantified using ImageJ software (Schneider et al., 2012).

**Bacterial Loads Analyses**

Bacterial loads were quantified by plating serial dilutions of lysates obtained from five individual cultures on nutrient agar (MRS). Biological triplicates were collected for each experimental condition. Homogenization of the samples was performed using the Precellys 24 tissue homogenizer (Bertin Technologies) and 0.75–1 mm glass beads in 500 µl of PBS.

**RNA Extraction and qPCR Analysis**

RNA extraction of three biological replicates of ten midguts (foregut, hindgut, and malphigian tubules removed) for each condition was performed as described in Erkosar et al. (2014). qPCR was performed using gene-specific primer sets (sequences provided in Table S2) and as described in Erkosar et al. (2014). Results were represented either as the value of ∆Ctref/∆CtRef ratios or as the relative fold induction of the ∆CtRef/∆CtRef ratios among conditions tested.

**Azocasein Assay**

Three biological replicates of ten midguts per condition were dissected in 50 µl of PBS and homogenized as for RNA extraction. 1 µl of sample were mixed with 300 µl of Azocasein solution (2.5 mg/ml in water, Sigma, ref. #A2765) and processed according to supplier’s instructions. For an extended protocol, see Supplemental Experimental Procedures.

**Metabolomics**

Axenic embryos were inoculated with PBS or 10^6 CFUs of LpWJL and reared on low-nutrition diet. Larvae were sampled at day 2.5 AED, snap frozen, and sent to Metabolon Inc. (http://www.metabolon.com). Five biological replicates were used containing each approximately 300 early L2 larvae of the same size. Samples were then extracted, normalized, and prepared for analysis using Metabolon’s standard solvent extraction method. The extracted samples were split into equal parts for analysis with GC/MS and LC/MS/MS. Compounds were identified by comparison to library entries of purified standards or recurrent unknown entities.

**Statistical Analysis**

Statistical calculations were made using R; details are provided in Supplemental Experimental Procedures. Detailed statistics are provided for each panel figures in Table S1.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes four figures, two tables, and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.chom.2015.09.001.

**AUTHOR CONTRIBUTIONS**

F.L. supervised the work. B.E., G.S., and F.L. designed the experiments. B.E., G.S., M.M., L.B., and N.B. performed experiments. B.E. performed statistics. F.L., B.E., and G.S. analyzed data. B.E. and F.L. wrote the paper.

**ACKNOWLEDGMENTS**

We thank S. Guillemin for technical help; M. Strigini and D. Ma for critical reading of the manuscript; J. Royet and T. Kawecki for support during this work; I. Miguel-Alia, N. Silverman, B. Charroux, S. Merabet, FlyORF, and the Bloomingtont stock center for fly lines; B. Lemaitre and W. J. Lee for bacterial strains; and the Arthro-Tools platform of the SFR Biosciences Gerland/Lyon-sud for Fly work. This work was funded by the ANR (ANR 2010 JCJC 1304 01) and an ERC starting grant (FP7/2007-2013-N.309704). G.S. is funded by the “Fondation pour la Recherche Medicale.” The lab of F.L. is sponsored by EMBO YIP, ATIP/AVENIR, FINOVI, and FSER.

Received: April 20, 2015

Revised: August 13, 2015

Accepted: September 8, 2015

Published: October 1, 2015

**REFERENCES**

Aggarwal, K., Rus, F., Vriesema-Magnuson, C., Ertürk-Hasdemir, D., Paquette, N., and Silverman, N. (2008). Rudra interrupts receptor signaling complexes to negatively regulate the IMD pathway. PLoS Pathog. 4, e1000120.

Ayres, J.S., and Schneider, D.S. (2009). The role of anorexia in resistance and tolerance to infections in Drosophila. PLoS Biol. 7, e1000150.

Basset, A., Tzou, P., Lemaitre, B., and Boccard, F. (2003). A single gene that promotes interaction of a phytopathogenic bacterium with its insect vector, Drosophila melanogaster. EMBO Rep. 4, 205–209.
Blum, J.E., Fischer, C.N., Miles, J., and Handelsman, J. (2013). Frequent replenishment sustains the beneficial microbiome of Drosophila melanogaster. MBio 4, e00860–e13.

Bosco-Drayon, V., Poidevin, M., Boneca, I.G., Narbonne-Reveau, K., Royet, J., and Charroux, B. (2012). Peptidoglycan sensing by the receptor PGRP-LE in the Drosophila gut induces immune responses to infectious bacteria and tolerance to microbiota. Cell Host Microbe 12, 153–165.

Broderick, N.A. (2015). A common origin for immunity and digestion. Front. Immunol. 6, 72.

Broderick, N.A., Buchon, N., and Lemaître, B. (2014). Microbiota-induced changes in drosophila melanogaster host gene expression and gut morphology. MBio 5, e01117–e14.

Brummel, T., Ching, A., Seroude, L., Simon, A.F., and Benzer, S. (2004). Drosophila lifespan enhancement by exogenous bacteria. Proc. Natl. Acad. Sci. USA 101, 12974–12979.

Buchon, N., Broderick, N.A., Chakrabarti, S., and Lemaître, B. (2009a). Invasive and indigenous microbiota impact intestinal stem cell activity through multiple pathways in Drosophila. Genes Dev. 23, 2333–2344.

Buchon, N., Broderick, N.A., Poidevin, M., Pradervand, S., and Lemaître, B. (2009b). Drosophila intestinal response to bacterial infection: activation of host defense and stem cell proliferation. Cell Host Microbe 5, 200–211.

Buchon, N., Broderick, N.A., and Lemaître, B. (2013). Gut homeostasis in a microbial world: insights from Drosophila melanogaster. Nat. Rev. Microbiol. 11, 615–626.

Chambers, M.C., Song, K.H., and Schneider, D.S. (2012). Listeria monocytogenes infection causes metabolic shifts in Drosophila melanogaster. PLoS ONE 7, e50679.

Clark, R.I., Tan, S.W., Péné, C.B., Roostalu, U., Vivancos, V., Bronda, K., Piltová, M., Fu, J., Walker, D.W., Berdeaux, R., et al. (2013). MEF2 is an in vivo immune-metabolic switch. Cell 155, 435–447.

De Gregorio, E., Spellman, P.T., Tzou, P., Rubin, G.M., and Lemaitre, B. (2002). Toll pathway receptors recruit lipid rafts to mediate diverse mammalian and insect immune responses. EMBO J. 21, 2568–2579.

Dionne, M.S., Pham, L.N., Shirasu-Hiza, M., and Schneider, D.S. (2006). Akt and FOXO dysregulation contribute to infection-induced wasting in Drosophila. Curr. Biol. 16, 1977–1985.

Douglas, A.E. (2010). The Symbiotic Habit (Princeton University Press).

Erkosar, B., Storelli, G., Defaye, A., and Leulier, F. (2013). Host-intestinal microbiota mutualism: “learning on the fly”. Cell 152, 8–14.

Erkosar, B., Defaye, A., Bozonnet, N., Puthier, D., Royet, J., and Leulier, F. (2014). Drosophila microbiota modulates host metabolic gene expression via IMD/NF-κB signaling. PLoS ONE 9, e94729.

Guo, L., Karpac, J., Tran, S.L., and Jasper, H. (2014). PGRP-SC2 promotes gut immune homeostasis to limit commensal dysbiosis and extend lifespan. Cell 156, 109–122.

Hoppier, S., and Bienz, M. (2014). How microbiomes influence metazoan development: insights from history and Drosophila modeling of gut-microbe interactions. Annu. Rev. Cell Dev. Biol. 29, 571–592.

Lemaître, B., and Migué-Aliaia, I. (2013). The digestive tract of Drosophila melanogaster. Annu. Rev. Genet. 47, 377–404.

Leulier, F., Rodriguez, A., Khush, R.S., Abrams, J.M., and Lemaître, B. (2000). The Drosophila caspase Dredd is required to resist gram-negative bacterial infection. EMBO Rep. 1, 353–358.

Lhocine, N., Ribeiro, P.S., Buchon, N., Wepf, A., Wilson, R., Tenev, T., Lemaître, B., Gstaiger, M., Meier, P., and Leulier, F. (2008). PIMS modulates immune tolerance and regulation by negatively regulating Drosophila innate immune signaling. Cell Host Microbe 4, 147–158.

McFall-Ngai, M., Hadfield, M.G., Bosch, T.C., Carey, H.V., Domazet-Loiò, T., Douglas, A.E., Dubilier, N., Eberl, G., Fukami, T., Gilbert, S.F., et al. (2013). Animals in a bacterial world, a new imperative for the life sciences. Proc. Natl. Acad. Sci. USA 110, 3229–3236.

Myllymäki, H., Valanne, S., and Rämö, M. (2014). The Drosophila immunity signaling pathway. J. Immunol. 192, 3455–3462.

Neyen, C., Poidevin, M., Roussel, A., and Lemaître, B. (2012). Tissue- and ligand-specific sensing of gram-negative infection in drosophila by PGRP-LC isoforms and PGRP-LE. J. Immunol. 189, 1866–1897.

Paredes, J.C., Welchman, D.P., Poidevin, M., and Lemaître, B. (2011). Negative regulation by amidas PGRP-L shapes the Drosophila antibacterial response and protects the fly from innocuous infection. Immunity 35, 770–779.

Phillips, M.D., and Thomas, G.H. (2006). Brush border spectrin is required for early endosome recycling in Drosophila. J. Cell Sci. 119, 1361–1370.

Ren, C., Webster, P., Finkel, S.E., and Tower, J. (2007). Increased internal and external bacterial load during Drosophila aging without life-span trade-off. Cell Metab. 6, 144–152.

Ryu, J.H., Kim, S.H., Lee, H.Y., Bai, J.Y., Nam, Y.D., Bae, J.W., Lee, D.G., Shin, S.C., Ha, E.M., and Lee, W.J. (2008). Innate immune homeostasis by the homeobox gene caudal and commensal-gut mutualism in Drosophila. Science 319, 777–782.

Schneider, C.A., Rasband, W.S., and Eliceiri, K.W. (2012). NIH Image to ImageJ: 25 years of image analysis. Nat. Methods 9, 671–675.

Sharon, G., Segal, D., Ringo, J.M., Heftzet, A., Zilber-Rosenberg, I., and Rosenberg, E. (2010). Commensal bacteria play a role in mating preference of Drosophila melanogaster. Proc. Natl. Acad. Sci. USA 107, 20051–20056.

Shin, S.C., Kim, S.H., You, H., Kim, B., Kim, A.C., Lee, K.A., Yoon, J.H., Ryu, J.H., and Lee, W.J. (2011). Drosophila microbiome modulates host developmental and metabolic homeostasis via insulin signaling. Science 334, 670–674.

Shirasu-Hiza, M.M., Dionne, M.S., Pham, L.N., Ayres, J.S., and Schneider, D.S. (2007). Interactions between circadian rhythm and immunity in Drosophila melanogaster. Curr. Biol. 17, R353–R355.

Storelli, G., Defaye, A., Erkosar, B., Hols, P., Royet, J., and Leulier, F. (2011). Lactobacillus plantarum promotes Drosophila systemic growth by modulating hormonal signals through TOLL-dependent nutrient sensing. Cell Metab. 14, 403–414.

Takehana, A., Yano, T., Mita, S., Tomi, A., Oshima, Y., and Kurata, S. (2004). Peptidoglycan recognition protein (PGRP)-LE and PGRP-LC act synergistically in Drosophila immunity. EMBO J. 23, 4690–4700.

Tzou, P., Ohresser, S., Ferrandon, D., Capovilla, M., Reichhart, J.M., Lemaître, B., Hoffmann, J.A., and Imler, J.L. (2000). Tissue-specific inducible expression of antimicrobial peptide genes in Drosophila surface epithelia. Immunity 13, 737–748.

Venu, I., Durisko, Z., Xu, J., and Dukas, R. (2014). Social attraction mediated by fruit flies’ microbiome. J. Exp. Biol. 217, 1346–1352.

Wong, A.C., Dobson, A.J., and Douglas, A.E. (2014). Gut microbiota dictates the metabolic response of Drosophila to diet. J. Exp. Biol. 217, 1894–1901.

Yamada, R., Deshpande, S.A., Bruce, K.D., Mak, E.M., and Ja, W.W. (2015). Microbes Promote Amino Acid Harvest to Rescue Undernutrition in Drosophila. Cell Rep. Published online February 12, 2015. http://dx.doi.org/10.1016/j.celrep.2015.01.018.
Supplemental Information

Pathogen Virulence Impedes Mutualist-Mediated Enhancement of Host Juvenile Growth via Inhibition of Protein Digestion

Berra Erkosar, Gilles Storelli, Mélanie Mitchell, Loan Bozonnet, Noémie Bozonnet, and François Leulier
Supplemental Information

Supplementary Figure 1, related to Figure 2: The difference in the proteolytic activity represented in Fig 2A cannot be explained by the mere proteolytic activity of \( L. \) \( plantarum^{W/L} \) on its own. Dotplots representing the relative proteolytic activity obtained from larval intestines either germ free or containing a maximum of \( 10^7 \) \( L. \) \( plantarum^{W/L} \) cells, \( L. \) \( plantarum^{W/L} \) culture pellet (\( 10^9 \) cells), and an over-night \( L. \) \( plantarum^{W/L} \) culture supernatant (400ul). All values were relativized (%) to the proteolytic activity obtained from 1mg/ml Trypsin solution. Values from gut samples were not normalized to total protein quantity for the consistency within the analysis and represent absolute values obtained from 10 dissected guts.
**Supplementary Figure 2, related to Figure 5:** Increased proteolytic activity and growth promotion is observed upon *L. plantarum* association in *Dredd* mutants similarly to wild type larvae.

(A-C) Line graphs representing mean±SEM units of proteolytic activity measured by azocasein assay normalized to total protein quantity for each sample. Each condition at every time point contains at least three biological replicates of ten larval guts dissected.

(D) Boxplots (n>61) represent the larval size in germ free and *L. plantarum* associated larvae, 7 days after egg deposition in wild type (*) and *Dredd* mutant (y,w,Dredd) genotypes. p values obtained by two-way ANOVA are indicated.
**Supplementary Figure 3, related to Figure 6:** *Ecc15* infection does not alter growth of GF animals

(A) Boxplots (n>55) representing the size of GF larvae at 9 days AED that were sham treated or infected with *Ecc15* at 5 days AED (at this time GF larvae are size matched with 3 days old *Lp*<sup>Wjl</sup>-associated larvae). (B) Timings of association, infections and sampling are indicated. p-values obtained by one-way ANOVA are indicated. A statistically significant size reduction is observed, however the growth inhibition observed in this setting is biologically not relevant as compared to the effect observed on *Lp*<sup>Wjl</sup>-associated larvae.
Supplementary Figure 4, related to Figure 6: Ecc15 suppresses *L. plantarum* mediated growth promotion in *Dredd* mutants as in wild-type larvae.

(A) Boxplots (n>41) represent the size of 7 days old, germ free or *L. plantarum* associated, *Dredd* (*y,w,Dredd*) larvae which were infected with increasing quantities of *Ecc15* (DO 10/100) 3 days after egg deposition. p-values obtained by Student’s T-Test with Welch correction and one-way ANOVA are indicated.
Supplementary Table 1, related to all Figures.
Statistical tests and p values for data represented in all Figures.

Supplementary Table 2, related to Figure 1 and 4.
Primers sequence for RT-qPCR

| Gene Name  | Forward Primer      | Reverse Primer      |
|------------|---------------------|---------------------|
| Jon66Cii   | aaactgaccgccgtccac  | cctcctcagccggtagc  |
| Jon66Ci    | cgtcggctgggtttagc  | acccactctttgagatgat|
| Jon44E     | acagcgcataacatgtct | ggtgtactgggcctcttg |
| Jon65Ai    | caaaataacacaaggtgttg| gccctatcgaggtcttt  |
| Jon99Cii   | tccataatcgaacatcgg | cagtaaggctatcagac  |
| CG18179    | accgatggcaaatccttt | ggcgtgtcatggtaacga |
| CG18180    | cgcttggaactcactcgg | acggacgctgtccgta   |
| PGRP-SC1   | aagcgatcgtcaactatt | gagagccacttggaaacca|
| AttD       | gtcactaggtcttcag   | gcggagatggacttg    |

Supplementary Methods

_Drosophila diets, stocks and breeding_

Fresh food was prepared every week to avoid desiccation, and no yeast paste was added to the medium. GF stocks were established by bleaching and cultivating embryos on autoclaved conventional medium supplemented with a cocktail of four antibiotics (final concentration: 50 μg/mL ampicillin, 50 μg/mL kanamycin, 15 μg/mL erythromycin, 50 μg/mL tetracyclin) for a few generations. In experimental settings GF animals were used without antibiotics. Germ-free-ness was routinely tested by plating serial dilution of animal lysates on nutrient agar plates. The following _Drosophila_ lines were used: _y,w_ - _y,w,Dredd^{64} (Leulier et al., 2000) ; _y,w, PGRP-LE^{112} (Takehana et al., 2004), _labial-GAL4 (Hoppler and Bienz, 1994) (BL#43651) , _mex-GAL4 (Phillips and Thomas, 2006), UAS-Jon66Cii-3xHA (Bischof et al., 2013) and UAS-Pirk (Aggarwal et al., 2008).
Bacterial culture conditions

*Lp*<sup>WJL</sup> was cultivated in Man, Rogosa and Sharpe (MRS) broth medium (Difco, ref. #288110) over night at 37°C without shaking and *Ecc15* in Luria-Bertani broth medium (Difco, ref. #244610) over night at 30°C with agitation. Note that *Ecc15* does not grow in liquid MRS or on MRS-agar plates.

Azocasein Assay

10μl of sample were mixed with 300μl of Azocasein solution (2,5mg/ml in water, Sigma, ref.#A2765). Samples were incubated for 4 hours at 37°C. 300μl of 20% Trichloroacetic acid (TCA, Sigma, ref.#T6399) was used to stop the enzymatic reaction and precipitate the undigested Azocasein. Recombinant trypsin (serial dilutions from 1mg/ml stock solution in PBS, Sigma, ref.#T1426) was used to calibrate the assay and as a positive control in each reaction. The reaction blank was generated using 10μl of 1mg/ml of Trypsin mixed with TCA prior to incubation. Samples were centrifuged for 5 minutes at 13 krpm. 600μl of the supernatant was transferred to cuvettes and completed with 200μl 2M NaOH to reveal the color. Absorbance at 440nm was measured using a spectrophotometer. The reference arbitrary unit of proteolytic activity.ml<sup>-1</sup> = 0.01 A<sup>440</sup> units / hour. Protein concentration of samples was measured using a Nanodrop apparatus, reading the absorbance at 280nm and was used for normalization.

Statistical analysis

For pairwise comparisons Student’s T-Test was applied with Welch correction. For group comparisons, one or two-Way Analysis of Variance (ANOVA) was applied regarding the experimental design. Application conditions for ANOVA were tested using Shapiro-Wilk normality test and Levene test in order to check the equal variances
among datasets. Log transformation was applied when it was necessary. Tukey's HSD test was used as a post-hoc test for pairwise comparisons post-ANOVA (multcomp package). Upon more than three comparisons p-values were corrected using Holm-Bonferroni method. For multivariate analysis, Multivariate ANOVA (MANOVA, when applicable) and Principal Component Analysis (PCA) were performed (ade4 package). Student’s T test with Welch correction and/or Wilcoxon’s rank sum tests were performed to determine the significance of differences in metabolites levels between \( Lp^{WJL}\)-associated and GF samples. The False Detection Rate (FDR) for a given compound is estimated using the \( q\)-value (Storey and Tibshirani, 2003).

**Supplementary References**

Bischof, J., Bjorklund, M., Furger, E., Schertel, C., Taipale, J., and Basler, K. (2013). A versatile platform for creating a comprehensive UAS-ORFeome library in Drosophila. Development 140, 2434-2442.

Schneider, C.A., Rasband, W.S., and Eliceiri, K.W. (2012). NIH Image to ImageJ: 25 years of image analysis. Nature methods 9, 671-675.

Storey, J.D., and Tibshirani, R. (2003). Statistical significance for genomewide studies. Proceedings of the National Academy of Sciences of the United States of America 100, 9440-9445.