**Probiotic Enterococcus faecalis Symbioflor®** down regulates virulence genes of enterohemorrhagic *Escherichia coli* O157:H7 (EHEC) and decreases pathogenicity in a *Caenorhabditis elegans* model

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File S1. Description and further background to virulence associated genes regulated in EHEC during co-culture with *E. faecalis* Symbioflor®.

An increasingly recognized fact for pathogenic interactions is that metabolic factors and involved genes are important for successful infection and that those can be considered as virulence factors (Staib & Fuchs, 2014). However, we restrict this report to “classical” and often better known virulence genes or complexes thereof, e.g., the locus of enterocyte effacement, Stx genes and prophage encoded effectors, genes of the O-islands #43 and #48, flagellar and chemotaxis genes, adhesion associated genes, acid stress and quorum sensing genes.

**Prophages and Shiga toxin.** Shiga toxin, especially Stx1 was shown to be essential for EHEC caused *C. elegans* killing (Chou, et al., 2013). Probiotic *Bifidobacterium breve* was shown to inhibit Stx production and to reduce Stx concentration in the cecal content of EHEC-infected mice (Asahara, et al., 2004). Shiga toxin’s influence on the host does not only depend on its expression, but also on lysis of the bacterial cell. While both toxins were expressed at somewhat consistent levels, the antitermination proteins N and Q of BP-933W (Stx2 encoding) and the antitermination protein N of cryptic prophage CP-933V (Stx1) were significantly upregulated in SYM. The altered transcription of the antitermination genes may indicate induction of EHEC lysis and increased Stx exposure (Yarnell & Roberts, 1999, Kimmitt, et al., 2000, Waldor & Friedman, 2005). Similarly, *L. acidophilus* was also unable to decrease stx expression in EHEC, but its lysate neutralized the Stx-mediated cytotoxic effects
(Kim, et al., 2006). Since C. elegans mechanically destroys bacteria while eating, Symbioflor®-fragments might cause a similar protective effect by binding of Shiga toxin.

**O-islands #43 and #48.** The induction of phage-triggered EHEC suicide is additionally indicated by the transcriptional pattern of several O-island encoded genes. Among them are two AlpA homologues, Z1124 and Z1563 of the islands 43 and 48, respectively, which were both upregulated. AlpA stimulates cryptic prophage excision in E. coli K-12 by upregulating the expression of an integrase located on the prophage (Kirby, et al., 1994). In accordance, the transcription of proteins with predicted integrase and protease activity (e.g., Z1150, Z1192, Z1589 and Z1632) is increased in SYM, suggesting excision of the mobile elements (Luck, et al., 2004).

**Adhesion.** EHEC colonization of the C. elegans intestinal tract is a prerequisite for nematode death according to Lee et al. (2008). Therefore, adhesion and biofilm genes are critical targets for probiotic modulation in terms of C. elegans disease development. In our study, transcription of several genes for flagella, curli and other adhesion modules was downregulated or even switched off in SYM. This might be a major factor for reduced EHEC virulence, as these genes have been shown to be important for adhesion and host colonization for a number of cases (Doughty, et al., 2002, Pawar, et al., 2005, Erdem, et al., 2007, Mahajan, et al., 2009, Saldana, et al., 2009, Lloyd, et al., 2012).

**Acid stress related genes.** Symbioflor® decreases the pH of the LB in the LB-Symbioflor® agar, thus, EHEC are exposed to acid stress. EHEC induces the GAD-system genes, which are characteristic for survival in the stomach (Price, et al., 2004). Furthermore, low pH serves as signal to induce virulence genes (House, et al., 2009). Despite that, virulence genes appear to be downregulated in SYM compared to REF. Interestingly, GadE is upregulated in SYM. This repressor decreases transcription of T3SS genes, leading to subsequently reduced cell adhesion capacity (Tatsuno, et al., 2003, Tree, et al., 2011). Thus, low pH exerted by Symbioflor® via secreted organic acids seems not to have the same effect as hydrochloric acid after a stomach passage on EHEC virulence genes (House, et al., 2009), since the former can enter the cell in its uncharged form by passive diffusion and after dissociation in the cytoplasm cause an adverse pH shift (van de Guchte, et al., 2002).

However, in later worm killing assays we wanted to exclude the influence of the pH response and used buffered agar. In doing so, we could omit low pH stress in both, EHEC and the worms, and effects observed are not attributable to any acid response.
**Quorum sensing.** The expression of essential EHEC virulence factors depends on quorum sensing (QS) via auto-inducer (Al). QS systems stimulate flagella gene expression via Al-2 and Al-3, or the LEE gene via Al-3 (Sperandio, et al., 1999, Sperandio, et al., 2001, Sperandio, et al., 2002), and Al-2 activity in EHEC is associated with enhanced virulence in *C. elegans* (Kim, et al., 2007). The presence of Symbioflor® downregulated the synthesis of the key components of the Al-2 system, LsrB and TqsA (Wang, et al., 2005, Hegde, et al., 2011). Other lactic acid bacteria were found to interfere with EHEC Al-2 communication by decreasing the extracellular Al-2 levels or inhibiting Al-2 like activity. Subsequently, adhesion and virulence-gene expression is reduced (Medellin-Peña, et al., 2007, Kim, et al., 2008). A similar mechanism can be assumed for Symbioflor® as well.

Transcription of Al-3 QS-system components was also modified by Symbioflor®, suggesting a reduced adhesion capacity and virulence. For instance, the Al-3 key sensor kinase QseC was downregulated in SYM. Interestingly, an EHEC qseC mutant is unable to sense external signals and, therefore, is reduced in virulence (Clarke, et al., 2006). Normally, QseC would activate the response regulator QseF upon contact with Al-3 or mammalian (nor-)epinephrine. The activated QseF subsequently switches T3SS expression through Ler (Hughes, et al., 2009). Further, the dominant negative regulator QseD was induced in SYM. QseD downregulates LEE and flagellar genes (Habdas, et al., 2010).

**Additional references**


