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Virulence gene induction of Enterohaemorrhagic *Escherichia coli* by *Caenorhabditis elegans*, *Acanthamoeba castellanii* and *Arabidopsis thaliana*

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Summary

Enterohaemorrhagic *Escherichia coli* is an emerging food pathogen in industrial countries. Their natural reservoir is the colon of ruminants, especially cows. In humans, EHEC can lead to life-threatening diseases like haemolytic-uremic syndrome. Most victims are small children. The mechanisms of human infection of EHEC are well known, but it remains unclear how the bacteria are spread in the environment, traveling from one host to another.

In this work, a total number of 65 promoter regions of genes of EHEC O157:H7 have been investigated concerning their possible activation in contact with different alternative host or vector organisms. Among these 65 genes were the genes encoded on the locus of enterocyte effacement, a pathogenicity island that EHEC have in common with EPEC.

As possible interaction partners, the soil nematode *Caenorhabditis elegans*, the waterborne protozoa *Acanthamoeba castellanii* and the thale cress *Arabidopsis thaliana* were chosen.

Promoter regions were defined the 100bp upstream regions for the LEE-encoded genes and 500bp upstream regions for all other tested genes. Promoter-*luxCDABE* fusions were generated on a plasmid and transformed into EHEC cells. The transformed EHEC were brought in contact with the chosen organisms in 96-well plates and emitted luminescence was monitored using a Victor^3 multilabel counter.

The results of this study indicate that an interaction similar to that occurring in human infection could take place in the *C. elegans* model.

In contact with the amoeba *A. castellanii* only little activation of promoter regions was observed. However, the interaction increases with dead *A. castellanii*, indicating that, in this interaction process, the enterohaemorrhagic *E. coli* are effectively rejected by living amoebae.

The most interesting outcome of this work is the ability of EHEC to cause a virulence-associated phenotype in the only plant submitted to the assays, *A. thaliana*. In addition to that, the luminescence emitted by transgenic EHEC was highest in contact with this test organism, and most of the tested promoter regions were activated.

From these findings I conclude that the type of interaction between EHEC and *C. elegans* is similar to that taking place in mammalian hosts, making *C. elegans* a suitable model organism which might serve as a vector for EHEC in the environment. The amoeba *A. castellanii* does not seem to play an important role in the spreading of EHEC. Contact with the thale cress *A. thaliana* leads to a strong genetic response in EHEC, involving several virulence factors. In addition to that, EHEC cause a phenotype in infected *A. thaliana* plants. The activation of genes is even stronger than with mammals. This indicates that *A. thaliana* is a new host organism for EHEC.

Zusammenfassung

Enterohämorrhagische *Escherichia coli* sind ein wichtiges Nahrungsmittelpathogen in Industrieländern. Ihr natürliches Reservoir ist der Darm von Wiederkäuern, vor allem Kühen. Beim Menschen kann EHEC zu lebensbedrohlichen Symptomen wie dem hämolytisch-uremischen Syndrom führen. Die meisten Opfer sind Kleinkinder. Der Mechanismus der Infektion im Menschen ist bekannt, aber man weiß nicht genau, wie EHEC in der Umwelt verbreitet werden und so von einem Wirt zum anderen gelangen.

Hier wurden insgesamt 65 Promoterregionen von Genen des EHEC-Serotyps O157:H7 bezüglich einer möglichen Aktivierung bei Kontakt mit verschiedenen alternativen Wirts- oder Vektororganismen untersucht. Darunter befanden sich auch die Gene der Locus of Enterocyte Effacement Pathogenitätsinsel, die EHEC und EPEC gemeinsam ist.

Als mögliche Interaktionspartner wurden die Nematode *Caenorhabditis elegans*, die Protozoe *Acanthamoeba castellanii* und die Kresse *Arabidopsis thaliana* ausgewählt.

Promoterregionen wurden definiert als die 100bp upstream Region für die LEE-Gene und die 500bp upstream Region für alle anderen getesteten Gene. Auf einem Plasmid wurden Promoter-*luxCDABE* Fusionskonstrukte erzeugt und in EHEC transformiert. Die transformierten EHEC wurden in 96-Well-Platten mit den Testorganismen in Kontakt gebracht. Die emittierte Lumineszenz wurde in einem Victor^3 Plattenlesegerät gemessen.

Die Ergebnisse dieser Studie zeigen, daß im *C. elegans* Modell eine ähnliche Interaktion wie im menschlichen Wirt stattzufinden scheint.

Kontakt mit *A. castellanii* führte zu nur geringer Aktivierung einiger Promoterregionen. Diese Interaktion steigert sich allerdings mit toten *A. castellanii*, was darauf schließen läßt, daß lebende Amöben sich effektiv gegen EHEC zur Wehr setzen können.

Das interessanteste Ergebnis dieser Arbeit ist die Fähigkeit von EHEC, in der Pflanze *A. thaliana* einen Phänotyp hervorzurufen. Zudem wurden in Kontakt mit diesem Organismus die meisten Promoterregionen aktiviert, und die Aktivierungsrate war am höchsten.

Also scheint die Art der Interaktion zwischen EHEC und *C. elegans* derjenigen in Säugetieren zu ähneln. Das würde *C. elegans* zu einem guten Modellorganismus machen, der vielleicht auch als Vektor für EHEC in der Umwelt dienen könnte. Die Amöbe *A. castellanii* dagegen scheint keine Rolle bei der Verbreitung von EHEC zu spielen. Ein Kontakt mit *A. thaliana* führt zu einer starken genetischen Reaktion bei EHEC, die auch mehrere Virulenzfaktoren betrifft. Zudem erzeugen EHEC einen Phänotyp in infizierten *A. thaliana*-Pflanzen. Die Aktivierung der Gene ist hier sogar stärker als im Säugetier. Dies alles deutet darauf hin, daß *A. thaliana* ein bisher unbekannter Wirtsorganismus für EHEC ist.

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1 Introduction

1.1 Enterohaemorrhagic *Escherichia coli* and human infection

Enterohaemorrhagic *Escherichia coli* O157:H7 (EHEC) is one of the worlds emerging food pathogens, especially in industrial countries [Mellmann et al. 2005]. It causes severe and often bloody diarrhea and can also lead to life-threatening diseases like haemolytic uremic syndrome (HUS) [Karch et al. 2005]. Most victims are small children because they do not have antibodies against EHEC. Although EHEC are sensitive to antibiotics, the infection can not be treated that way because that would lead to a lysis of the bacteria, which produce the shiga toxins StxI and StxII [Bielaszewska 1993; O'Brien et al. 1992]. Those classical AB-toxins, composed of one A- and five B-subunits each (see Fig. 1), would be released into the colon of the host at the moment of bacterial lysis. The more important of the two Shiga toxins for the development of HUS seems to be Stx II [Carey CM 2008]. Once present in the bloodstream, the B subunit pentamer of the Stxs binds to the human globotriaosylceramide receptor Gb_3, which is present in the membranes of numerous human cells including renal endothelial cells, monocytes, platelets and colonic and cerebral microvascular endothelial cells. The Shiga toxins are transported via platelets in the bloodstream to the glomerular endothelial renal cells where they can cause fatal renal failure. Shiga toxins bound to blood platelets also contribute to the development of thrombocytopenia by aggregating the platelets [Nolasco L 2005; Sandvig et al. 1993]. As the Shiga toxins are encoded on prophages in the EHEC genome and can only be secreted by cell lysis because EHEC have no secretion system for the Shiga toxins, it has been proposed that those prophages are transferred to the commensal human gut *E. coli* which are recognized by EHEC via quorum sensing, so that the EHEC bacteria do not have to undergo lysis themselves and let the commensals produce the shiga toxins leading to haemolytic-uremic syndrome [Brussow et al. 2004].

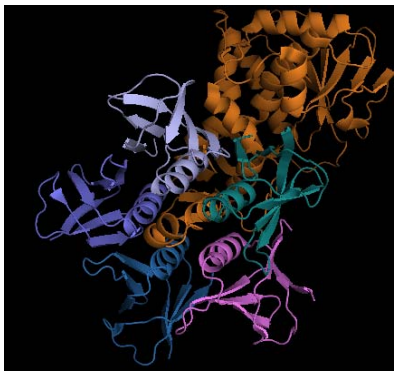


Figure 1: molecular structure of Stx2. The A subunit is depicted in orange, the pentameric B subunits in different shades of blue.

EHEC share some virulence factors with enteropathogenic *E. coli* (EPEC), including a type three secretion system which is encoded on a pathogenicity island called the locus of enterocyte effacement (LEE) [McDaniel et al. 1995; Pallen et al. 2005; Porter et al. 2005], which is organized in five major operons. The EHEC LEE and that of EPEC are very similar to each other [Schmidt and Hensel 2004; Viswanathan et al. 2004], but there are differences regarding the regulation of transcription of the LEE genes. There is a LEE-external master regulator called *per* in EPEC, which could not be found in EHEC so far [Porter et al. 2004; Schmidt and Hensel 2004]. The next stage of regulation, however, seems to be identical in both *E. coli* strains, as the LEE-encoded regulator, *ler*, is present in both EHEC and EPEC, being the first gene of the LEE1 operon [Brady et al. 2007]. Ler is a positive regulator of the whole LEE-PAI and also autoregulates its own expression [Barba et al. 2005]. There is evidence that it also regulates virulence factors that are not encoded on the LEE [Elliott et al. 2000]. Ler has also been proposed to act as a negative regulator for the LEE1 operon, which would indicate that Ler can act both activating and repressing on LEE transcription [Berdichevsky et al. 2005]. Additionally, another activator of LEE-gene expression, *grlA*, and a repressor of LEE-gene expression, *grlR*, are encoded on the LEE [Iyoda et al. 2006; Jobichen et al. 2007].

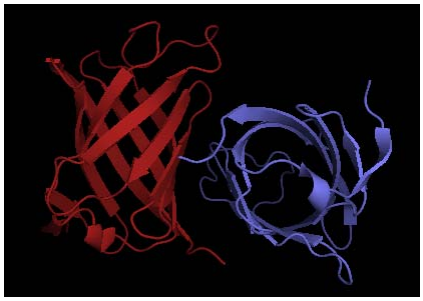


Figure 2: molecular structure of GrlR.

Obviously, EHEC and EPEC differ regarding the *ler*-upstream regulatory cascades. Several regulatory genes and pathways have already been found to play a role in regulation of EHEC-LEE gene expression, e.g. genes involved in quorum sensing, the *pch*-genes, *dksA*, *clpXP* and H-NS [Iyoda and Watanabe 2004; Laaberki et al. 2006; Nakanishi et al. 2006; Sperandio et al. 1999; Tomoyasu et al. 2005].

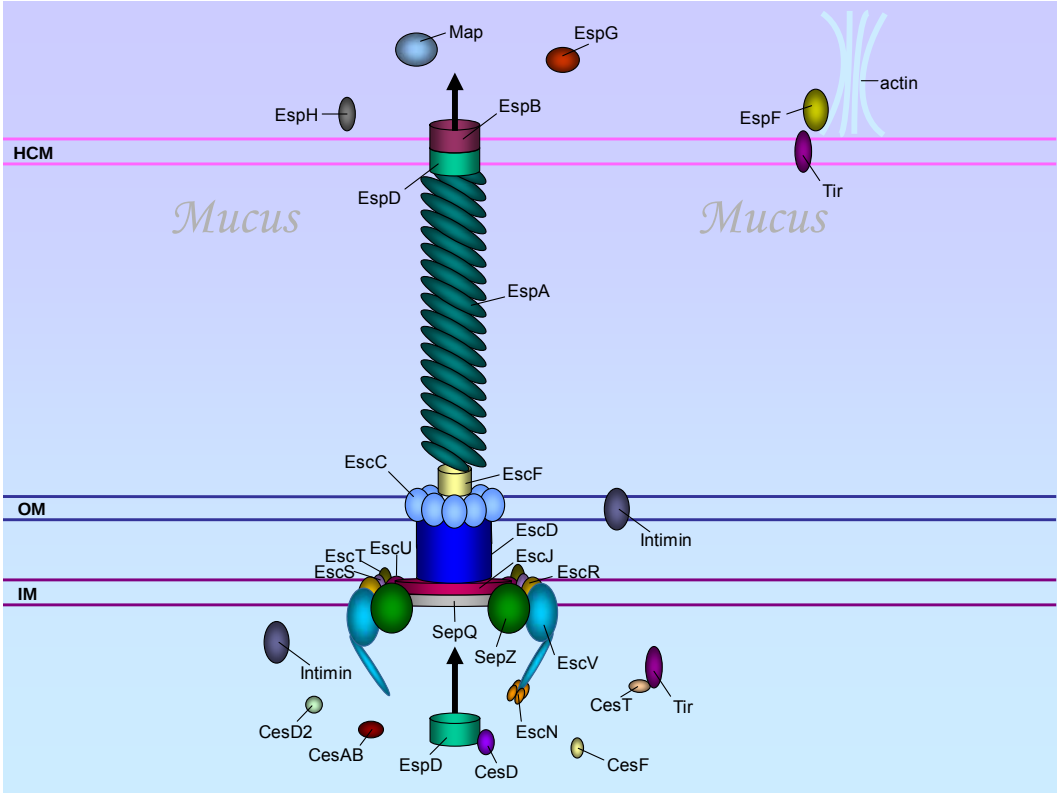


Figure 3: schematic picture of the LEE-encoded Type Three secretion System of EHEC.

The LEE-encoded TTSS-secreted effector proteins, like EspH, EspA and EspB, lead to a disarrangement of the actin filaments of the colon epithelium host cell cytoskeleton [Ebel et al. 1998; Kodama et al. 2002; Tu et al. 2003]. The actin is remodeled so that pedestals below each EHEC cell are formed, resulting in a tight adherence, which is called the attaching and effacing lesion phenotype [Caron et al. 2006; Kresse et al. 2001]. The first contact between EHEC and their host cells in the colon epithelium is mediated by the LEE-encoded protein Intimin and its translocated receptor Tir. Tir has to be secreted by the EHEC-TTSS into the host cell, where it integrates into the outer membrane, allowing the Intimin in the EHEC outer membrane to bind [Kenny et al. 1997]. Tir interactions with the cell cytoskeleton are mediated by the non-LEE encoded effector protein TccP [Garmendia et al. 2004]. It has been shown that EHEC and EPEC Tir act in different manners to reorganize the actin cytoskeleton of host cells [DeVinney et al. 2001].

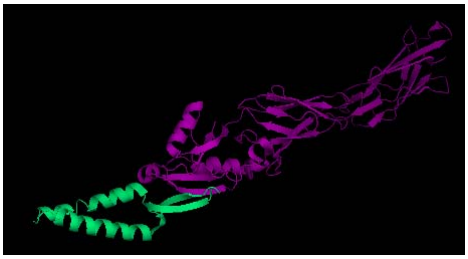


Figure 4: molecular structure of the C-terminal region of Intimin (violet) associated with the Tir binding domain (green).

There is evidence that Intimin can also bind to some eukaryotic host cell outer membrane proteins like Stx2-induceable Nucleolin and Integrin β1 [Robinson et al. 2006; Sinclair et al. 2006; Sinclair and O'Brien 2002], which allows EHEC cells to bind to host cells that do not yet have integrated Tir into their outer membrane. The EHEC TTSS also secretes some effector proteins that are not encoded on the LEE pathogenicity island, e. g. EspE, EspJ, EspK, the Nle genes and TccP [Chen 2008; Deibel et al. 1998; Garmendia and Frankel 2005; Gruenheid et al. 2004; Marches et al. 2005; Sallee 2008; Vlisidou et al. 2006b].
Once present in the colon, EHEC produce several additional virulence factors like the Shiga toxins Stx I and II, the EHEC-haemolysin Hly and the cytolethal distending toxin Cdt [Orth et al. 2006; Wieler et al. 1996].

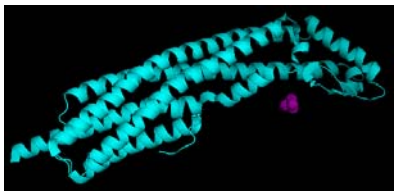


Figure5 : molecular structure of HlyE, a putative pore-forming haemolysin of EHEC O157:H7 (turquoise) in complex with SO_4 (violet).

There is evidence that other organisms present in the colon like the yeast *Saccharomyces boulardi* are capable of interfering with the signaling cascades induced by EHEC by preventing apoptosis of experimentally infected T84 cells [Dahan et al. 2003; Dalmasso et al. 2006].
First recognized as a threat to human health in 1983, when it was found to be the causative agent of the so-called “hamburger disease”, where the infectious dose turned out to be as low as less than 700 bacteria [Tuttle 1999], EHEC can be found in a broad range of food products and beverages like beef, sausages, cheese, plant products like lettuce, apple cider and milk [Caro et al. 2006; Cody et al. 1999; Sharma et al. 2004; Wachtel et al. 2002a; Wahi et al. 2006] and is responsible for a growing amount of clinical outbreaks during the last decades [Paton and Paton 1998].

1.2 Natural reservoirs and animal hosts of EHEC

EHEC are usually found in the colon epithelium of ruminants, among which cows are recognized to be the main reservoir of EHEC [Blanco et al. 1996]. In most cases, there is no sign of a pathogenic phenotype in cows, except for some young calves developing severe diarrhea [Vlisidou et al. 2006a]. EHEC have also been found in goats, sheep, pigs, cats, water buffalos, pigeons etc. [Oliveira et al. 2007; Sonntag et al. 2005; Vettorato et al. 2003; Vu-

Khac and Cornick 2007; Vu-Khac et al. 2007]. Just like cows, those animals do not show signs of illness, in general. EHEC are shed with the feces and thereby spread throughout the environment of the infected animal population [Robinson et al. 2004]. The bacteria are capable of persisting in a viable state on ground soil for up to 217 days [Islam M 2004] and seem to be able to colonize the surface of plants and survive under the low nutrition conditions given there [Heaton and Jones 2007; Solomon et al. 2003]. In water, EHEC can form biofilms [Lee et al. 2007]. However, it is still unknown how a population of cows can be infected with EHEC which was not in vicinity to any other (infected) population and did not graze on the same meadows as infected cattle [Grif et al. 2005]. These circumstances indicate that there might be another, probably much smaller, organism serving either as an additional reservoir or as a vector, transferring EHEC from one cattle herd to another. Another possible explanation could be the transfer of EHEC from contaminated soil or plants to healthy cattle, infecting them with EHEC. If the transfer happened underground, that would indicate the possibility of plants taking up EHEC through their roots and the ability of the bacteria to persist inside the plant for a longer time period and the transport of EHEC through the plant tissue to leaves that are then ingested by grazing cattle. Of course, there is also the possibility of contaminated water serving as a transport medium for EHEC, as EHEC have been shown to be able to survive in water without any host present [Halabi et al. 2007; Ram 2008].

1.3 Possible interaction partners of EHEC in the environment: *Caenorhabditis elegans, Acanthamoeba castellanii* and *Arabidopsis thaliana*

In order to find out more about possible reservoir or vector organisms beside the above mentioned mammalian species, we decided to concentrate on organisms which are found in soil and water environments. Fortunately, some of those are genetically well characterized and easy to handle under laboratory conditions. We chose the ubiquitous soil nematode *Caenorhabditis elegans*, which has already been shown to ingest and spread EHEC. Ingested EHEC are shed within the worm pellets and are able to survive inside the worms for a prolonged time period of at least one week [Anderson et al. 2006]. It has been shown for EPEC, that the LEE-encoded regulator Ler is necessary for a colonization of the nematode gut [Mellies et al. 2006]. There is also evidence that some pathogenic bacteria including EHEC O157:H7 can be transmitted within a population of nematodes, crossing the generation barrier [Kenney et al. 2005]. Nematodes feeding on EHEC lawns have a shorter live span than worms fed on OP50 food bacteria, indicating some virulence-associated interaction between

the bacteria and the nematode. This interaction seems to require several genes of the locus of enterocyte effacement and the *tnaA*-gene [Anyanful et al. 2005]. *C. elegans* are slowly migrating in ground soil while foraging, moving approximately one meter in five hours [Anderson et al. 2006], which makes them and other nematodes a prime candidate organism that could serve as a new reservoir or vector for EHEC [Kenney et al. 2006].

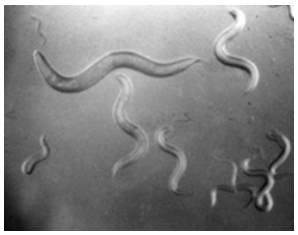


Figure 6: Microscopy picture of *C. elegans*. Taken from www.whyfiles.org. Picture by Bill Love, NIH /National Center for Research Resources.

The waterborne protozoa *Acanthamoeba castellanii* might be another interaction partner of EHEC in the environment. They prey on bacteria of different species some of which are able to survive inside the amoeba and become even more resistant to other stresses once passaged through the amoeba [Cirillo et al. 1997]. This effect has also been observed for pathogenic *E. coli* cells [Alsam et al. 2006; Barker et al. 1999]. Other examples for this phenomenon include *Legionella pneumophila*, *Vibrio cholerae*, *Listeria monocytogenes*, *Campylobacter jejunii*, *Mycobacteria* and *Salmonella* [Abd et al. 2007; Anderson et al. 2003; Snelling et al. 2005; Taylor et al. 2003; Tezcan-Merdol et al. 2004; Zhou et al. 2007]. In recent years, it has even been discovered that several uncharacterized small bacteria of different origin live inside some *A. castellanii* strains in perpetual and obligate endosymbiosis [Horn M 2000]. Although there are other amoebae like *Hartmanella veriformis* that also have been shown to prey on *E. coli*, only *A. castellanii* was included in this study [Pickup et al. 2007]. *A. castellanii* is the causative agent for keratitis in humans, infecting the keratocytes and corneal cells of the human eye, especially if they are already weakened, e.g. by the abusive use of contact lenses [Kinnear 2004]. *A. castellanii* mediated keratitis can lead to complete blindness of the infected eye, if not recognized and treated soon enough [Fiori et al. 2006].

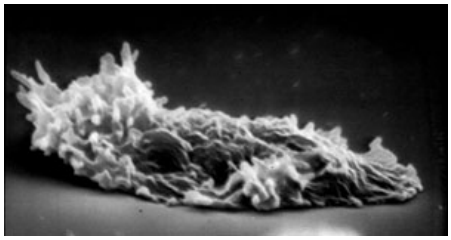


Figure 7: electron microscopy of *A. castellanii.* Taken from www.bms.ed.ac.uk. Picture by Dr. Steven Dobberstein.

The third organism chosen was the thale cress *Arabidopsis thaliana*, which includes a plant species into the studies, in order to elucidate the possibility of EHEC interacting with plants and examine the invasion of plant tissues. *A. thaliana* is a well known laboratory organism. EHEC and other enteric bacteria like *Salmonella* have already been shown to adhere to the surface of different plants, e.g. tomatoes, lettuce and alfalfa sprouts [Guo et al. 2002; Solomon et al. 2003; Torres et al. 2005], and there is evidence that the bacteria can even invade and survive inside the plant tissue of e.g. lettuce [Solomon et al. 2002b] and the roots and seed of *A. thaliana* [Cooley et al. 2003]. Some of these processes, like the adherence of EHEC O157:H7 do not seem to require living bacteria, as adhesion has also been shown when dead bacteria were placed on plant surfaces [Solomon and Matthews 2006].

Figure 8: the thale cress *A. thaliana*. Taken from www.mindfully.org. Picture by Peter Gwin for National Geographic.

There are numerous other small organisms that have been found as possible vectors or hosts for EHEC but could not be included in this study.

Flies like *Musca domestica* have also been proposed as possible interaction partners or vector organisms for human pathogenic bacteria but were not included into this study [De Jesús 2004; Iwasa 1999; Mylonakis and Aballay 2005; Sasaki 2000]. *E. coli* O157:H7 have also been detected in filth flies [Szalanski 2004].

There seems to be a great potential of insects in general, as unknown reservoirs for EHEC, as *E. coli* O157:H7 have also been found in the dung beetle *Catharsius molossus* [Xu 2003].

In addition to that, it has been proven that EHEC-TTSS secreted effector proteins have the ability to affect the actin cytoskeleton of *Saccharomyces cerevisiae*, which to some extend could even make yeast a suitable model organism for EHEC infections in humans [Rodriguez-Escudero et al. 2005].

1.4 Aims of this work

In this study, I wanted to find out more about the survival of EHEC in environmental organisms and possible ways of transmission from one mammalian reservoir or host to another. I was particularly interested in the genes of the locus of enterocyte effacement and the role of the TTSS in interactions with *C. elegans*, *A. castellanii* and *A. thaliana*. For this purpose, I conducted promoter-*lux*-fusion assays with the promoter regions of all LEE-encoded genes and a selection of other EHEC genes. The majority of these other genes are regulators and virulence factors, including genes of the quorum sensing system, flagellar genes and subunits of the Shiga toxins I and II. I transformed the promoter-*lux*-constructs of the chosen genes into EHEC and brought the transgenic bacteria in contact with *C. elegans*, *A. castellanii* and *A. thaliana*. From the bioluminescence readout, I aimed to draw conclusions about the activated genes and their possible role in the interaction process between EHEC and the tested organisms.

There are three possible ways of interaction between EHEC and the tested organism:

First, infection of the organism after ingestion or, in case of *A. thaliana*, invasion into plant tissue. This seemed most likely to happen in *C. elegans*, which is already known to be infected by *Salmonella typhimurium*, EHEC O157:H7 and other bacteria.

The second possibility is ingestion or invasion of EHEC and persistence of the bacteria inside the organism without any signs of infection, which would indicate the possibility that the tested organism might serve as another reservoir for EHEC in the environment. This seems most likely for *A. castellanii*, because a number of different bacteria have already been shown to persist and replicate inside the vacuoles of these protozoa. The above mentioned two possible ways of interaction should lead to an activation of virulence-associated genes of some kind.

The third possibility is that EHEC serve as prey for the worms and amoeba and are ingested and digested completely, without the ability to persist inside or do any harm to the tested organism. In this case, we would expect the activation of genes with defensive functions and general regulator genes, but not of virulence-associated genes like those of the LEE.

Of course, there is also the possibility that no interaction at all takes part between EHEC and the chosen organisms, which would leave both the bacteria and the tested organism completely unaffected. This possibility was most probable in the *A. thaliana* experiments, because EHEC has not been known to be a plant pathogen so far.

2 Material and Methods

2.1 Strains and plasmids

2.1.1 Bacterial strains and growth conditions

EHEC strain O157:H7 EDL933 (obtained from the Collection de l'Institute Pasteur, Paris, France, Collection Number CIP106327) and TOP10$^-$, a K-12 derivative (obtained from Invitrogen GmbH, Karlsruhe, Germany) were maintained on LB agar at 37°C.

OP50 were obtained from B. Spanier (TUM, Germany) and grown on NGM-Nys-agar at 37°C.

Plant pathogenic *Pseudomonas syringae* pv. tomato DC3000 was a gift from Jutta Ludwig-Müller, Technical University of Dresden, Germany and kept on King`s B agar at 30°C.

All bacterial cultures were every two months refreshed from glycerol stocks stored at -80°C.

2.1.2 Alternative host organism strains and growth conditions

Caenorhabditis elegans wild type strain Bristol N2 was a gift from B. Spanier (TUM, Germany). Nematodes were kept on an OP50 lawn on NGM-Nys-agar at 22°C and passaged to fresh medium every four days using a binocular microscope (Wild Heerbrugg, Gais, Switzerland).

Acanthamoeba castellanii was obtained from Klaus Heuner, Department of Molecular Infection Biology, University of Würzburg, Germany. Amoebae were kept axenically in PYG-medium in cell culture flasks at 22°C without shaking and were passaged to fresh medium every week.

Arabidopsis thaliana CV Columbia was obtained from Jutta Ludwig-Müller, Technical University of Dresden, Germany and was seeded one week before an assay either on MS agar or into liquid MS medium. Plants were grown at 22°C below an HQI-lamp at 35-50µmol light.

2.1.3 Plasmids

For the generation of *luxCDABE*-promoter fusions, the pSL-1 plasmid was used (constructed by M. Käser, Technical University of Munich, Germany). For the LEE-genes, the 100bp upstream region of each gene was defined as promoter region. For the other tested genes, I chose 500bp upstream promoter fragments because the other regions of the EHEC genome are not as densely packed with coding sequences as the locus of enterocyte effacement.

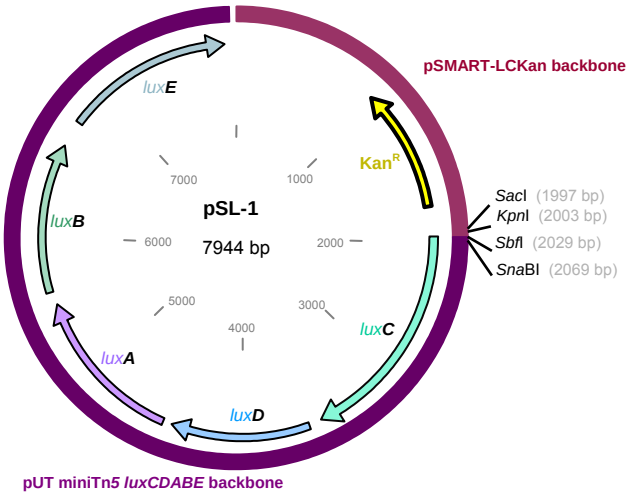


Figure 9: pSL-1 plasmid. The promoter regions were inserted into the SacI/KpnI-restriction site. The luxCDABE-cassette derived from Photorhabdus luminescens.

2.2 Cultivation media

All media were prepared using Milli-Q-water (Millipore Corporation, Billerica, USA). All autoclaving steps were conducted at 121°C for 20min. All agar plates and SOC medium were stored at 4°C, other media at room temperature (22°C). All chemicals used in this study were purchased from Merck (Darmstadt, Germany), Roth (Karl Roth GmbH & Co. KG, Karlsruhe, Germany) and Sigma (Sigma-Aldrich Co., Taufkirchen, Germany) in the highest purity available.

2.2.1 King`s B Medium

20g glycerol

40g proteose peptone

10ml K_2HPO_4 (10%)

10ml $MgSO_4$ (10%)

50mg rifampicin

980ml H_2O (add 15 g/l agar for plates)

→autoclave

2.2.2 LB-Medium

10g tryptone

5g yeast extract

5g NaCl

16g agar (for LB agar plates only)

ad 1l H_2O

→ pH 7.4 was adjusted using NaOH.

→autoclave

After cooling down to 50°C, the medium was supplemented with the following antibiotics and supplements when needed:

Kanamycin	100mg /l
Ampicillin	100mg /l
Erythromycin	200mg /l
Gentamycin	200mg /l
IPTG	100mM

2.2.3 NGM-Agar

3g NaCl

2.5g peptone

17g agar (high strength)

970ml H_2O

→ autoclave

after autoclaving, the following supplements were added:

1ml cholesterol (0.5g in 100ml ethanol)

0.5ml 1M $CaCl_2$ (autoclaved)

1ml 1M $MgSO_4$ (autoclaved)

25ml 1M kaliumphosphate buffer (pH6), autoclaved

5ml nystatin solution (Sigma-Aldrich Co., Taufkirchen, Germany)

2.2.4 S-Medium

The final S-medium consists is a mixture of the following:

S-basal medium:

5.9g NaCl

50ml 1M KH_2PO_4 (pH6)

950ml H_2O

→autoclave

1ml cholesterol (0.5g in 100ml ethanol) was added.

Trace element solution:

1.86g Na_2EDTA

0.69g $FeSO_4$ x 7 H_2O

0.2g $MnCl_2$ x 4 H_2O

0.29g $ZnSO_4$ x 7 H_2O

0.016g $CuSO_4$

ad 1l H_2O

→autoclave

final S-medium:

1l S-basal medium

10ml kaliumcitrate (pH6.0), autoclaved

10ml trace element solution

3ml 1M $CaCl_2$ (autoclaved)

3ml 1M $MgSO_4$ (autoclaved)

The medium was supplemented with

10ml nystatin suspension (Sigma-Aldrich Co., Taufkirchen, Germany)

10ml pen/ strep/ neo-solution (Sigma-Aldrich Co., Taufkirchen, Germany),

when necessary.

2.2.5 MS-Medium

The final MS medium is a mixture of the following:

macroelement solution:

16.5g NH_4NO_3

1.7g KH_2PO_4

19g KNO_3

3.32g $CaCl_2$

1.81g $MgSO_4$

ad 1l H_2O →autoclave

microelement solution:

3.37g Na_2EDTA

2.78g $FeSO_4$ x 7 H_2O

0.62g H_3BO_3

1.69g $MnSO_4$ x H_2O

0.86g $ZnSO_4$ x 7 H_2O

0.083g KI

0.025g Na_2MoO_4 x 2 H_2O

0.0025g $CuSO_4$ x 5 H_2O

0.0025g $CoCl_2$ x H_2O →autoclave

vitamine solution:

10mg nicotinic acid

100mg thiamine-HCl

10mg pyridoxin-HCl

1g meso-inositol

40mg glycerol

100ml H_2O

→sterile filtration

final MS medium:

100ml macroelement solution

10ml microelement solution

10ml vitamine solution

100ml saccharose (100g /l H_2O)

→adjust pH 5.8 with NaOH

For MS agar, 12g /l agar were added.

2.2.6 PYG-Medium

1g Na_3-citrate

20g proteose peptone

1g yeast extract

10ml 0.4M $MgSO_4$ (autoclaved)

10ml 0.25M Na_2HPO_4 (autoclaved)

10ml 0.25M KH_2PO_4 (autoclaved)

8ml 0.05M $CaCl_2$ (autoclaved)

902ml H_2O

→autoclave, cool down

50ml 2M glucose (autoclaved)

10ml 0.005M $(NH_4)_2Fe^{II}(SO_4)_2$ (sterile filtered)

2.2.7 SOC Medium

20g tryptone

5g yeast extract

0.5g NaCl

0.186g KCl

10ml 1M MgCl (autoclaved)

4g glucose

990ml H_2O

→autoclave

adjust to pH 7.0 with NaOH.

2.2.8 CHROMagar™ *E. coli* O157

Chromogenic agar was purchased from Mast Diagnostica (Reinfeld, Germany) and prepared as recommended by the manufacturer.

2.3 Kits and standard protocols

2.3.1 Kits

The following kits were used in this study:

Plasmid minipreps were conducted using a GenElute Plasmid Miniprep Kit by Sigma (Sigma-Aldrich Co., Taufkirchen, Germany).

Plasmid maxipreps were conducted using a GenElute HP Plasmid Maxiprep Kit by Sigma (Sigma-Aldrich Co., Taufkirchen, Germany).

DNA was cleaned from agarose gel excisions and from enzymatic reactions using a QIAquick Gel Extraction Kit by Qiagen (Qiagen GmbH, Hilden, Germany).

DNA was desalted using micropore filters with a pore diameter of 0.025µm (Millipore Corporation, Billerica, USA). DNA was left on the membrane floating on fresh Milli-Q-water for 20min.

2.3.2 Standard protocols

Pictures of molecular structures

All pictures showing molecular structures of proteins are generated using PyMOL-software.

PCR

The standard PCR protocol used in this work is

20s 95°C	denaturation	
10s 94°C	denaturation	24x
15s 50°C	annealing	
20s 72°C	elongation	
30s 72°C	elongation	

Cyclers used were a Primus96 advanced (PeqLab Biotechnologie GmbH, Erlangen, Germany) and a Primus (MWG Biotech AG, Ebersberg, Germany).

Agarose gels

Agarose gels were prepared with 1.2% agarose (Genaxxon bioscience GmbH, Stafflangen, Germany). Standard running conditions for the gels were 90V, 1h. Gels were stained in an ethidiumbromide bath. Results were documented using an Image Master VDS (Amersham Pharmacia Biotech, Vienna, Austria).

Determination of DNA concentration

DNA concentrations were determined using a ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, USA).

Restriction enzyme digests

Restriction enzyme digests were carried out in ultrapure water (Biochrom AG, Berlin, Germany). The volume was 40µl containing 4µl restriction buffer. After adding 1µl of *Kpn*I, the digest was incubated for 80min at 37°C without shaking. Subsequently, 1µl *Sac*I was added and incubation at 37°C continued for another hour. Enzymes were inactivated at 65°C for 20min and DNA was purified. All DNA-ladders, enzymes and corresponding buffers used in this work were purchased from Fermentas (Fermentas GmbH, St. Leon-Rot, Germany).

Ligations

Ligations of PCR-amplified promoter fragments into pSL-1 were carried out as follows:

20µl *Kpn*I /*Sac*I digested promoter fragment

3µl *Kpn*I /*Sac*I digested pSL-1 maxiprep DNA

3µl 10x ligation buffer

1µl T4-ligase

3µl H_2O ultrapure (Biochrom AG, Berlin, Germany)

The ligation took place at 4°C over night. All ligations were desalted before transformation into electrocompetent cells.

Preparation of electrocompetent *E. coli* cells

Electrocompetent cells were prepared as follows:

A 5ml over night culture of *E. coli* in LB was diluted in 500ml LB medium and shaken at 37°C until OD_{600} of 0.5 to 0.6 was reached.

Cells were pelleted at 6000rpm, 10min, 4°C and resuspended in 300ml sterile 2.5% glycerol.

Resuspended cells were centrifuged as described above and diluted in 150ml MilliQ-water.

Cells were centrifuged as described above and resuspended in 10ml sterile 10% glycerol.

Cells were pelleted again and resuspended in 500µl sterile 10% glycerol. Aliquots of 40µl each were shock frozen in liquid ice and stored at -80°C.

Transformation of electrocompetent *E. coli* cells

Electrocompetent *E. coli* were transformed with plasmid DNA using a Biorad Micropulser Electroporator and electroporation cuvettes with a 0.2 cm gap (Bio-Rad Laboratories GmbH, Munich, Germany). The program used was giving one pulse of 2.5 kV. Transformed bacteria were resuspended in 1 ml SOC medium and left shaking at 37°C for 1h, then 1:1 and 1:10 dilutions were plated on corresponding antibiotic LB agar and incubated over night at 37°C.

Preparation of bacterial glycerol stocks

Glycerol stocks of bacteria were prepared as follows:

A single colony was picked from an agar plate and diluted in 5ml medium. After an over night incubation on a shaker, 740µl of the resulting culture were supplemented with 260µl of sterile 50% glycerol and incubated for 30min at 8°C. Cultures were stored at -80°C.

Preparation of *A. castellanii* glycerol stocks

A 2-3 days old *A. castellanii* culture in PYG medium was counted and then centrifuged (5min, 1000rpm). Cells were resuspended in PYG medium to a final concentration of $1x10^8$ cells/ ml. The cells were incubated on ice for 10min in order to cool down. Subsequently, the cell suspension was slowly enriched with Dimethylsulfoxide to a final concentration of 5%. Aliquots of 1ml were frozen at -20°C for 2h and then stored at -80°C.

2.4 Primer design

Primers defining the 100- or 500bp promoter regions were constructed as follows: all specific regions of primers used are 20bp long and included in the corresponding promoter region, with the forward primer at the end of the defined promoter region further away from the start codon of the gene of interest and the reverse primer binding directly to the 20bp upstream of the transcription start site [Perna et al. 2001]. Each forward primer was constructed with a *Sac*I restriction site at its end and each reverse primer with a *Kpn*I restriction site at its end producing sticky end overlaps, so that the resulting PCR fragments are inserted into pSL-1 in the correct orientation.

gene N°	gene name	forward primer	reverse primer
z0012		AGGAGCTCCAGACCCAGTTTCGGCATCA	GAGGTACCCGGCAGCTCCTATCGCGTGA
z0034	*lytB*	AGGAGCTCTCGTAGGCCGCATCCGGCAA	GAGGTACCGTTACGCCTCCAGTGCCGGA
z0301	*crl*	AGGAGCTCCGGCCTTTGGTTTCCGTTTC	GAGGTACCTGCTATCTCCTGTTGTGATG
z0539	*bolA*	AGGAGCTCGCAGATCGCGGGAGGCGGTC	GAGGTACCCCATCCCTTTAAATACTAGC
z0573	*hha*	AGGAGCTCTTATAGTTAAGCTAACAAAC	GAGGTACCACTTCTACCCATGGTTGTAA
z1078	*mdaA*	AGGAGCTCATTTCTCAGCCAGATCTTTT	GAGGTACCTATCTTTTTCTCTTTCTGAA
z1464	*stx2A*	AGGAGCTCCCGTAGCGTCAAAGCAGCAA	GAGGTACCATACAGGTGTTCCTTTTGGC
z1944	*hlyE*	AGGAGCTCATCAACAGTTGATTCAGATC	GAGGTACCTCGCCTCTTTAAATATATAA
z2030	*tonB*	AGGAGCTCTTTCACTGAAACGTGTTCAT	GAGGTACCTGAAGTCATAATCATTTCAG
z2170	*marA*	AGGAGCTCTACCCCAAATCCCGTAGCCA	GAGGTACCTGACGTTGTCACGTTTTCAA
z2433	*fnr*	AGGAGCTCGCCGGTAATCTTAGCATTAT	GAGGTACCAGGTCTGCTCAAGCCGTAAT
z2745	*rplT*	AGGAGCTCCGAAGGCGGCCATTAGCCAG	GAGGTACCAGCTCCCTCTTTCAACTGGC
z2946	*flhD*	AGGAGCTCGTTGTGCGGTAAGTGTCTGT	GAGGTACCCCAGAATAACCAACTTTATT
z3012	*fliA*	AGGAGCTCCACCAACCTGAACAACACCA	GAGGTACCGATAAACAGCCCTGCGTTAT
z3013	*fliC*	AGGAGCTCACGCGGTGGTGCTGCTGGTG	GAGGTACCGATTCGTTATCCTATATTGC
z3276		AGGAGCTCGAACCGTCTTTCCGAAACGT	GAGGTACCATTTCACCTCTCACCGACAG

z3277	*yehB*	AGGAGCTCAAAAGATTCAGGTTCCTTTC	GAGGTACCAGGCAGCTCCTGCAATTAAA
z3279	*yehD*	AGGAGCTCCTACAACATTAACCATCAAT	GAGGTACCGATGCATTCCTTAATAAGTT
z3343	*stx1B*	AGGAGCTCATCGCCATTCGTTGACTACT	GAGGTACCTTTACCCCCTCAACTGCTAA
z4326		AGGAGCTCTGTAAGCTCAGTGGCATGGA	GAGGTACCATGTTTGTATCCTTTCTATA
z4377	*qseB*	AGGAGCTCAATTCCAGTCTTTATCGACT	GAGGTACCTTTTTCATCCCTGCGATAAC
z4378	*qseC*	AGGAGCTCACCTTACCAGGCATGGATGG	GAGGTACCTTCTCACCTAATGTGTAGCC
z4379	*mdaB*	AGGAGCTCGGTGATGGATATTTACCACA	GAGGTACCTTTTTAACCTCAAGGTGAGA
z4982		AGGAGCTCAATTAAACTGGCGATGCAGG	GAGGTACCAGAGGGGCCTCACTTTGATT
z5100	*espF*	AGGAGCTCGGTACGAGGATTTTAATTTA	GAGGTACCAATTTATGCCTCTTTATATC
z5102	*orf29*	AGGAGCTCCGGTTCAGCAATATTCTGCT	GAGGTACCTAAAATTAAAAACTACGGTT
z5103	*escF*	AGGAGCTCGAAAGGGTGCCGTGCAGCAA	GAGGTACCTAAATTTCCCTCAGAAGTTA
z5104	*cesD2*	AGGAGCTCTATCACGACTCGTCTGCGTG	GAGGTACCGATCAATTACCCAGCTAAGC
z5105	*espB*	AGGAGCTCCGAGCTATTTACAAAGTGTT	GAGGTACCAATAAAATTCTCTTTAATAA
z5106	*espD*	AGGAGCTCGAATACATTAAATCTCTTAA	GAGGTACCAGTTATCTCCGGTTATTTAC
z5107	*espA*	AGGAGCTCATGATTGGTAAAGTTATCGA	GAGGTACCCTATATACCTCTTGATAATT
z5108	*sepL*	AGGAGCTCATATGGTGAACTTACATCGA	GAGGTACCTGGAAACTCACGTAATCATT
z5109	*escD*	AGGAGCTCAGTACTGTACAACAGCTTCC	GAGGTACCTTTTAAATTTTATTCATCCT
z5110	*eae*	AGGAGCTCTCAGTTCAAGCGACAATAAA	GAGGTACCGTTATGGCTCCACCACAATG
z5111	*cesT*	AGGAGCTCGGGAAGTCTAACTAACGTCA	GAGGTACCTGTATTTTCTCTTAATTAAA
z5112	*tir*	AGGAGCTCTTCAGCCGTTTATCGACTAC	GAGGTACCAAATATCTCCTTTTTATTTT
z5113	*map*	AGGAGCTCATAATTCAAATATAAATACA	GAGGTACCACCATAAACCTTTATAAATT
z5114	*cesF*	AGGAGCTCATTCCCGTAATCTTATATCT	GAGGTACCATTGTAGTCCTGCCATTCTT
z5115	*espH*	AGGAGCTCGATAGCATACGGGAGTATTG	GAGGTACCGATACATCTCCCTATATAAC
z5116	*sepQ*	AGGAGCTCATTTATCTGTCTATTAAAGT	GAGGTACCCAGGAAAGATTTCTTTTAAC
z5117	*orf16*	AGGAGCTCTTCTTTTCGGCCATCAGATG	GAGGTACCTCGTATGAACAACGTATCAG
z5118	*orf15*	AGGAGCTCAAAATAGGAAATTAATTCAG	GAGGTACCACTCAGGCAACCACTTTGAA
z5119	*escN*	AGGAGCTCTATGTGAAGAAAATGATTGA	GAGGTACCTTACCGTTCCTAATACTTTA
z5120	*escV*	AGGAGCTCTGTGCTTAAGTTGTTTGTTA	GAGGTACCGATGTCATCCTGCGAACGCG
z5121	*orf12*	AGGAGCTCAATAGTTGCAATGTACATAA	GAGGTACCCTGCAGGCTCTGAAGTAAGT
z5122	*sepZ*	AGGAGCTCGTACATTGCAACTATTTAAA	GAGGTACCTGATCTTTCTCCTTTTGTCT
z5123	*rorf8*	AGGAGCTCGTATTACTTCTGGCGTACTA	GAGGTACCTACCTCTATCTTTTATTTAT
z5124	*escJ*	AGGAGCTCAAAAGAATGTGCTCAACGCA	GAGGTACCTTTTATTGCATCGAAACTAA
z5125	*sepD*	AGGAGCTCTTCAGATAAAAAACCTAGTT	GAGGTACCACATATTACCCGTCCTGTCC
z5126	*escC*	AGGAGCTCCTTTGCTCGATAAGCAATTT	GAGGTACCTACACAATTCGTCCTATATC
z5127	*cesD*	AGGAGCTCTTCTCCAGTACGGTAAAACA	GAGGTACCAAAGCCTCATTTATTATTCG
z5128	*grlA*	AGGAGCTCATTAGTTATTAAAAACACAG	GAGGTACCTTTCCATATTCTTTTTATTT
z5129	*grlR*	AGGAGCTCATTGCAATCTGGAGAAAAAG	GAGGTACCAACTCACTCAGTTTCATACT
z5131	*rorf3*	AGGAGCTCGCAATGTAATATATCATTAA	GAGGTACCTTTTACGTTGTTACTCAATA
z5132	*escU*	AGGAGCTCGCAATCTTTATATTGCTATT	GAGGTACCTAATCATGCTCGGTAACGAT
z5133	*escT*	AGGAGCTCAGTGTTTGCTACGCTTGCC	GAGGTACCTAGCCGTTCACCTTCGGAAT
z5134	*escS*	AGGAGCTCTGGTATCGCCAGTAACAATT	GAGGTACCCAATTCACCACCAACAGAAA
z5135	*escR*	AGGAGCTCAATGAATATATTAGAATAAC	GAGGTACCATCATCATTCCTGAATAATG
z5136	*orf5*	AGGAGCTCATAAAACCAACTCATATTAA	GAGGTACCTAAATCAATTTTTATATTTT
z5137	*orf4*	AGGAGCTCCCCTAAGTAAGATTGAAAAA	GAGGTACCACTATTTTTCTATTATTTCT
z5138	*cesAB*	AGGAGCTCTAAAGTTACCTACAATGAAG	GAGGTACCCCTCTATTTATTATTAATCC
z5139	*orf2*	AGGAGCTCAAGAAGCACTGTTGAATGGA	GAGGTACCTTAATTATTTCATGTTAAAT

z5140	*ler*	AGGAGCTCTCCTAATTTGATAGATAAAC	GAGGTACCGCTTTAATATTTTAAGCTAT
z5142	*espG*	AGGAGCTCAATATTTTCACTTATTTTTC	GAGGTACCATAGCATCATATAGTGTCAA
z5143	*rorf1*	AGGAGCTCAATAGAAACCACACCCATAA	GAGGTACCTTTCTCTATTTGCTGTAAAT
z5358	*tatA*	AGGAGCTCGCTTCAATATGGAAGTGCAG	GAGGTACCTATACGTTACACGGCCCGCC

Table 1: list of primers used for the generation of 500- and 100bp (for the LEE genes) upstream regions of EHEC genes tested in this work. Introduced *Sac*I restriction sites are printed in blue, introduced *Kpn*I sites in violet. All primers are shown in 5'-3' direction. Primer names are given as follows: gene number-fragment length-F for forward or R for reverse-corresponding restriction enzyme (*Kpn*I or *Sac*I). For example, the forward primer for the z0012 500bp promoter region is named z0012-500F-*Sac*I.

*Sac*I cutting scheme

*Kpn*I cutting scheme

Figure 10: scheme of the restriction sites recognized by *Sac*I and *Kpn*I . In both cases, a DNA-overlap of four bases is produced.

2.5 Generation of transgenic EHEC

Hundred bp upstream fragments of each operon or gene of the locus of enterocyte effacement and 500bp upstream fragments of the other tested genes were PCR-amplified from EHEC genomic DNA. These fragments were cloned into pSL-1 using the *Sac*I- and *Kpn*I-restriction sites upstream of the promoterless *luxCDABE* cassette. The resulting promoter probe vectors were transformed into TOP10^{-} cells via electroporation and plasmid identity was confirmed by colony PCR. For this purpose, the upstream forward primer of each 100bp promoter fragment and the reverse primer *luxC*+59R, binding within the *lux* cassette, were used. Verified plasmids were re-isolated, transformed into EHEC O157:H7 via electroporation and the plasmid identity was verified by colony PCR again.

primer name	sequence
luxC+59R	5'-GATTGCACTAAATCATCACTTTCGG-3'

Table 2: sequence of *luxC*+59R, the primer used for verification of plasmid identities.

2.6 Promoter-*lux* studies with EHEC and *C. elegans*

Over night cultures of transgenic EHEC and wild type EHEC as a control were grown in LB-broth shaking at 37°C. Medium was supplemented with kanamycin for transgenic bacteria. The cultures were harvested by centrifugation at 6000rpm for 2min, the supernatant was discarded. Bacteria were washed twice and finally resuspended in S-medium supplemented with nystatin. Luminescence was measured for 2.5h in a Victor^3 1420 multilabel counter (Perkin Elmer, Überlingen, Germany) in white opaque 96-well plates with transparent bottoms (Greiner Bio-One GmbH, Frickenhausen, Germany) in order to adapt the bacteria to the new medium and to exclude later measurement of adaptation effects generating false positive results. For each promoter region, six wells were occupied with 150µl bacteria suspension each.

C. elegans were rinsed from 4d old NGM-Nys-plates with OP50, grown at 22°C, using S-medium supplemented with nystatin, penicillin, neomycin and streptomycin in order to kill the sensitive OP50 food bacteria. The worms were washed twice in the same medium by centrifugation at 5000rpm. Two additional washing steps were carried out in S-medium supplemented with nystatin in order to wash out the antibiotics. The nematodes were resuspended in S-medium supplemented with nystatin and 150µl /well added to the EHEC-suspensions in the 96-well plates. As a control, three of the six wells containing the same transgenic EHEC over night culture were added S-medium supplemented with nystatin containing no nematodes. Luminescence was measured every 30min in the Victor^3 for at least 6.5h at room temperature. The 96-well-plates were left on a Titromax1000 plate shaker (Heidolph Instruments, Schwabach, Germany) shaking 800 times/min between the measurements. Luminescence measurements were also conducted with heat killed worms (30min at 40°C) and with conditioned S-medium (spent medium which had kept worms for 1.5h and was subsequently sterile filtered), to elucidate whether effects of a direct contact to the live nematode or reactions to some secreted substance were measured. All measurements were carried out at least three times independently and the average was determined, setting the luminescence amount measured for the control without nematodes to 1 for each time point.

2.7 Promoter-*lux* studies with EHEC and *A. castellanii*

Over night cultures of transgenic EHEC and wild type EHEC as a control were grown in PYG-broth shaking at 37°C, which for the transgenic bacteria was supplemented with kanamycin. The over night cultures were directly transferred (150µl /well) to 96-well plates with transparent bottoms (Greiner Bio-One GmbH, Frickenhausen, Germany) for the luminescence measurements following the same scheme as for the assays with *C. elegans*. After a short adaptation time, luminescence was measured once without amoebae in a Victor^3 multilabel counter. Subsequently, 6d old cultures of *A. castellanii* grown in PYG supplemented with kanamycin were added to the bacterial cultures (150µl /well). For the three control wells for each promoter region, PYG-Kan-medium was added without amoebae. Luminescence was measured every 30min for at least 7h under the same conditions as described for the *C. elegans* assays. Luminescence measurements were also conducted with heat killed amoebae (30min at 50°C) and with conditioned PYG-medium (supplemented with kanamycin), which had kept the amoebae for 6d and was then sterile filtered, to elucidate whether effects of a contact to the live organism or reactions to some secreted substance were measured. All measurements were carried out at least three times independently. Evaluation of the results was conducted the same way as for the *C. elegans* assay.

2.8 Promoter-*lux* studies with EHEC and *A. thaliana*

Over night cultures of transgenic EHEC and wild type EHEC as a control were grown in LB-broth shaking at 37°C, which for the transgenic bacteria was supplemented with kanamycin. For luminescence measurements, the cultures were washed twice in MS medium and then resuspended in fresh MS medium. After an adaptation time of 2.5h, they were transferred (150µl /well) to *A. thaliana* plants which had been allowed to grow in 96-well plates with transparent bottoms with MS medium for one week (approximately 3-5 plants /well in 150µl MS medium) below an HQI-lamp (35-50 µmol light) at room temperature (approximately 22°C). There were at least four wells loaded with the same bacterial culture, because luminescence measurements using plants are difficult to conduct due to the plant tissue disturbing the experiment, shielding the bacteria in the shadow of the leaves from the luminescence measurements. Luminescence was measured every hour in a Victor^3 plate reader. The 96-well-plates were left below the HQI-lamp at 22°C without shaking between the measurements. Experiments were also carried out with spent MS medium, which had kept *A. thaliana* for one week. All experiments were carried out three times independently. Evaluation of the results was conducted the same way as for the *C. elegans* assay.

2.9 Promoter-*lux* studies with EHEC and Caco-2 cells

Caco-2 colon epithelial cells were grown in 96-well plates in DMEM 1640 medium (Biochrom AG, Berlin, Germany) to a density of 2.5 x 10^4 cells /well at 37°C and 5% CO_2.
5ml cultures of transgenic EHEC were grown over night in DMEM (Dulbecco's modified eagle medium) at 37°C, shaking. The following day, the cultures were diluted in DMEM supplemented with 44mM $NaHCO_3$ as described by Abe et al [Abe et al. 2002]. After an additional growth phase of 90-120 min, the transgenic EHEC were centrifuged onto the Caco-2 cells for an optimal adherence of EHEC to the Caco-2 Monolayer. Luminescence was measured every 30 min in a Victor^3 plate reader. The 96-well plates were kept at 37°C and 5%CO_2 between the measurements. pH values were controlled in test wells containing Caco-2 cells in DMEM supplemented with 44mM $NaHCO_3$ in order to make sure the Caco-2 cells stayed alive. Measurements were conducted for 4.5 h.

2.10 *A. thaliana* growth assay

A. thaliana plants were grown in 500ml glasses with transparent lids on MS agar for one week. Each glass contained four spots of *A. thaliana*, consisting each of about five individual plants. EHEC O157:H7 were added to the one week old plants (150µl over night culture per plant spot) and plants were photographed every two days using a casio exilim EX-Z600 camera in order to observe the phenotype of the *A. thaliana* plants. Each glass was supplemented with only one bacterial culture in order to avoid cross-contamination. TOP10$^-$ as a negative control and the plant pathogenic *Pseudomonas syringae* as a positive control were also submitted to this test.

2.11 Abbreviation list

Amp	ampicillin
Ces	chaperone for *E. coli* secreted proteins
EHEC	enterohaemorrhagic *E. coli*
EPEC	enteropathogenic *E. coli*
Esc	*E. coli* secretion apparatus component sharing high homology with a component of the *Yersinia* TTSS
Esp	*E. coli* secreted protein
Gb_3	human globotriaosylceramide receptor
GFP	green fluorescent protein
HCM	host cell membrane

IM	inner membrane
IPTG	isopropyl-β-D-thiogalactopyranosid
Kan	kanamycin
LB	Luria-Bertani broth
LEE	locus of enterocyte effacement
Neo	neomycin
NGM	nematode growth medium
Nys	nystatin
OM	outer membrane
orf	open reading frame
ori	origin of replication
PAI	pathogenicity island
Pen	penicillin
PCR	polymerase chain reaction
PYG	proteose N^{o} 3, yeast extract, glucose
QS	Quorum sensing
Sep	*E. coli* secretion apparatus component without homology to components of the *Yersinia* TTSS
Strep	streptomycin
TTSS	type three secretion system
UV	ultraviolet
wt	wild type

2.12 Gene name list

cdt	cytolethal distending toxin
eae	intimin
hly	EHEC haemolysin
ler	LEE-encoded regulator
luxCDABE	luminescence gene cassette from *Photorhabdus luminescens*
stxI	Shiga toxin I
stxII	Shiga toxin II
tir	translocated intimin receptor
tnaA	tryptophanase A gene of EHEC

3 Results

3.1 The LEE1 operon

The LEE1 operon consists of nine genes (*ler*, *orf2*, *cesAB*, *orf4*, *orf5*, *escR*, *escS*, *escT*, *escU*), the first of which is the gene for the LEE-encoded regulator *ler*. Ler is the positive regulator of all other LEE operons and monocistronic genes and also autoregulates its own expression [Laaberki et al. 2006]. Ler has been proposed to antagonize an H-NS dependent repression of the LEE PAI [Bustamante et al. 2001]. The other eight genes encode for structural elements of the activation machinery for the export through the bacterial inner membrane (*escR*, *escS*, *escT*, *escU*), a chaperone (*cesAB*) and several genes of unknown function (*orf2*, *orf4*, *orf5*). The most important promoter region is that upstream of *ler*, because, as the LEE1 operon is transcribed polycistronically, an upregulation of this region indicates activation of the whole operon, while nothing is known so far about promoter regions for the other genes of LEE1 and their importance regarding the regulation of the whole operon or single genes.

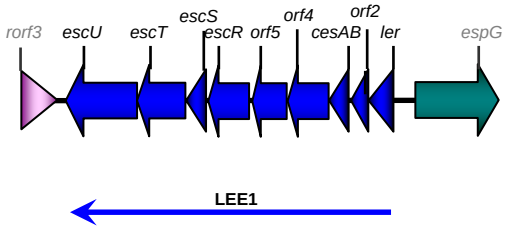


Figure 11: genomic organization of the LEE1 operon and neighbouring LEE-orfs.

3.1.1 LEE1 genes and *C. elegans*

In contact with the soil nematode *Caenorhabditis elegans*, the 100bp upstream region of each gene of the LEE1 operon was induced more than threefold. This is especially important for the *ler* 100bp upstream promoter region because an upregulation of *ler* indicates positive regulation of the whole LEE pathogenicity island. There was no induction of any promoter region in the control assays with spent S-medium which had kept nematodes for 1.5h and was subsequently sterile filtered (data in supplement) or with heat-killed nematodes (30min at 40°C).

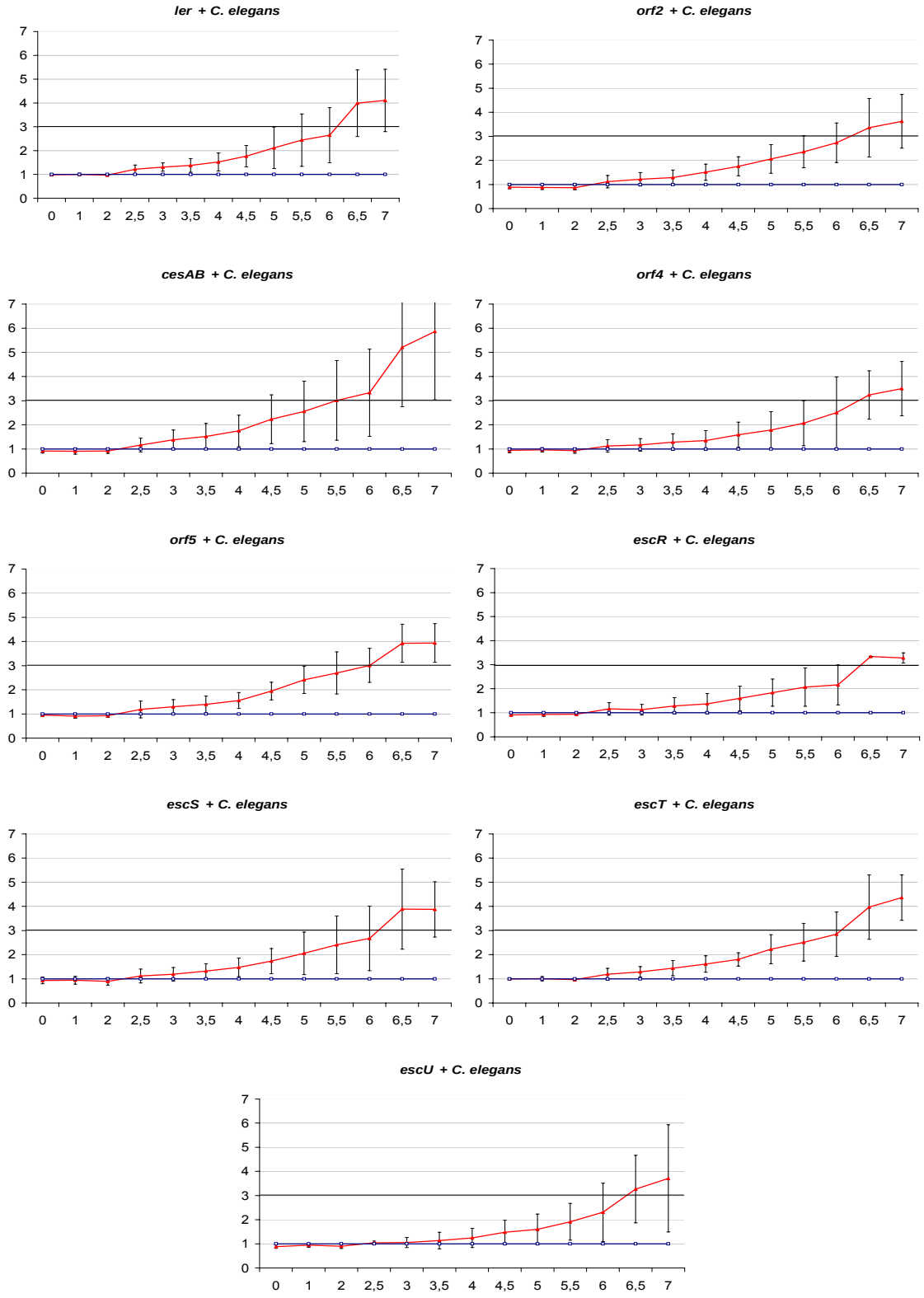


Figure 12: activation of 100bp upstream promoter regions of the genes of the LEE1 operon. C. elegans were added at t = 2.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *C. elegans*, which is defined 1 for each time point (blue line). Red lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. Experiments were carried out in triplicate.

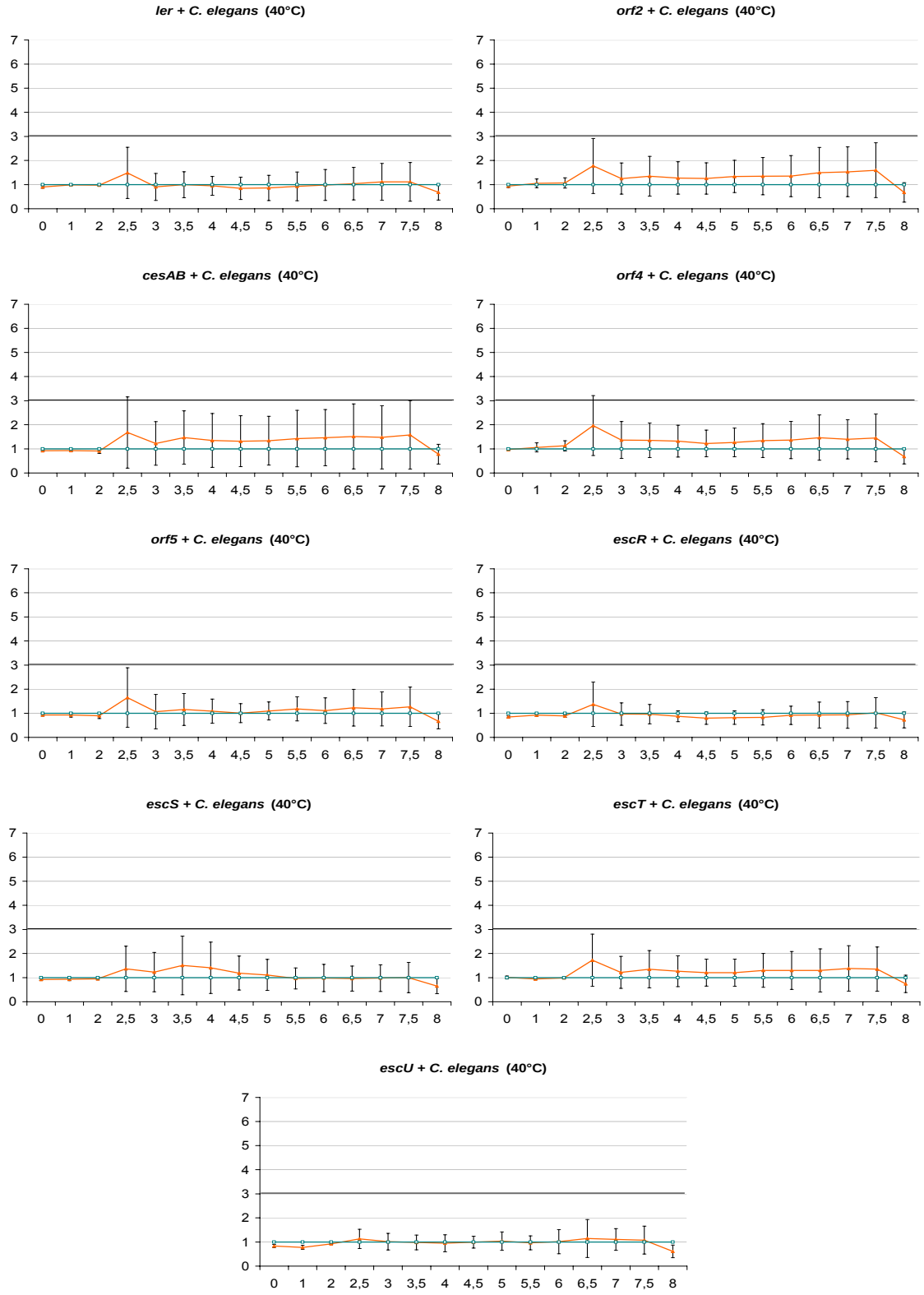
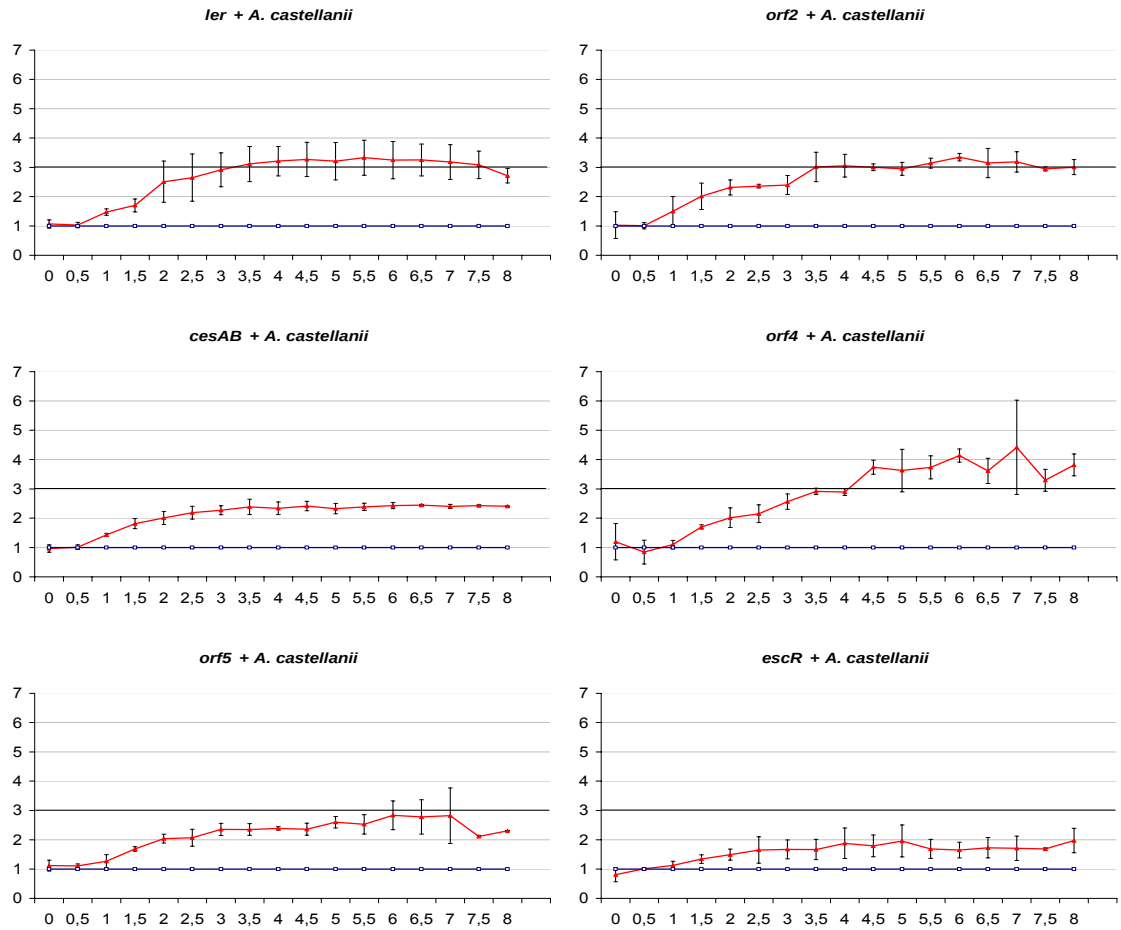


Figure 13: activation of 100bp upstream promoter regions of the genes of the LEE1 operon. Heat killed *C. elegans* (30min at 40°C) were added at t = 2.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *C. elegans*, which is defined 1 for each time point (turquoise line). Orange lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. Experiments were carried out in triplicate.

3.1.2 LEE1 genes and *A. castellanii*

In contact with the waterborne protozoa *Acanthamoeba castellanii*, the 100bp upstream promoter region of *ler*, the first gene of the LEE1 operon, was upregulated. This indicates an upregulation of the whole LEE1 operon, which is transcribed polycistronically, although *cesAB*, *orf5* and *escR* were not induced above the threshold of a threefold activation compared to the bioluminescence emitted without the presence of *A. castellanii*. In contact with spent PYG medium, which had kept amoebae for six days and was then sterile filtered, no induction of any promoter region of the LEE1-genes above threshold was observed (data in supplement). In contrast to this, when heat-killed amoebae (30min at 50°C) were submitted to the assay with the LEE1-genes, the activation pattern was the same as that with live amoebae, although in most cases the threshold of three-fold activation was reached slower than with live amoebae.



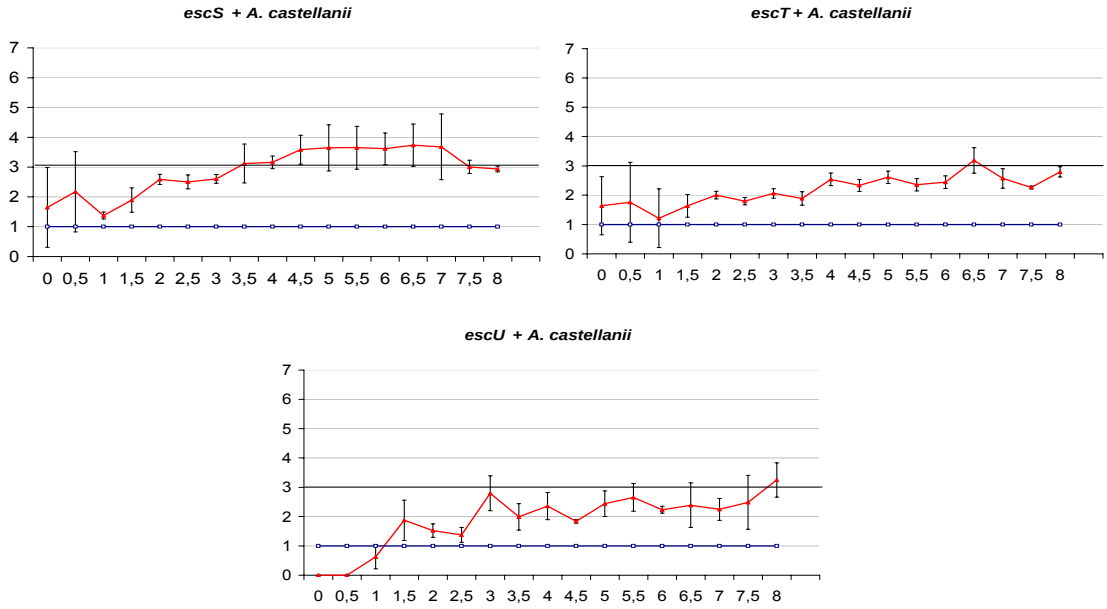
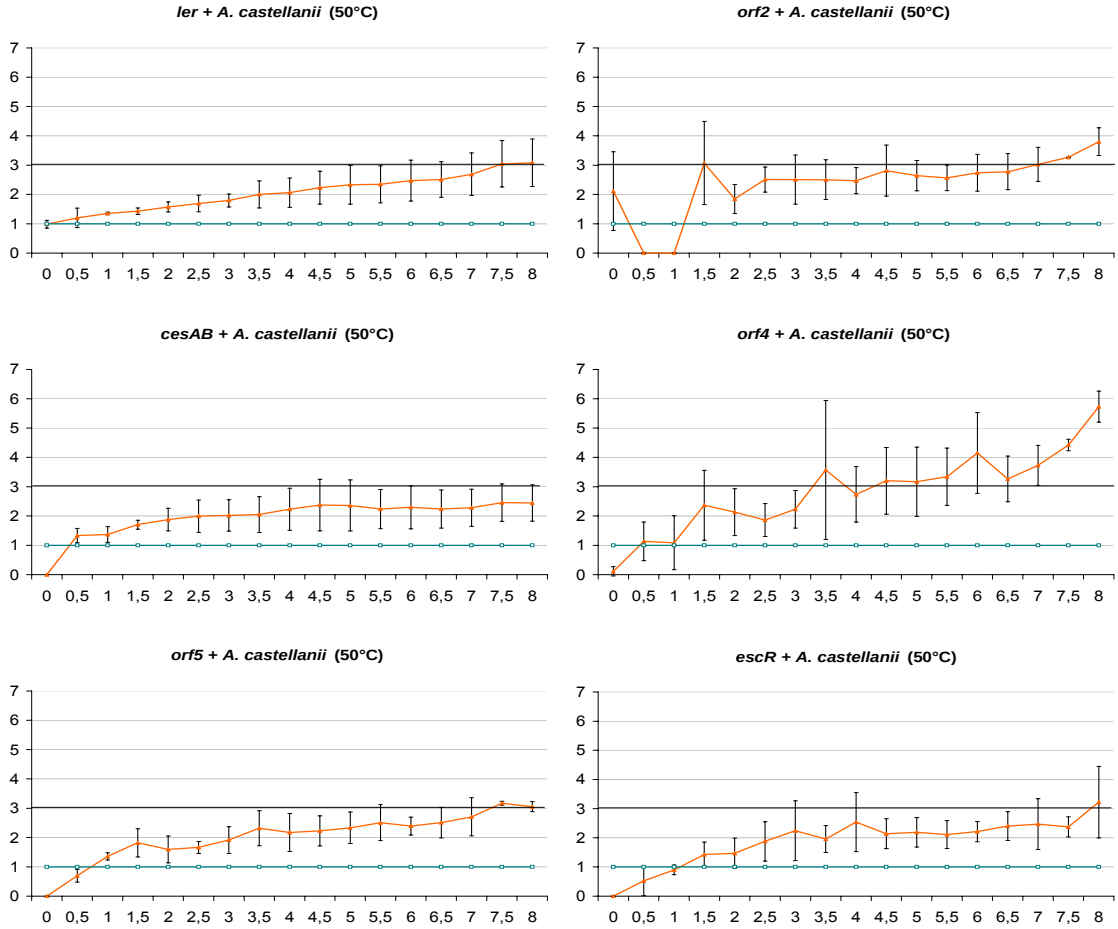


Figure 14: activation of 100bp upstream promoter regions of the genes of the LEE1 operon. *A. castellanii* were added at t = 0.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *A. castellanii*, which is defined 1 for each time point (blue line). Red lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. Experiments were carried out in triplicate.



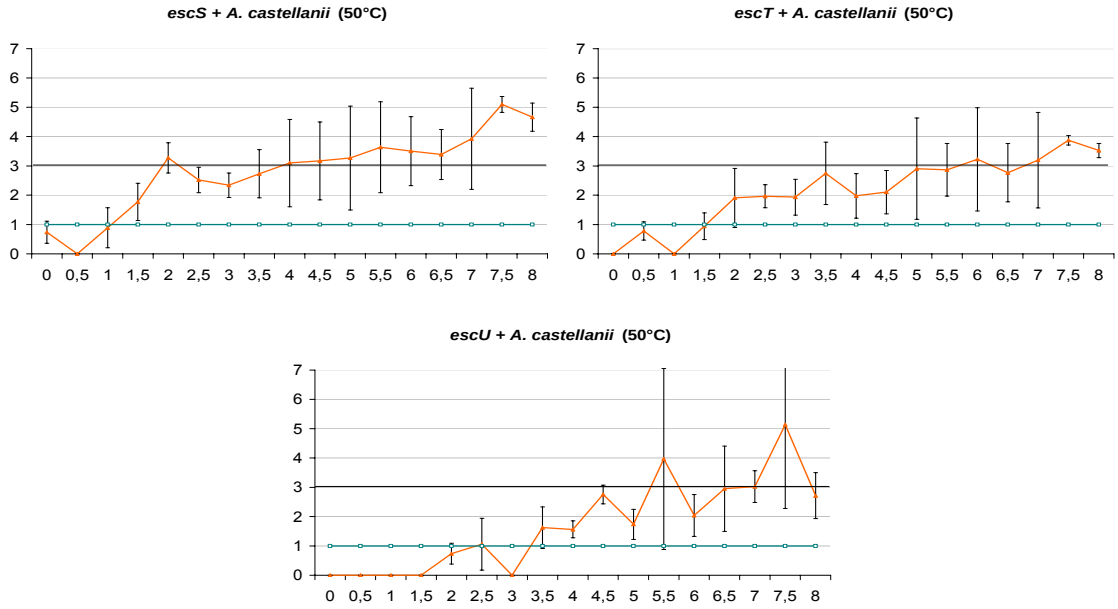
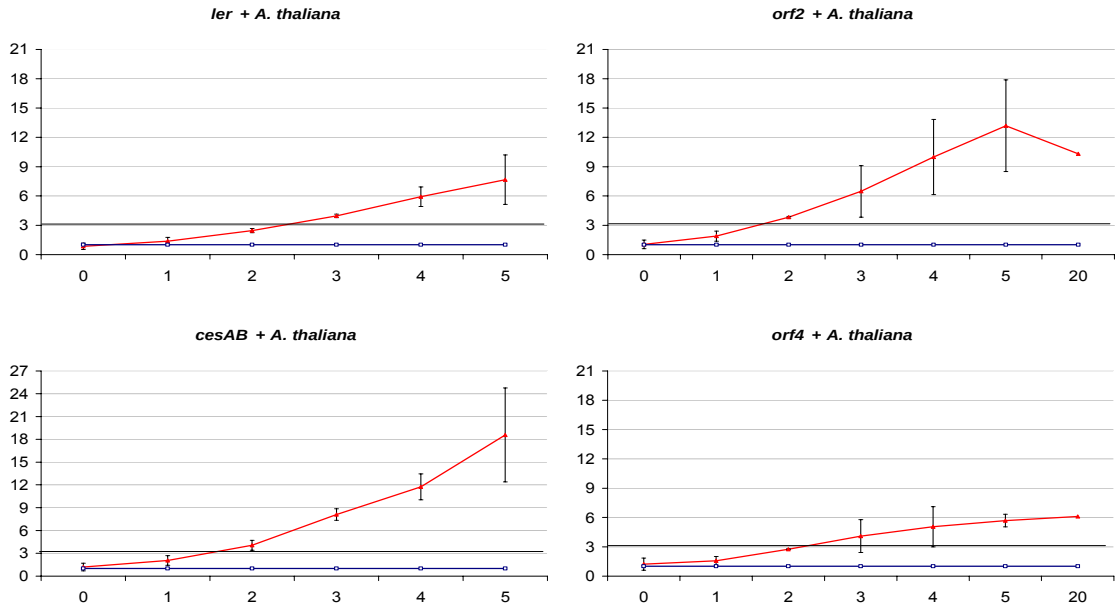


Figure 15: activation of 100bp upstream promoter regions of the genes of the LEE1 operon. Heat killed *A. castellanii* (30min at 50°C) were added at t = 0.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *A. castellanii*, which is defined 1 for each time point (turquoise line). Orange lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. Experiments were carried out in triplicate.

3.1.3 LEE1 genes and *A. thaliana*

In contact with the thale cress *Arabidopsis thaliana*, the 100bp upstream promoter regions of all LEE1-genes were upregulated remarkably above the threshold of threefold induction in comparison to the control wells without *A. thaliana*. There was no activation of any LEE1 promoter region with spent MS-medium, which had kept *A. thaliana* for one week and was subsequently sterile filtered.



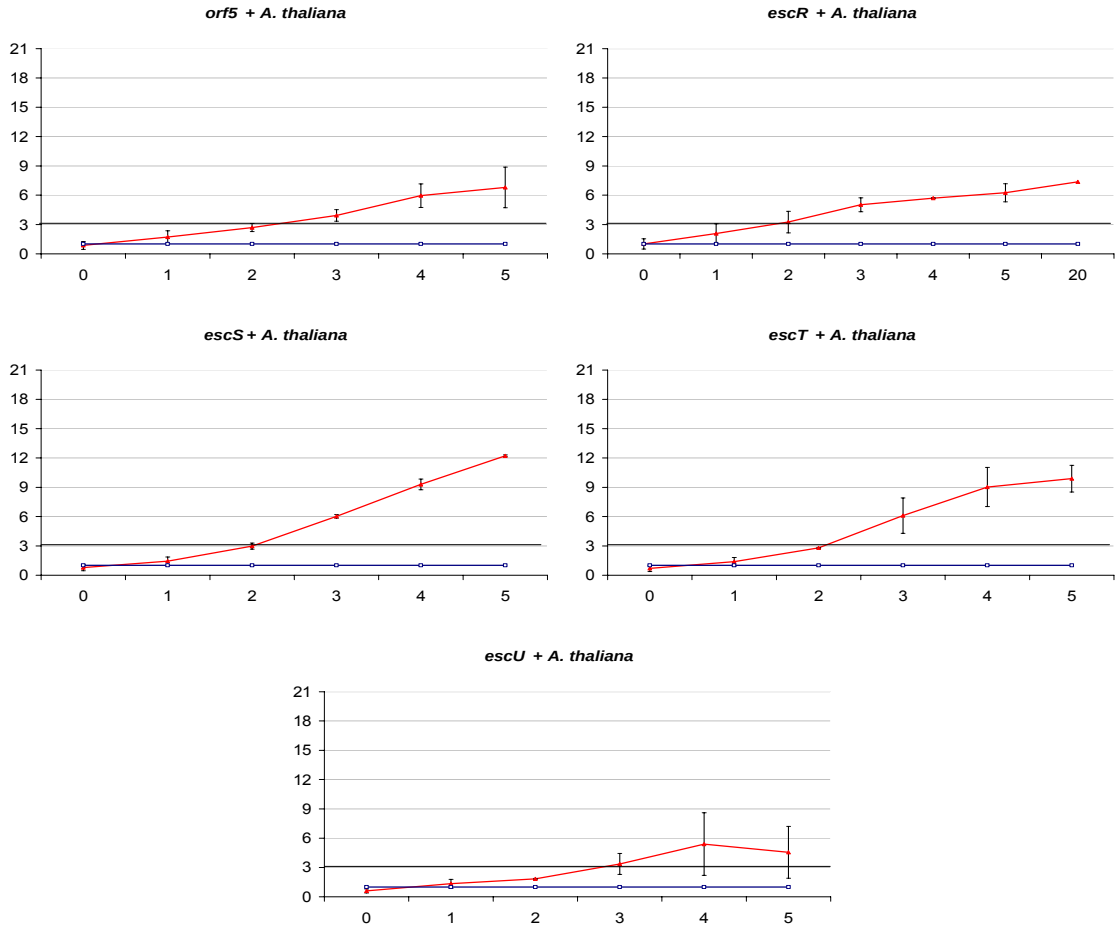


Figure 16: activation of 100bp upstream promoter regions of the genes of the LEE1 operon. *A. thaliana* were added at t = 0 h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *A. thaliana*, which is defined 1 for each time point (blue line). Red lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. Experiments were carried out in triplicate.

3.2 The LEE2 operon

The LEE2 operon consists of six genes (*sepZ*, *rorf8*, *escJ*, *sepD*, *escC*, *cesD*). The first gene of LEE2 is *sepZ*, encoding for another structural component of the TTSS which is needed for the export of effector and translocator proteins through the EHEC inner membrane. SepZ has also been proposed to be secreted as an effector protein by the TTSS and is therefore sometimes referred to as EspZ [Gilmour et al. 2006]. One of the other five LEE2 genes is *sepD*, which, together with *sepL*, encodes for a molecular switch regulating the secretion hierarchy of TTSS-effected translocators and effectors [Deng et al. 2005]. The remaining four genes encode for structural components (*escJ*, *escC*), the chaperone for the translocator EspD (*cesD*) and a gene product of unknown function (*rorf8*), which has been shown to interact with the *rorf3* gene product in EPEC [Creasey 2003].

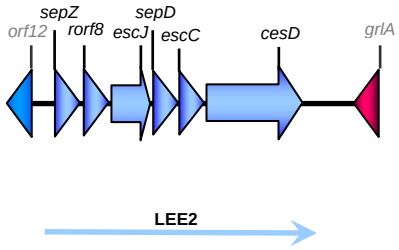
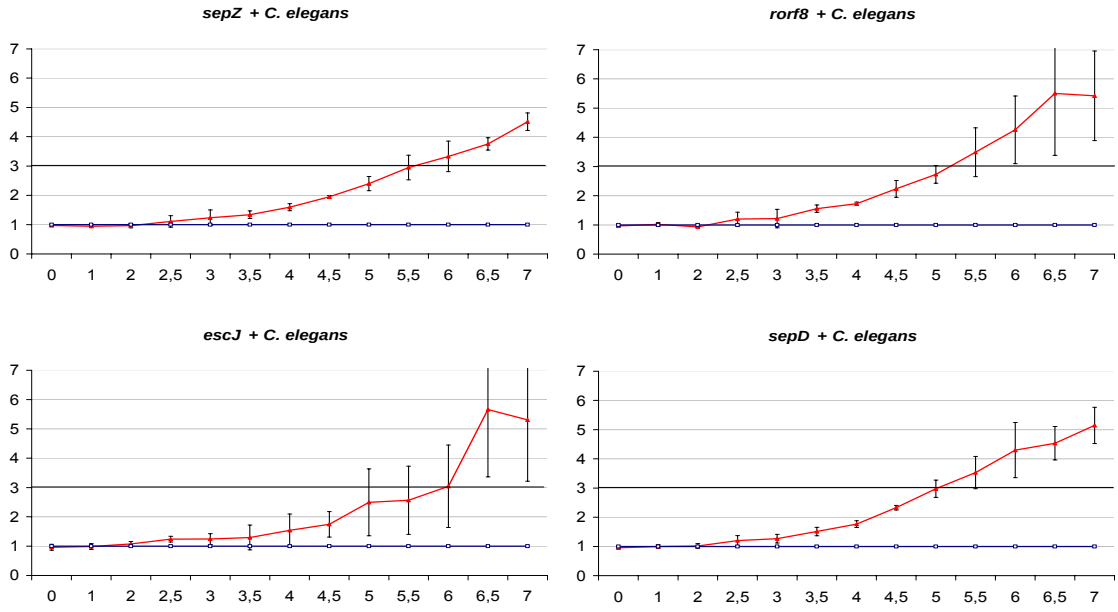


Figure 17: genomic organization of the LEE2 operon and neighbouring LEE-orfs.

3.2.1 LEE2 genes and *C. elegans*

In contact with *C. elegans*, all 100bp upstream regulatory regions of the genes of the LEE2 operon were activated in EHEC. There was no induction of any promoter region in the control assays with spent S-medium which had kept nematodes for 1.5h and was subsequently sterile filtered (data not shown) or with heat-killed nematodes (30min at 40°C).



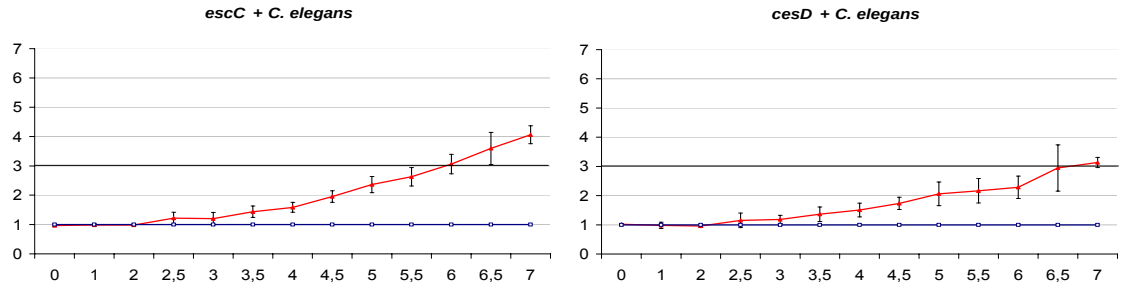


Figure 18: activation of 100bp upstream promoter regions of the genes of the LEE2 operon. *C. elegans* were added at t = 2.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *C. elegans*, which is defined 1 for each time point (blue line). Red lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. Experiments were carried out in triplicate.

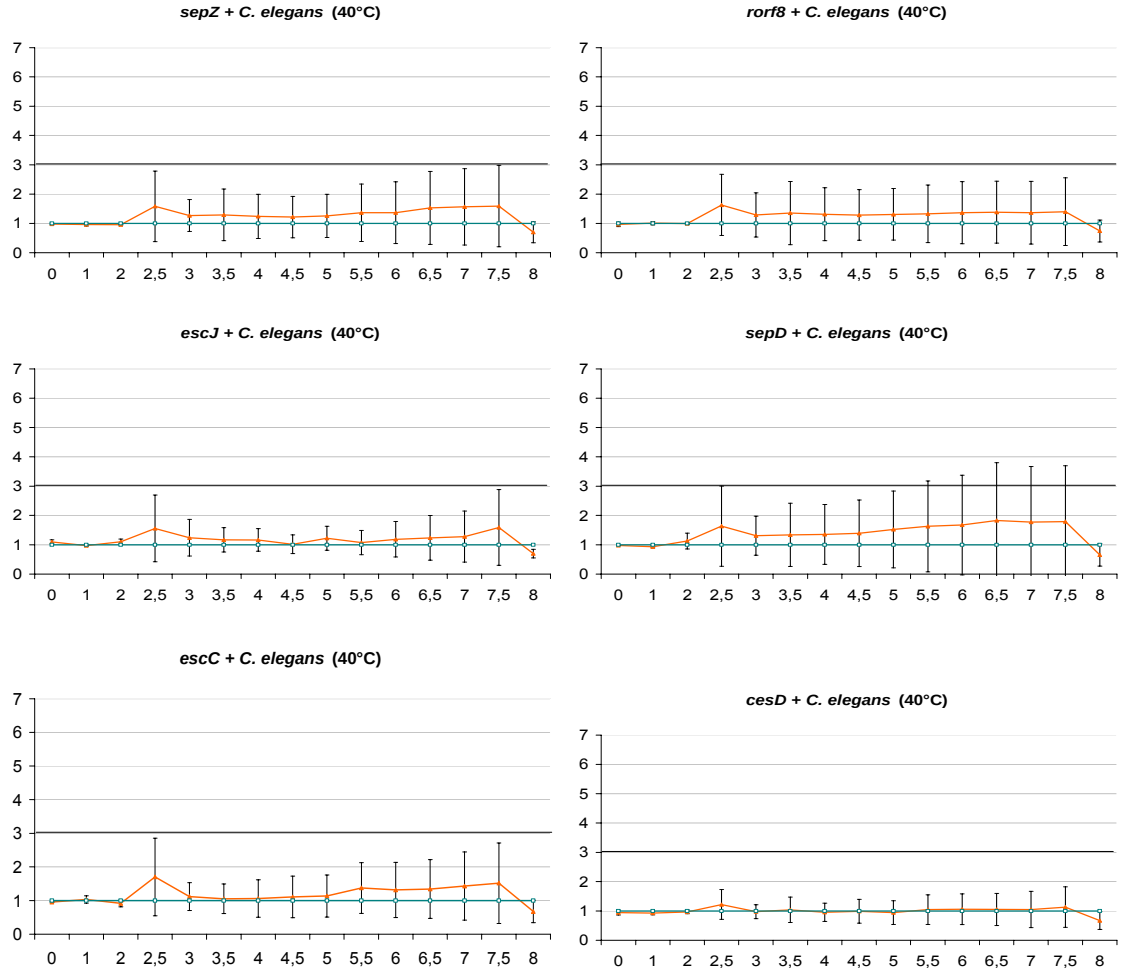


Figure 19: activation of 100bp upstream promoter regions of the genes of the LEE2 operon. Heat killed *C. elegans* (30min at 40°C) were added at t = 2.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *C. elegans*, which is defined 1 for each time point (turquoise line). Orange lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. Experiments were carried out in triplicate.

3.2.2 LEE2 genes and *A. castellanii*

In contact with *A. castellanii*, all the promoter regions of LEE2 genes were activated except for those of *sepD* and *cesD*. In contact with spent PYG medium, which had kept amoebae for six days and was then sterile filtered, no induction of any promoter region of the LEE2-genes above threshold was observed (data in supplement). In contrast to this, when heat-killed amoebae (30min at 50°C) were submitted to the assay with the LEE2-genes, the activation pattern was the same as that with living amoebae. In the case of the promoter region of *rorf8* and *cesD*, the activation seems to be even stronger with heat killed amoebae than with live *A. castellanii*.

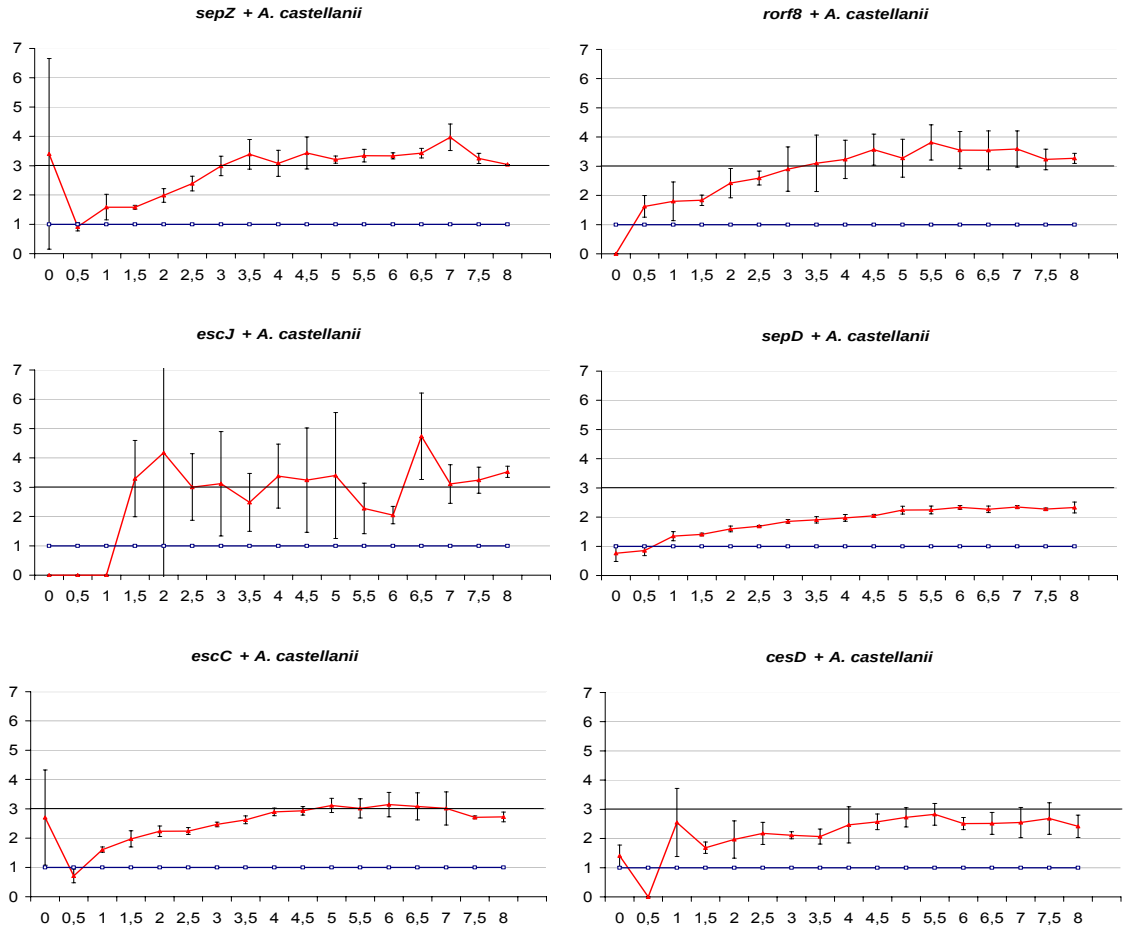


Figure 20: activation of 100bp upstream promoter regions of the genes of the LEE2 operon. *A. castellanii* were added at t = 0.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *A. castellanii*, which is defined 1 for each time point (blue line). Red lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. Experiments were carried out in triplicate.

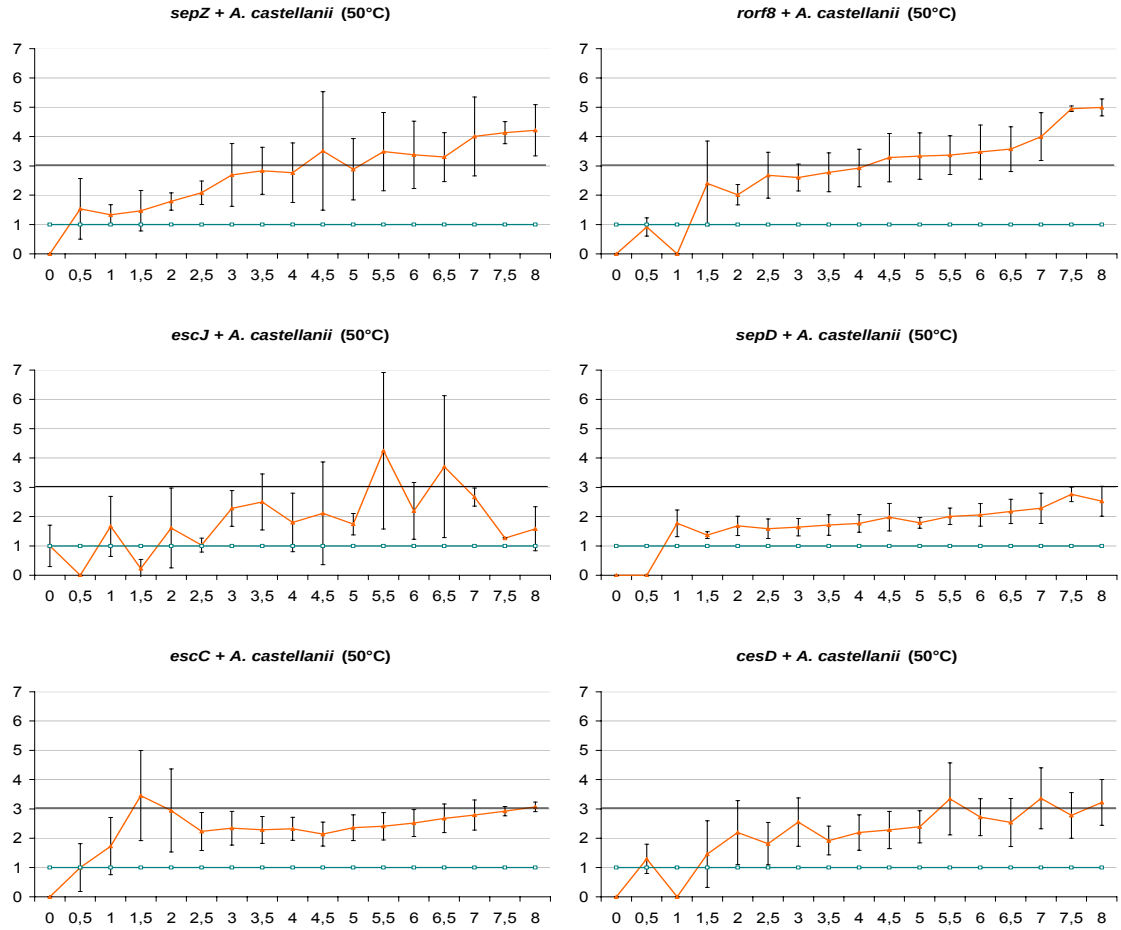


Figure 21: activation of 100bp upstream promoter regions of the genes of the LEE2 operon. Heat killed *A. castellanii* (30min at 50°C) were added at t = 0.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *A. castellanii*, which is defined 1 for each time point (turquoise line). Orange lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. Experiments were carried out in triplicate.

3.2.3 LEE2 genes and *A. thaliana*

In contact with the thale cress *A. thaliana*, all promoter regions of genes of the LEE2 operon were activated. There was no activation of any LEE2 promoter region with spent MS-medium, which had kept *A. thaliana* for one week and was subsequently sterile filtered.

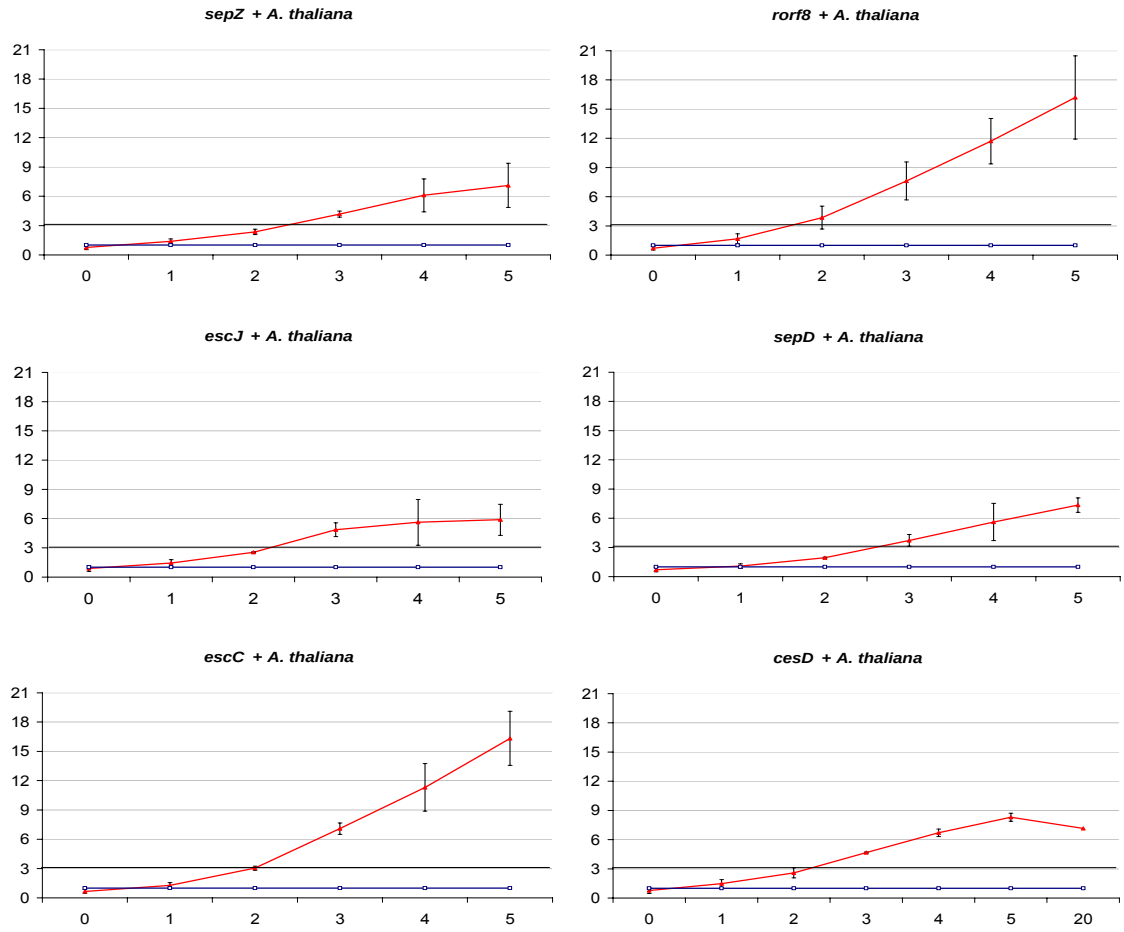


Figure 22: activation of 100bp upstream promoter regions of the genes of the LEE2 operon. *A. thaliana* were added at t = 0 h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *A. thaliana*, which is defined 1 for each time point (blue line). Red lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. Experiments were carried out in triplicate.

3.3 The LEE3 operon

In the LEE3 operon, seven genes are encoded (*orf12*, *escV*, *escN*, *orf15*, *orf16*, *sepQ*, *espH*). The function of the first gene of the operon is still not known exactly, it is referred to as *orf12*. In the LEE3 operon, the genes for the ATPase component of the EHEC-TTSS, *escN*, and its direct interaction partner, *escV* are encoded. The function of the gene products of *orf15* and *orf16* is still unknown. *sepQ* encodes for a pore-forming protein needed to transport translocators and effectors through the EHEC inner membrane. In this study, the 100bp upstream promoter region of the last gene of LEE3, *espH*, encoding for a putative cytoskeleton modulating factor, could not be submitted to the *lux*-promoter fusion assays with the chosen test organisms *C. elegans*, *A. castellanii* and *A. thaliana*, because the 100bp upstream region of this gene could not be PCR-amplified with any of the primers and conditions I tried.

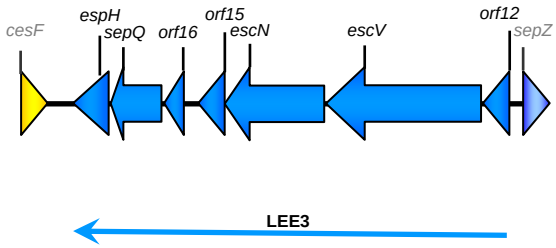
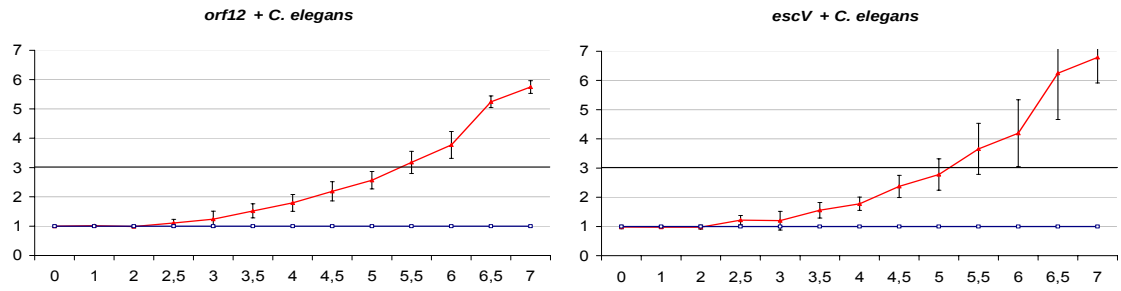


Figure 23: genomic organization of the LEE3 operon and neighbouring LEE-orfs.

3.3.1 LEE3 genes and *C. elegans*

In the *C. elegans* assays, all 100bp promoter regions of genes belonging to the LEE3 operon were activated above threshold. There was no induction of any promoter region in the control assays with spent S-medium which had kept nematodes for 1.5h and was subsequently sterile filtered (data not shown) or with heat-killed nematodes (30min at 40°C).



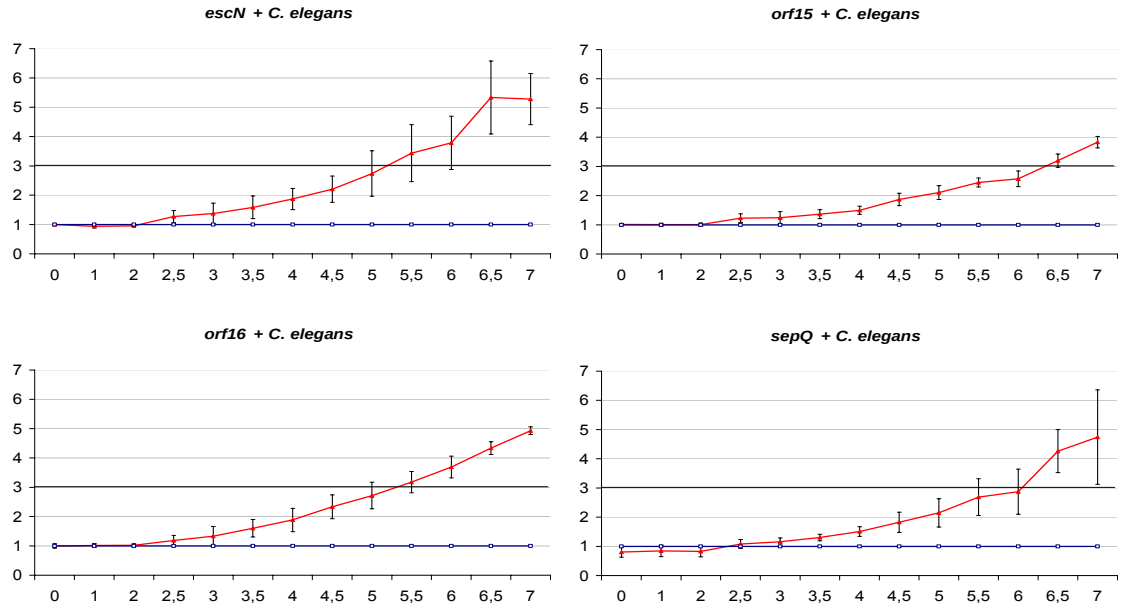
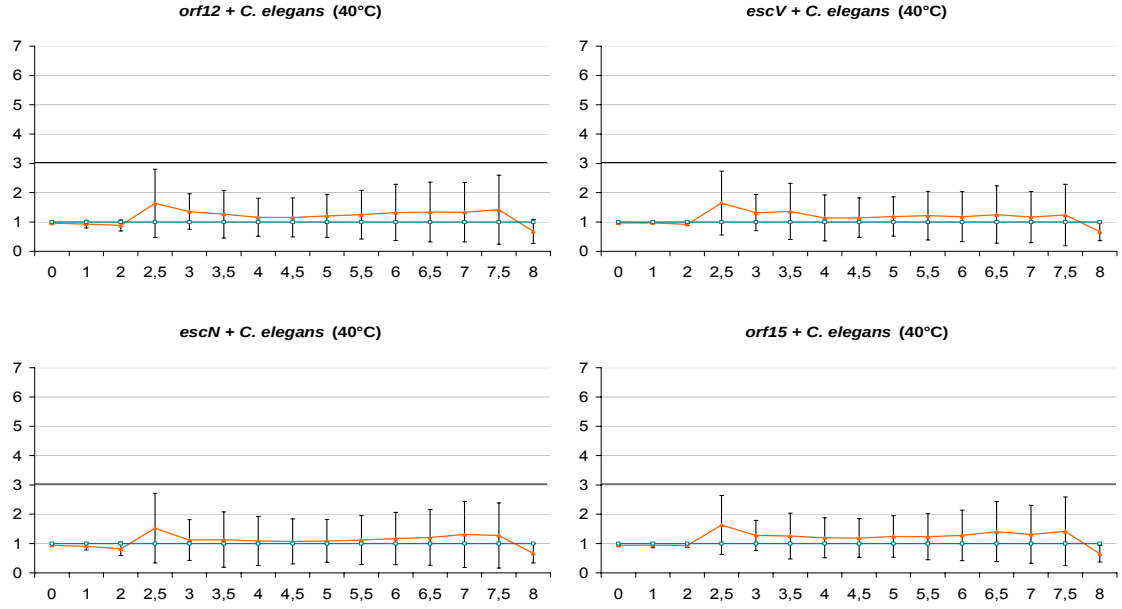


Figure 24: activation of 100bp upstream promoter regions of the genes of the LEE3 operon. *C. elegans* were added at t = 2.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *C. elegans*, which is defined 1 for each time point (blue line). Red lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. Experiments were carried out in triplicate.



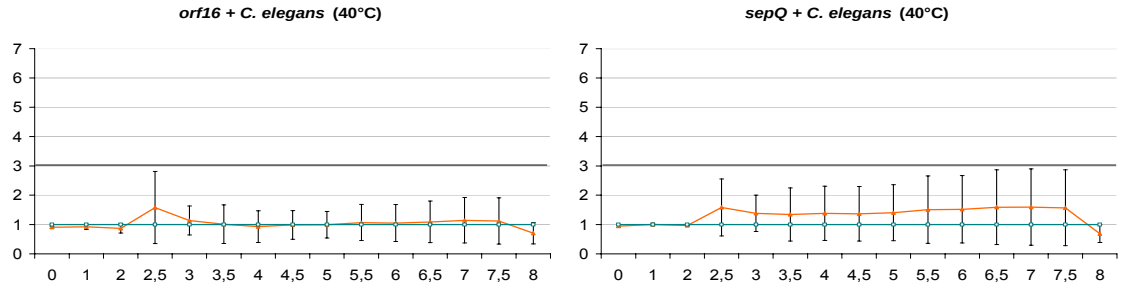
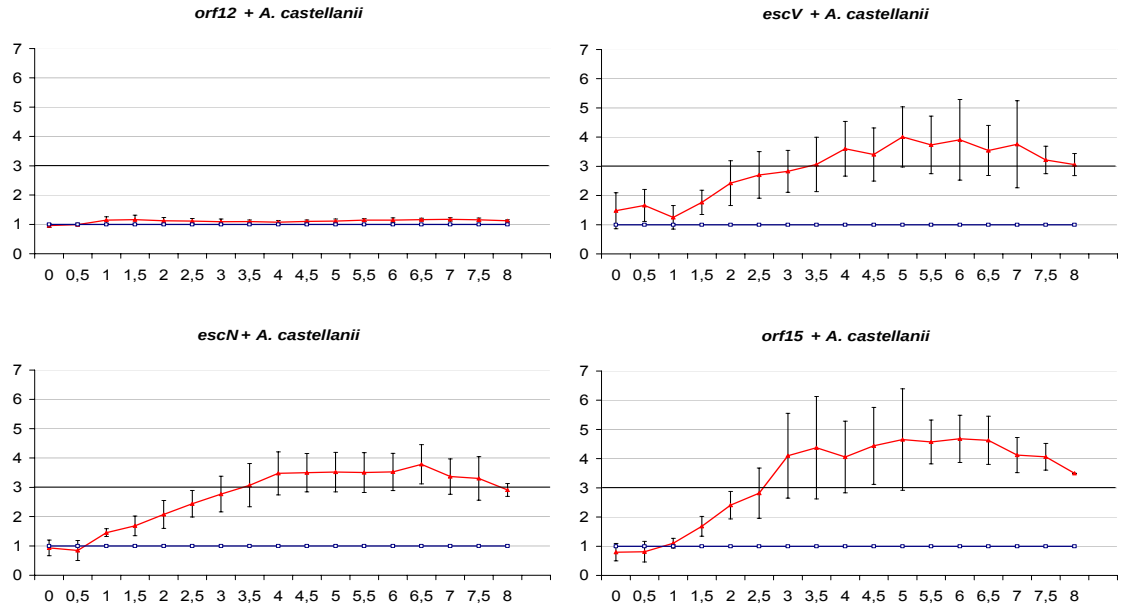


Figure 25: activation of 100bp upstream promoter regions of the genes of the LEE3 operon. Heat killed *C. elegans* (30min at 40°C) were added at t = 2.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *C. elegans*, which is defined 1 for each time point (turquoise line). Orange lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.

3.3.2 LEE3 genes and *A. castellanii*

In contact with the amoeba *A. castellanii*, the very first promoter region of the LEE3 operon, that of *orf12*, which should also activate the transcription of the whole operon, is not affected at all. Surprisingly, all other 100bp upstream regions in this operon except for that of *orf16* are activated more than threefold in comparison to the control without *A. castellanii*. In contact with spent PYG medium, which had kept amoebae for six days and was then sterile filtered, no induction of any promoter region of the LEE3-genes above threshold was observed (data in supplement). In contrast to this, when heat-killed amoebae (30min at 50°C) were submitted to the assay with the LEE3-genes, the activation pattern was the same as that with living amoebae.



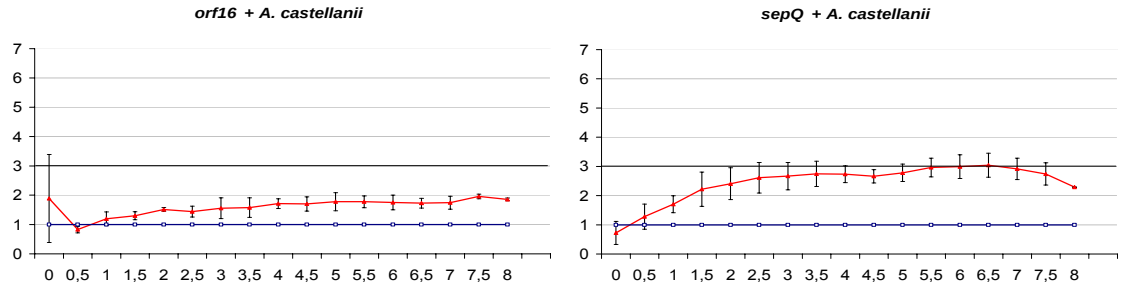


Figure 26: activation of 100bp upstream promoter regions of the genes of the LEE3 operon. *A. castellanii* were added at t = 0.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *A. castellanii*, which is defined 1 for each time point (blue line). Red lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.

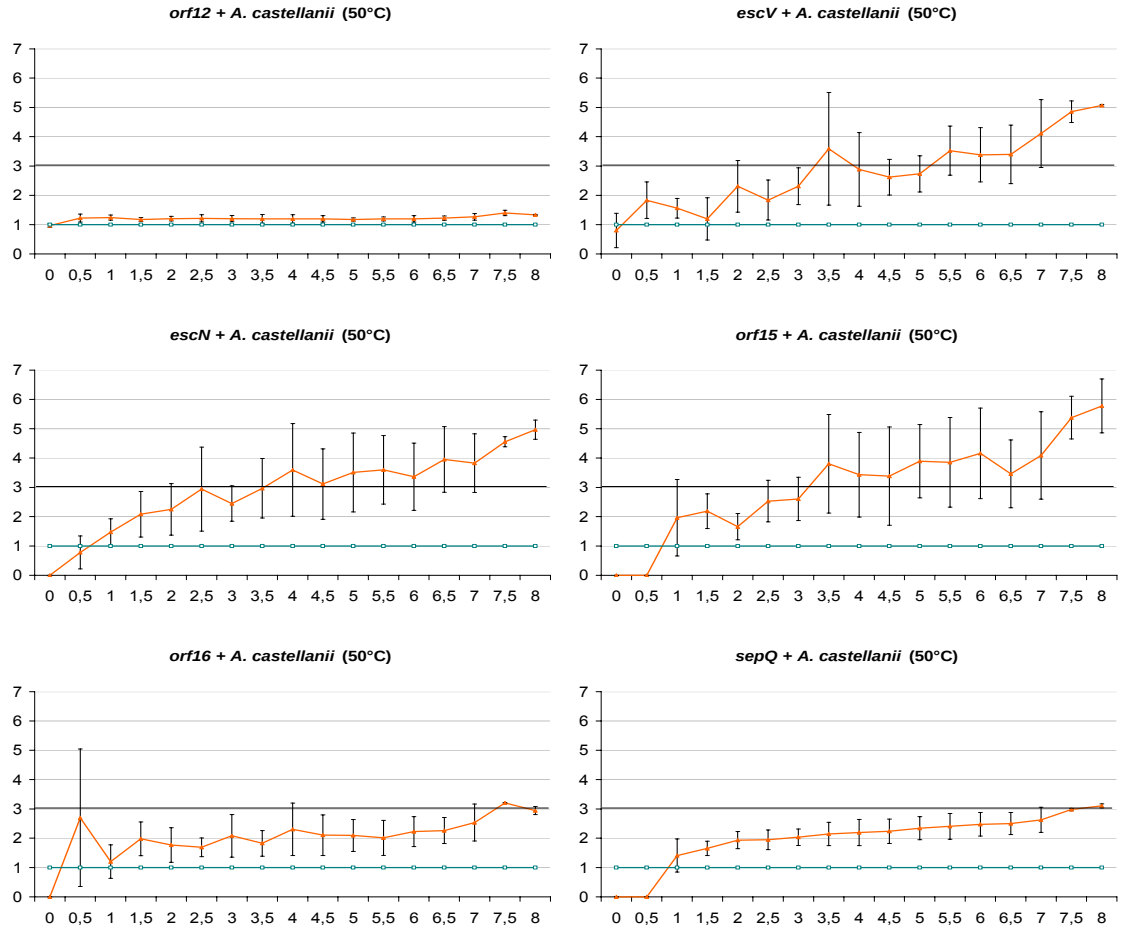


Figure 27: activation of 100bp upstream promoter regions of the genes of the LEE3 operon. Heat killed *A. castellanii* 30min at 50°C) were added at t = 0.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *A. castellanii*, which is defined 1 for each time point (turquoise line). Orange lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control.

3.3.3 LEE3 genes and *A. thaliana*

In contact with *A. thaliana* plants, the promoter region of every gene encoded in the LEE3 operon was activated more than threefold. There was no activation of any LEE3 promoter region with spent MS-medium, which had kept *A. thaliana* for one week and was subsequently sterile filtered.

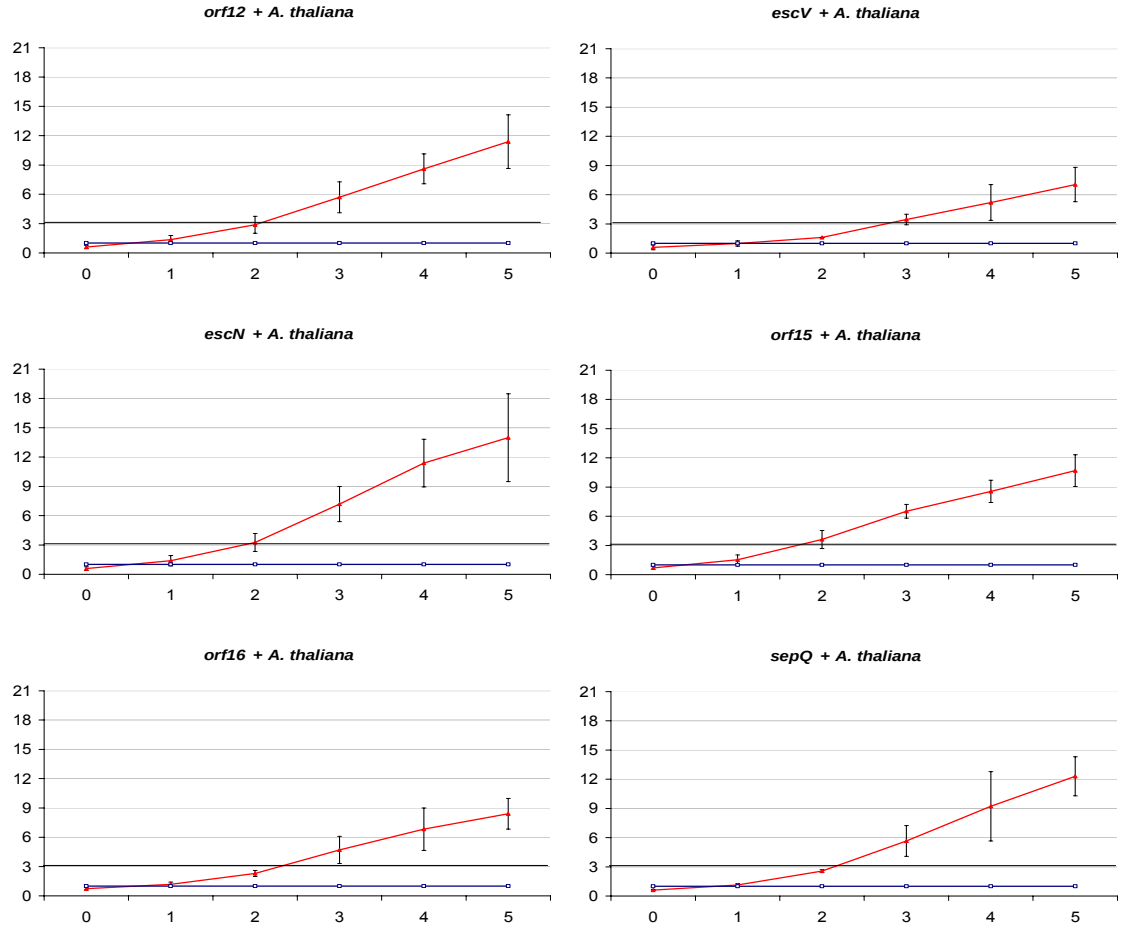


Figure 28: activation of 100bp upstream promoter regions of the genes of the LEE3 operon. *A. thaliana* were added at t = 0h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *A. thaliana*, which is defined 1 for each time point (blue line). Red lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control.

3.4 The LEE4 operon

The LEE4 consists of eight genes (*sepL*, *espA*, *espD*, *espB*, *cesD2*, *escF*, *orf29*, *espF*), the majority of which encode for secreted translocator and effector proteins. First gene of the operon is *sepL*, the second part of the switch controlling the secretion hierarchy between translocators and effectors. This gene has already been shown to be transcribed monocistronically as well. It is unclear, whether *sepL* is a part of the LEE4 operon or if the first gene of the operon is really the following gene, *espA* [Kresse et al. 2000]. The only structural components encoded in the LEE4 operon are the translocators *escF*, *espA*, *espB* and *espD*, which have to be secreted before assembling to form the needle complex of the TTSS. EspD is inserted into the host cell membrane and leads to the formation of bacterial surface appendages [Kresse et al. 1999]. EspF is a secreted protein which directly interacts with the host cell actin filaments to form tight actin pedestals below each EHEC cell and alters the function of the epithelial barrier [Viswanathan et al. 2004]. *cesD2* encodes for another chaperone for translocator proteins while the function of *orf29* is not known yet.

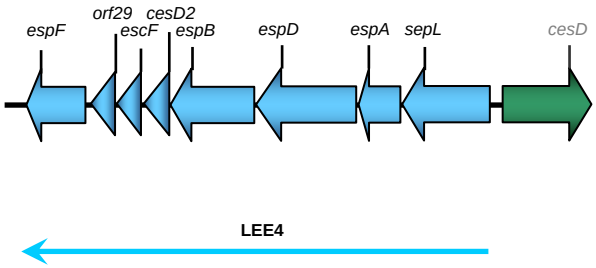


Figure 29: genomic organization of the LEE4 operon and neighbouring LEE-orfs.

3.4.1 LEE4 genes and *C. elegans*

In contact with the nematode *C. elegans*, all promoter regions in the LEE4 operon except for that upstream of *orf29* were activated more than threefold. Remarkably, the activation reached for no gene of this operon levels above 4.5fold, and no gene of this operon hit the threshold earlier than 3.5h after first contact with the nematodes. There was no induction of any promoter region in the control assays with spent S-medium which had kept nematodes for 1.5h and was subsequently sterile filtered (data in supplement) or with heat-killed nematodes (30min at 40°C).

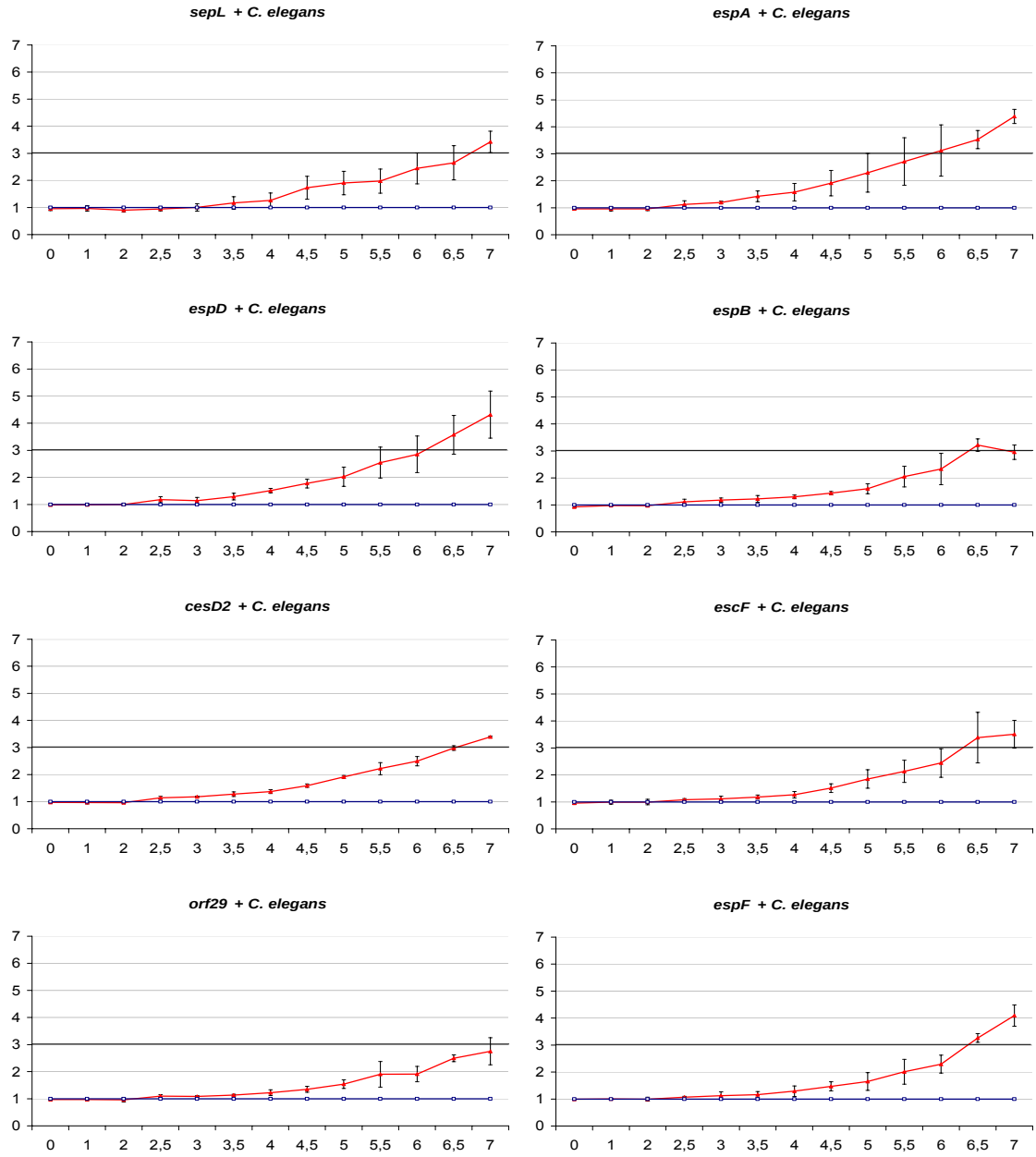
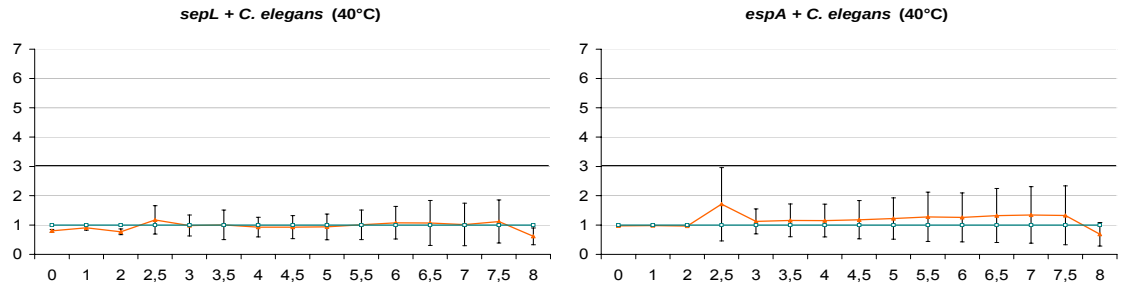


Figure 30: activation of 100bp upstream promoter regions of the genes of the LEE4 operon. *C. elegans* were added at t = 2.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *C. elegans*, which is defined 1 for each time point (blue line). Red lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. Experiments were carried out in triplicate.



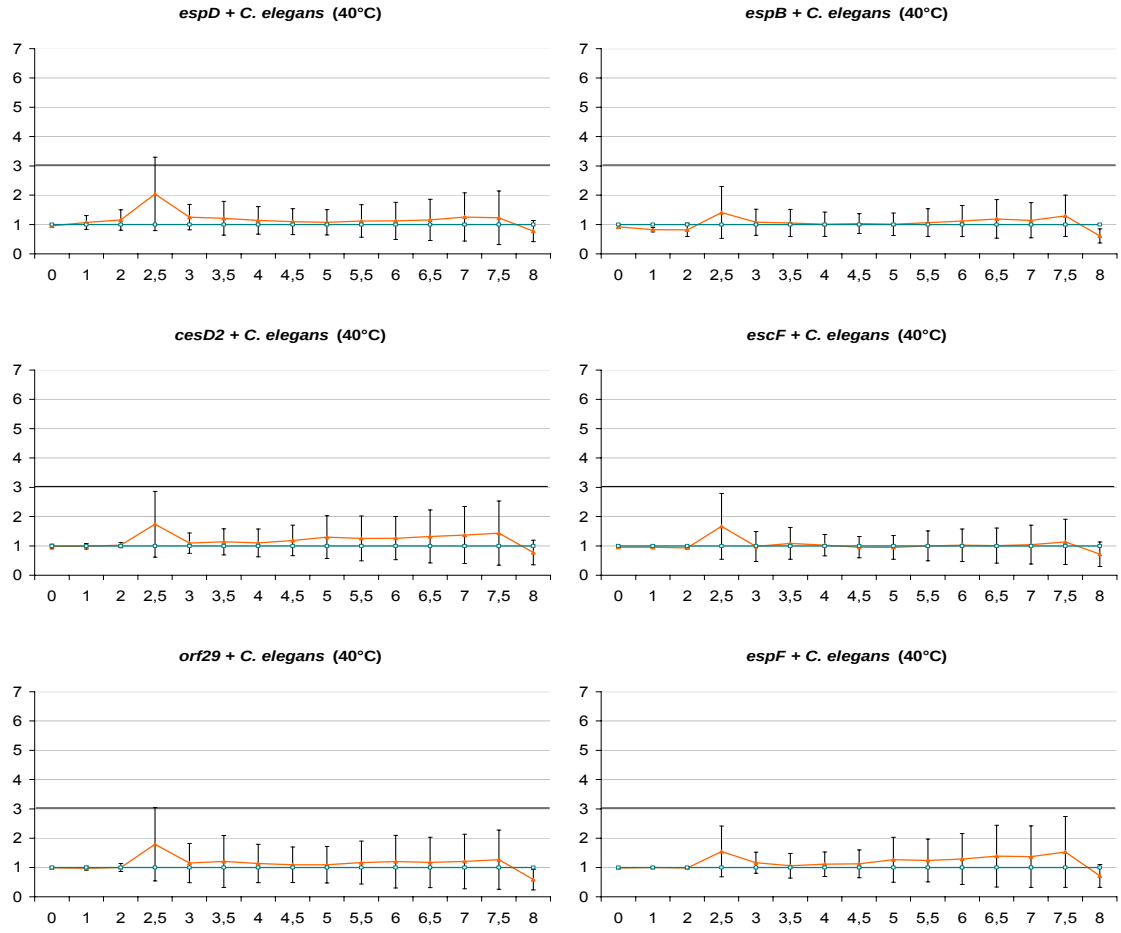


Figure 31: activation of 100bp upstream promoter regions of the genes of the LEE4 operon. Heat killed *C. elegans* (30min at 40°C) were added at t = 2.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *C. elegans*, which is defined 1 for each time point (turquoise line). Orange lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.

3.4.2 LEE4 genes and *A. castellanii*

In contact with *A. castellanii*, the *sepL* 100bp upstream region is not activated. The promoter region of *espA* is activated more than threefold. If the hypothesis that the LEE4 operon starts with *espA* is correct, transcription of the whole operon should be activated, too. If the operon starts with *sepL*, there is be no increased polycistronic transcription of the complete LEE4 operon. The other genes of LEE4 not meeting the threshold in contact with *A. castellanii* are *espD*, *cesD2* and *espF*. In contact with spent PYG medium, which had kept amoebae for six days, no induction of any promoter region of the LEE4-genes above threshold was observed (data in supplement). When heat-killed amoebae (30min at 50°C) were submitted to the assay with the LEE4-genes, the activation pattern was comparable to that with living amoebae. The activation resulting from a contact with heat killed amoebae seemed to be stronger than the activation in contact with living *A. castellanii*. In contrast to this observation, contact with living amoebae in most cases leads to an earlier response than with heat killed *A. castellanii*.

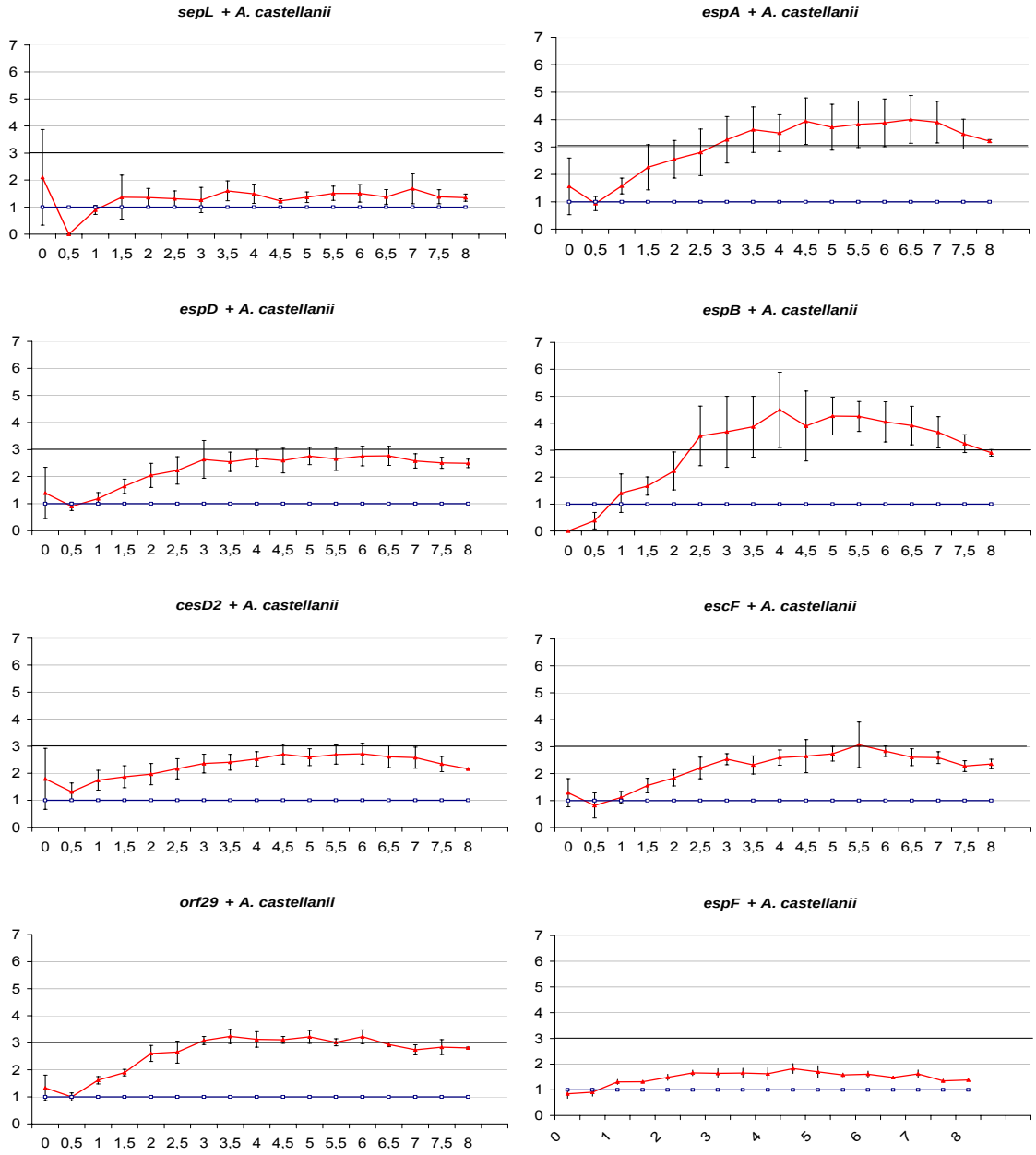


Figure 32: activation of 100bp upstream promoter regions of the genes of the LEE4 operon. *A. castellanii* were added at t = 0.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *A. castellanii*, which is defined 1 for each time point (blue line). Red lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.

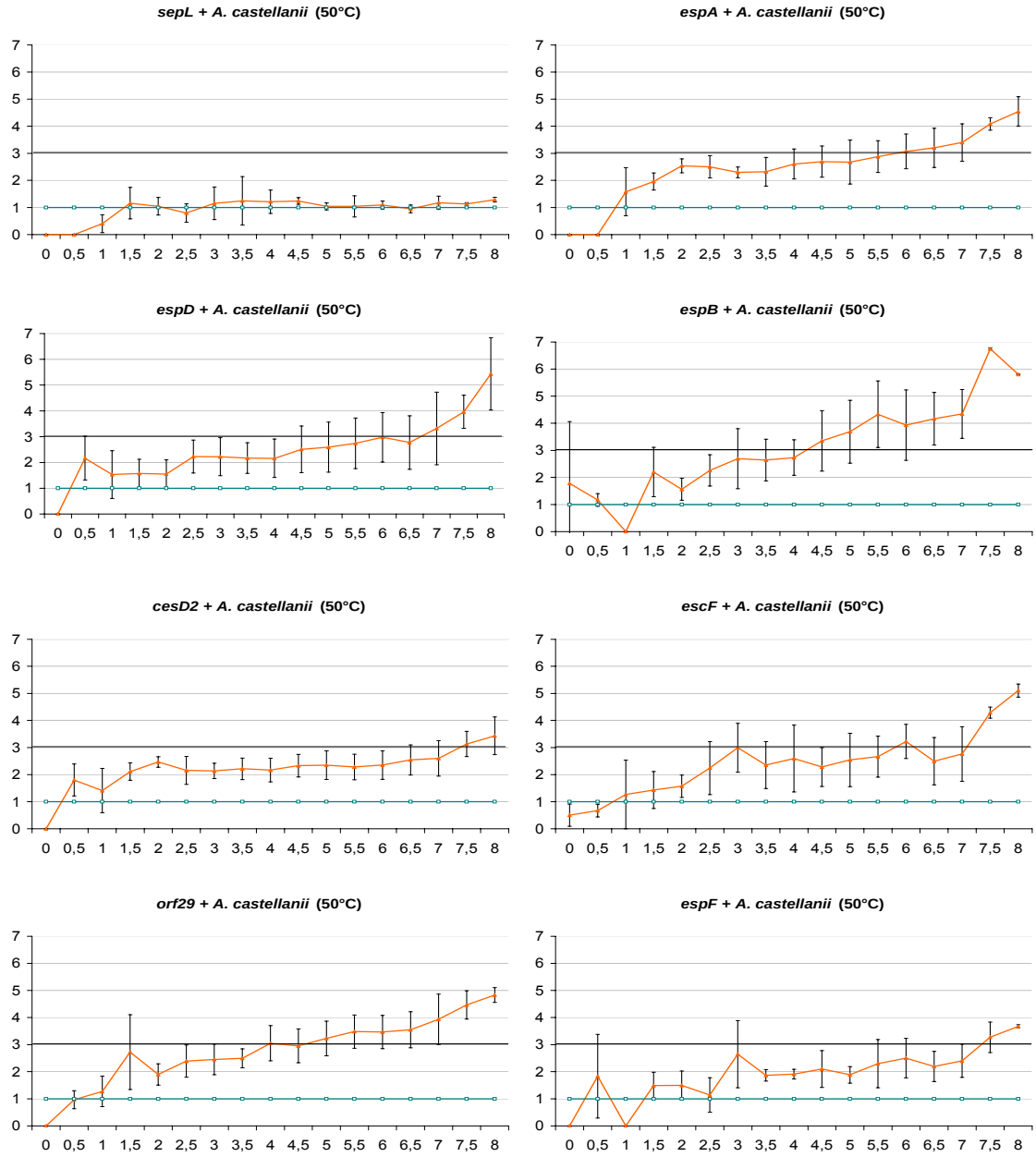


Figure 33: activation of 100bp upstream promoter regions of the genes of the LEE4 operon. Heat killed *A. castellanii* (30min at 50°C) were added at t = 0.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *A. castellanii*, which is defined 1 for each time point (turquoise line). Orange lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. Experiments were carried out in triplicate.

3.4.3 LEE4 genes and *A. thaliana*

In contact with *A. thaliana*, all 100bp upstream regions tested in the LEE4 operon were activated more than threefold, although, in comparison to the other genes of the operon, the activation of *sepL* was remarkably weak and occurred later than the activation of the other LEE4 genes. There was no activation of any LEE4 promoter region with spent MS-medium, which had kept *A. thaliana* for one week and was subsequently sterile filtered.

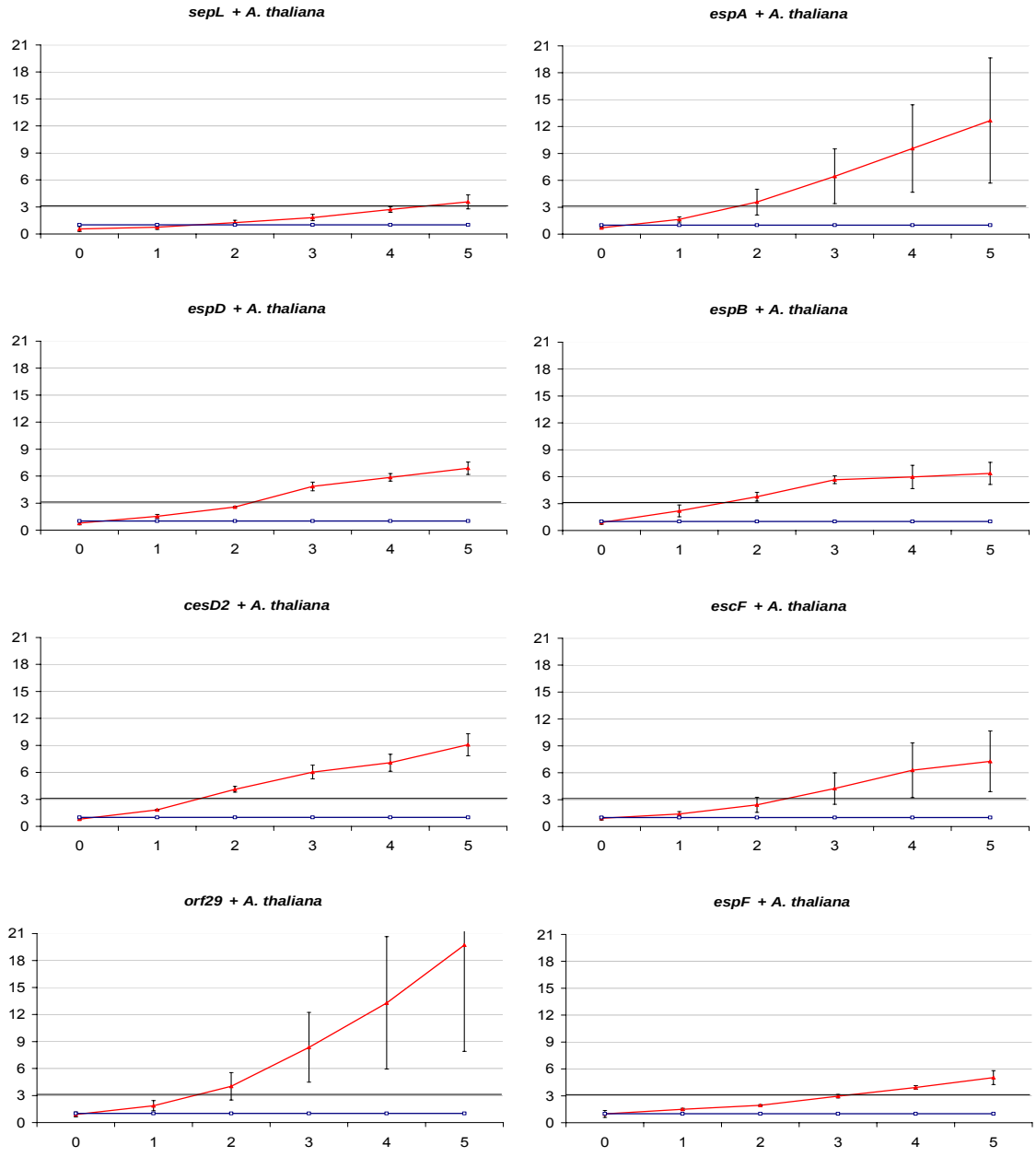


Figure 34: activation of 100bp upstream promoter regions of the genes of the LEE4 operon. *A. thaliana* were added at t = 0h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *A. thaliana*, which is defined 1 for each time point (blue line). Red lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. Experiments were carried out in triplicate.

3.5 The LEE5 operon

The LEE5 operon consists of only three genes: for the translocated intimin receptor *tir*, its chaperone *cesT* and its binding partner in the EHEC outer membrane intimin, the gene for which is named *eae*. The chaperone CesT has been shown to be also the chaperone for the secreted effector protein Map in EPEC [Creasey et al. 2003]. The LEE5 operon is essential for the potential of the EHEC-TTSS as a virulence factor, as the most important proteins needed for the development of an attaching and effacing lesion phenotype are encoded here.

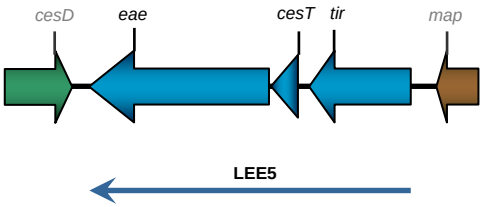


Figure 35: genomic organization of the LEE5 operon and neighbouring LEE-orfs.

3.5.1 LEE5 genes and *C. elegans*

In contact with *C. elegans*, all three genes of the LEE5 operon were activated more than threefold. There was no induction of any promoter region in the control assays with spent S-medium which had kept nematodes for 1.5h and was subsequently sterile filtered (data in supplement) or with heat-killed nematodes (30min at 40°C).

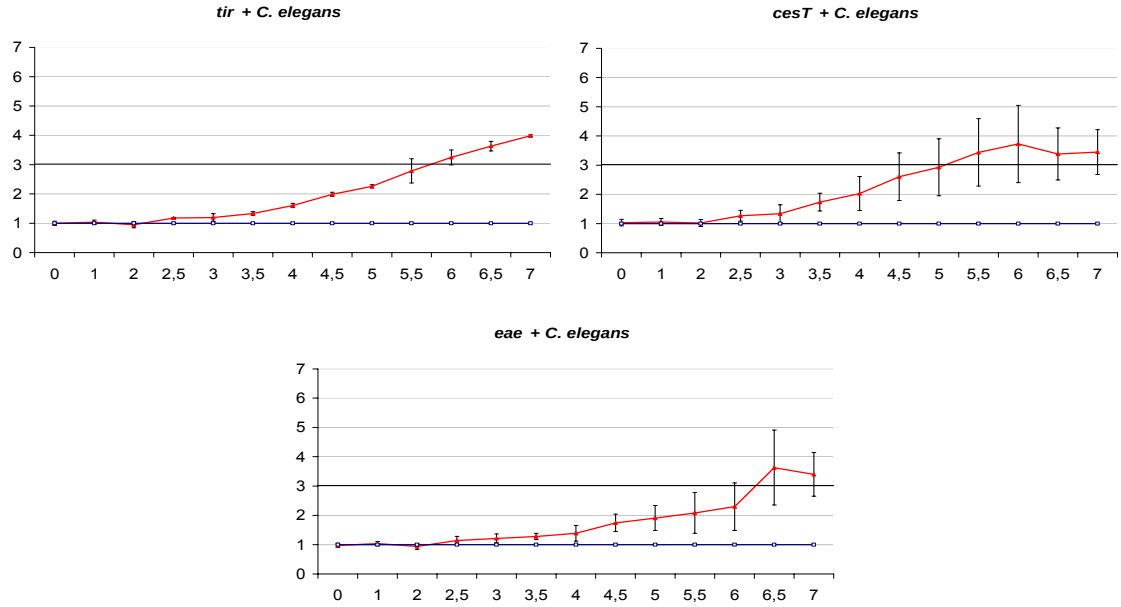


Figure 36: activation of 100bp upstream promoter regions of the genes of the LEE4 operon. *C. elegans* were added at t = 2.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *C. elegans*, which is defined 1 for each time point (blue line). Red lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.

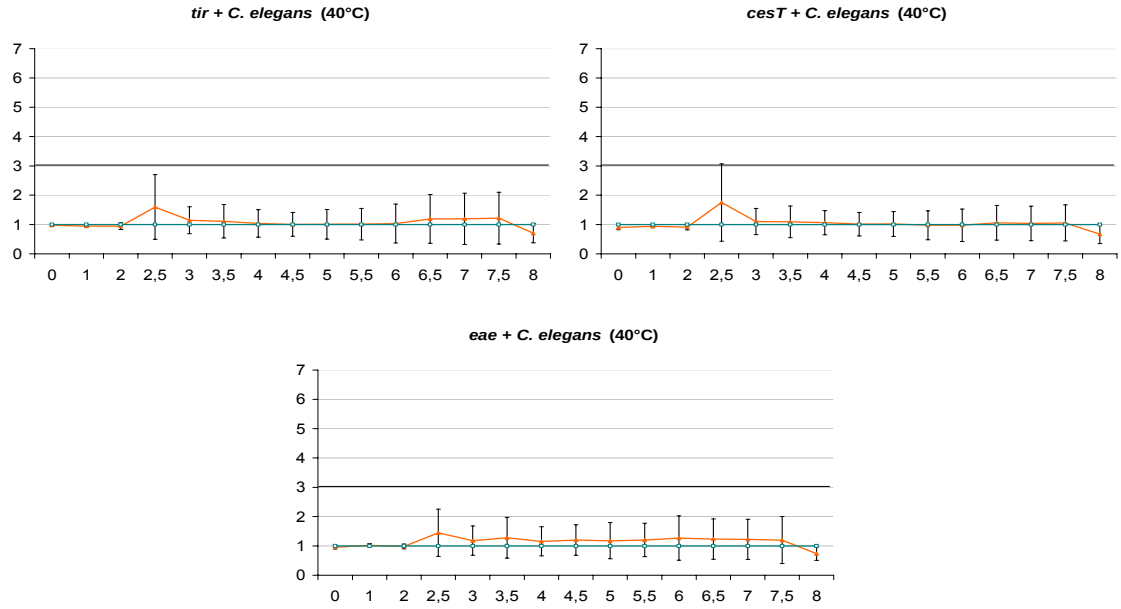
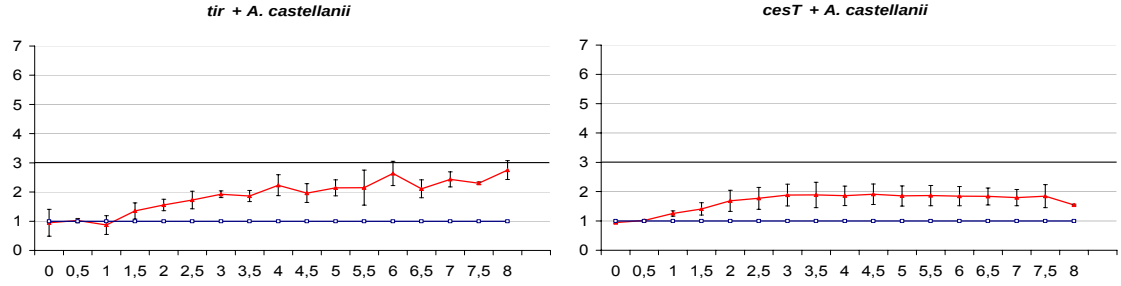


Figure 37: activation of 100bp upstream promoter regions of the genes of the LEE4 operon. Heat killed *C. elegans* (30min at 40°C) were added at t = 2.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *C. elegans*, which is defined 1 for each time point (turquoise line). Orange lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to the control line. All experiments were carried out in triplicate.

3.5.2 LEE5 genes and *A. castellanii*

In contact with *A. castellanii*, the only gene encoded in the LEE5 operon that met the threshold was *eae*, the gene encoding for the secreted intimin adherence protein. In contact with spent PYG medium, which had kept amoebae for six days and was then sterile filtered, no induction of any promoter region of the LEE5-genes above threshold was observed (data in supplement). In contrast to this, when heat-killed amoebae (30min at 50°C) were submitted to the assay with the LEE5-genes, the activation pattern was similar to that with living amoebae. With heat-killed amoebae, also the 100bp upstream region of *tir* was activated slightly above threshold.



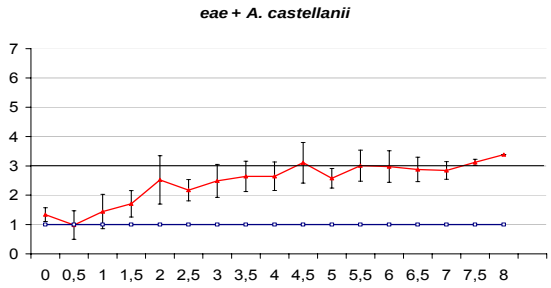


Figure 38: activation of 100bp upstream promoter regions of the genes of the LEE4 operon. *A. castellanii* were added at t = 0.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *A. castellanii*, which is defined 1 for each time point (blue line). Red lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. Experiments were carried out in triplicate.

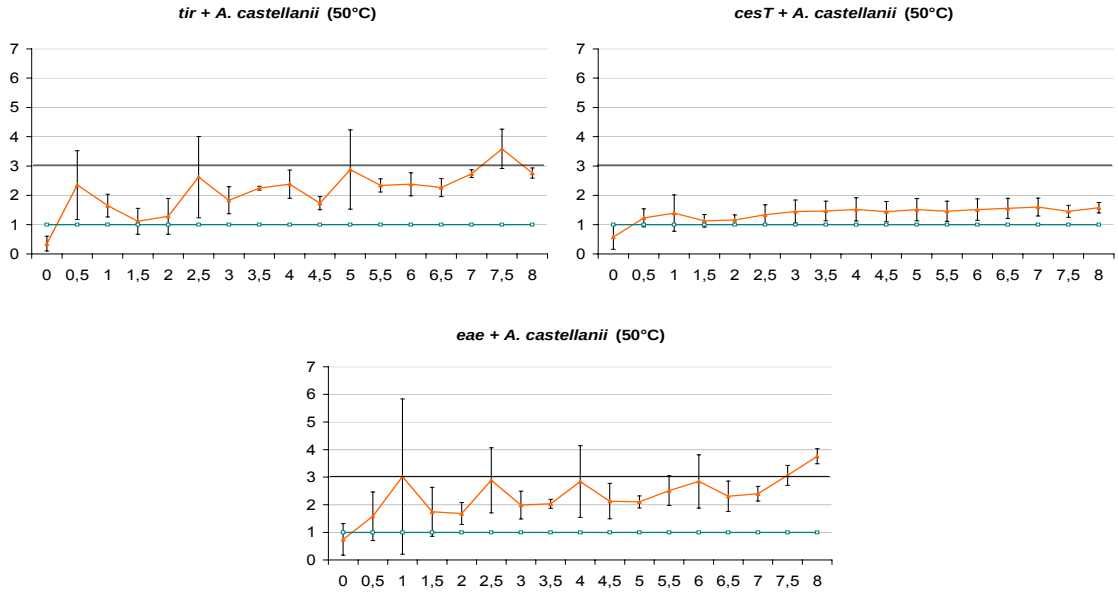
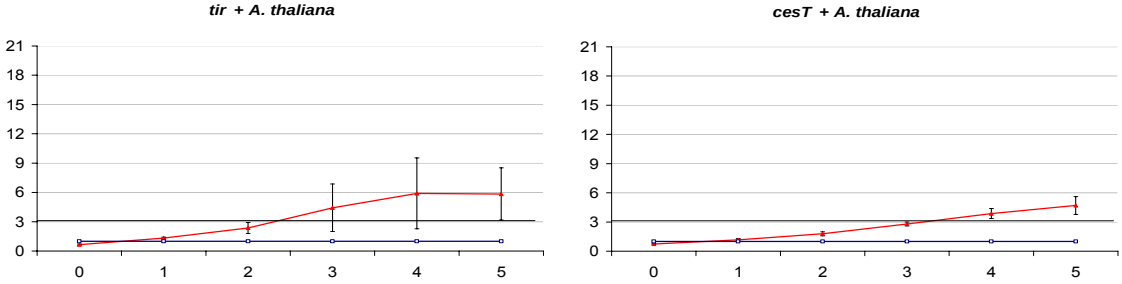


Figure 39: activation of 100bp upstream promoter regions of the genes of the LEE4 operon. Heat killed *A. castellanii* (30min at 50°C) were added at t = 0.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *A. castellanii*, which is defined 1 for each time point (turquoise line). Orange lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. Experiments were carried out in triplicate.

3.5.3 LEE5 genes and *A. thaliana*

In the *A. thaliana* assays, all genes of the LEE5 operon were activated above threshold. There was no activation of any LEE5 promoter region with spent MS-medium, which had kept *A. thaliana* for one week and was subsequently sterile filtered.



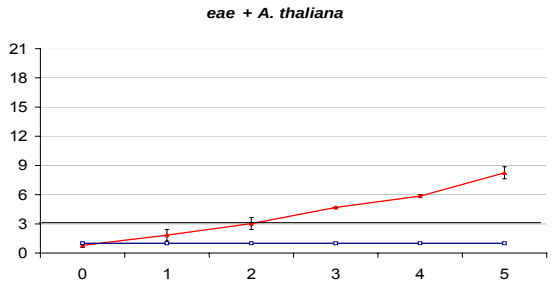


Figure 40: activation of 100bp upstream promoter regions of the genes of the LEE4 operon. *A. thaliana* were added at t = 0h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *A. thaliana*, which is defined 1 for each time point (blue line). Red lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.

3.6 The espG operon

The espG operon is a very small operon consisting of only two genes: *espG*, which encodes for a secreted effector protein, and the *rorf1* gene product, which has been proposed to play a role in quorum sensing regulation of the LEE [Pallen et al. 2005].

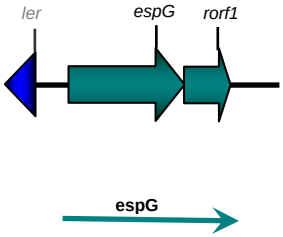


Figure 41: genomic organization of the espG operon and neighbouring LEE-orfs.

3.6.1 espG genes and *C. elegans*

In contact with *C. elegans*, both genes encoded in the espG operon were activated more than threefold in comparison to the control without nematodes. There was no induction of any promoter region in the control assays with spent S-medium which had kept nematodes for 1.5h and was subsequently sterile filtered (data in supplement) or with heat-killed nematodes (30min at 40°C).

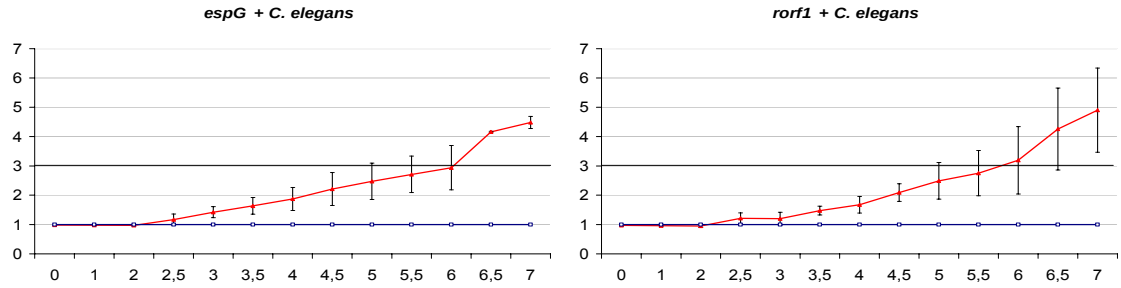


Figure 42: activation of 100bp upstream promoter regions of the genes of the espG operon. *C. elegans* were added at t = 2.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *C. elegans*, which is defined 1 for each time point (blue line). Red lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. Experiments were carried out in triplicate.

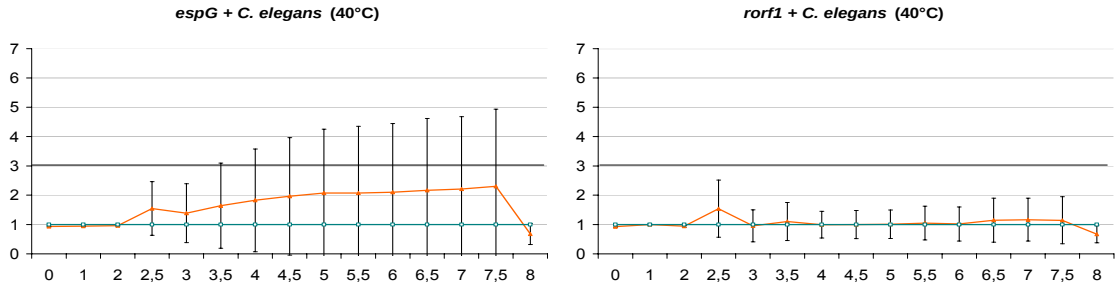


Figure 43: activation of 100bp upstream promoter regions of the genes of the espG operon. Heat killed *C. elegans* (30min at 40°C) were added at t = 2.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *C. elegans*, which is defined 1 for each time point (turqoise line). Orange lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. Experiments were carried out in triplicate.

3.6.2 espG genes and *A. castellanii*

In contact with the amoeba *A. castellanii*, none of the 100bp promoter regions of the espG operon was activated as much as threefold compared to the control. In contact with spent PYG medium, which had kept amoebae for six days and was then sterile filtered, no induction of any promoter region of the espG-genes above threshold was observed (data in supplement). When heat-killed amoebae (30min at 50°C) were submitted to the assay with the espG-genes, there was also no activation above threshold of any gene of this operon.

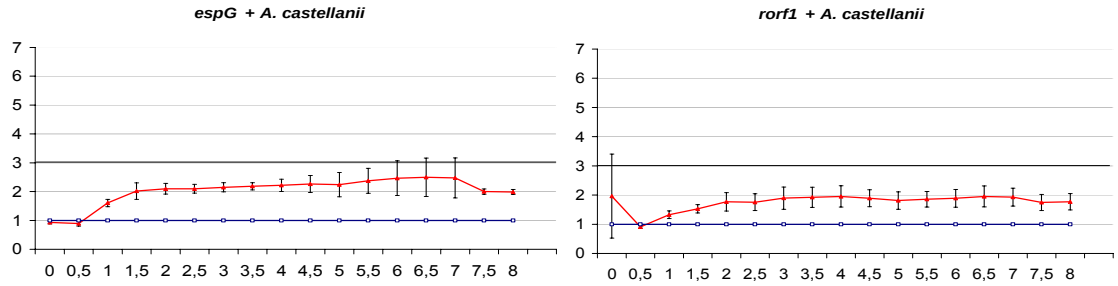


Figure 44: activation of 100bp upstream promoter regions of the genes of the espG operon. *A. castellanii* were added at t = 0.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *A. castellanii*, which is defined 1 for each time point (blue line). Red lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. Experiments were carried out in triplicate.

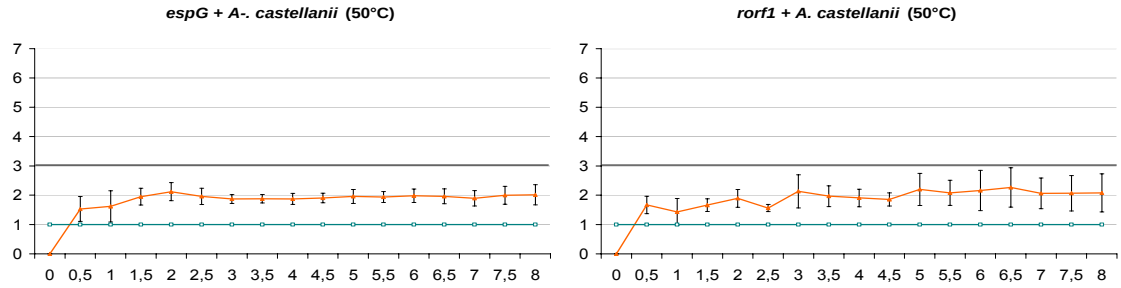


Figure 45: activation of 100bp upstream promoter regions of the genes of the espG operon. Heat killed *A. castellanii* (30min at 50°C) were added at t = 0.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *A. castellanii*, which is defined 1 for each time point (turquoise line). Orange lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. Experiments were carried out in triplicate.

3.6.3 espG genes and *A. thaliana*

Both genes of the espG operon were activated more than threefold in contact with *A. thaliana*. There was no activation of any espG promoter region with spent MS-medium, which had kept *A. thaliana* for one week and was subsequently sterile filtered.

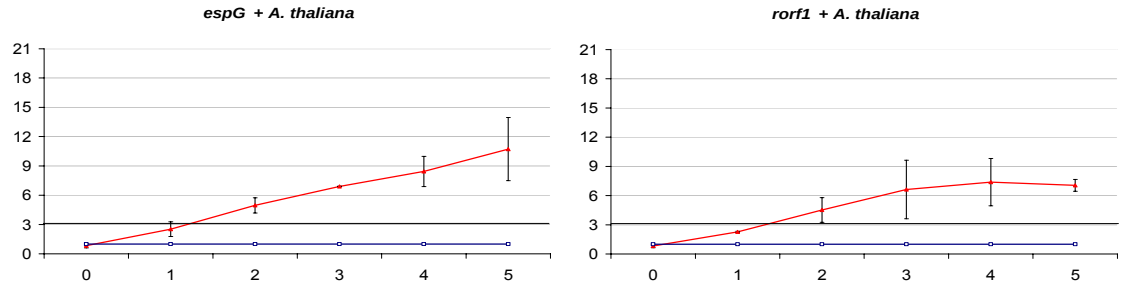


Figure 46: activation of 100bp upstream promoter regions of the genes of the espG operon. *A. thaliana* were added at t = 0h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *A. thaliana*, which is defined 1 for each time point (blue line). Red lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.

3.7 *escD* and other monocistronically transcribed LEE genes

There are in total six genes in the locus of enterocyte effacement that are transcribed monocistronically: *escD*, which is necessary for the transport of secreted components from the bacterial inner membrane to the outer membrane, *map*, encoding for a secreted effector protein, *cesF*, encoding for a chaperone, the antagonistic *grlA* and *grlR*, encoding for a global LEE-activator and a global LEE-repressor which are additional LEE-regulators to Ler, and *rorf3*, the exact function of which could not be determined yet. Rorf3 has been shown to interact with Rorf8 and has been proposed to be secreted by the general secretion pathway to disrupt the peptidoglycan layer in the periplasm in EPEC [Creasey 2003].

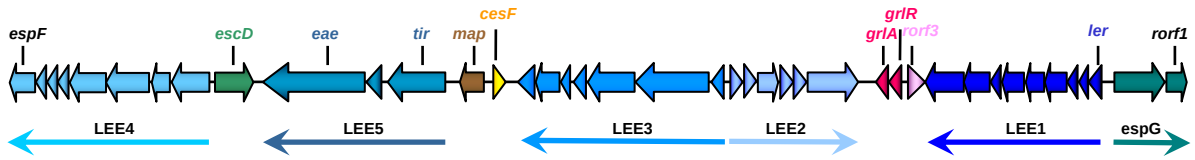
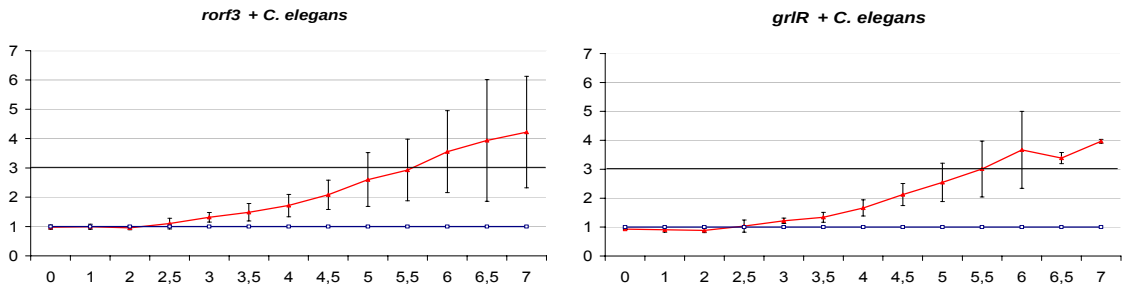


Figure 47: genomic organization of the EHEC-LEE. All genes belonging to the same operon have corresponding colors. *grlA* and *grlR* do not constitute an operon, but encode for antagonistic regulators of LEE gene expression.

3.7.1 Monocistronically transcribed LEE genes and *C. elegans*

All monocistronically transcribed LEE-genes were activated in contact with *C. elegans*. Most of those genes showed not much more than three-fold activation compared to the control assays, however. One exception to this was the *map* gene, encoding for a secreted effector protein, which was five times as active as the control without *C. elegans*. There was no induction of any promoter region in the control assays with spent S-medium which had kept nematodes for 1.5h and was subsequently sterile filtered (data in supplement) or with heat-killed nematodes (30min at 40°C).



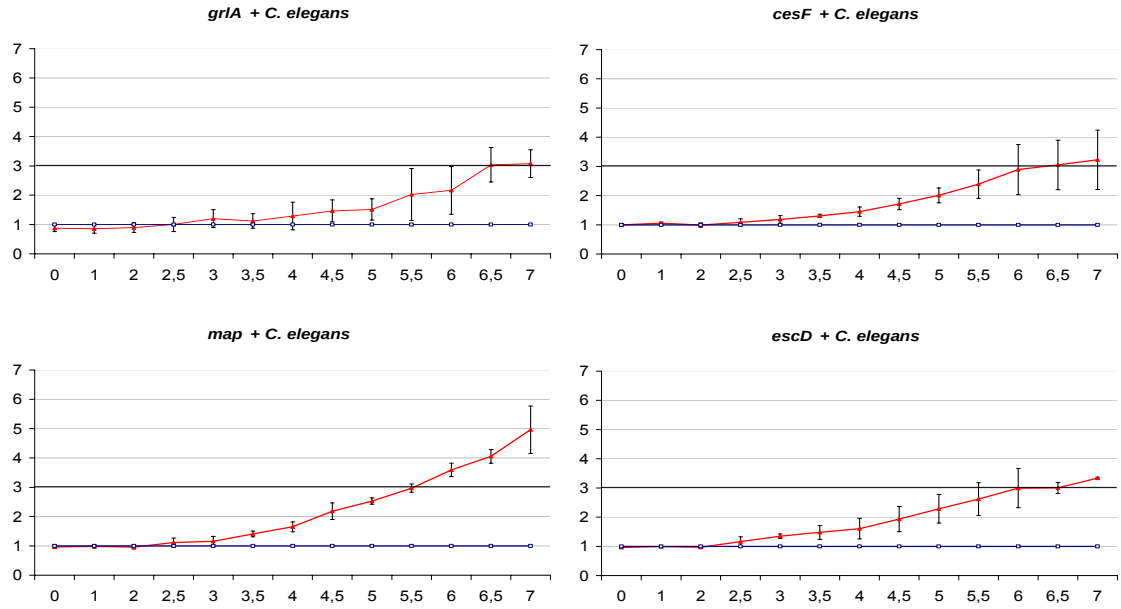
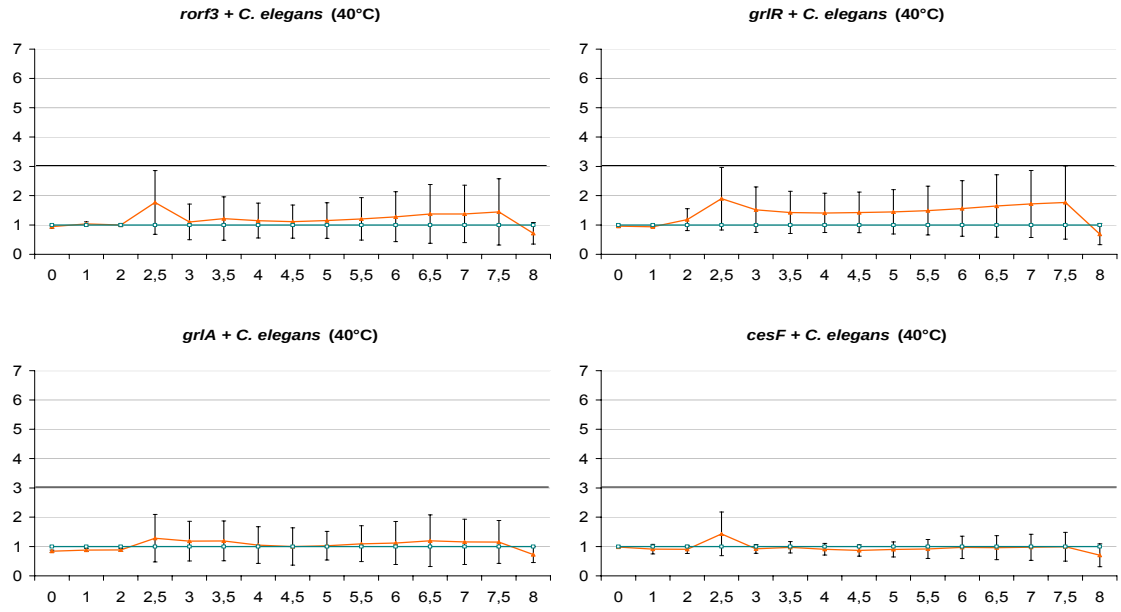


Figure 48: activation of 100bp upstream promoter regions of the genes of the monocistronically transcribed LEE-genes. *C. elegans* were added at t = 2.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *C. elegans*, which is defined 1 for each time point (blue line). Red lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.



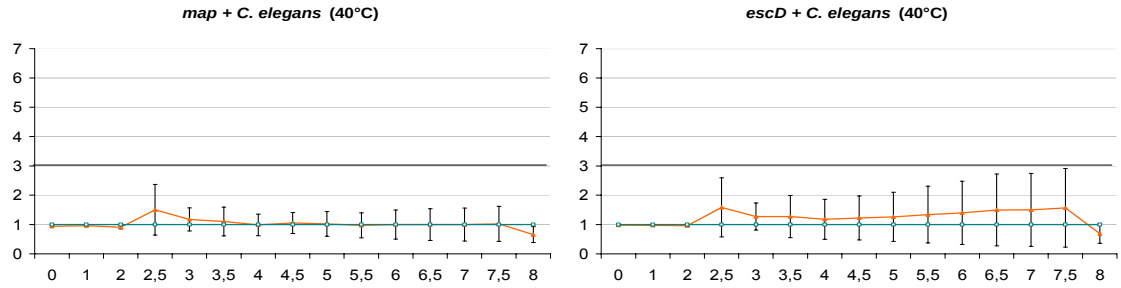
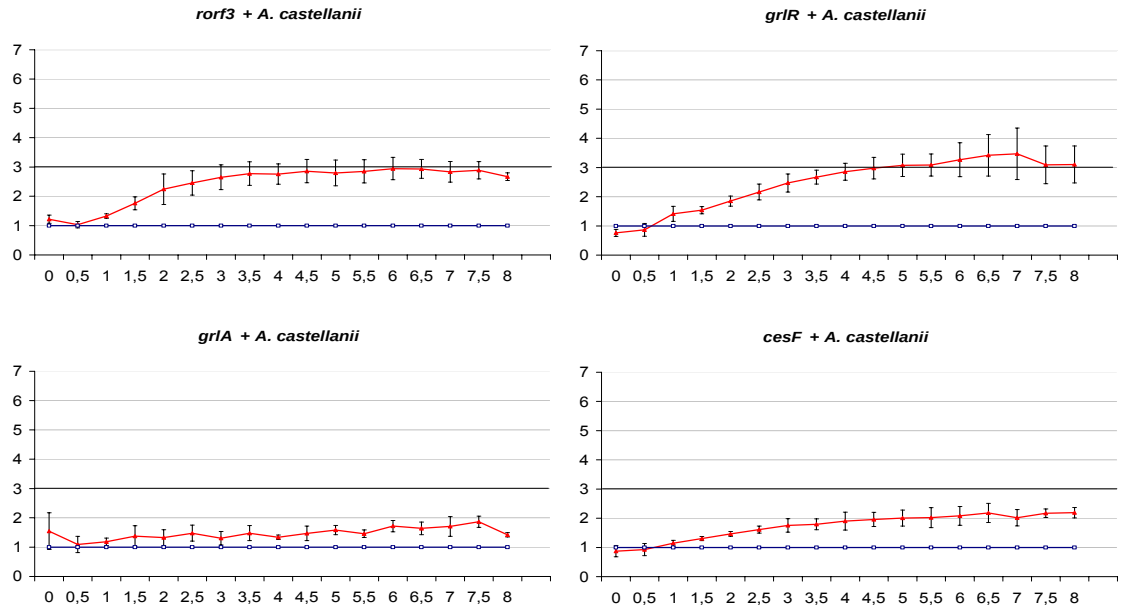


Figure 49: activation of 100bp upstream promoter regions of the genes of the monocistronically transcribed LEE-genes. Heat killed *C. elegans* (30min at 40°C) were added at t = 2.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *C. elegans*, which is defined 1 for each time point (turquoise line). Orange lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. Experiments were carried out in triplicate.

3.7.2 Monocistronically transcribed LEE genes and *A. castellanii*

Only two monocistronically transcribed genes hit the activation threshold in the *A. castellanii* assays: *rorf3* and *grlR*. Both were activated only little more than threefold, however. In contact with spent PYG medium, which had kept amoebae for six days and was then sterile filtered, no induction of any promoter region of the monocistronically transcribed genes above threshold was observed (data in supplement). When heat-killed amoebae (30min at 50°C) were submitted to the assay with the monocistronically transcribed genes, the activation pattern was almost the same as that with living amoebae, with the exception of the *escD* promoter region which was upregulated above threshold only in contact with heat-killed amoebae.



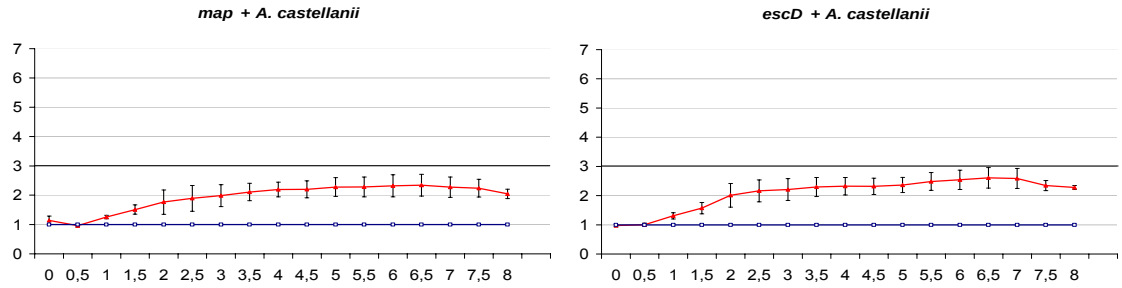


Figure 50: activation of 100bp upstream promoter regions of the genes of the monocistronically transcribed LEE-genes. *A. castellanii* were added at t = 0.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *A. castellanii*, which is defined 1 for each time point (blue line). Red lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.

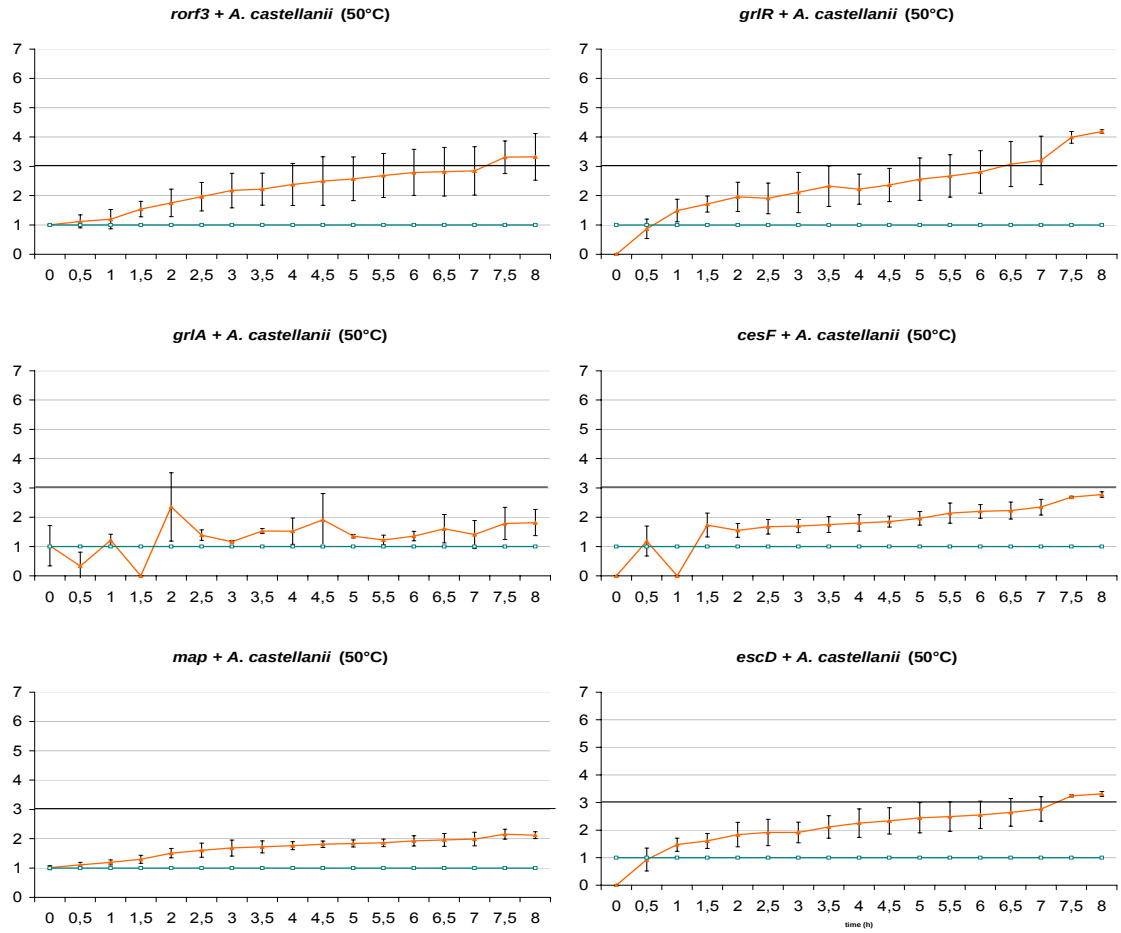


Figure 51: activation of 100bp upstream promoter regions of the genes of the monocistronically transcribed LEE-genes. Heat killed *A. castellanii* (30min at 50°C) were added at t = 0.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *A. castellanii*, which is defined 1 for each time point (turquoise line). Orange lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. Experiments were carried out in triplicate.

3.7.3 Monocistronically transcribed LEE genes and *A. thaliana*

The 100bp upstream regions of all monocistronically transcribed LEE-genes were activated in EHEC in contact with the thale cress *A. thaliana*. There was no activation of any monocistronically transcribed promoter region in the LEE-PAI with spent MS-medium, which had kept *A. thaliana* for one week and was subsequently sterile filtered.

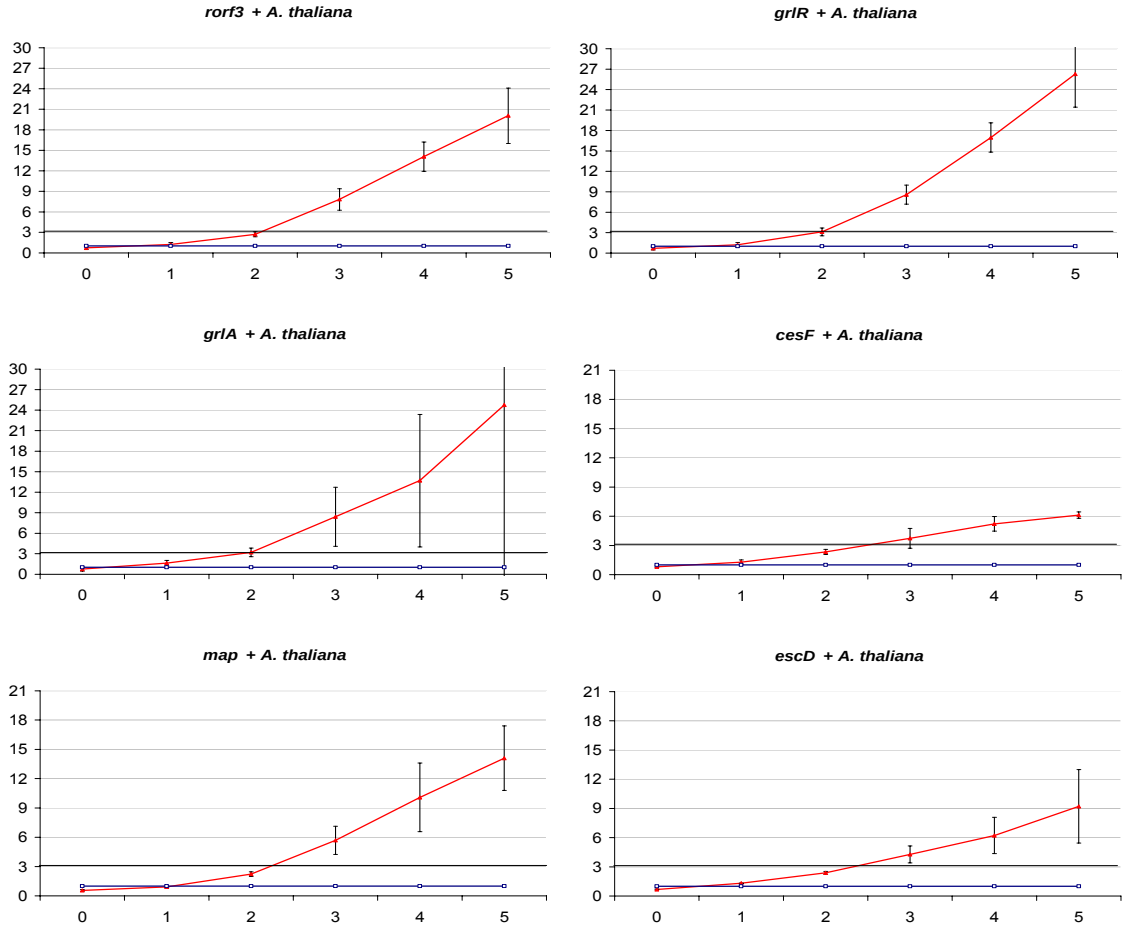


Figure 52: activation of 100bp upstream promoter regions of the genes of the monocistronically transcribed LEE-genes. *A. thaliana* were added at t = 0h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *A. thaliana*, which is defined 1 for each time point (blue line). Red lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.

3.8 Non-LEE genes

Several genes of EHEC that are not encoded on the LEE PAI were also submitted to the promoter-*lux* fusion assays in contact with *C. elegans*, *A. castellanii* and *A. thaliana*. For those genes, the promoter regions were defined as the 500bp upstream regions of the start codon. The genes chosen are all of regulatory or virulence associated importance:

gene number	gene name	gene function
z0012	-	positive regulator for σ-32 heat shock promoters
z0034	*lytB*	control of stringent response; penicillin tolerance
z0301	*crl*	transcriptional regulator for cryptic csgA gene for curli fibers
z0539	*bolA*	putative regulator of murein genes
z0573	*hha*	haemolysin expression modulating protein
z1078	*mdaA*	modulator of drug activity A
z1464	*stx2A*	*A* subunit of Shiga toxin II (on prophage BP-933W)
z1944	*hlyE*	putative pore forming haemolysin
z2030	*tonB*	sensitivity to phages, iron uptake, energy transducer
z2170	*marA*	multiple antibiotic resistance, activator of defense systems
z2433	*fnr*	transcriptional regulator of osmotic balance& respiration
z2745	*rplT*	50S ribosomal subunit protein L20, regulatory functions
z2946	*flhD*	regulator of flagella biosynthesis
z3012	*fliA*	regulator of fagellar operons, alternative σ-factor 23
z3013	*fliC*	flagella biosynthesis, filament structure protein flagellin
z3276	-	putative fimbrial protein
z3277	*yehB*	putative outer membrane protein
z3279	*yehD*	putative fimbrial-like protein
z3343	*stx1B*	*B* subunit of Shiga toxin I (on prophage CP-933V)
z4326	-	putative enterotoxin
z4377	*qseB*	Quorum Sensing *Escherichia coli* regulator B
z4378	*qseC*	Quorum Sensing *Escherichia coli* regulator C
z4379	*mdaB*	modulator of drug activity B
z4982	-	putative membrane permeability altering protein
z5358	*tatA*	twin arginine translocation, *sec*-independent protein export

Table 3: list of non-LEE genes submitted to study and functions of the encoded proteins. Red: virulence associated functions; turquoise: membrane-associated, fimbria, flagella; yellow: regulatory functions

3.8.1 Virulence-associated non-LEE genes and *C. elegans*

There was no induction of any promoter region. In the control assays with spent S-medium which had kept nematodes for 1.5h and was subsequently sterile filtered (data in supplement) or with heat-killed nematodes (30min at 40°C), there was also no activation of any tested promoter region. The promoter region of *hlyE* even seems to be repressed under the tested conditions.

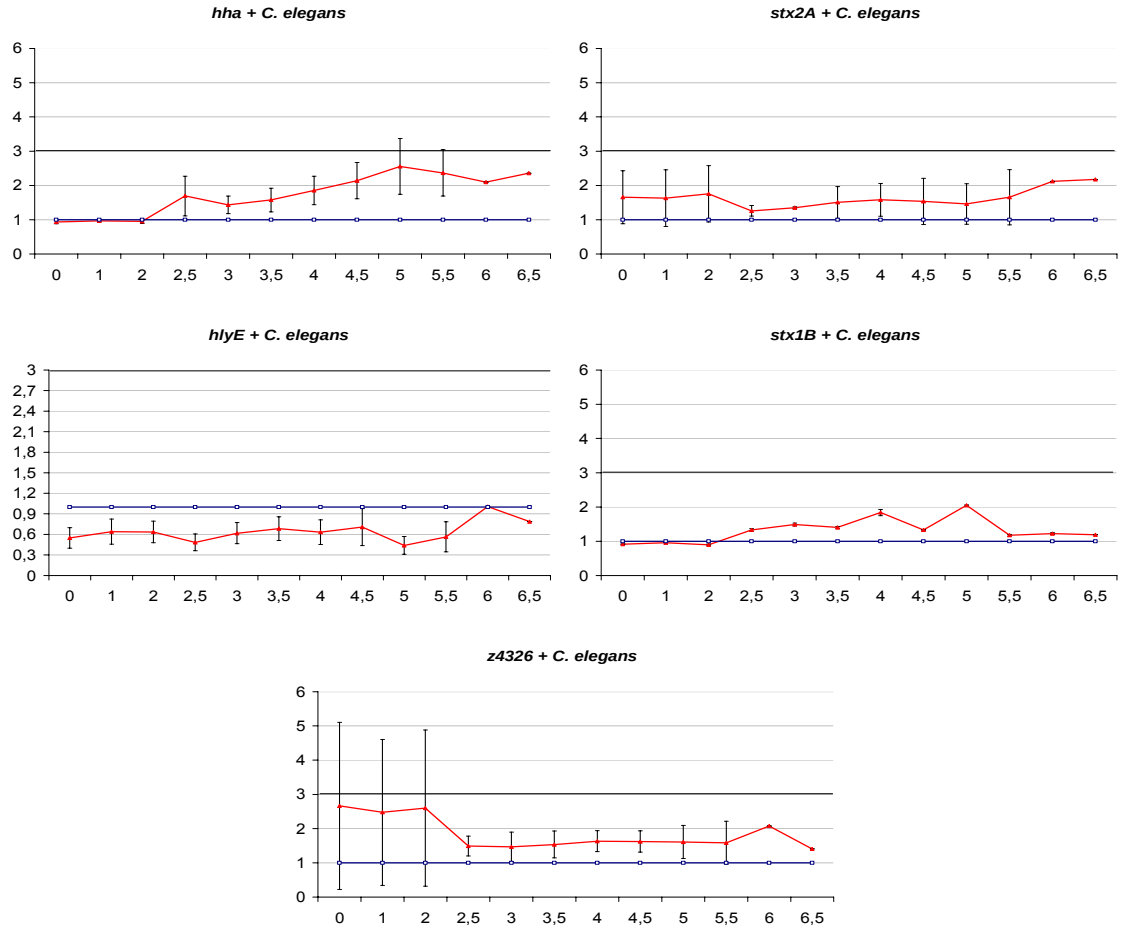
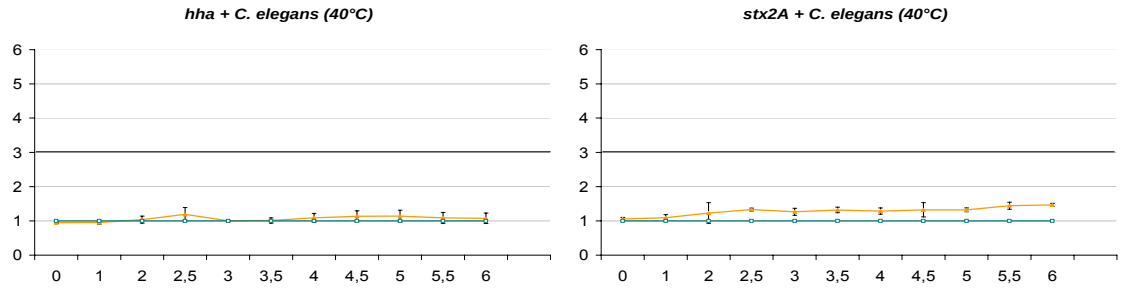


Figure 53: activation of 100bp upstream promoter regions of virulence associated genes. *C. elegans* were added at t = 2.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *C. elegans*, which is defined 1 for each time point (blue line). Red lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.



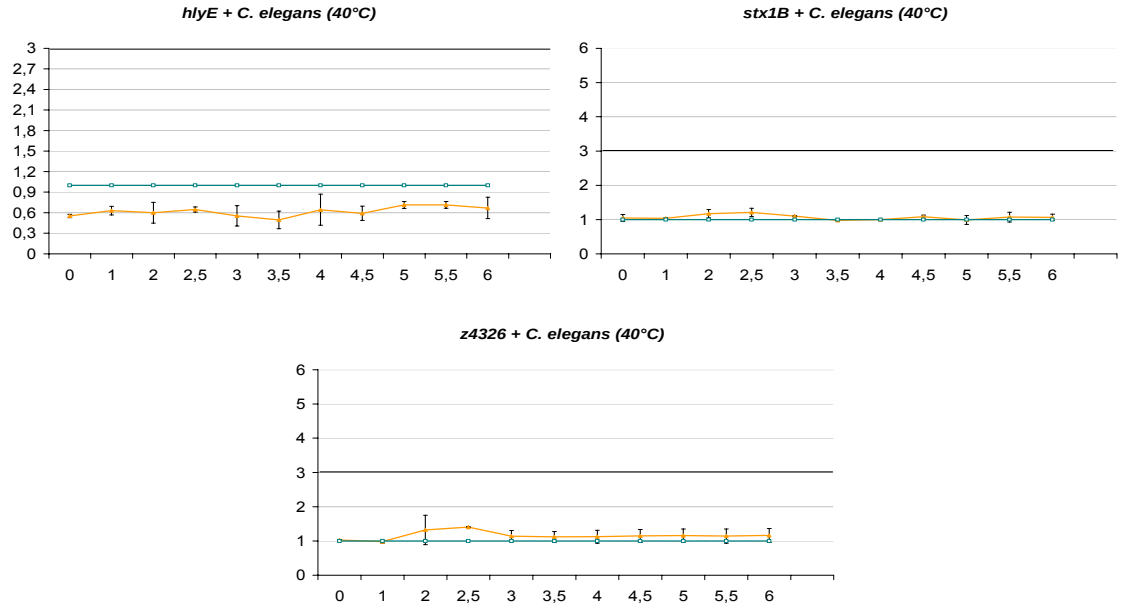
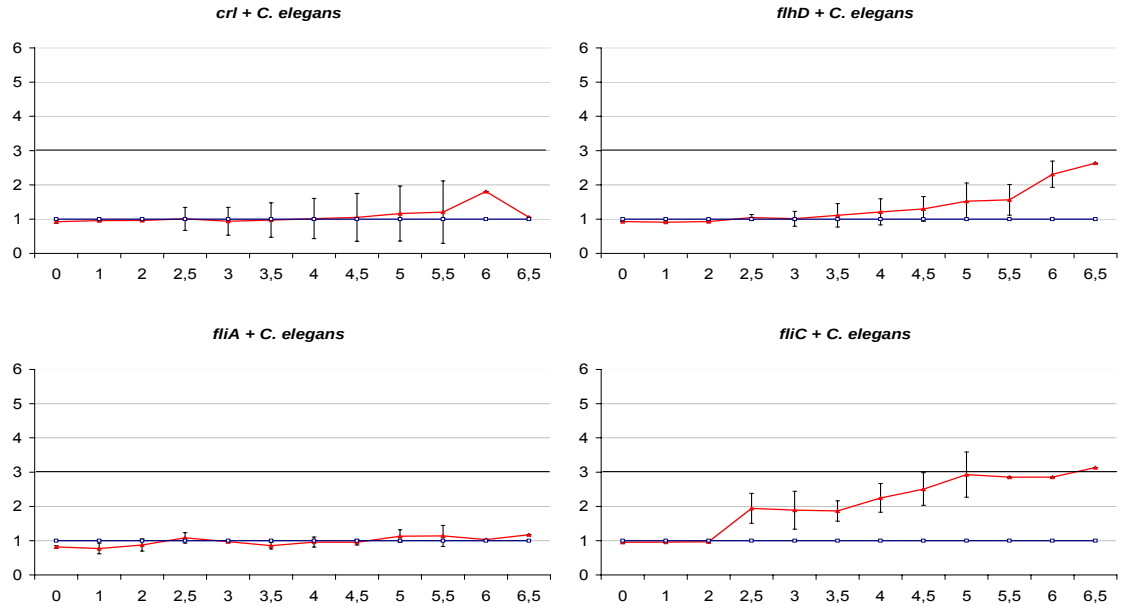


Figure 54: activation of 100bp upstream promoter regions of virulence associated genes. Heat-killed *C. elegans* were added at t = 2.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *C. elegans*, which is defined 1 for each time point (turquoise line). Orange lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in duplicate.

3.8.2 Membrane structure-associated non-LEE genes and *C. elegans*

The only genes activated in the *C. elegans* assay were *fliC* and *yehD*.There was no induction of any promoter region in the control assays with spent S-medium which had kept nematodes for 1.5h and was subsequently sterile filtered (data not shown) or with heat-killed nematodes (30min at 40°C).



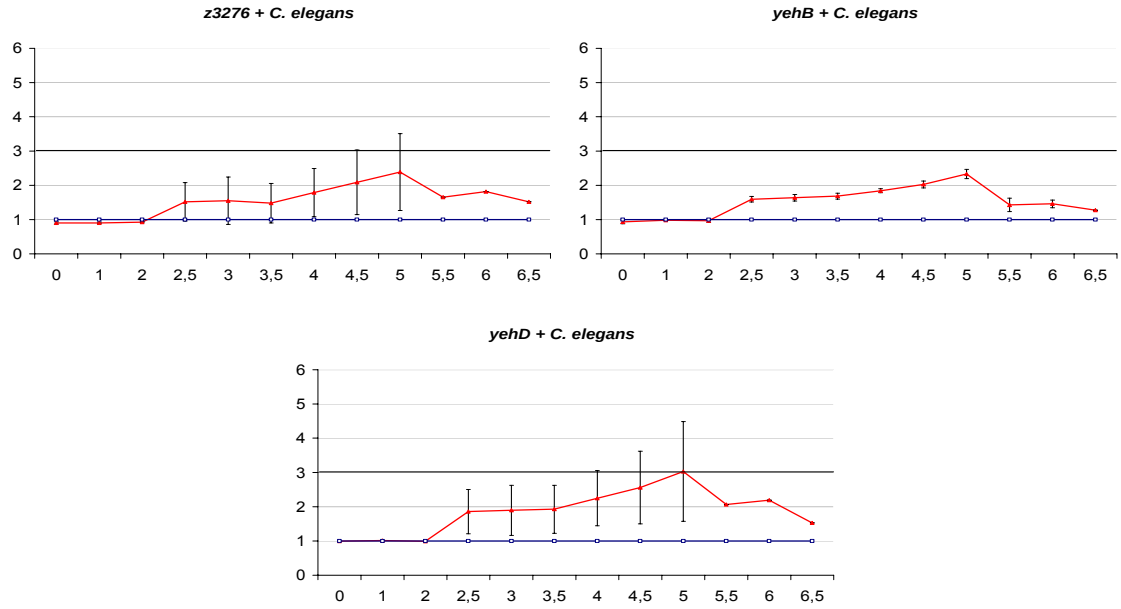
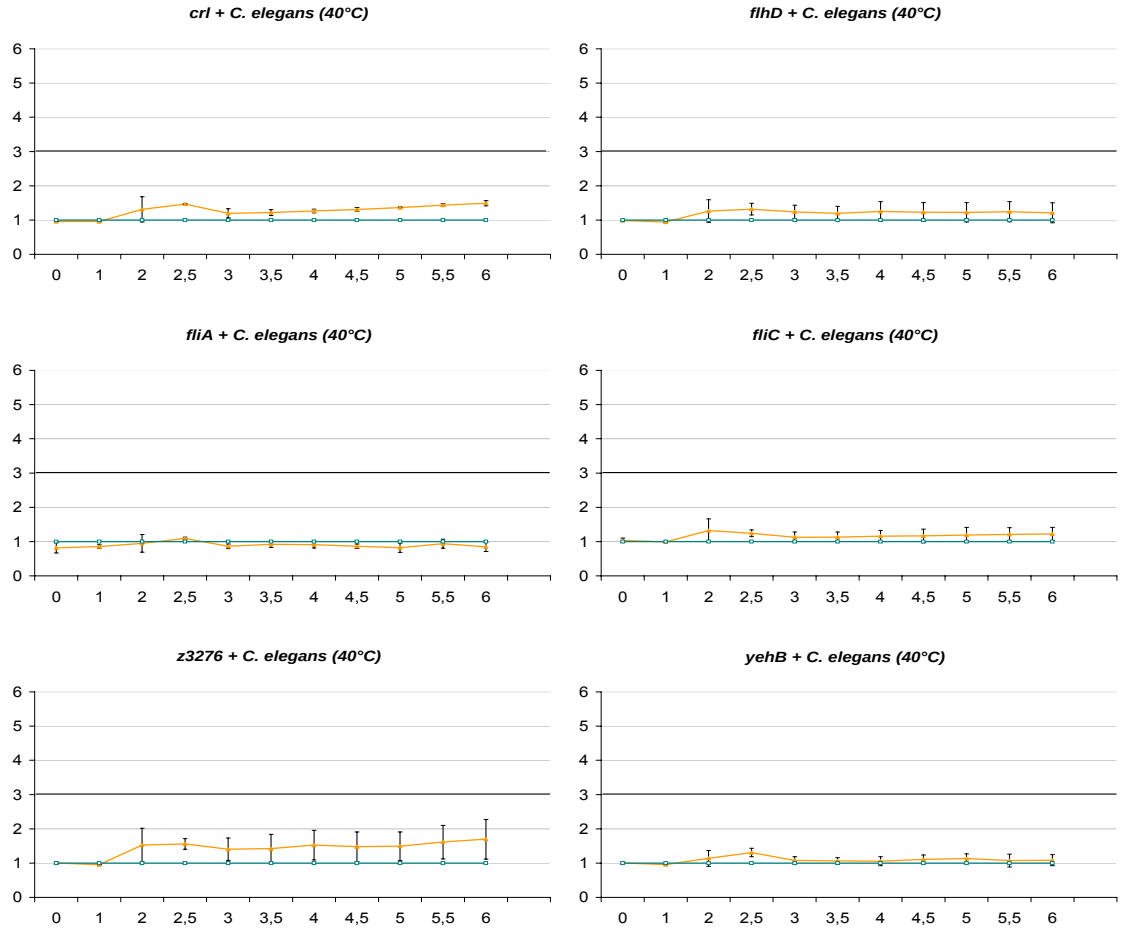


Figure 55: activation of 100bp upstream promoter regions of membrane structure associated genes. *C. elegans* were added at t = 2.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *C. elegans*, which is defined 1 for each time point (blue line). Red lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.



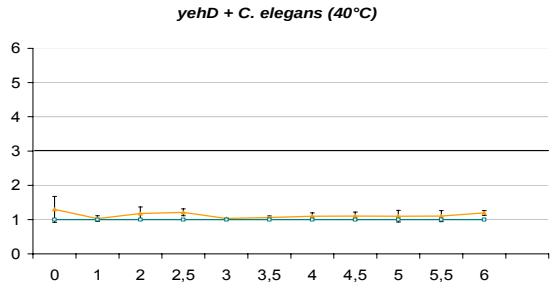
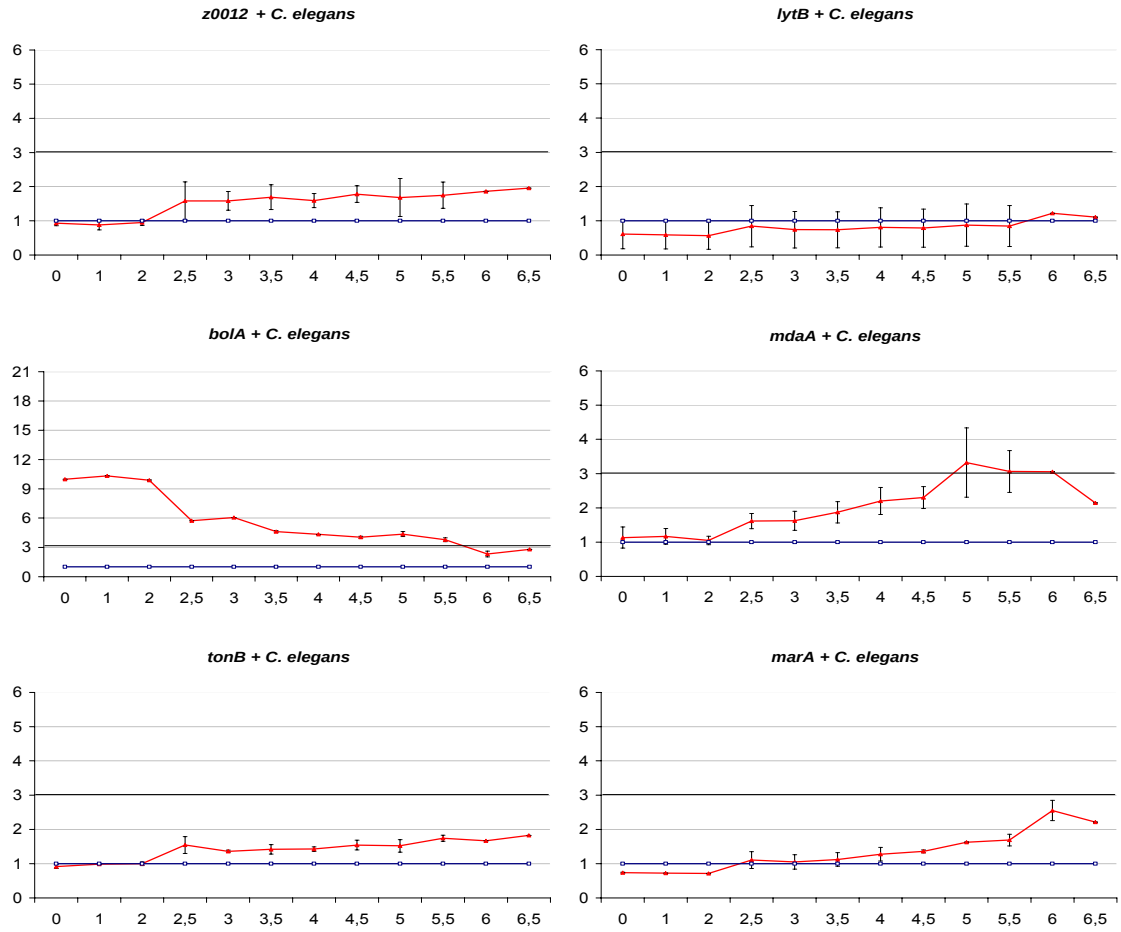


Figure 56: activation of 100bp upstream promoter regions of membrane structure associated genes. Heat-killed *C. elegans* were added at t = 2.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *C. elegans*, which is defined 1 for each time point (turquoise line). Orange lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in duplicate.

3.8.3 Regulatory active non-LEE genes and *C. elegans*

The promoter regions of *bolA*, *mdaA*, *qseB* and *z4982* were upregulated in contact with *C. elegans*. There was no induction of any promoter region in the control assays with spent S-medium which had kept nematodes for 1.5h and was subsequently sterile filtered (data in supplement) or with heat-killed nematodes (30min at 40°C).



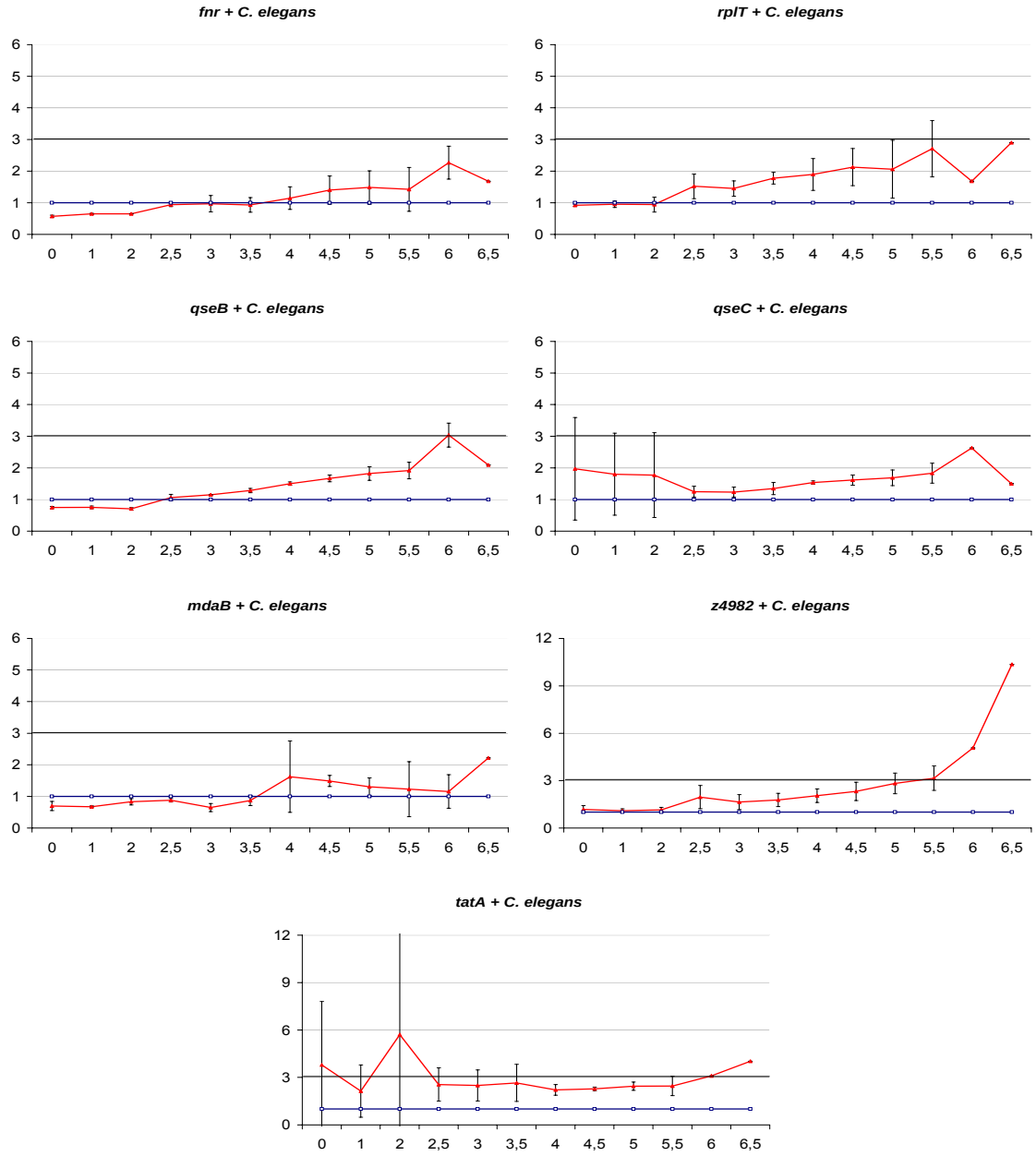
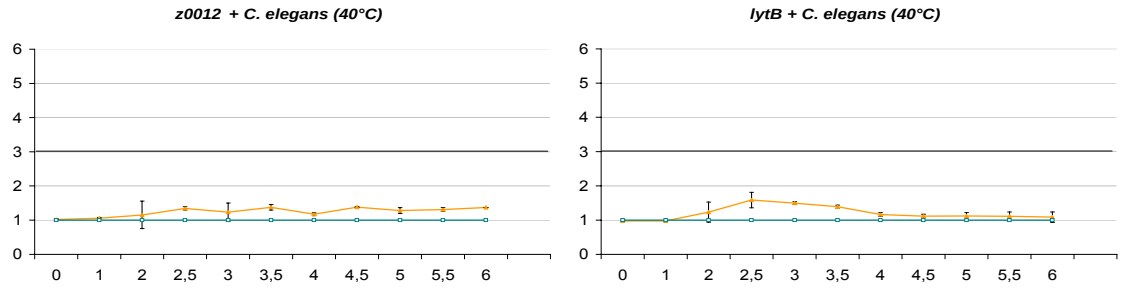
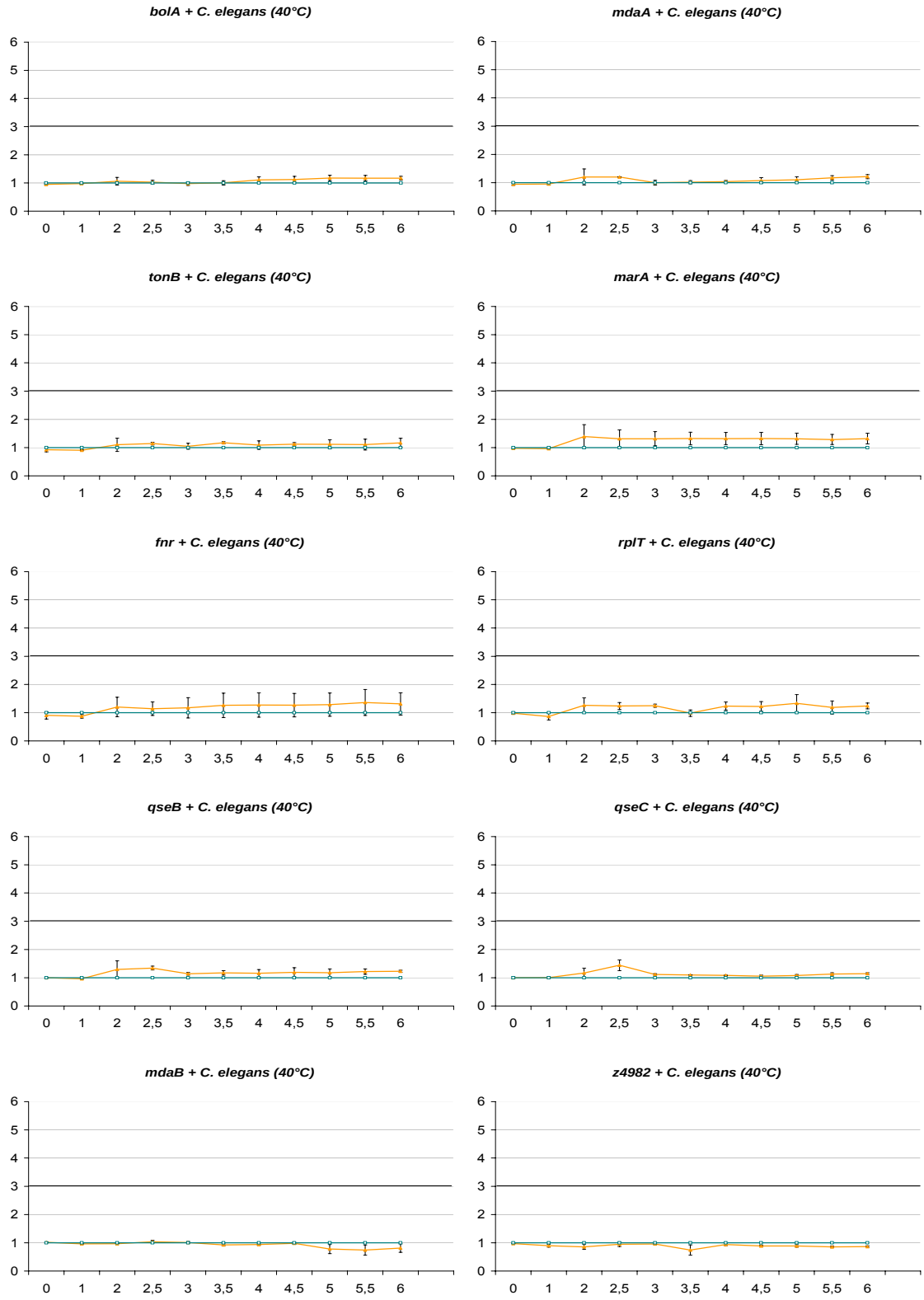


Figure 57: activation of 100bp upstream promoter regions of genes with regulatory functions. *C. elegans* were added at t = 2.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *C. elegans*, which is defined 1 for each time point (blue line). Red lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.




bolA + C. elegans (40°C)
mdaA + C. elegans (40°C)
tonB + C. elegans (40°C)
marA + C. elegans (40°C)
fnr + C. elegans (40°C)
rplT + C. elegans (40°C)
qseB + C. elegans (40°C)
qseC + C. elegans (40°C)
mdaB + C. elegans (40°C)
z4982 + C. elegans (40°C)
0 1 2 2,5 3 3,5 4 4,5 5 5,5 6
0 1 2 3 4 5 6

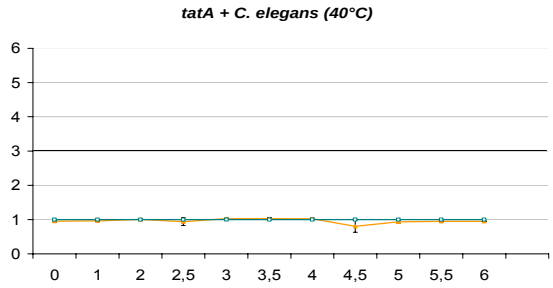
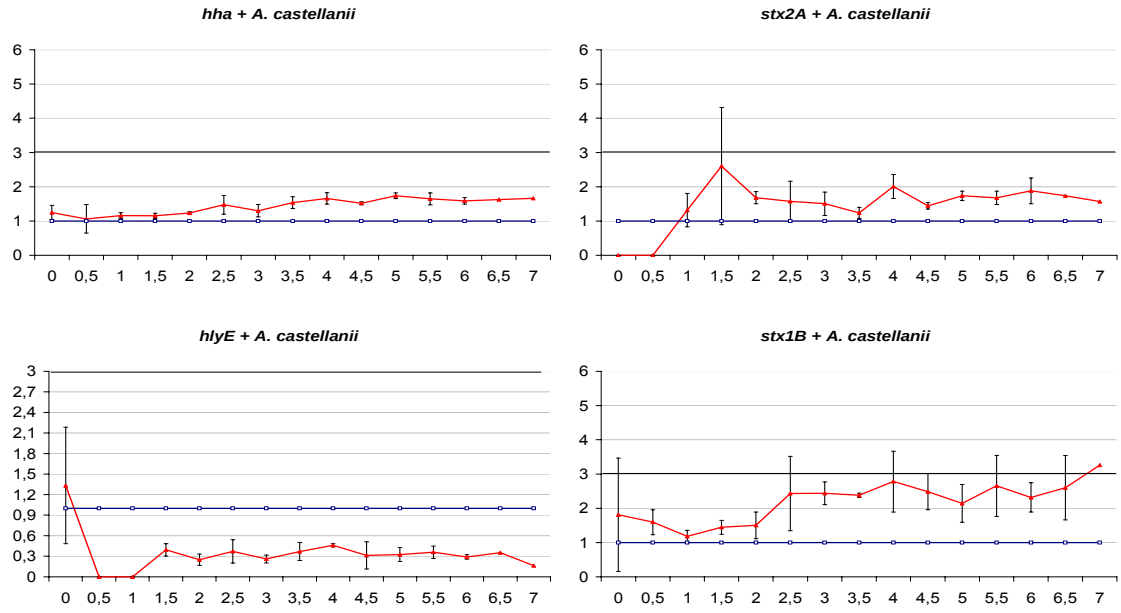


Figure 58: activation of 100bp upstream promoter regions of genes with regulatory functions. Heat-killed *C. elegans* were added at t = 2.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *C. elegans*, which is defined 1 for each time point (turquoise line). Orange lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in duplicate.

3.8.4 Virulence-associated non-LEE genes and *A. castellanii*

There was no induction of any of the tested promoter regions with living *A. castellanii*. Surprisingly, when heat-killed amoebae were submitted to the assay, all of the tested promoter regions except for that of *hlyE* were activated. The *hlyE* promoter seemed, like in the *C. elegans* assay, even slightly repressed. There was a strong activation of the B subunit of Shiga Toxin I with dead *A. castellanii*.



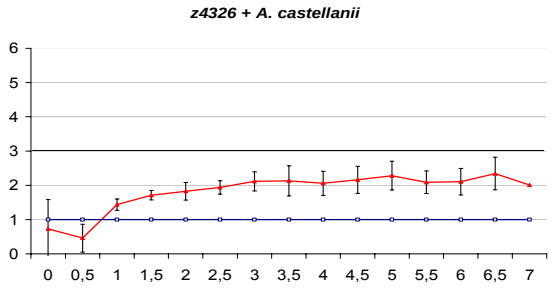


Figure 59: activation of 100bp upstream promoter regions of virulence associated genes. *A. castellanii* were added at t = 0.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *A. castellanii*, which is defined 1 for each time point (blue line). Red lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.

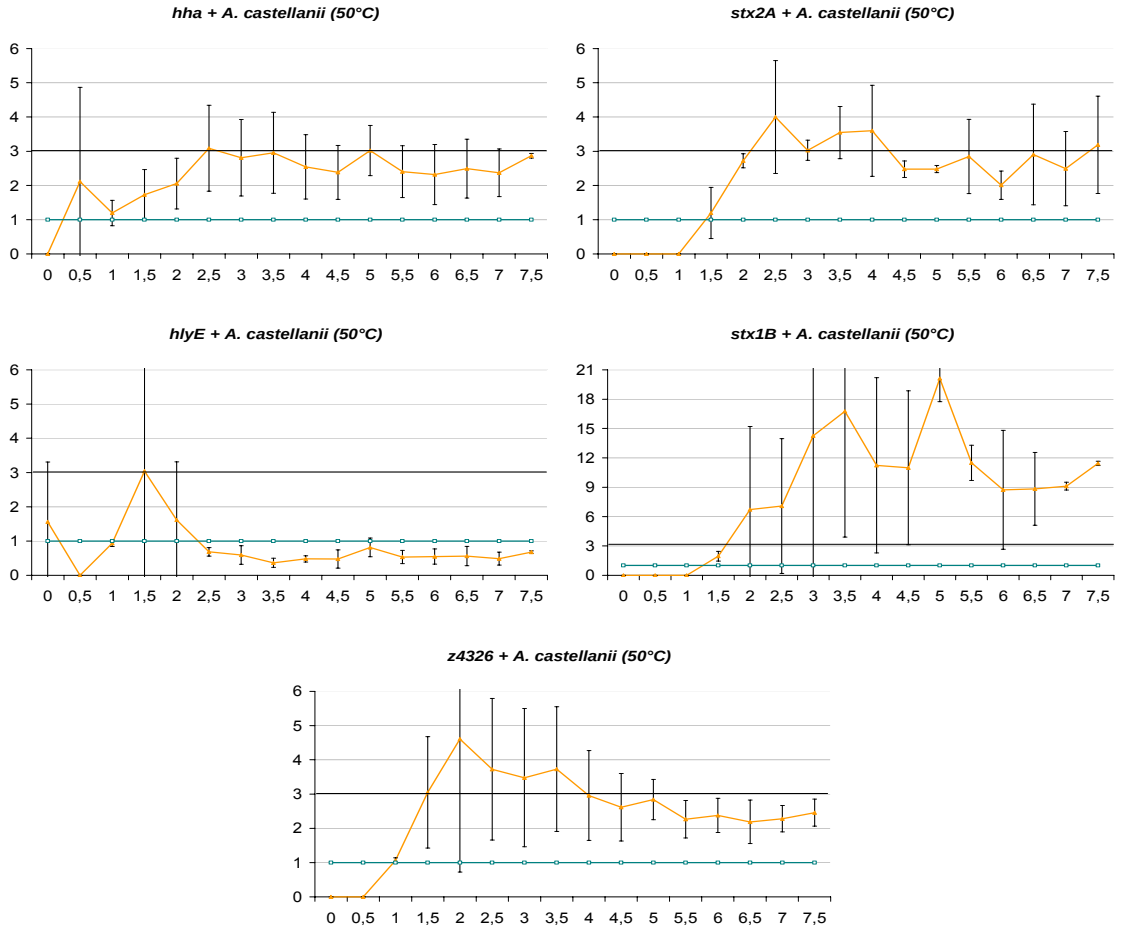


Figure 60: activation of 100bp upstream promoter regions of virulence associated genes. Heat-killed *A. castellanii* were added at t = 0.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *A. castellanii*, which is defined 1 for each time point (turquoise line). Orange lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.

3.8.5 Membrane structure-associated non-LEE genes and *A. castellanii*

In contact with living *A. castellanii*, only the promoter region of *yehB* was activated above threshold. In the experimental setup with heat-killed amoebae, all tested 500bp upstream regions were activated at least threefold.

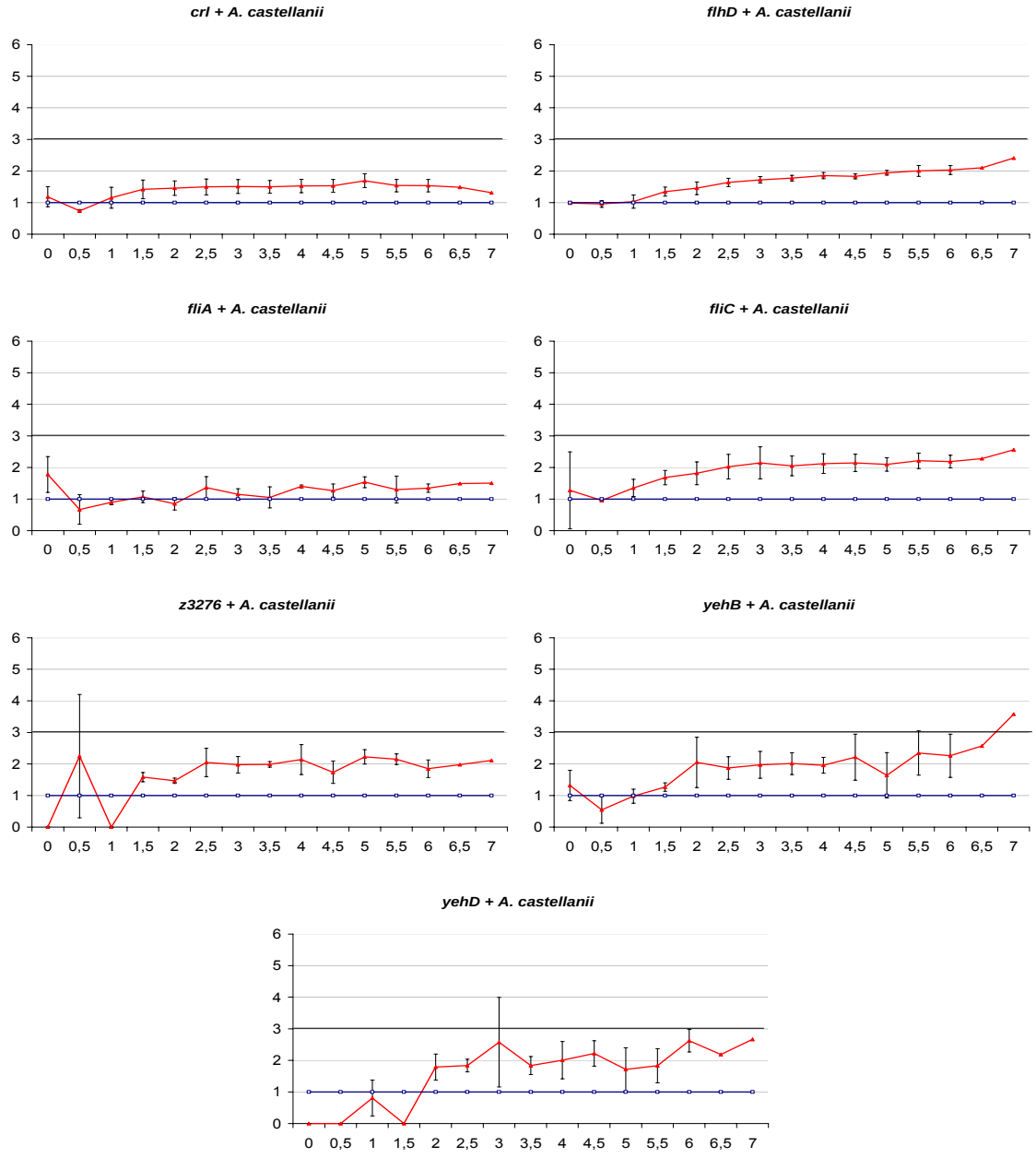


Figure 61: activation of 100bp upstream promoter regions of membrane structure associated genes. *A. castellanii* were added at t = 0.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *A. castellanii*, which is defined 1 for each time point (blue line). Red lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.

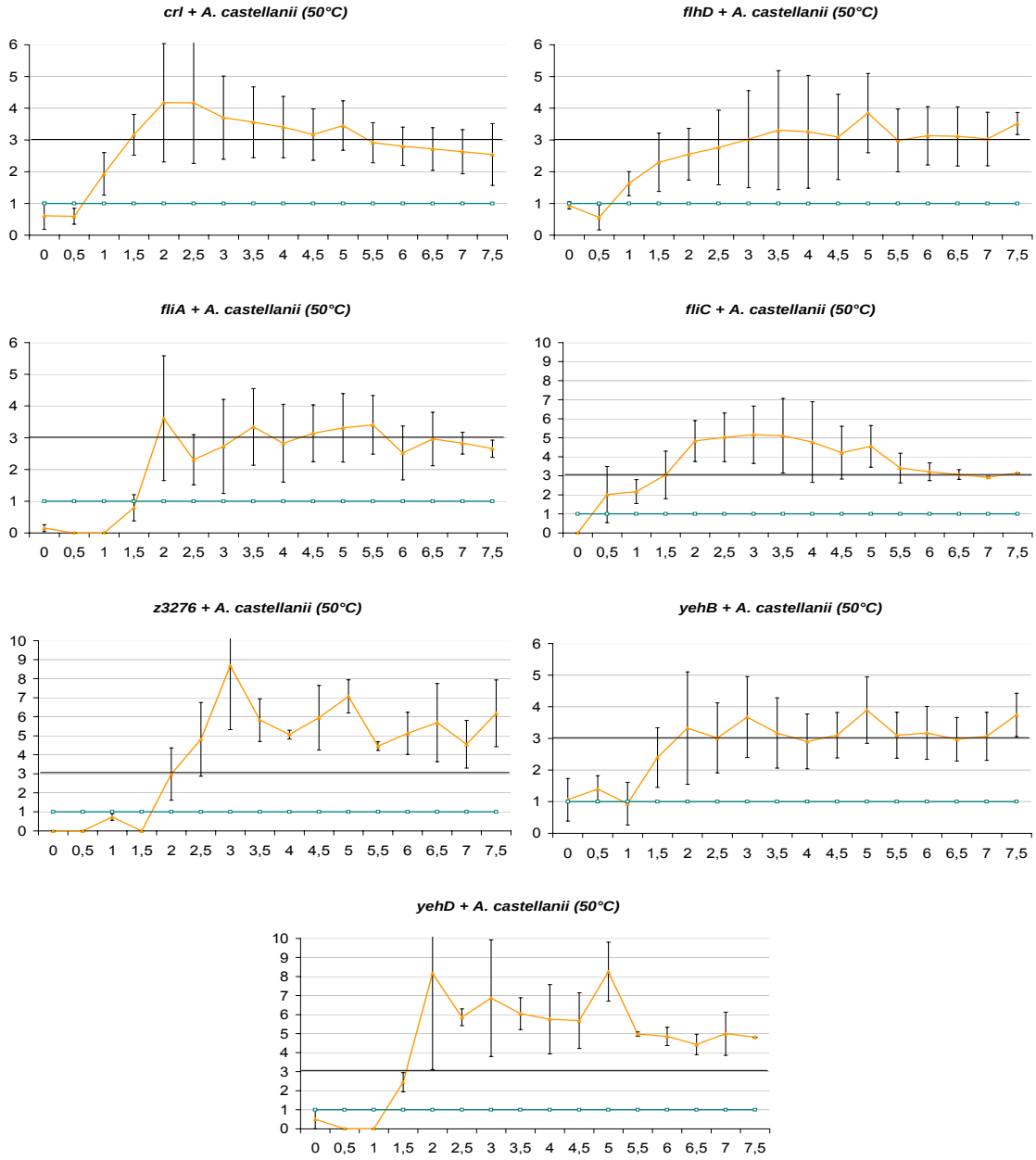
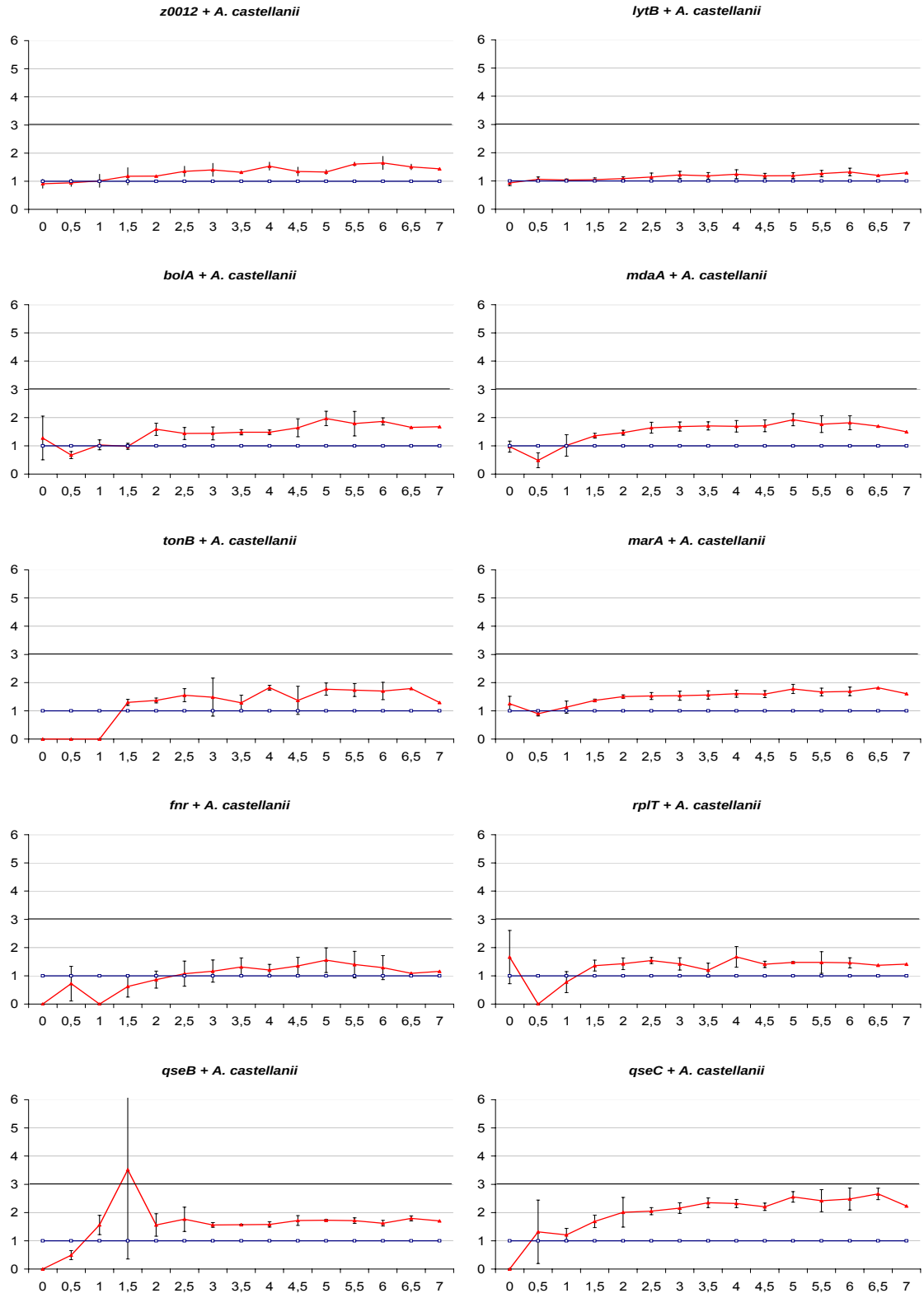


Figure 62: activation of 100bp upstream promoter regions of membrane structure associated genes. Heat-killed *A. castellanii* were added at t = 0.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *A. castellanii*, which is defined 1 for each time point (turquoise line). Orange lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.

3.8.6 Regulatory active non-LEE genes and *A. castellanii*

The only 500bp upstream region slightly activated with living *A. castellanii* was that of *qseB*. In contact with heat-killed amoebae, the promoter regions of *z0012*, *bolA*, *mdaA*, *tonB*, *marA*, *fnr*, *rplT*, *qseB* and *qseC* were activated at least threefold compared to the control measurements.


z0012 + A. castellanii
lytB + A. castellanii
bolA + A. castellanii
mdaA + A. castellanii
tonB + A. castellanii
marA + A. castellanii
fnr + A. castellanii
rplT + A. castellanii
qseB + A. castellanii
qseC + A. castellanii
0 1 2 3 4 5 6
0 0,5 1 1,5 2 2,5 3 3,5 4 4,5 5 5,5 6 6,5 7

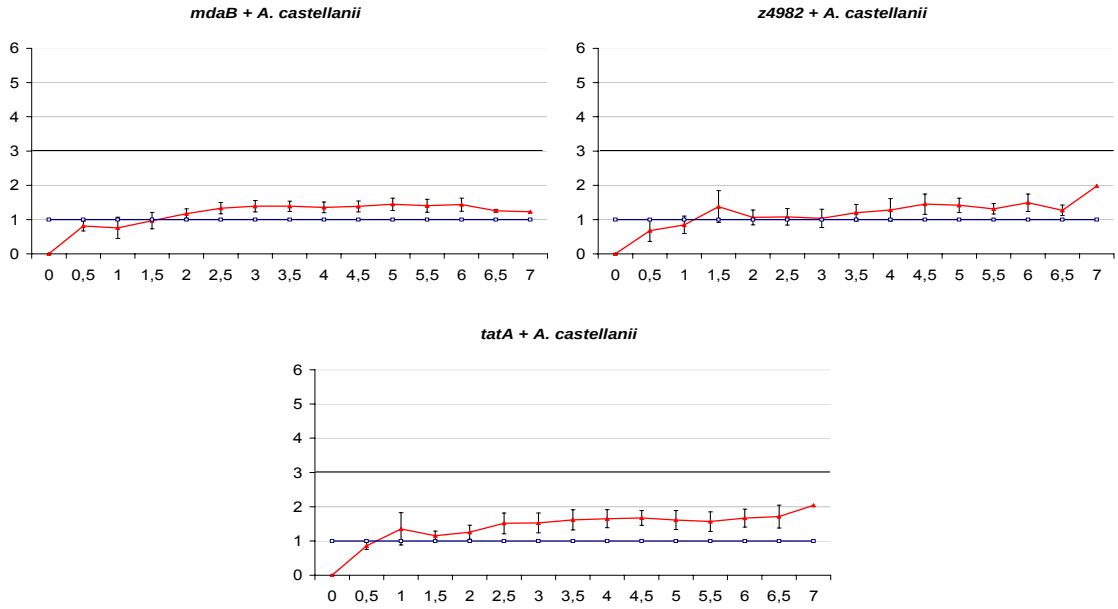
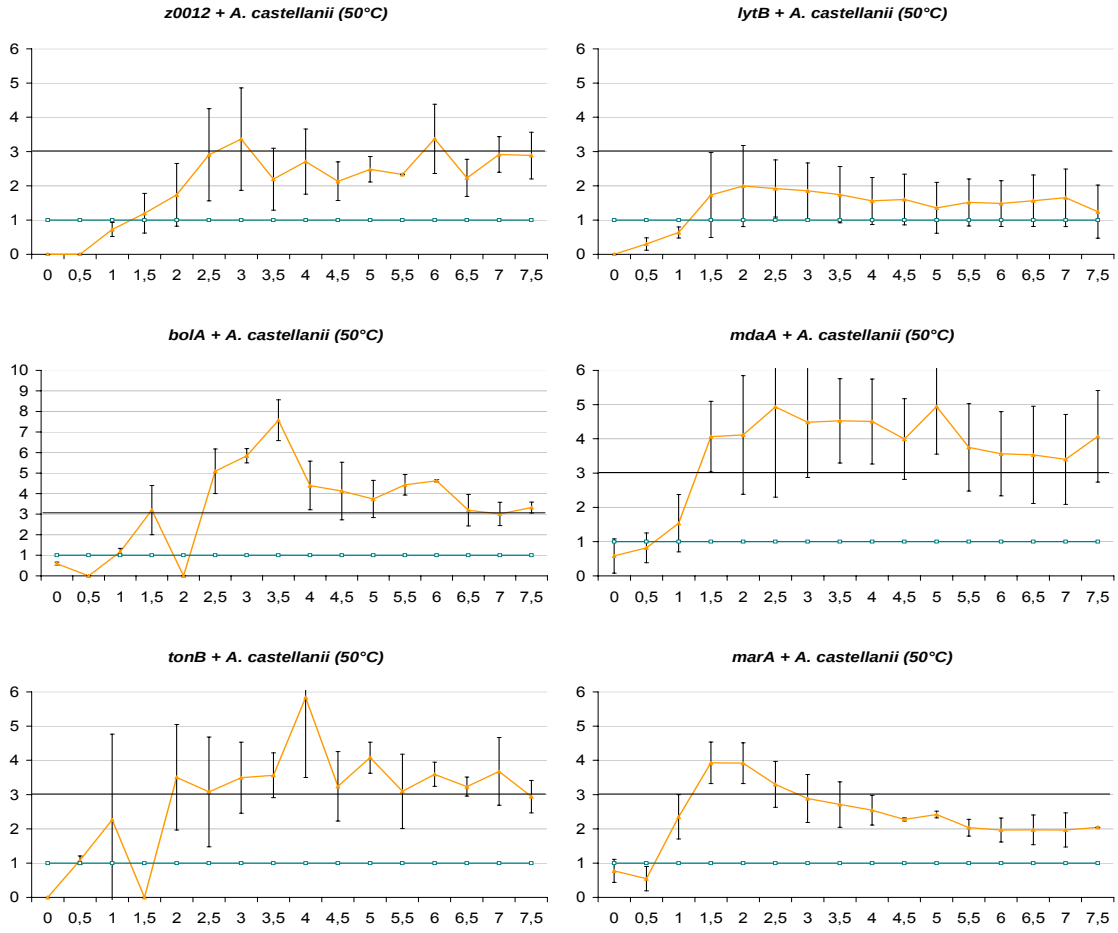


Figure 63: activation of 100bp upstream promoter regions of genes with regulatory functions. *A. castellanii* were added at t = 0.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *A. castellanii*, which is defined 1 for each time point (blue line). Red lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.



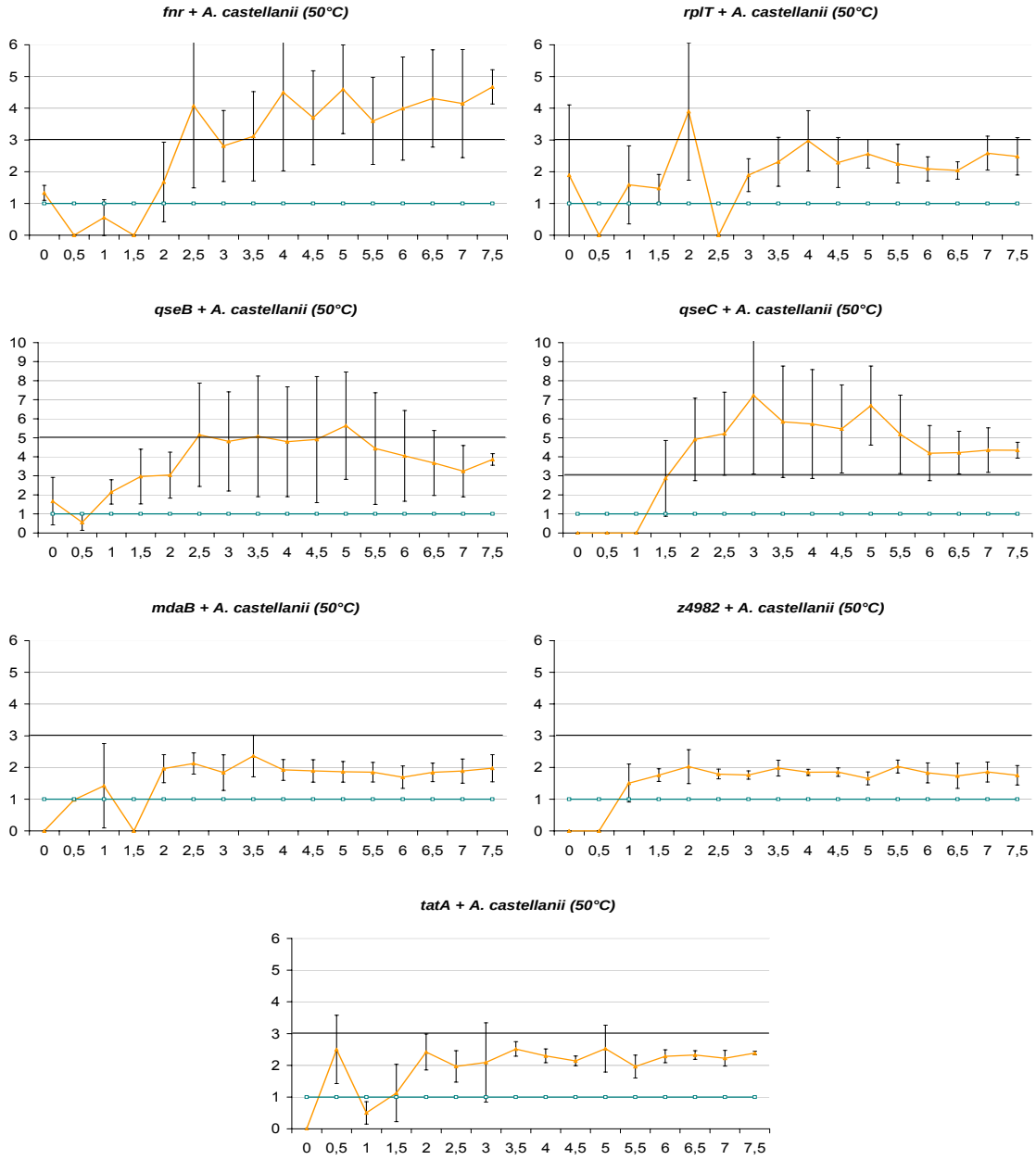


Figure 64: activation of 100bp upstream promoter regions of genes with regulatory functions. Heat-killed *A. castellanii* were added at t = 0.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *A. castellanii*, which is defined 1 for each time point (turquoise line). Orange lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.

3.8.7 Virulence-associated non-LEE genes and *A. thaliana*

The only promoter region not activated in contact with *A. thaliana* was that of *hha*. When the assays were repeated with spent MS medium, no activation of any promoter region was observed.

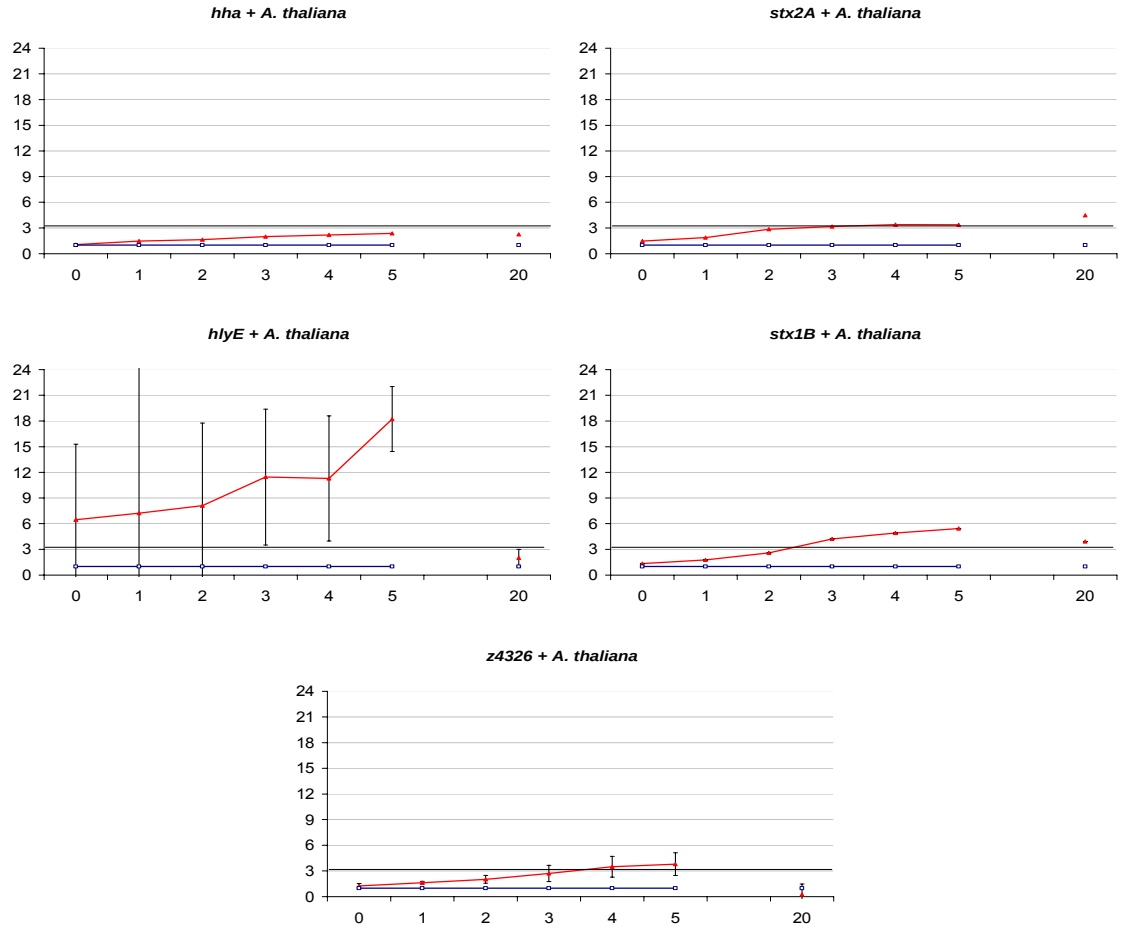


Figure 65: activation of 100bp upstream promoter regions of virulence associated genes. *A. thaliana* were added at t = 0h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *A. thaliana*, which is defined 1 for each time point (blue line). Red lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.

3.8.8 Membrane structure-associated non-LEE genes and *A. thaliana*

The only membrane associated gene tested that was not activated above threshold was *fliA*. When the assays were repeated with spent MS medium, no activation of any promoter region was observed.

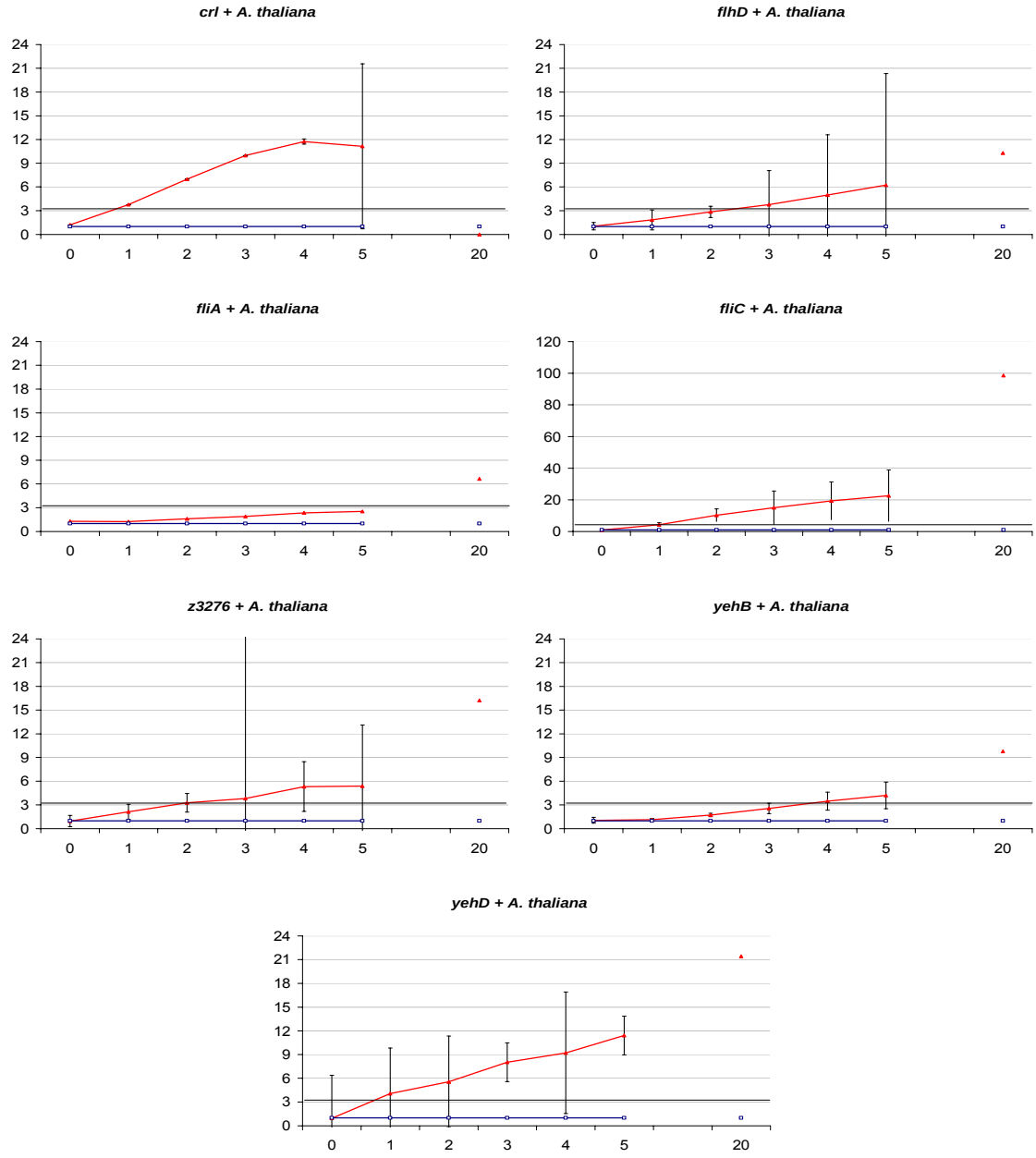
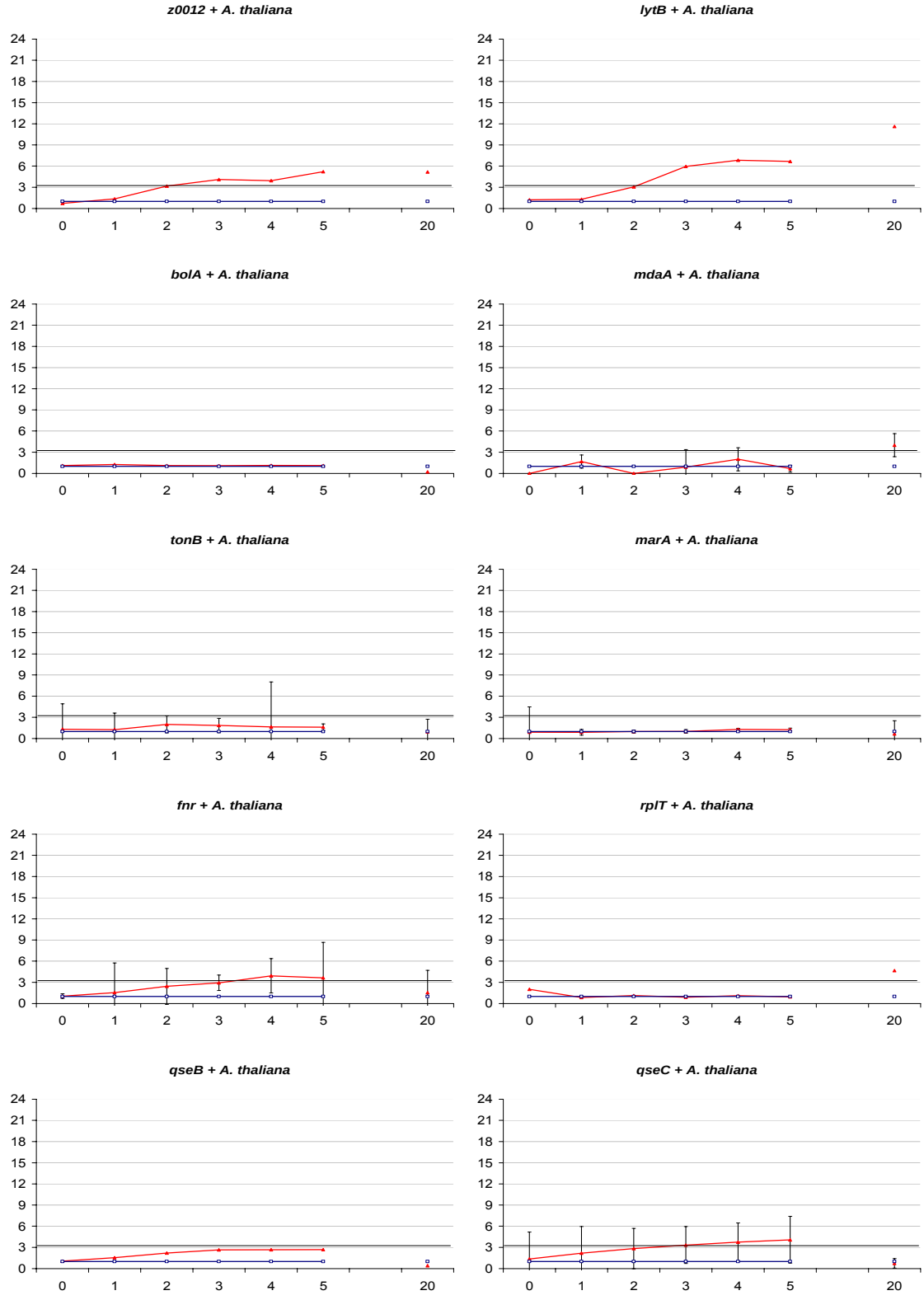


Figure 66: activation of 100bp upstream promoter regions of membrane structure associated genes. *A. thaliana* were added at t = 0h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *A. thaliana*, which is defined 1 for each time point (blue line). Red lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.

3.8.9 Regulatory active non-LEE genes and *A. thaliana*

In contact with *A. thaliana*, the 500bp upstream regions of the following genes were activated: *z0012*, *lytB*, *fnr*, *qseC*, *mdaB*, *z4982* and *tatA*. When the assays were repeated with spent MS medium, no activation of any promoter region was observed.



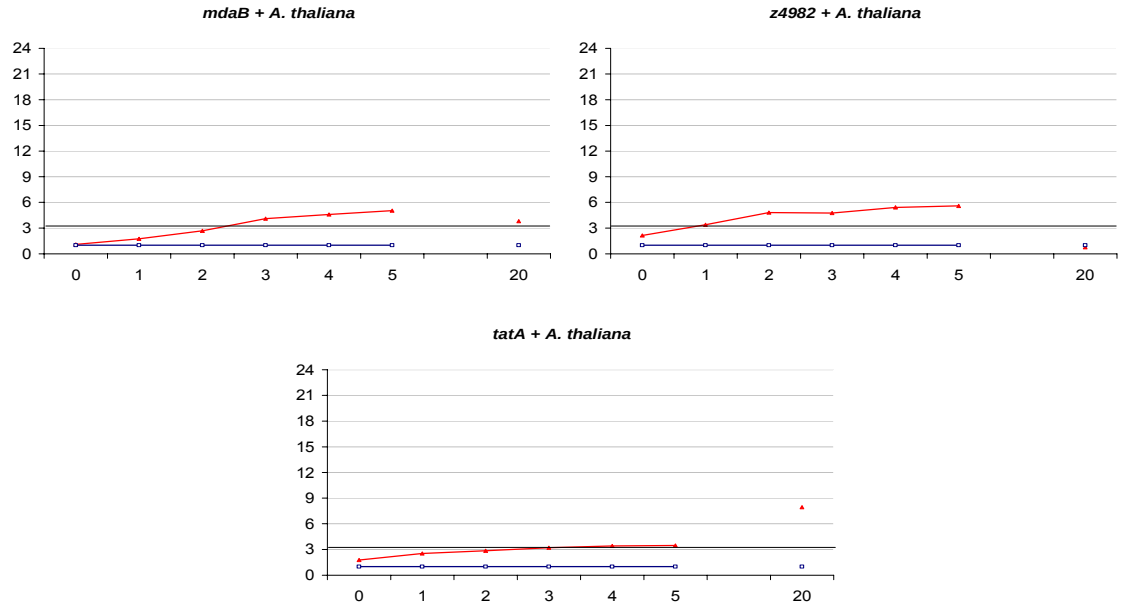


Figure 67: activation of 100bp upstream promoter regions of genes with regulatory functions. *A. thaliana* were added at t = 0h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *A. thaliana*, which is defined 1 for each time point (blue line). Red lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.

3.9 *A. thaliana* growth assay with EHEC

A. thaliana plants inoculated with EHEC O157:H7 developed a virulence-associated phenotype that does not ressemble the phenotype of a *P. syringae* infection. Plants inoculated with TOP10$^-$ cells or LB medium supplemented with kanamycin do not show any signs of illness. The plants that are infected with EHEC have small, red-brownish leaves. They seem to develop even more flowers than healthy plants. Plants inoculated with *P. syringae* are small with normally sized, reddish-yellow leaves and almost no flowers. First signs of illness can be observed after 5-7 days both with EHEC and *P. syringae*, but the *P. syringae*-infected plants look worse than those infected with EHEC after 21 days on the last date of observation. In addition to that, another *E. coli* strain was found that also induces the same phenotype as EHEC O157:H7 in *Arabidopsis thaliana* plants. This unknown strain was named *Escherichia coli* AS and has to be determined closer yet in order to find out whether it is an already well known strain of *E. coli* or not. The phenotype observed in *A. thaliana* inoculated with this strain looks a little more like the *P. syringae* mediated phenotype than that produced by EHEC, as the leaves of the infected plants with *E. coli* AS look more reddish than with O157:H7.

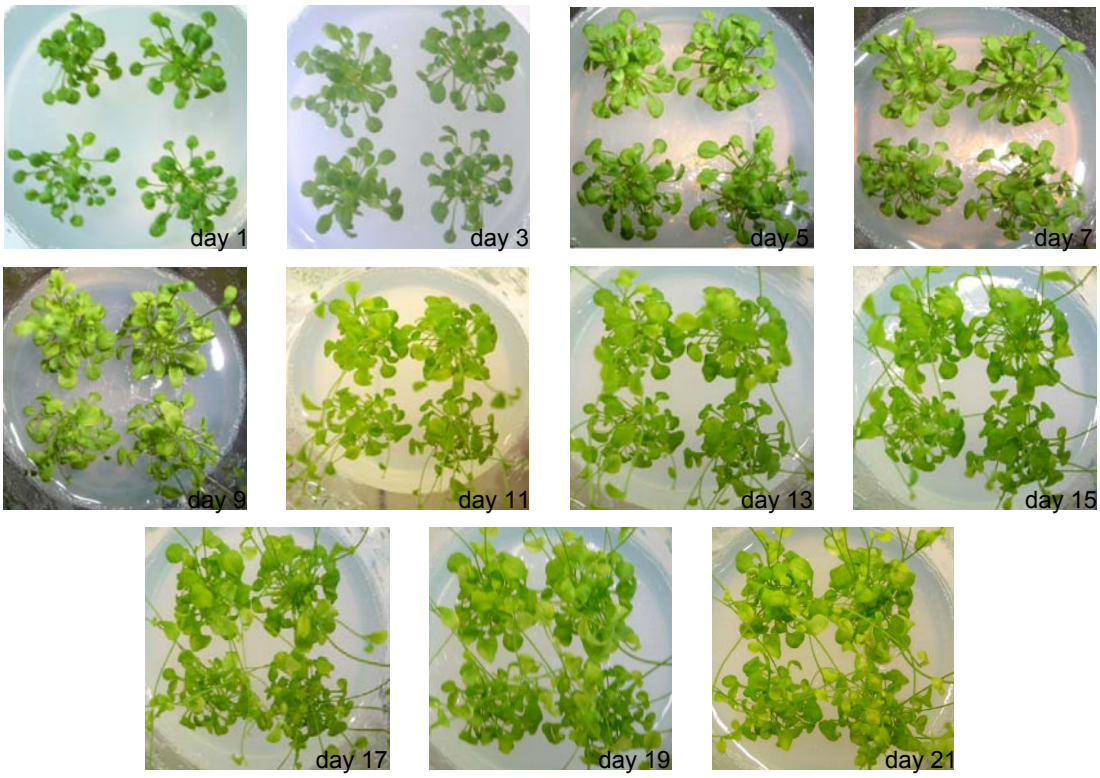


Figure 68: A. thaliana plants inoculated with LB medium (+kanamycin). There are no growth defects.

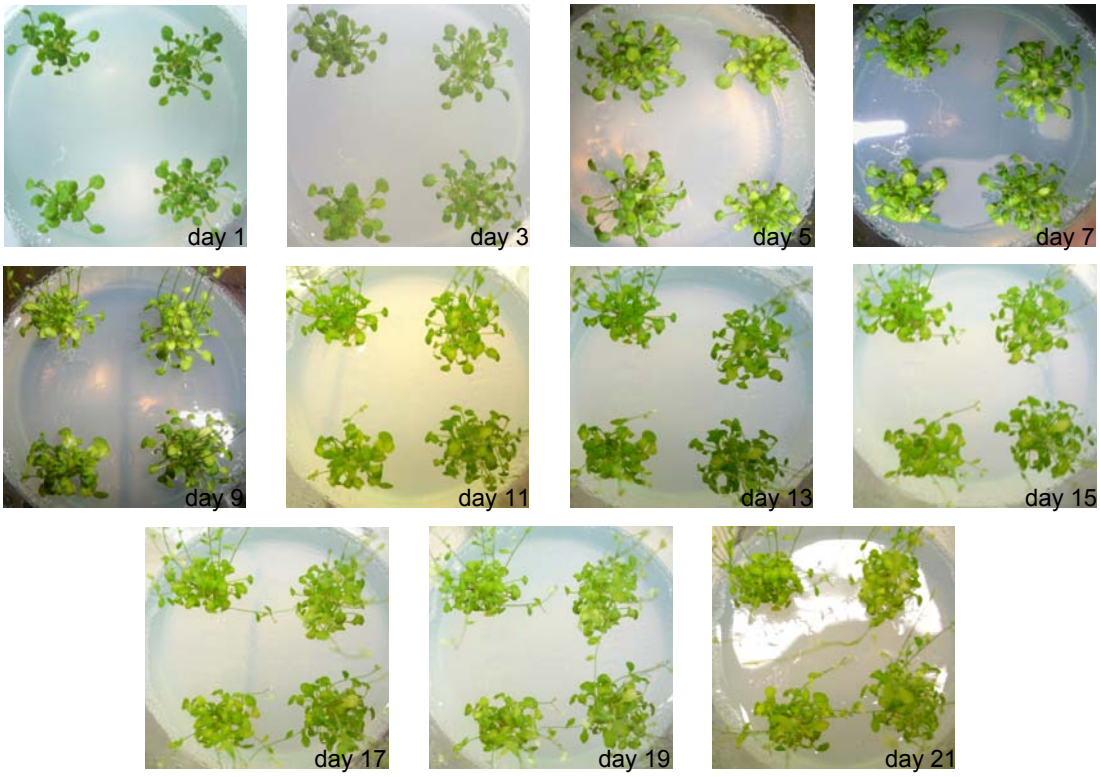


Figure 69: A. thaliana plants inoculated with TOP10^{-} cells. There are no severe growth defects.

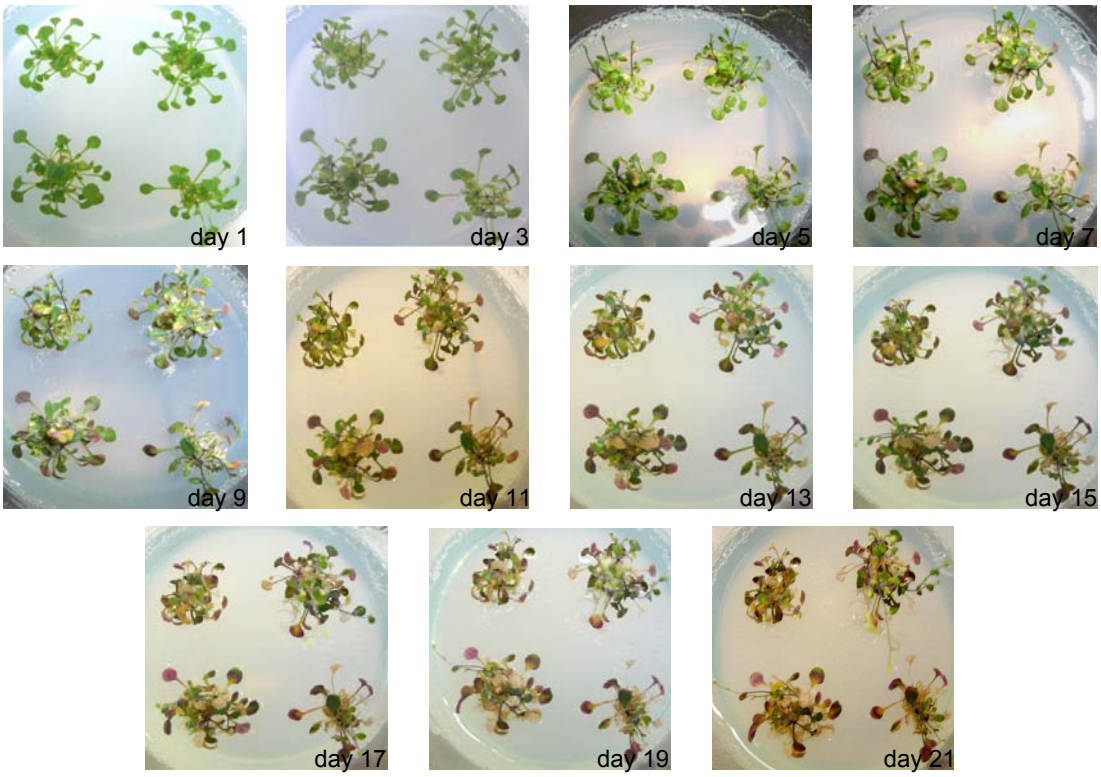


Figure 70: A. thaliana plants inoculated with *Pseudomonas syringae*. There are severe growth defects.

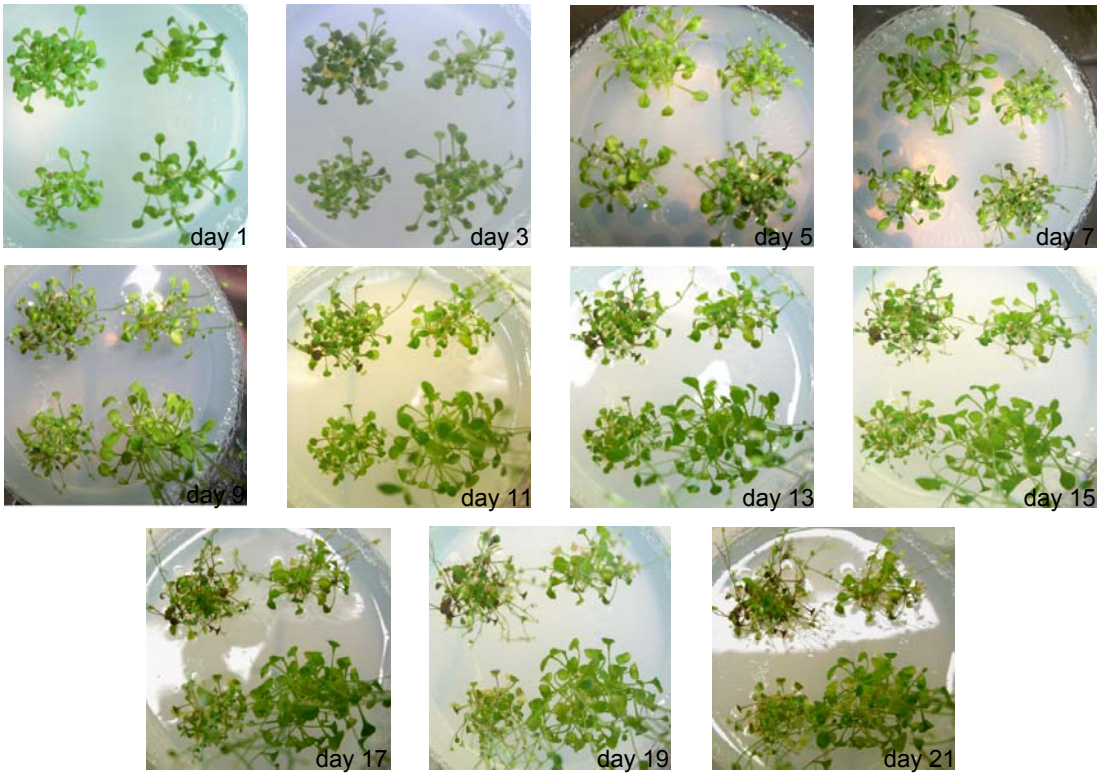


Figure 71: EHEC O157:H7 cells on *A. thaliana plants*. Pictures were taken every two days.

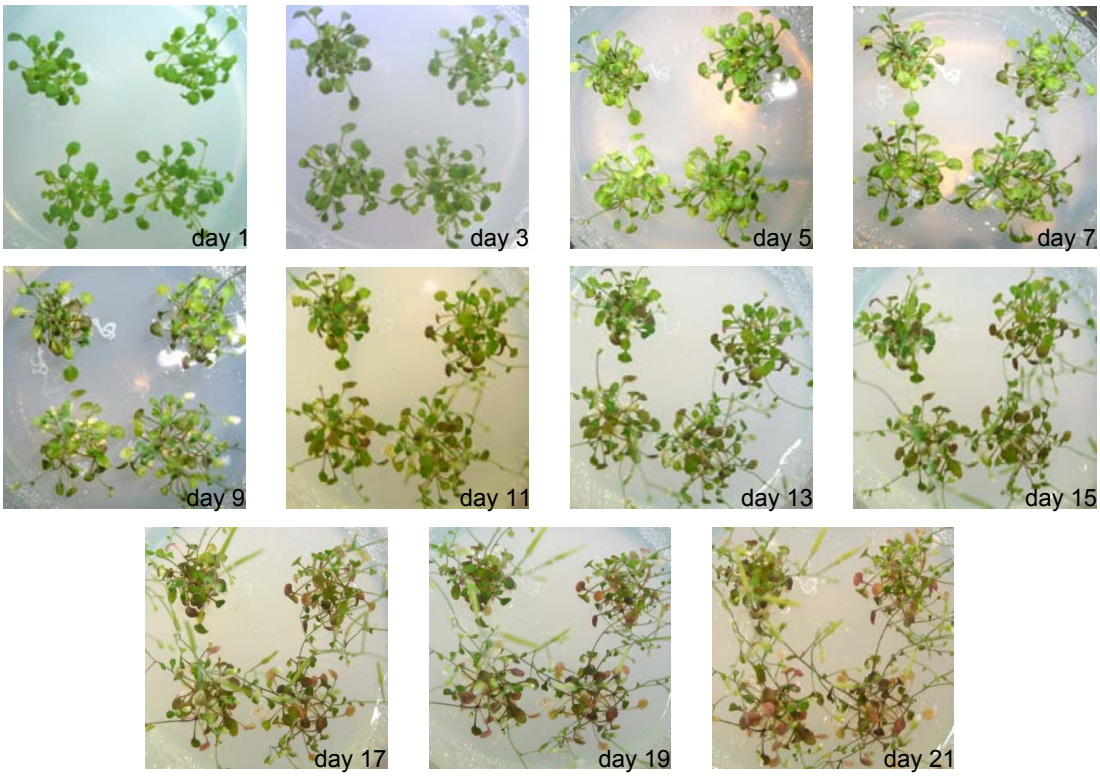


Figure 72: *Escherichia coli* AS (undetermined *E. coli* strain) cells on *A. thaliana* plants. Pictures were taken every two days.

3.10 Overview LEE genes

Surprisingly, all the 100bp upstream regions of genes encoded in the locus of enterocyte effacement were activatable in our experimental setup. The assays with *A. thaliana* showed the highest amount of total activation (Fig. 77, 78). In addition to that, contact with *A. thaliana* resulted in an upregulation of each tested promoter region.

In contact with *C. elegans*, almost every tested 100bp upstream region of LEE-genes was activated as well, with the exception of the *orf29* promoter region which did not meet the threshold of threefold induction. The multitude of activation was in general lower than in the *A. thaliana* assays, however. In addition to that, the amount of total luminescence emitted was on a lower level compared to the *A. thaliana* experiments (Fig. 73, 74).

In contact with the amoeba *A. castellanii*, the activation pattern of the tested 100bp upstream regions was quite different to those observed with *C. elegans* and *A. thaliana*. Only about half of the LEE-encoded genes were activated at least threefold in contact with *A. thaliana*. The pattern of activation was almost the same, when heat-killed amoebae were submitted to the assay. The amount of total luminescence measured was similar to that observed in the *C. elegans assay* (Fig. 75, 76). No activation of any LEE promoter region was observed in contact with Caco-2 cells (data in supplementary).

gene number	gene name	*A. castellanii* assay		*C. elegans* assay		*A. thaliana* assay
		alive	dead (50°C)	alive	dead (40°C)	
z5100	*espF*	/	/	+	-	+
z5102	*orf29*	+	+	/	-	+
z5103	*escF*	+	+	+	-	+
z5104	*cesD2*	/	/	+	-	+
z5105	*espB*	+	+	+	-	+
z5106	*espD*	/	+	+	-	+
z5107	*espA*	+	+	+	-	+
z5108	*sepL*	-	-	+	-	+
z5109	*escD*	/	/	+	-	+
z5110	*eae*	+	/	+	-	+
z5111	*cesT*	/	-	+	-	+
z5112	*tir*	/	/	+	-	+
z5113	*map*	/	/	+	-	+
z5114	*cesF*	/	/	+	-	+
z5116	*sepQ*	+	/	+	-	+
z5117	*orf16*	/	-	+	-	+
z5118	*orf15*	+	+	+	-	+
z5119	*escN*	+	+	+	-	+
z5120	*escV*	+	+	+	-	+
z5121	*orf12*	-	-	+	-	+
z5122	*sepZ*	+	/	+	-	+
z5123	*rorf8*	+	+	+	-	+
z5124	*escJ*	+	/	+	-	+
z5125	*sepD*	/	/	+	-	+
z5126	*escC*	+	/	+	-	+
z5127	*cesD*	/	/	+	-	+
z5128	*grlA*	/	-	+	-	+
z5129	*grlR*	+	+	+	-	+
z5131	*rorf3*	/	/	+	-	+
z5132	*escU*	+	+	+	-	+
z5133	*escT*	+	+	+	-	+
z5134	*escS*	+	+	+	-	+
z5135	*escR*	/	/	+	-	+
z5136	*orf5*	/	/	+	-	+
z5137	*orf4*	+	+	+	-	+
z5138	*cesAB*	/	/	+	-	+
z5139	*orf2*	+	+	+	-	+
z5140	*ler*	+	/	+	-	+
z5142	*espG*	/	/	+	-	+
z5143	*rorf1*	/	/	+	-	+

Table 4: overview of all LEE-encoded genes and their activation in contact with *C. elegans*, *A. castellanii* and *A. thaliana*. +: activation above threshold (threefold activation in comparison to control without tested organism); -: no activation; /: activation below threshold

The following graphs show the total level of luminescence emitted by the several transgenic EHEC at the time point of highest luminescence measured, which is different for each construct, and at the time point of lowest luminescence measured, which was the same for all constructs. Depending on the assay, this was either the first measurement (for the *A. castellanii* assay) or the last measurement (for the *C. elegans* and *A. thaliana* assays). These differences in the time point of lowest luminescence measured derive from the different conduction of the assays: as the PYG-medium for *A. castellanii* was the only medium used in the assays EHEC could use as a growth medium, the bacterial overnight cultures were directly submitted to the assay. Both the *C. elegans* S-medium and the *A. thaliana* MS-Medium were not suitable for EHEC as a growth medium, so that the overnight cultures were grown in LB medium, washed and transferred into the new, organism-related medium. This resulted in an activation of multiple promoter regions as a shock response to the changed environment. Due to this, in both the *C. elegans* and *A. thaliana* assays, the bacteria had to be adapted to the new medium for a certain time (see materials & methods). As the EHEC could not replicate well in the new media, the amount of luminescence measured grew smaller during the assays due to dying bacteria. In contrast to this, the bacteria in the *A. castellanii* assays grew further, resulting in generally growing amounts of luminescence during the assays.

All Bars shown results from the same assay; standard deviations in these graphs do not result from three independent measurements but from the three wells (eight in the *A. thaliana* assay) loaded with one transgenic EHEC strain each.

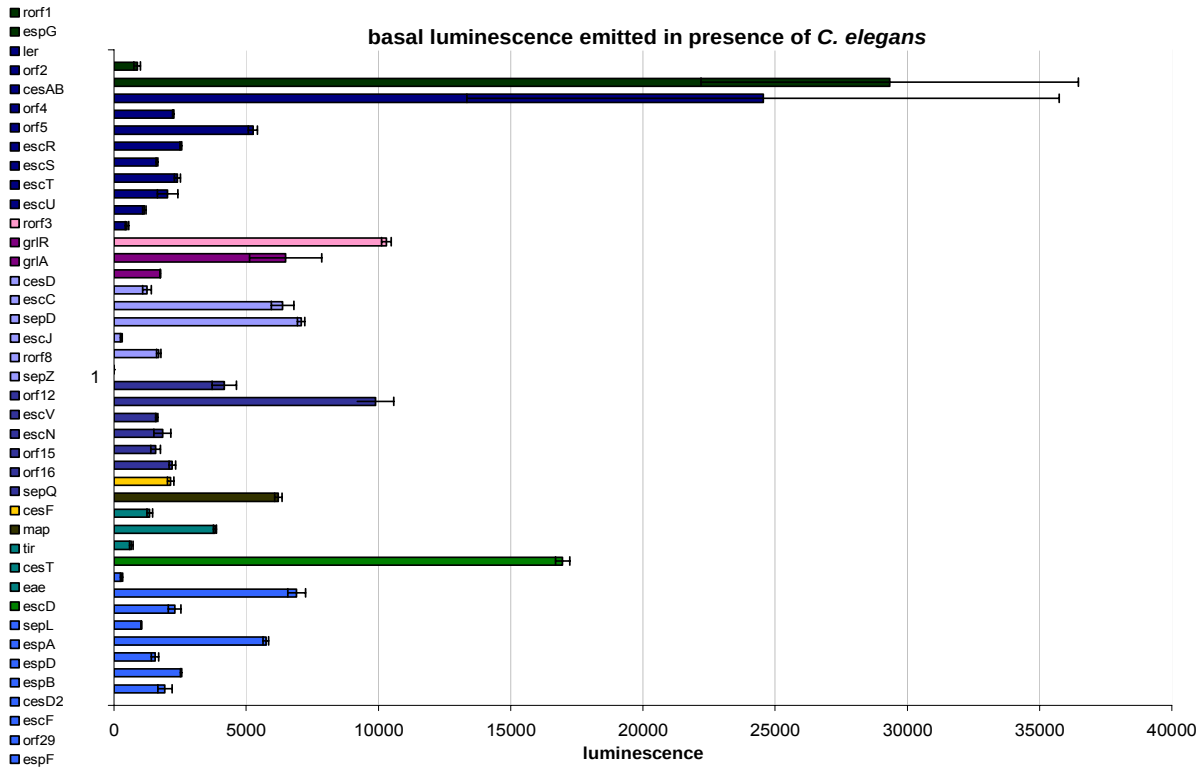


Figure 73: Comparison of the luminescence levels emitted by the different transgenic EHEC at the end of luminescence measurements at t 6h in the control wells without *C. elegans* in S-medium supplemented with nystatin.

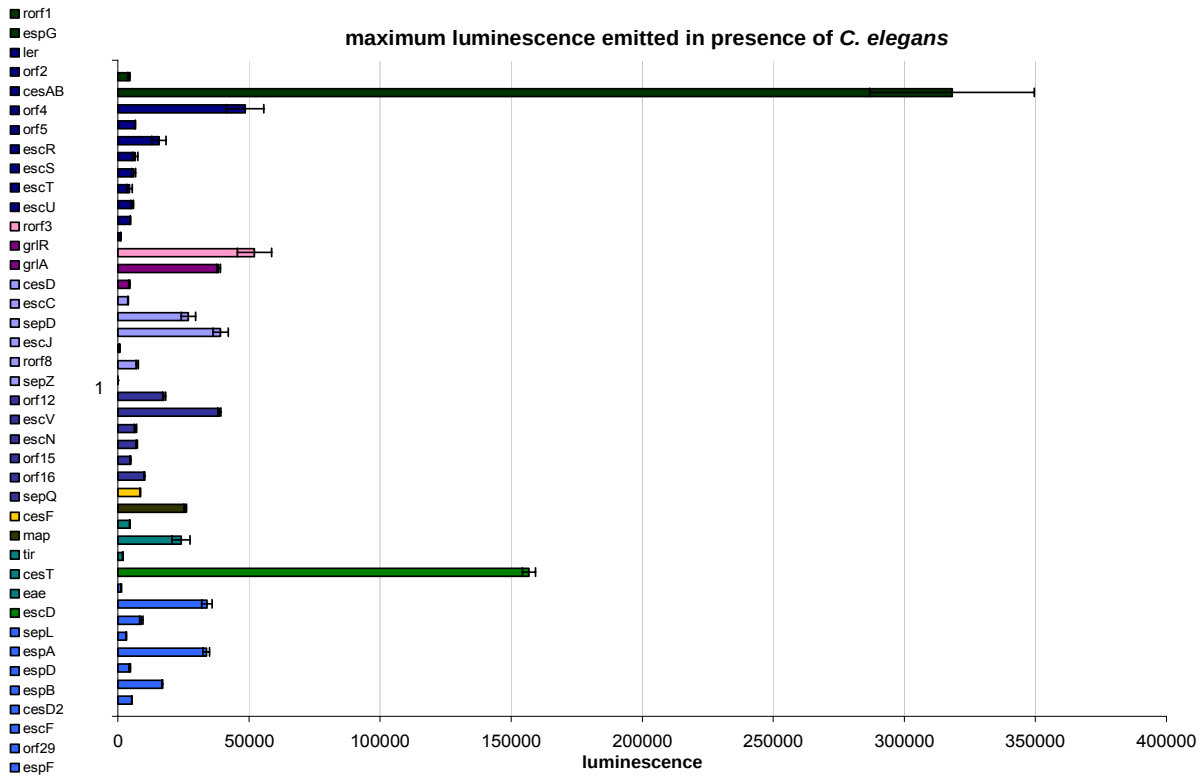


Figure 74: Comparison of the luminescence levels emitted by the different transgenic EHEC at the time point of maximal luminescence. Luminescence measurements in contact with *C. elegans*.

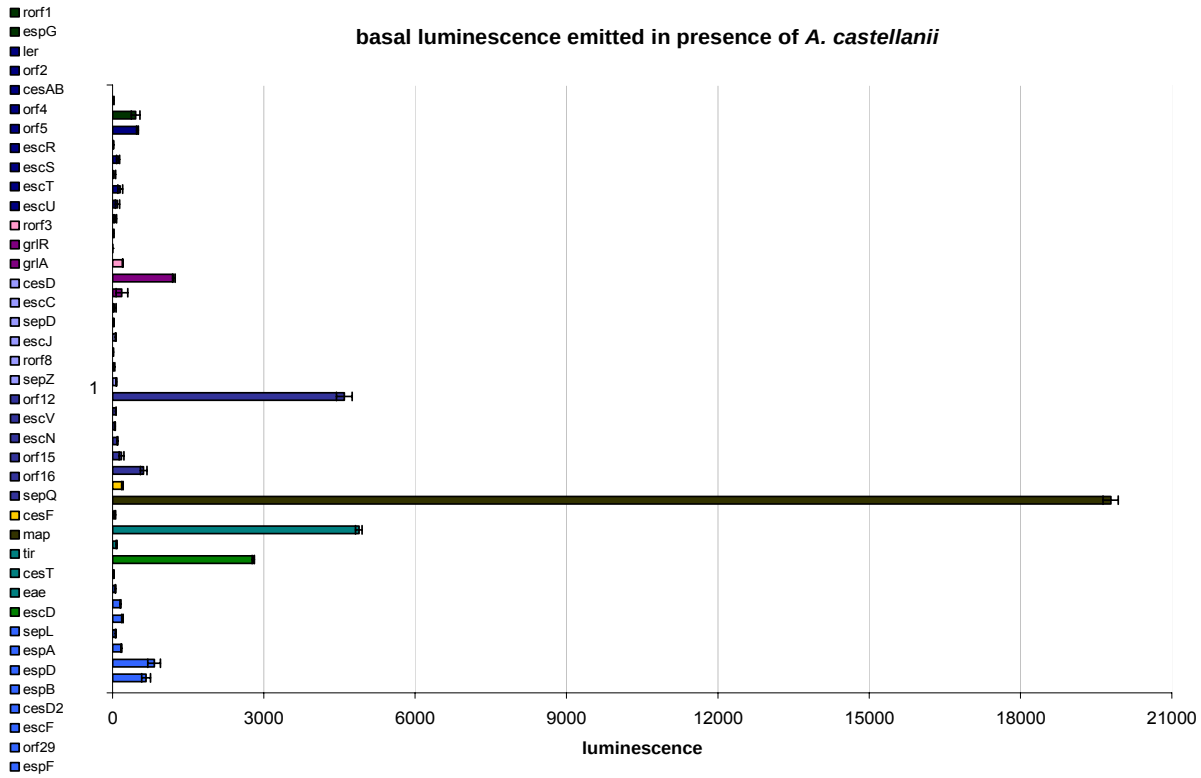


Figure 75: Comparison of the luminescence levels emitted by the different transgenic EHEC at the start of luminescence measurements at t=0h in the control wells without *A. castellanii* in PYG medium supplemented with kanamycin.

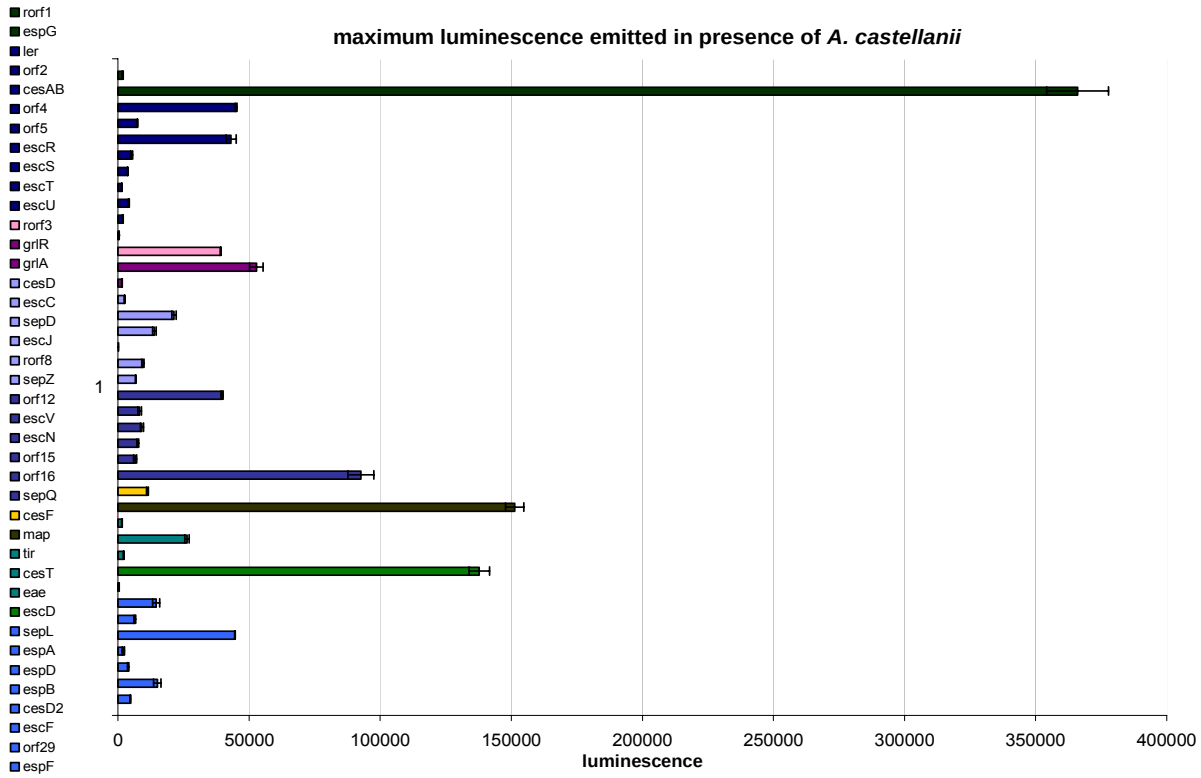


Figure 76: Comparison of the luminescence levels emitted by the different transgenic EHEC at the time point of maximal luminescence. Luminescence measurements in contact with *A. castellanii*.

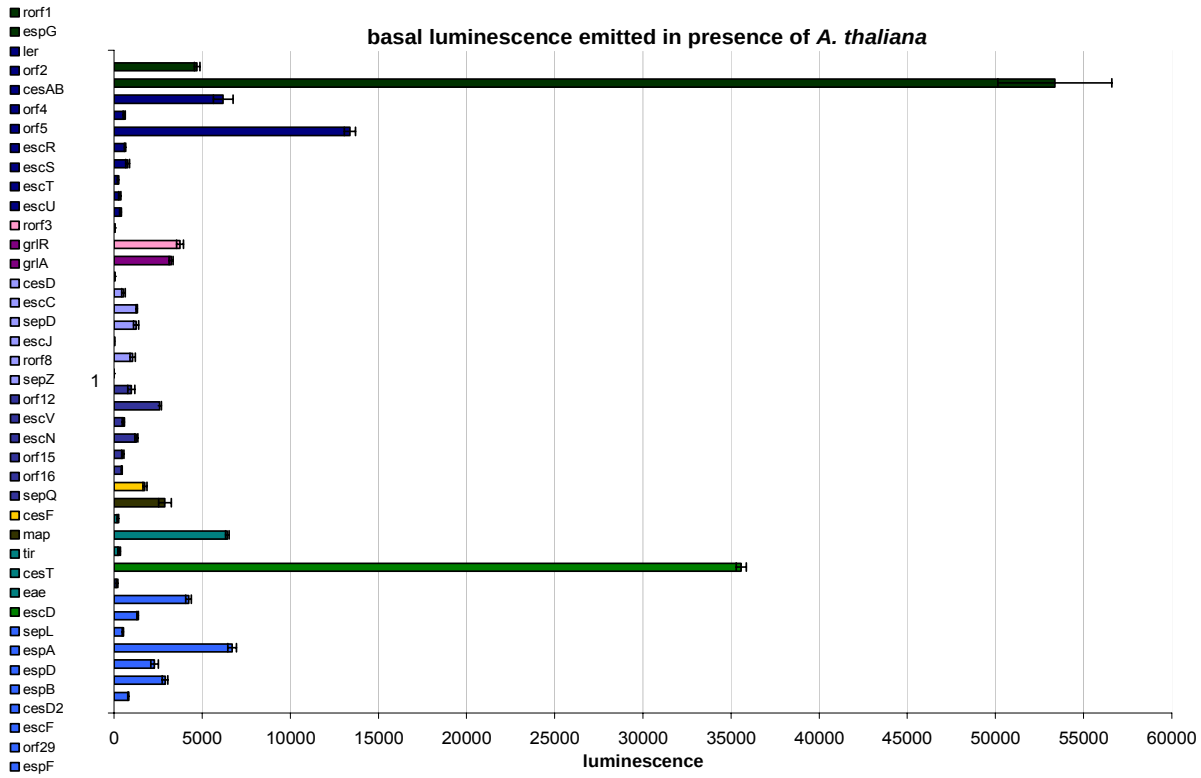


Figure 77: Comparison of the luminescence levels emitted by the different transgenic EHEC at the end of luminescence measurements at t=6h in the control wells without *A. thaliana* in MS medium.

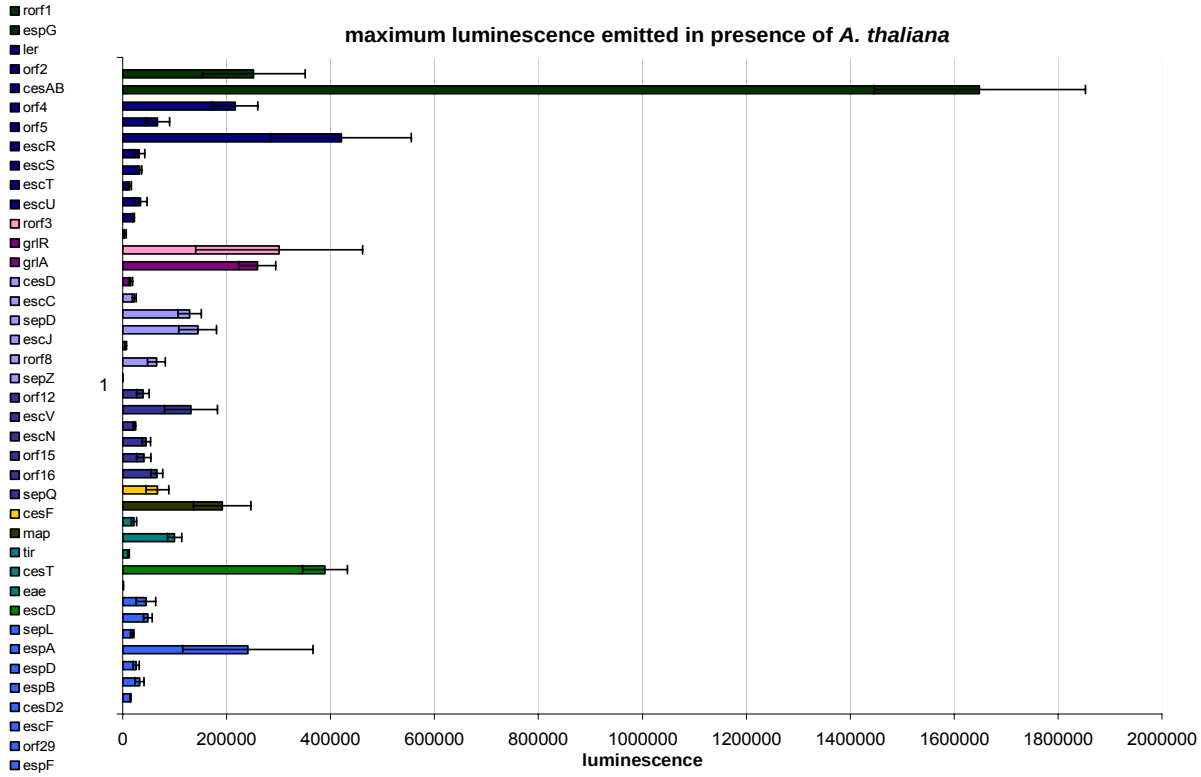


Figure 78: Comparison of the luminescence levels emitted by the different transgenic EHEC at the time point of maximal luminescence. Luminescence measurements in contact with *A. thaliana*.

3.11 Overview Non-LEE genes

The genes not encoded on the LEE submitted to the assays are, in general, not as much affected as those encoded on the LEE. Only seven of the 25 tested genes were activated more than threefold in contact with *C. elegans*, most of them regulatory genes (*bolA*, *mdaA*, *qseB*, *z4982* and *tatA*). In addition to that, *yehD*, a putative fimbrial gene, and *fliC* encoding for a flagellar gene, were affected. No activation of any gene occurred in contact with heat-killed *C. elegans*.

In contact with living *A. castellanii*, only two of the 25 tested genes were upregulated. These were the putative outer membrane component *yehB* and the gene encoding for the B subunit of Shiga toxin I, *stx1B*. In contact with heat-killed *A. castellanii*, 19 of the 25 tested genes were activated more than threefold.

In the *A. thaliana* assay, 17 of the 25 tested non-LEE genes were induced above threshold. In contact with *A. thaliana*, the amount of total luminescence measured was highest, and also the relative activation in comparison to the control measurements without organism was higher than in the other two assays (Fig. 83, 84). This was similar to the results of the tested LEE-genes. In contrast to the tested LEE-encoded genes, there are genes that are not affected by the presence of *A. thaliana* among the genes not encoded on the LEE.

Interestingly, *hlyE*, encoding for a putative pore forming haemolysin, is only affected in presence of *A. thaliana*. In contact with *C. elegans* and *A. castellanii*, this gene seems to be repressed in comparison to the control measurements.

No tested promoter region was activated by contact with Caco-2 cells (data in supplementary).

gene number	gene name	*A. castellanii* assay		*C. elegans* assay		*A. thaliana* assay
		alive	dead (50°C)	alive	dead (40°C)	
z0012		-	+	/	-	+
z0034	*lytB*	-	/	-	-	+
z0301	*crl*	/	+	-	-	+
z0539	*bolA*	/	+	+	-	-
z0573	*hha*	/	+	/	-	/
z1078	*mdaA*	/	+	+	-	-
z1464	*stx2A*	/	+	/	-	+
z1944	*hlyE*	--	-	--	-	+
z2030	*tonB*	/	+	/	-	-
z2170	*marA*	/	+	/	-	-
z2433	*fnr*	-	+	/	-	+
z2745	*rplT*	-	/	/	-	-
z2946	*flhD*	/	+	/	-	+
z3012	*fliA*	-	+	-	-	/
z3013	*fliC*	/	+	+	-	+
z3276		/	+	/	-	+
z3277	*yehB*	+	+	/	-	+
z3279	*yehD*	/	+	+	-	+
z3343	*stx1B*	+	+	/	-	+
z4326		/	+	/	-	+
z4377	*qseB*	/	+	+	-	/
z4378	*qseC*	/	+	/	-	+
z4379	*mdaB*	-	/	/	-	+
z4982		-	/	+	-	+
z5358	*tatA*	/	/	+	-	+

Table 5: overview of all genes not encoded on the LEE submitted to the assays and their activation in contact with *C. elegans*, *A. castellanii* and *A. thaliana*. +: activation above threshold (threefold activation in comparison to control without tested organism); -: no activation; /: activation below threshold; --: possible repression

The following graphs show the total level of luminescence emitted by the several transgenic EHEC at the time point of highest luminescence measured, which is different for each construct, and at the time point of lowest luminescence measured, which was the same for all constructs. Time points taken are the same as in chapter 3.10 for the different assays.

All Bars shown results from the same assay; standard deviations in these graphs do not result from three independent measurements but from the three wells (eight in the *A. thaliana* assay) loaded with one transgenic EHEC strain each.

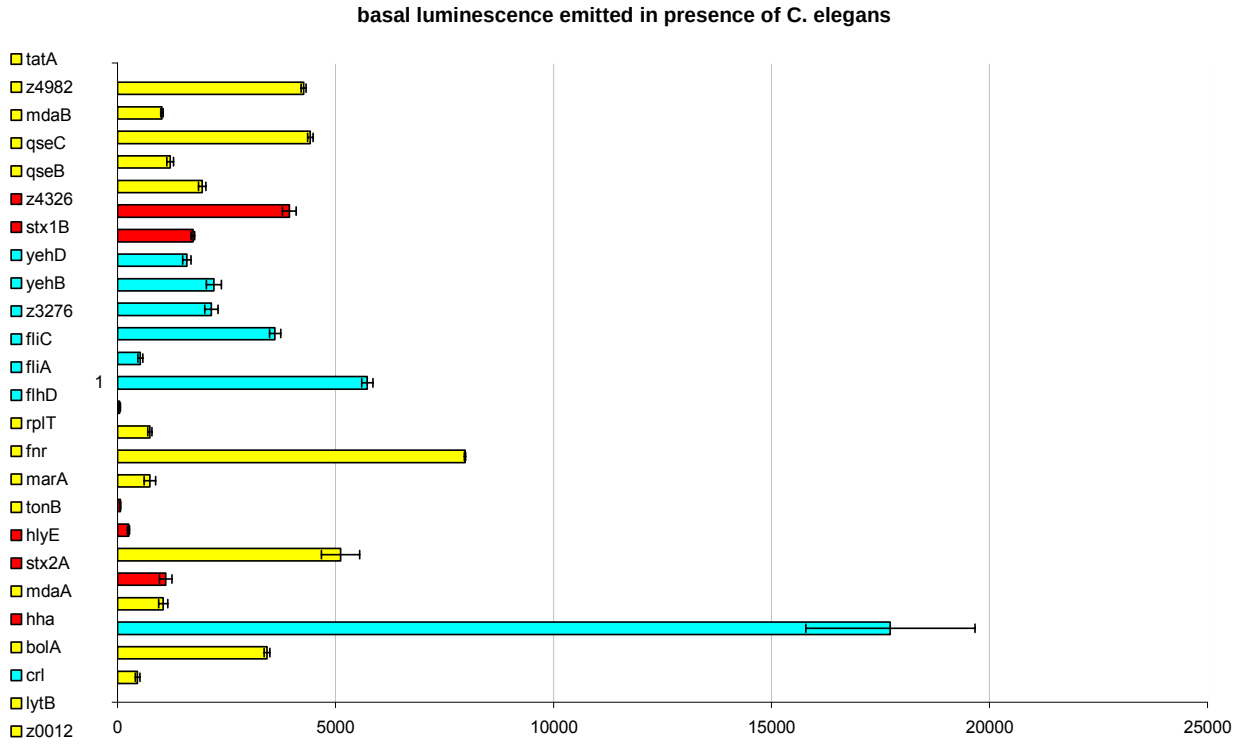


Figure 79: Comparison of the luminescence levels emitted by the different transgenic EHEC at the end of luminescence measurements at t=6h in the control wells without *C. elegans*. Red: genes with virulence related functions; turquoise: genes with membrane structure related functions; yellow: genes with regulatory functions.

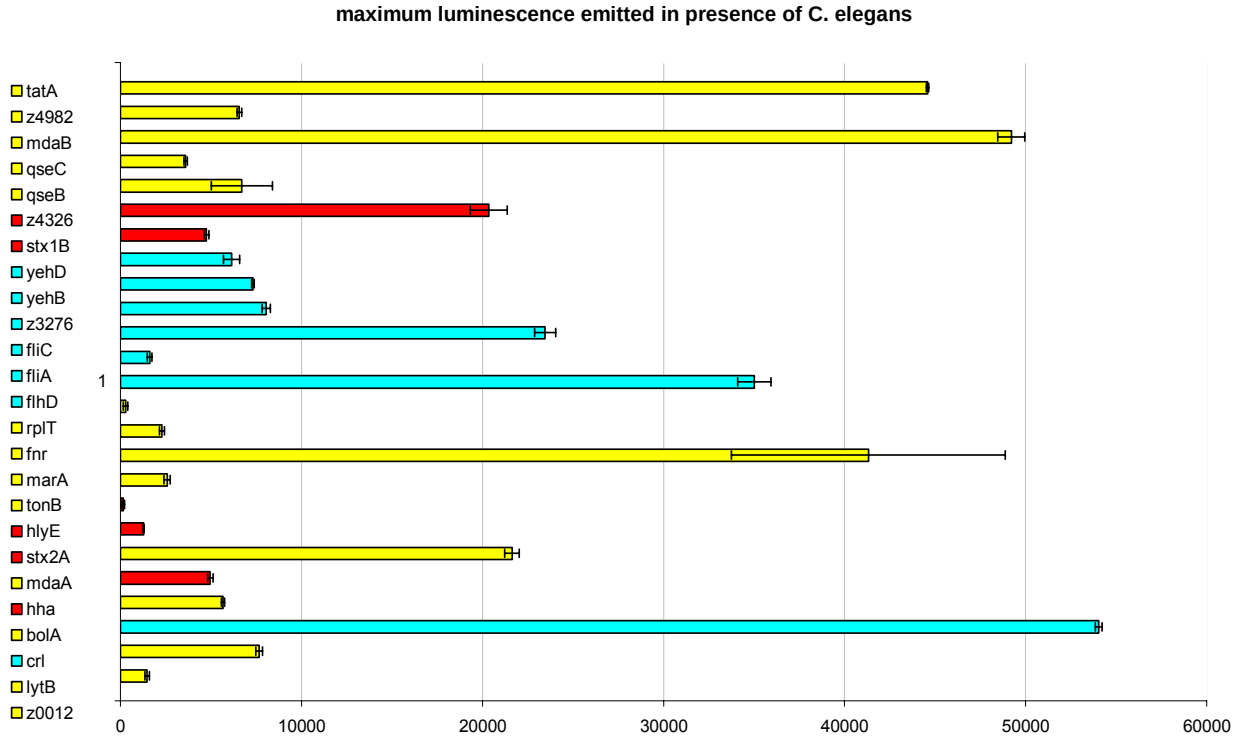


Figure 80: Comparison of the luminescence levels emitted by the different transgenic EHEC at the time point of maximal luminescence. Luminescence measurements in contact with *C. elegans*. Red: genes with virulence related functions; turquoise: genes with membrane structure related functions; yellow: genes with regulatory functions.

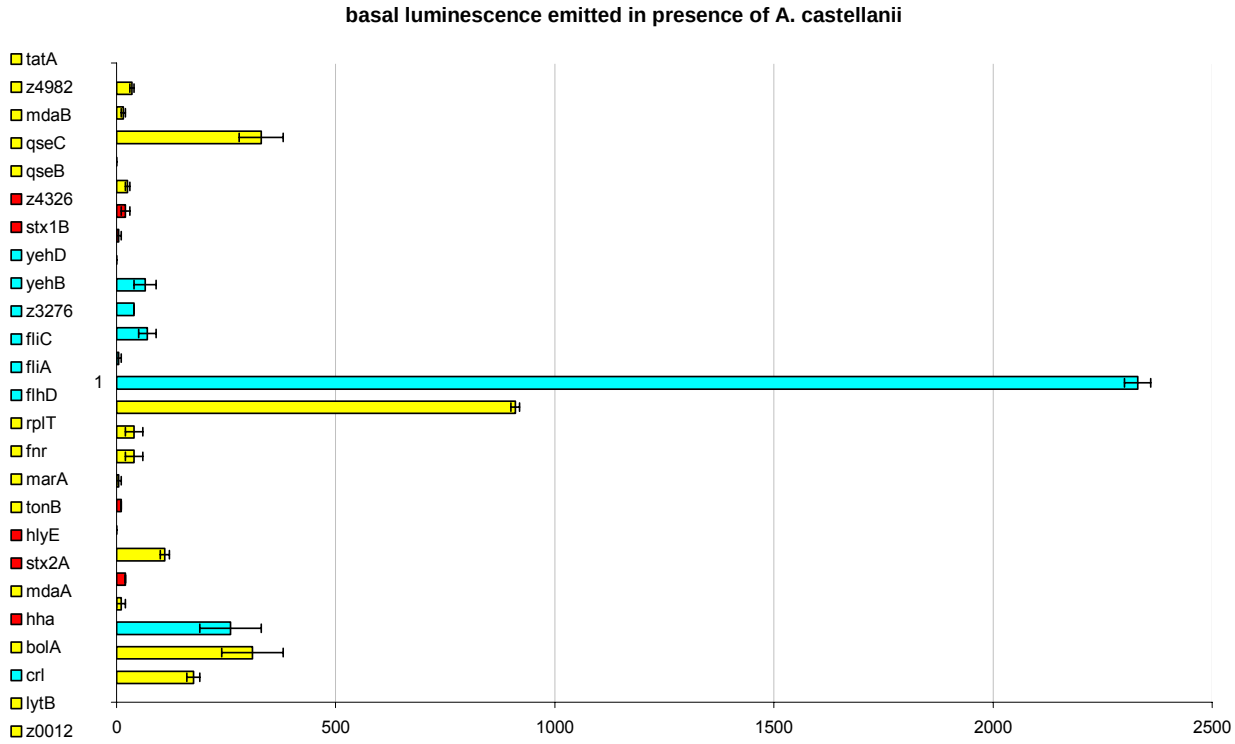


Figure 81: Comparison of the luminescence levels emitted by the different transgenic EHEC at the start of luminescence measurements at t=0h in the control wells without *A. castellanii*. Red: genes with virulence related functions; turquoise: genes with membrane structure related functions; yellow: genes with regulatory functions.

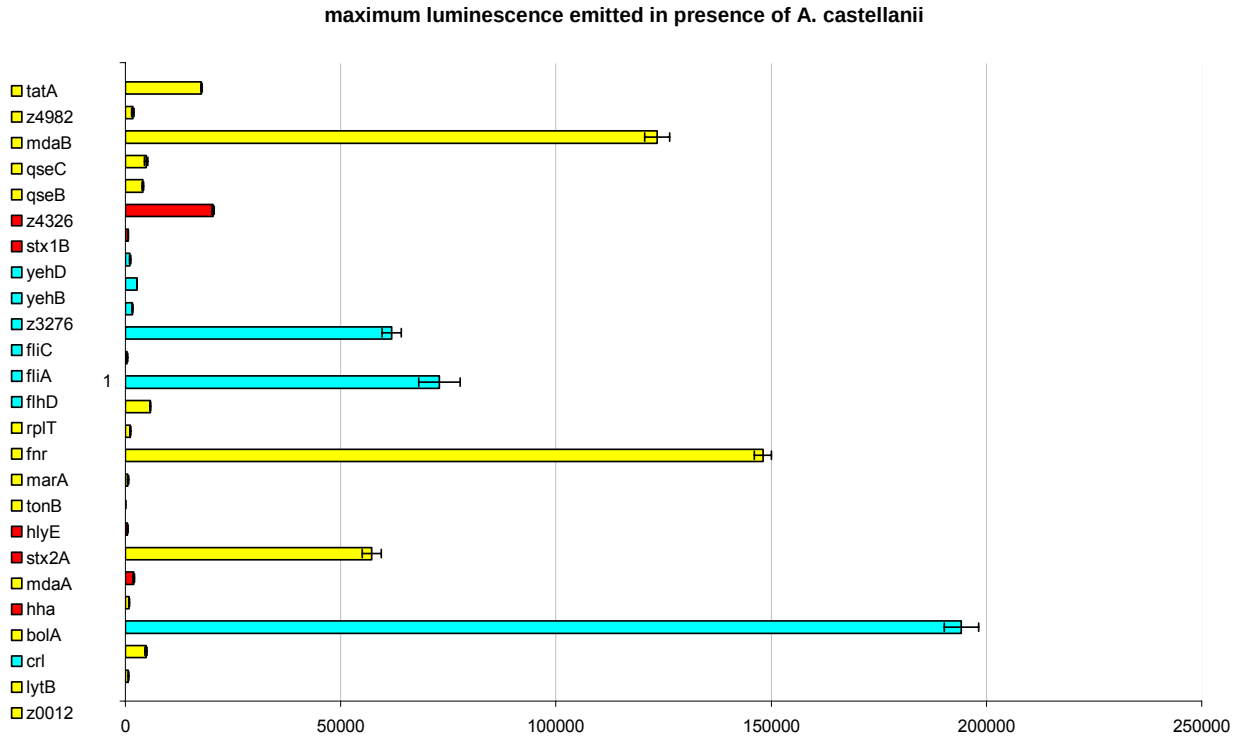


Figure 82: Comparison of the luminescence levels emitted by the different transgenic EHEC at the time point of maximal luminescence. Luminescence measurements in contact with *A. castellanii*. Red: genes with virulence related functions; turquoise: genes with membrane structure related functions; yellow: genes with regulatory functions.

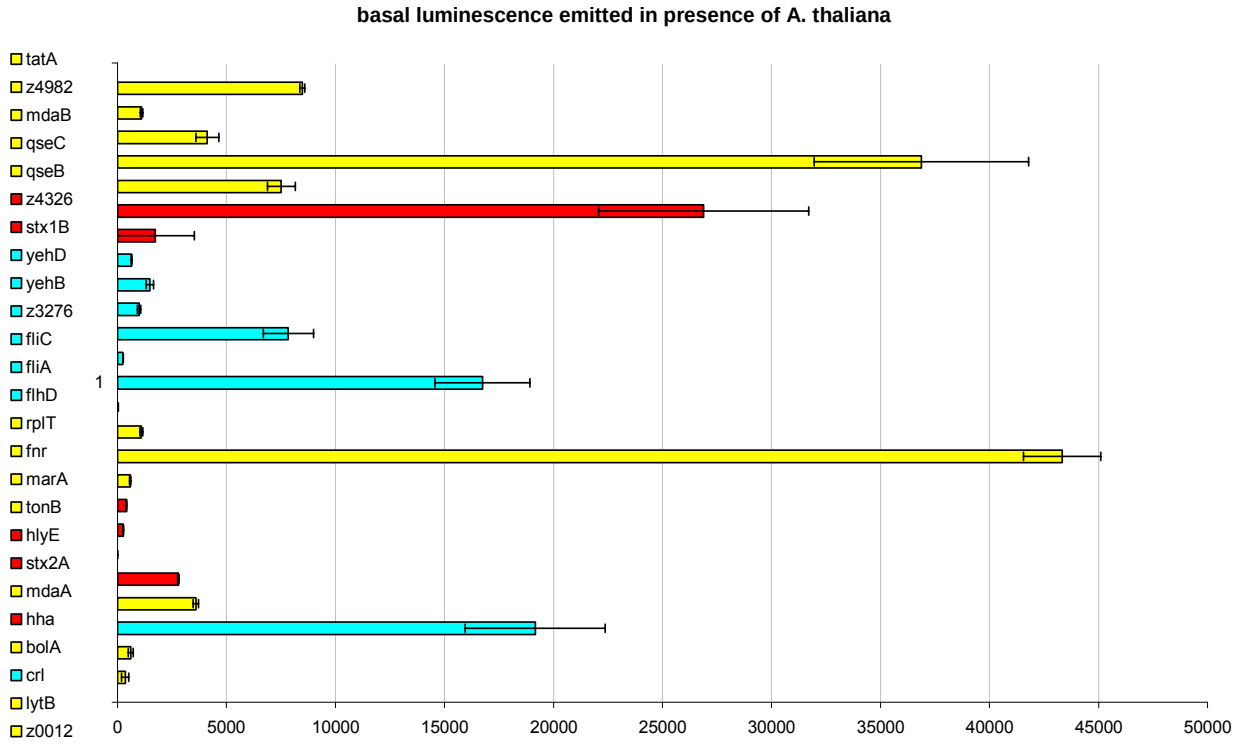


Figure 83: Comparison of the luminescence levels emitted by the different transgenic EHEC at the end of luminescence measurements at t=6h in the control wells without *A. thaliana*. Red: genes with virulence related functions; turquoise: genes with membrane structure related functions; yellow: genes with regulatory functions.

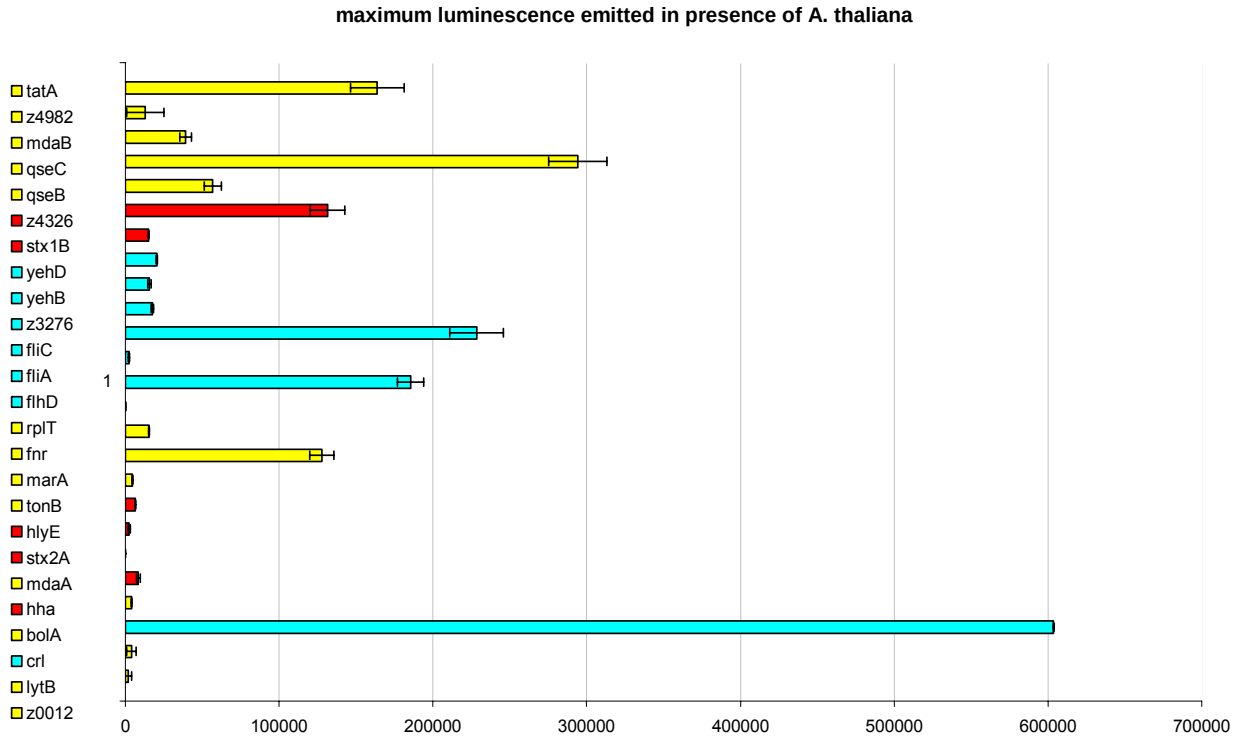


Figure 84: Comparison of the luminescence levels emitted by the different transgenic EHEC at the time point of maximal luminescence. Luminescence measurements in contact with A. thaliana. Red: genes with virulence related functions; turquoise: genes with membrane structure related functions; yellow: genes with regulatory function.

4 Discussion

4.1 Activation of LEE genes

In this study, activation of promoters in the 100bp upstream region of every gene of the locus of enterocyte effacement has been shown. This indicates that, in addition to the transcription of the operons of the LEE, there is also a regulation of gene activity on the single gene level. This has already been shown for the first gene of the LEE4 operon, *sepL*, which is transcribed monocistronically and probably not a part of an operon at all [Kresse et al. 2000]. From our findings, we conclude that in EHEC there is the possibility to regulate LEE gene transcription monocistronically in order to be able to adapt gene expression more exactly to changes in the environment. Our findings also in most cases show that the 100bp upstream promoter region of the first gene of each operon shows the strongest reaction to the tested environmental conditions when compared to the downstream genes of the same operon. This indicates that the more important way of regulation of gene expression is the polycistronic transcription of the LEE-operons.

The activation pattern of the LEE genes was different for each tested organism. Surprisingly, the overall strongest reaction was observed in contact with the thale cress *Arabidopsis thaliana*, where every single gene of the locus of enterocyte effacement was activated more than threefold compared to the control measurements without *A. thaliana* (see Tab. 4). Obviously, EHEC produce additional TTSS and secreted effector proteins in contact with this plant. This indicates the possibility that the EHEC TTSS is able to perforate plant cell walls and inject effector proteins into the plant tissue. Up to now, this is thought not be possible, as plant pathogenic bacteria like *Pseudomonas syringae* have a TTSS which encodes for structurally different TTSSs with longer needles than bacteria with mammalian hosts like EHEC [Journet et al. 2003]. A possible explanation for this phenomenon could be a mutation of the molecular ruler determining the needle length of the TTSS. In addition to that, the cell walls of very young plants are not as thick as those of older specimens. Perhaps the one week old *A. thaliana* used in this study still have cell walls thin enough to be penetrated by the EHEC needle.

In contact with the soil nematode *Caenorhabditis elegans*, all genes of the LEE except for one were upregulated as well (Tab 4). From this finding I conclude that the mechanism of EHEC infection in the nematodes might be similar to that found in the colon of mammalian hosts. This has also been proposed by other works, suggesting *C. elegans* as a model host organism for human infections [Aballay and Ausubel 2002].

Direct contact with the waterborne protozoa *Acanthamoeba castellanii* had a different effect on EHEC. There was also an upregulation of certain LEE-encoded genes observed, but the activation appeared much weaker than in the *C. elegans* and *A. thaliana* assays. In addition to that, there were important genes that remained unaffected by the presence of *A. castellanii*. These include the intimin gene *eae* and almost all genes encoding for the TTSS secreted effector proteins (Tab. 4). This leads us to the conclusion that, in contact with *A. castellanii*, the virulence related genes of the LEE PAI are not activated in EHEC O157:H7. A possible explanation for this could be a different type of interaction between EHEC and the amoebae. While *C. elegans* and *A. thaliana* can be considered as hosts for EHEC both showing an EHEC-dependent phenotype [Anyanful et al. 2005], *A. castellanii* might not be an adequate host for EHEC. That would make it necessary for the bacteria to activate defensive mechanisms rather than virulence-related genes like those encoded on the locus of enterocyte effacement in order to protect the EHEC from grazing amoebae. As the transcription of most of the structural components of the LEE-TTSS is upregulated in contact with *A. castellanii* anyway, there is still the possibility that, in contact with the amoebae, the LEE-encoded TTSS is still needed, e.g. for secretion of other, perhaps more defensive effector proteins that are not encoded on the LEE-PAI.

There could no induction of any tested gene be observed in contact with Caco-2 cells. As both the control without Caco-2 cells and the tested strain produced growing amounts of luminescence in this assay, I conclude that activation of virulence factors in the human colon might not be induceable by the presence of the host cells themselves, but by the conditions EHEC find in the colon (37°C, 5% CO^2). As these were the same for the control wells, no differences between control and test promoter region could be observed. The thesis that LEE-gene expression in EHEC might be stimulated not by cell contact but general environmental conditions has also been proposed by Yona-Nadler *et al.* [Yona-Nadler et al. 2003].

4.1.1 Activation of LEE genes with *C. elegans*

The only LEE gene that was not activated at least three-fold in contact with *C. elegans* is *orf29* (Tab. 4). As the function of this gene has not been determined yet, I conclude that *orf29* does probably not encode for an important structural component of the TTSS. This indicates that, in contact with *C. elegans*, the production and assembly of type three secretion systems is upregulated in EHEC. So, the TTSS mediated virulence pathway affecting the actin assembly of the host cells seems to play an important role in *C. elegans* virulence. Obviously, EHEC cells are able to adhere to and survive in the *C. elegans* gut [Kenney et al. 2005], which makes the nematode a promising candidate for an additional host organism that could

also serve as a vector for EHEC, transmitting the bacteria from one cattle herd to another. The bacteria that are transmitted by *C. elegans* could even be more virulent for mammalian hosts than EHEC originating from other sources, as my findings indicate that they might have an enhanced number of type three secretion systems on their surface compared to EHEC cells that have not been transmitted by *C. elegans*.

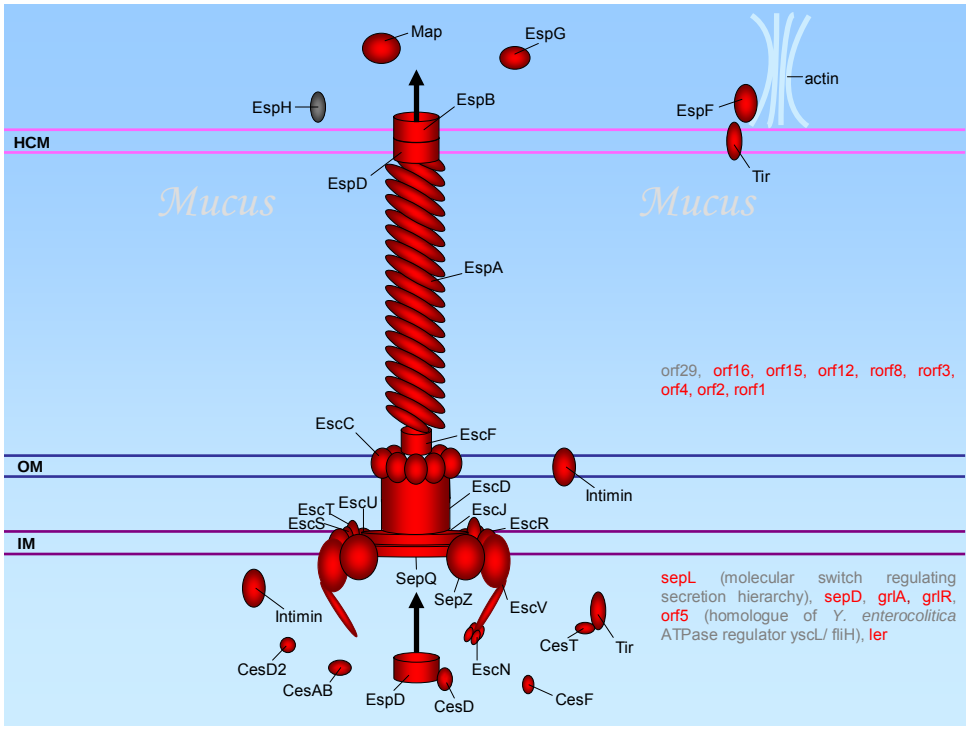


Figure 85: schematic picture of the EHEC-TTSS; genes upregulated in contact with *C. elegans* are depicted in red, genes not affected are depicted in grey. Genes of unknown function are written at the side of the TTSS, regulators below the inner membrane. EspH could not be submitted to the test.

A comparison of the amount of emitted luminescence in the background of the *C. elegans* assay shows that the gene with the highest amount of transcription activity in this assay was *espG*, encoding for a translocated effector protein. This was true with and without nematodes present (Fig. 73, 74). That indicates that the transcription of *espG* could either be activated by general changes of growth conditions or that the *espG* gene product is extremely unstable and has to be produced in higher amounts than the other LEE-encoded genes.

The amount of luminescence emitted by the LEE encoded promoter-*luxCDABE* fusions was, in general, increased about tenfold in the presence of *C. elegans*, while the pattern of activation among the different LEE-genes itself did not change remarkably when *C. elegans* were added (Fig. 73, 74). This is consistent with our finding that the whole LEE PAI seems to be affected by contact to the nematodes resulting in an increased production of TTSSs.

These findings indicate that *C. elegans* are infected by EHEC in a way involving the genes of the LEE PAI and therefore probably attaching and effacing lesions of *C. elegans* gut cells.

4.1.2 Activation of LEE genes with *A. castellanii*

In contact with *A. castellanii*, none of the tested LEE-encoded effectors or chaperones showed an induction above threshold. However, most structural components of the TTSS were activated, (Tab. 4). This indicates the possibility that a contact with *A. castellanii* might enhance the TTSS-production in EHEC, but not for the purpose of LEE-encoded effector secretion. Perhaps the TTSS injectisome is used in a different fashion here, e. g. in a defensive way. The needle could be used to secret proteins that shall protect the EHEC cells from *A. castellanii* phagocytosis. These findings do not give evidence for *A. castellanii* serving as a potent host or vector organism for EHEC O157:H7 involving the genes of the locus of enterocyte effacement.

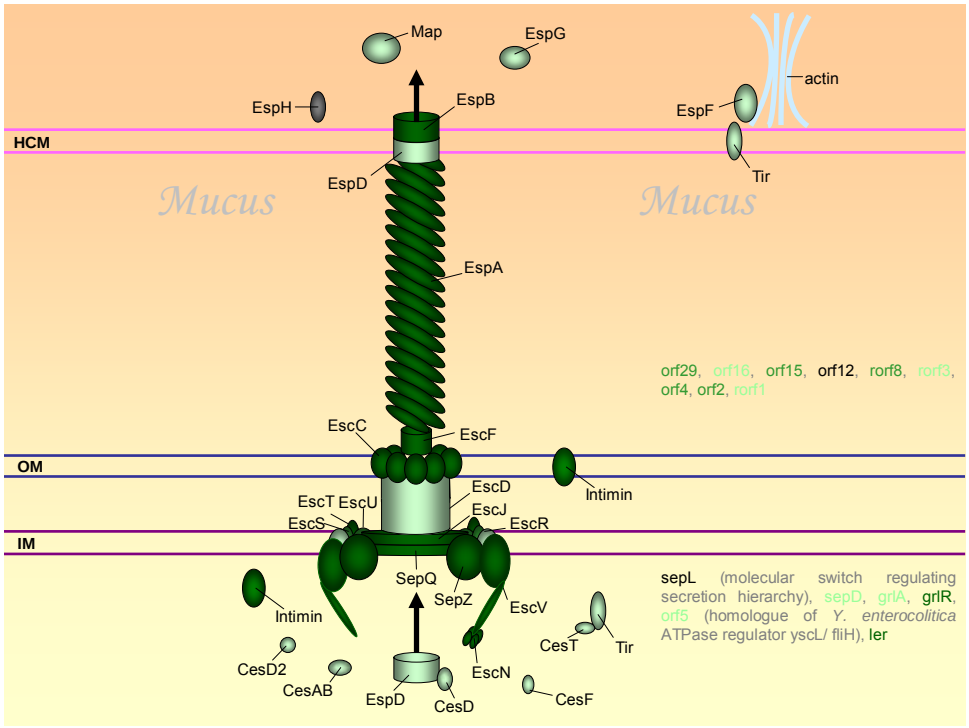


Figure 86: schematic picture of the EHEC-TTSS; genes upregulated in contact with *A. castellanii* are depicted in green, genes that are activated below threshold in light green, genes not affected are depicted in grey. Genes of unknown function are written at the side of the TTSS, regulators below the inner membrane. EspH could not be submitted to the test.

Interestingly, a comparison between the amounts of luminescence emitted in presence or absence of *A. castellanii* in PYG-medium supplemented with kanamycin revealed that the gene with the strongest transcription without *A. castellanii* present was encoding for the secreted effector Map (Fig. 75, 76). When *A. castellanii* were added, the general amount of luminescence increased about 10- to 20-fold. The gene-*luxCDABE* fusion emitting the highest amount of luminescence then was *espG* (Fig. 75, 76). This observation fits the above drawn thesis that *espG* might be activatable by general changes in the environment or nutrition

medium. In contrast to the other assays, the *A. castellanii* assays was conducted in the medium the EHEC cell over night cultures had grown in, as the PYG medium for *A. castellanii* was the only medium used in the assays that EHEC O157:H7 could use as a growth medium. Because of this, the *A. castellanii* assay is the only assay where the bacteria are not under the stress of a change of nutrition medium. The general level of emitted luminescence was very similar to that observed in the *C. elegans* assay, especially for the measurements where test organisms were present (Fig. 73-76).

4.1.3 Activation of LEE genes with *A. thaliana*

In contact with *A. thaliana*, the transcription of every single gene of the locus of enterocyte effacement was upregulated (Tab. 4). In comparison to the experimental setups with *C. elegans* and *A. castellanii*, the effect resulting from contact with *A. thaliana* was strongest. 17 of the tested LEE-encoded promoter regions showed an activation of at least tenfold compared to the control cells without *A. thaliana*. Among these are the genes encoding for the secreted effector proteins Map, EspA and EspG, the regulators GrlA and GrlR, the structural TTSS components SepQ, EscC, EscT, and EscS, the ATPase component of the EHEC TTSS EscN and the chaperone CesAB. Also, some of the genes the function of which has not been determined yet are affected more than tenfold: *orf12* as first gene of the LEE3 operon, *orf2*, *orf15* and *orf29*. Interestingly, both the *rorf3* and the *rorf8* gene product seem to be affected very strongly as well. As mentioned above, in EPEC these two gene products have been shown to interact, being secreted via the general secretion pathway to disrupt the peptidoglycan layer in the periplasm of the host. As EHEC and EPEC are quite similar regarding the LEE effectors, this interaction might also take place in EHEC. All other tested LEE-encoded promoter regions were upregulated at least threefold. These findings were rather unexpected as EHEC O157:H7 to our knowledge has never been shown to act as a plant pathogenic bacterium and the syringe of the EHEC-TTSS is not known to be fit for the penetration of plant cell walls. My findings indicate that there is some virulence related interaction between EHEC and *A. thaliana* anyhow, involving the EHEC TTSS. It has already been shown that EHEC are able to adhere to and persist on the surfaces of different plants [Solomon et al. 2003; Solomon et al. 2002b; Torres et al. 2005], but until now, no increased transcription of genes of the EHEC TTSS has been observed in contact with a plant species. But, interestingly, *Salmonella typhimurium* have recently been shown to be pathogenic for *A. thaliana* as well [Schikora 2008].

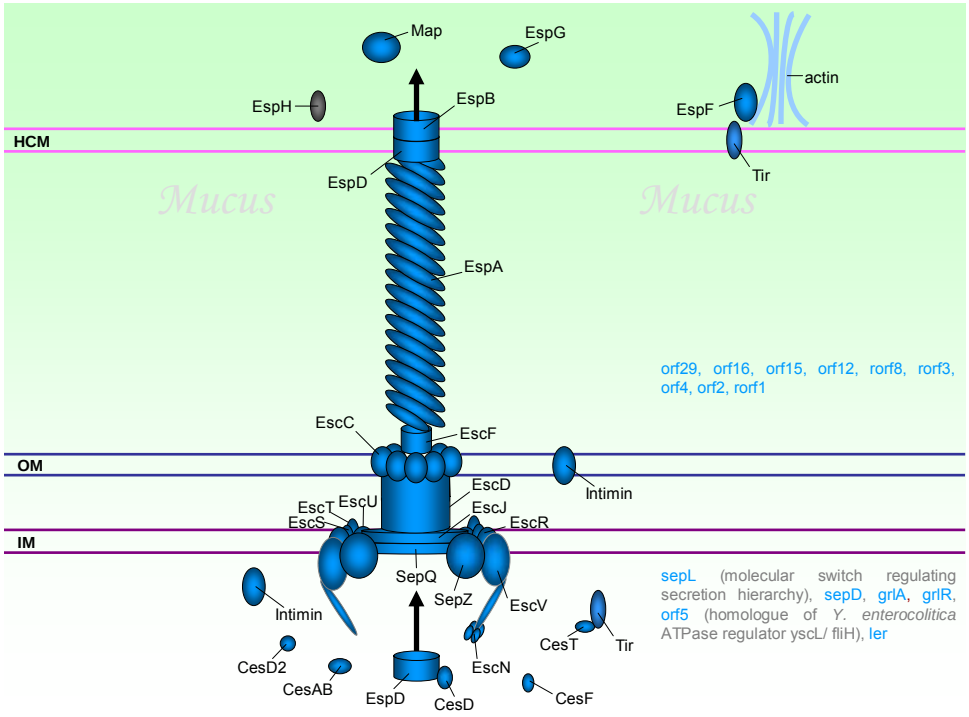


Figure 87: schematic picture of the EHEC-TTSS; genes upregulated in contact with *A. thaliana* are depicted in blue, genes not affected are depicted in grey. Genes of unknown function are written at the side of the TTSS, regulators below the inner membrane. EspH was not submitted to the test.

A comparison of the amount and pattern of activation in presence or absence *A. thaliana* revealed a picture very similar to that described for the *C. elegans* experiment: the promoter region of *espG* was affected most strongly, and the pattern of activation itself did not change remarkably when *A. thaliana* were added (Fig. 77, 78). The amount of activation was increased about 30-fold when *A. thaliana* were present. This is not consistent with the *C. elegans* assays any more, as the luminescence measured with *C. elegans* was only about 10-fold as high as without nematodes (Fig. 73, 74). That indicates that a contact with *A. thaliana* leads to a similar, but stronger genetic response in *E. coli* O157:H7 than contact with *C. elegans*. These results contribute to the thesis that both organisms might serve as alternative hosts for an infection. My observation of an EHEC-dependent phenotype of infected *A. thaliana* plants also indicates this possibility.

4.2 Activation of Non-LEE genes

The genes not encoded on the locus of enterocyte effacement that were submitted to the assays with *C. elegans*, *A. castellanii* and *A. thaliana* did not all have an activatable promoter in the 500bp upstream region of the corresponding start methionin codon. The 500bp upstream region of *rplT* could not induce luminescence to a level of activation above the threshold under any tested circumstance (Tab. 5). The *hlyE* 500bp upstream region even seems to act as a repressor in presence of *C. elegans* or *A. castellanii*, as, in both assays, the luminescence measured was even lower than in the control wells (Tab. 5). The luminescence readout reached in both assays only approximately 50% of the controls, which was also true when heat-killed organisms were tested. In the *A. thaliana* assay, however, the same 500bp region seems to act as a promoter. These findings indicate that there is a regulatory region in the tested 500bp fragment that can act either as a repressor or as an activator. One explanation for this could be a dose-dependent regulation of *hlyE* that depends on the amount of some unknown ligand which binds to the regulatory region.

In the *C. elegans* assay, the pattern of activation of non-LEE genes did not change very much when nematodes were present (Fig. 79, 80). In contrast to the assays conducted with the LEE-genes, the total amount of luminescence did not change that remarkably (only about twofold).

In contact with *A. castellanii*, the pattern of activation among the tested non-LEE genes changed remarkably, and also the level of the general luminescence increased about 100-fold (Fig. 81, 82). Without amoebae present, in the *A. castellanii* environment, almost no virulence related genes were transcribed strongly. The promoter region with the highest amount of luminescence measured by far was that of *flhD*, which encodes for the master regulator of flagellar proteins biosynthesis [Clarke and Sperandio 2005]. When *A. castellanii* were added, the picture changed: The gene transcribed most strongly now was *crl*, and one of the virulence related genes, *z4326*, encoding for a putative enterotoxin, was transcribed on a much higher level than all other virulence related genes. Crl has been reported to positively regulate the activity of σ^S, which has direct effects on the transcription rate of the *csgBA* operon encoding for the two subunits of curli fibers [Bougdour 2004]. In addition to that, the 500bp upstream regions of *marA*, *mdaA* and *mdaB* showed high levels of activation now (Fig. 81, 82). As those three genes encode for general regulators which have, at least in the case of *marA*, distinct defensive functions, this contributes to the thesis that a contact with *A. castellanii* might induce defensive mechanisms in EHEC in the first place. As the functions of *z4326*

have not been further investigated yet, it is still unclear whether it is really an enterotoxin or not.

Like it has been shown for the LEE genes, the general pattern of the luminescence level with *A. thaliana* changed in a way similar to that observed in contact with *C. elegans* (Fig. 83, 84). There were two exclusions, however: the *marA* construct emitted the highest amount of luminescence in MS medium without plants and was not even among the strongest five constructs when plants were present. In contrast to that, the promoter region of *crl* was emitting the highest amounts of luminescence in contact with *A. thaliana*, while it is only the fourth strongest construct without plants. This is particularly interesting because MarA as an activator of defense systems could be expected to be transcribed more when the environment is changed. The higher level of transcription of *crl* in presence of *A. thaliana* can be explained more easily, as *crl* encodes for a transcriptional regulator for the cryptic *csgA* gene for curli fibers, which are an important virulence factor. This fits the thesis that *A. thaliana* are attacked by EHEC and might serve as an additional host organism.

In contact with Caco-2 cells, the same phenomenon as with the LEE-encoded genes was observed; all wells, including the control wells without Caco-2 cells produced growing amounts of luminescence over a time period of at least 6h.

4.2.1 Activation of Non-LEE genes with *C. elegans*

In contact with *C. elegans*, a total of seven non-LEE genes showed activation above threshold (Tab. 5). The upregulated genes were *bolA*, *mdaA*, *fliC*, *yehD*, *qseB*, *z4982* and *tatA*. Most of these (*bolA*, *mdaA*, *qseB*, *z4982*, *tatA*) have regulatory functions, *fliC* and *yehD* encode for flagellar (*fliC*) and fimbrial (*yehD*) structure proteins.

BolA is a putative regulator for murein genes and general cell morphology [Santos 2002]. This indicates that the promoter region of this gene might be upregulated in contact with *C. elegans* for defensive reasons. It is possible that, in the nematode gut, the EHEC bacteria need a stronger cell wall containing more murein than without any predatory organism present.

mdaA encodes for the Modulator of Drug Activity A, a general drug resistance regulator with tumoricidal functions [Chatterjee 1995]. If this gene is involved in general stress responses, the activation of this promoter region was expectable.

fliC encodes for flagellin, forming the filament structure of flagella. Possibly, in contact with *C. elegans*, more flagella are formed on the EHEC surface. This would enhance the motility of the bacteria and therefore be an important virulence factor. Bacteria would be able to reach their target cells more quickly. In enterohaemorrhagic *E. coli* O113:H21, FliC plays an important role in the invasion of epithelial cells [Luck et al. 2006].

yehD encodes for a putative fimbrial-like protein [Perna et al. 2001]. It is probably upregulated in contact with the nematodes for the same reason as *fliC*.

The Quorum Sensing regulator B, *qseB*, is probably affected because the Quorum sensing system plays a role in regulation of the locus of enterocyte effacement [Sperandio et al. 1999]. In addition to that, QseB has also been found to be involved in the regulation of flagella and motility in EHEC O157:H7 [Sperandio et al. 2002].

The gene product of z4982 is not characterized well yet. It is a putative protein which is able to alter the permeability of the EHEC cell membrane. The transcription of this gene might be activated as a response to the different conditions EHEC meet in the nematode gut.

TatA finally is involved in the *sec*-independent export of proteins. This indicates that there are other protein export machineries than the LEE-encoded TTSS involved in nematode virulence. Additionally, the twin Arginine Translocation System *tatA* is the first gene of has been proven to be an important virulence factor of EHEC, playing a role in flagellin synthesis and Stx1 secretion [Pradel et al. 2003].

Taken together, these results suggest an important role of flagella and maybe other appendices of the bacterial membrane as virulence factors of EHEC O157:H7 in contact with *C. elegans*.

4.2.2 Activation of Non-LEE genes with *A. castellanii*

Only two of the tested genes, *stx1B* and *yehB*, were induced above threshold with living *A. castellanii*, while in contact with heat-killed *A. castellanii*, 19 of the tested genes were activated at least threefold in comparison to the control (Tab. 5). The only promoter region tested here that was not at all affected by the presence of dead *A. castellanii* was the above mentioned 500bp upstream region of *hlyE*.

These findings indicate that, in contrast to the results of the experiments with LEE-encoded virulence factors, the response of EHEC towards *A. castellanii* is not strictly defensive, as *stx1B*, encoding for the β-subunit of Shiga Toxin I, is upregulated in contact with the amoebae. On the other hand, production of Shiga toxins has recently been demonstrated to enhance the survival rate of *Escherichia coli* in the food vacuoles of grazing *Tetrahymena pyriformis* amoebae [Steinberg 2007]. If this mechanism also works in the vacuoles of *A. castellanii*, the upregulation of *stx1B* could still be a defensive reaction to the presence of *A. castellanii*.

Anyhow, the second affected gene, *yehB*, encodes for a putative outer membrane protein of EHEC. As this protein is not further characterized, it remains unclear whether its functions are defensive or offensive.

The finding that far more genes are upregulated in contact with dead *A. castellanii* than with living amoebae is consistent with my results for the LEE-encoded genes. Obviously, EHEC can interact with dead *A. castellanii* much better than with living amoebae, probably feeding on the dead cells, while living *A. castellanii* do not seem suitable as a food source for the bacteria. Whether this is due to the fact that *A. castellanii* phagocyte the EHEC and completely digest them, or the amoebae do simply have the better defense mechanisms is not clear. Interestingly, among the genes that are activated only below threshold in contact with dead *A. castellanii* are *mdaB*, *z4982* and *tatA*. These genes, or genes with similar functions in the case of *mdaB*, have been activated in contact with *C. elegans* (see chapter 4.2.1). In the case of *mdaA* and *mdaB*, it appears possible that those two general stress response proteins might be induceable by different kinds of stress. This would indicate that the general stress response of EHEC towards *C. elegans* and *A. castellanii* might be of the same kind, as both organisms induce an *mdaA*-mediated response in EHEC. This is another fact that strengthens the hypothesis that the interaction observed with *A. castellanii* is of a completely different kind than that with *C. elgans*. This seems to be true also with heat-killed *A. castellanii*. As *mdaB* is transcribed strongly in comparison to the other tested genes in the *A. castellanii* background anyway (Fig. 81, 82), there is probably no need to further activate the transcription of this gene.

There are only two more genes activated below threshold in the *A. castellanii* assay with dead amoebae, *lytB* and *rplT*. LytB plays a role in the stringent response of EHEC and penicillin tolerance, while Rplt is the 50S ribosomal subunit L20, which also has some general regulatory functions.

All other tested genes were activated at least threefold in contact with heat-killed *A. castellanii*, indicating that dead amoebae might serve as a food source for EHEC O157:H7. My findings indicate that *A. castellanii* is no suitable host or vector organism for EHEC and not able to transport or harbour viable EHEC cells for a longer time period because dead amoebae will disintegrate too fast to serve as a vector organism.

4.2.3 Activation of Non-LEE genes with *A. thaliana*

In contact with *A. thaliana*, 17 of the tested non-LEE genes were induced at least threefold. The only virulence-related gene tested here that did not meet the threshold was *hha*. Genes not affected at all were *bolA*, *mdaA*, *tonB*, *marA* and *rplT* (Tab. 5).

hha, encoding for a protein which regulates the expression of EHEC haemolysin, is probably only activated below threshold because the haemolysin does not display any toxic activity against plants and is therefore not needed as a virulence factor in contact with *A. thaliana*.

bolA and *mdaA* were both among the genes affected by contact with the nematode *C. elegans* (see chapter 4.2.1).

BolA plays a role in the regulation of murein genes. In chapter 4.2.1, I concluded that this gene might be upregulated for defensive reasons in order to protect the bacteria against the hostile environment of the nematode gut by rendering the EHEC membrane more stable or change of the whole cell morphology. This theory is consistent with the finding that *bolA* is not activated in contact with *A. thaliana*, because the plants do not possess any digesting proteins or acids that might be dangerous for EHEC in a *C. elegans* gut. Besides that, it is not yet clear whether the bacteria are able to invade the plant tissue at all or the interaction takes place only at the plant surface [Cooley et al. 2003; Solomon et al. 2002a].

MdaA has been proposed to trigger the general drug resistance in contact with *C. elegans* and *A. castellanii*. In the case of *A. thaliana*, this general response seems to be activated via *mdaB*, which has been shown to protect the cells against quinonoid compounds [Adams 2006]. This would make sense because both *C. elegans* and *A. castellanii* prey on EHEC and other bacteria, while *A. thaliana* as a passive plant organism remains inert when attacked by bacteria. The only immediate way of a plant to defend itself against bacterial infections is the secretion of chemicals or proteins that are toxic for the bacteria. This scenario generates a different kind of stress for the EHEC cells than the above mentioned predator-prey relationship. Interestingly, *mdaA* is not only unaffected by the contact with *A. thaliana*, but seems to be barely transcribed at all in the *A. thaliana* background (Fig. 83, 84).

TonB, another unaffected gene in contact with *A. thaliana*, plays a role in the iron uptake of EHEC. Probably, plants are not a good iron source at all, as the cell walls of plants are not that easy to disrupt and plant cells do not contain as much iron as eukaryotic cells.

MarA is an activator of EHEC defense systems and might therefore in contact with *A. thaliana* not be needed for the same reasons as BolA. *marA* is transcribed quite strongly in comparison to the other tested genes in the *A. thaliana* background, however. So, it is also possible that the amount of protein expression is sufficient for the bacteria without further activation of the transcription of this gene.

The ribosomal subunit protein RplT does not seem to be activatable at all by the environmental conditions I tested, as it did not hit the threshold in all assays.

In general, the genetic response of EHEC towards *A. thaliana* was more virulent than those provoked by contact to the other two tested organisms. Only one of the tested virulence genes, *hha*, was not affected in this assay. In addition to that, the whole LEE was activated in the *A. thaliana* assay very strongly in comparison to the other two assays. These findings, in

addition to the observed phenotype that EHEC-infected *A. thaliana* plants developed, lead me to the conclusion that *A. thaliana* might be a potent, yet unknown host organism for EHEC.

4.3 EHEC causes a virulence associated phenotype in *A. thaliana*

To my knowledge, this is the first work to report the ability of the human pathogen EHEC to cause signs of illness in a plant like *A. thaliana*. Recently, similar findings have been reported for the related bacterial species *Salmonella typhimurium* [Schikora 2008]. Here, I demonstrated that the observed phenotype results from an inoculation of the plants with EHEC bacteria, as it could not be observed when non-pathogenic TOP10$^-$ cells or LB medium supplemented with kanamycin were used (Fig. 68-72). This finding is especially striking as the TTSS encoded on the EHEC-LEE is suitable for the penetration of animal cells, which do not have a cell wall. The thick wall of plant cells should not be harmed by the EHEC syringe. Clearly, more work needs to be done here in order to elucidate whether the observed effects are TTSS-mediated or whether other, perhaps still unknown factors, play a role in EHEC plant pathogenicity. However, as suggested by Jutta Ludwig-Müller (TU-Dresden, personal communication), young plant cell lack the intense cell wall network, possibly enabling TTSS delivery of effector proteins into the plant tissue by EHEC. To find out more about this issue, knockout mutants of all LEE-operons and of the whole PAI should be generated and submitted to growth assays with *A. thaliana*. In addition to that, it could be helpful to submit different non-O157 EHEC strains and EPEC bacteria to the same tests. Also, it would be interesting whether the same phenotype can be observed with other plant species like e. g. alfalfa sprouts, crop or lettuce, which have already been shown to interact with EHEC or related bacteria in some fashion. The bacteria are able to persist on the surfaces of these plants for a prolonged time period [Guo et al. 2002; Solomon et al. 2002b; Wachtel and Charkowski 2002; Wachtel et al. 2003; Wachtel et al. 2002b]. As the phenotype caused by EHEC in *A. thaliana* looks different than the phenotype observed in an infection with *Pseudomonas syringae*, it is still possible that the EHEC-generated phenotype is TTSS-independent. However, the main virulence factor of the plant pathogen *Pseudomonas syringae* is a TTSS that is related to the EHEC TTSS encoded on the LEE [Fu et al. 2006; Guo et al. 2005]. Future experiments will have to show whether the TTSS encoded on the LEE is able to penetrate plant cell walls or not, due to unsufficient needle length and stability [Hueck 1998; Mota et al. 2005]. Anyway, a cryptic second TTSS gene cluster has been found in EHEC O157:H7, the regulators of which directly interact with the LEE-encoded TTSS [Zhang et al. 2004]. If some of the structural genes encoded there are functional, it could be possible that

two or even more different types of Type Three Secretion Systems are assembled in EHEC when the components of both clusters are mixed. If this is true, the second TTSS could perhaps encode for a needle structure that is more suitable for the disruption of plant cell walls than the better investigated LEE-encoded TTSS. In addition to that, the second *E. coli* strain AS that was found to be pathogenic for *A. thaliana* has to be further characterized, in order to be able to draw conclusions about the mechanism of virulence and genes involved in the development of the plant phenotype observed here. This could be done by comparing the virulence factors present in both plant pathogenic strains and those by which the two strains differ.

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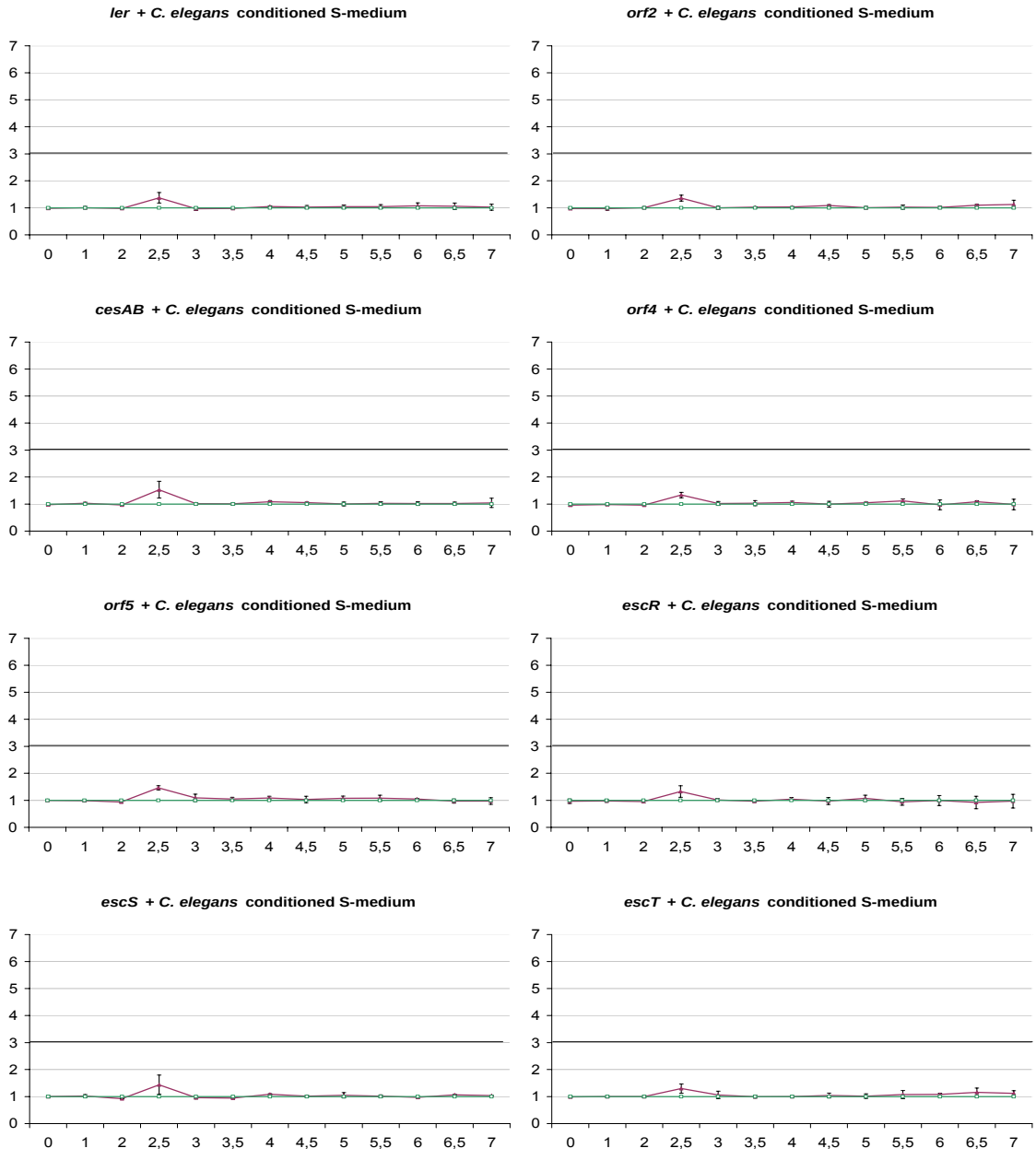
6 Appendix

6.1 Supplementary Data

6.1.1 Promoter-*lux* studies with EHEC and conditioned *C. elegans* medium



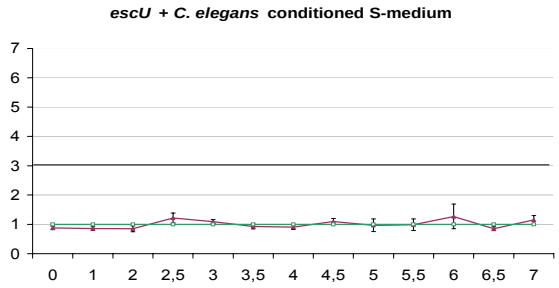


Figure α: activation of 100bp upstream promoter regions of LEE1 genes. *C. elegans* conditioned S-medium was added at t = 2.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *C. elegans*, which is defined 1 for each time point (green line). Violet lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.

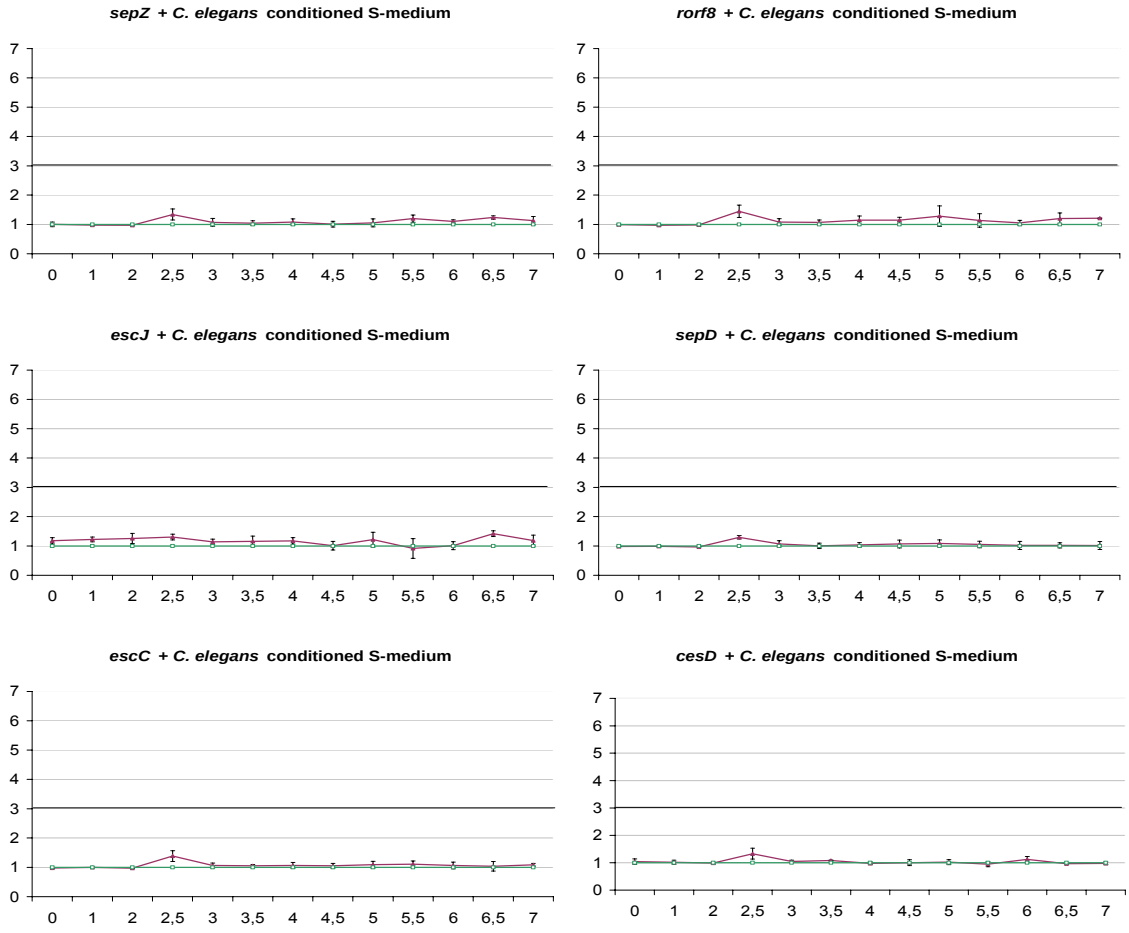


Figure β: activation of 100bp upstream promoter regions of LEE2 genes. *C. elegans* conditioned S-medium was added at t = 2.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *C. elegans*, which is defined 1 for each time point (green line). Violet lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.

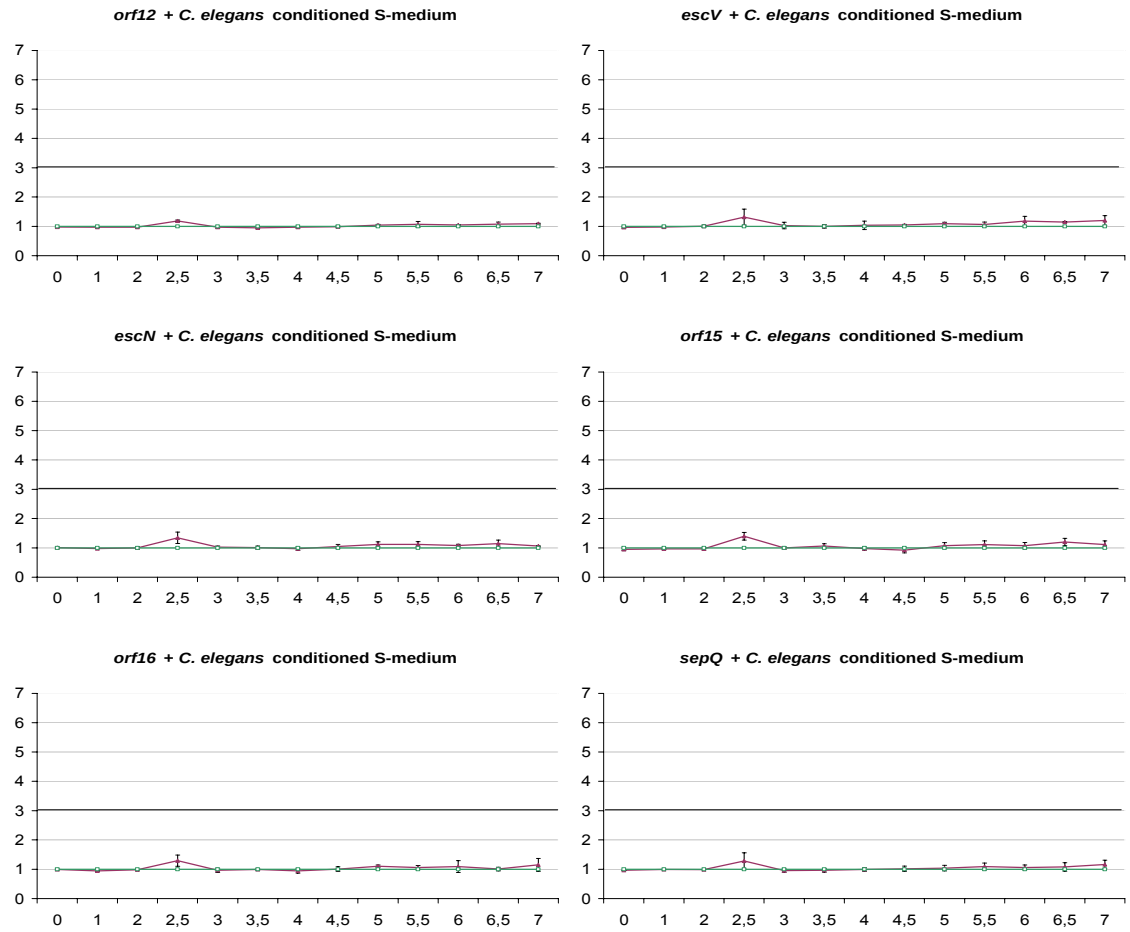
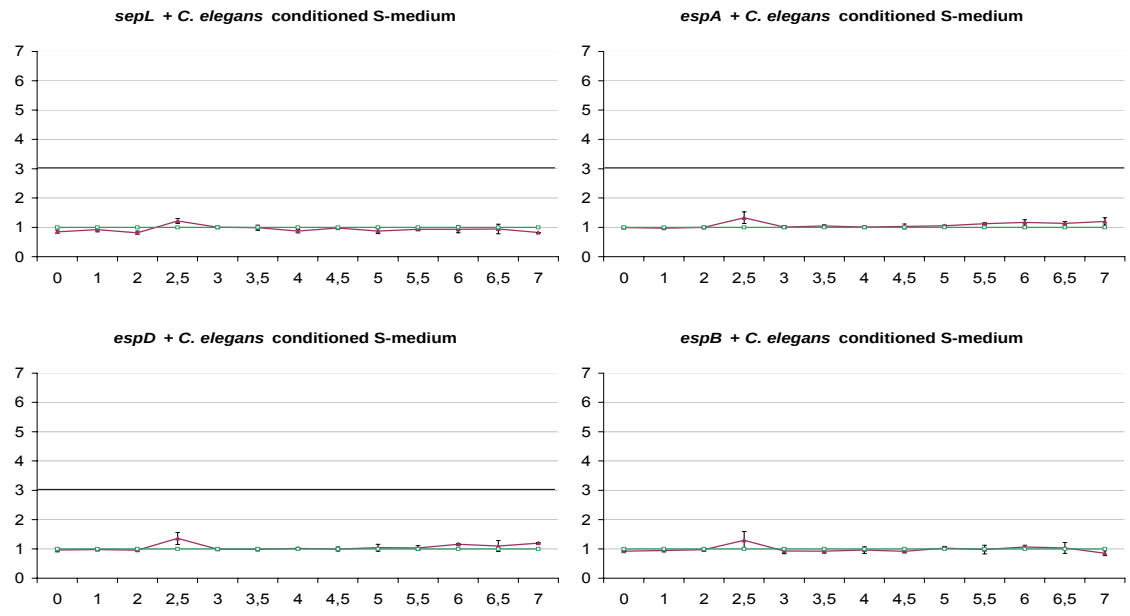


Figure γ: activation of 100bp upstream promoter regions of LEE3 genes. *C. elegans* conditioned S-medium was added at t = 2.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *C. elegans*, which is defined 1 for each time point (green line). Violet lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.



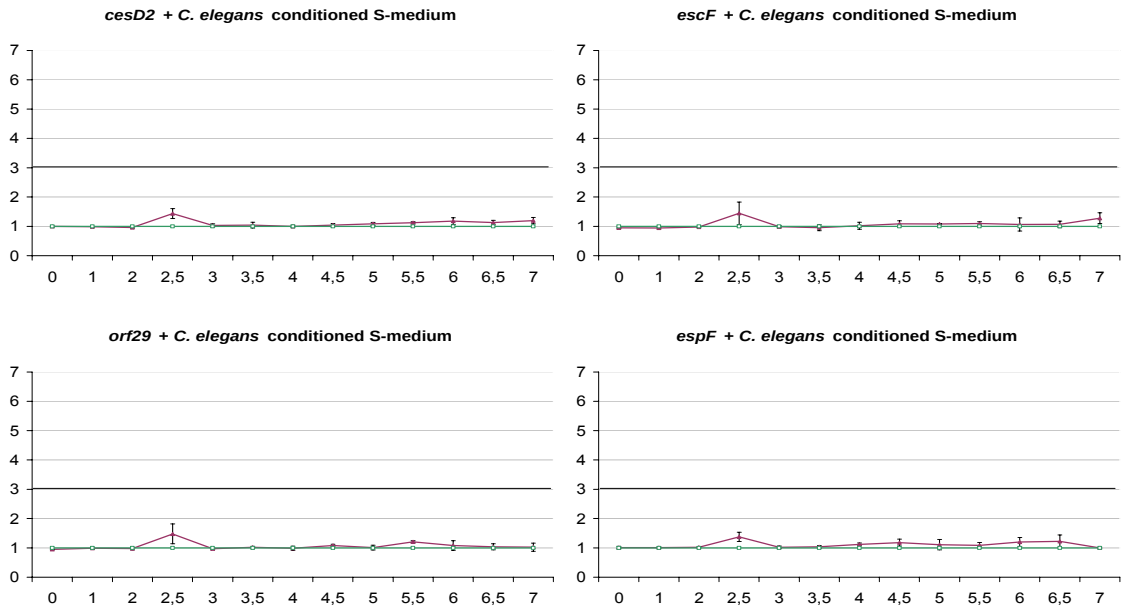


Figure δ: activation of 100bp upstream promoter regions of LEE4 genes. *C. elegans* conditioned S-medium was added at t = 2.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *C. elegans*, which is defined 1 for each time point (green line). Violet lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.

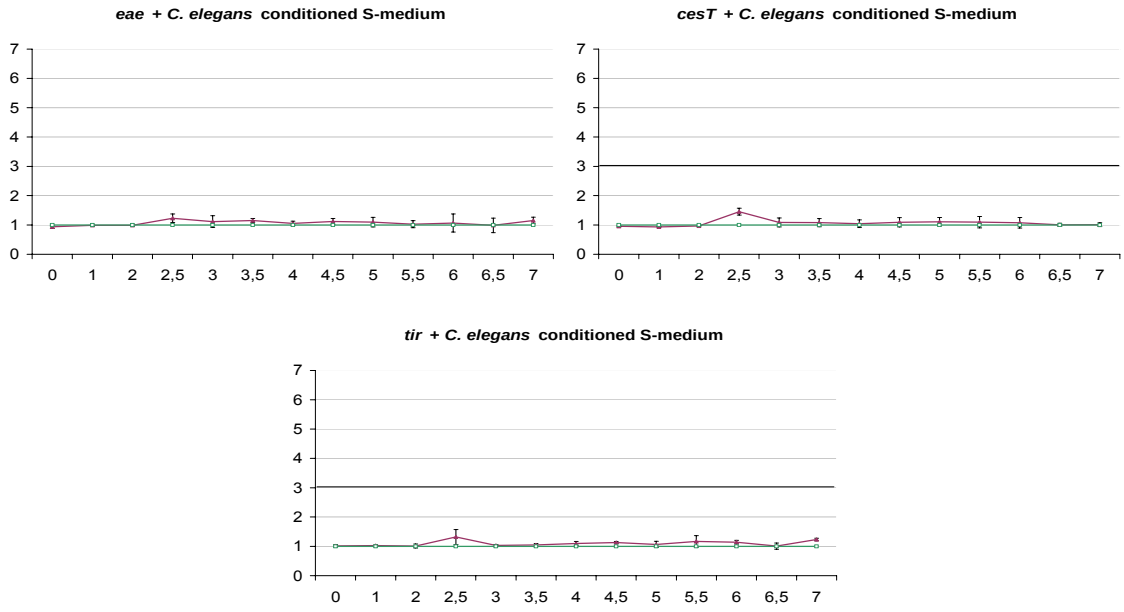


Figure ε: activation of 100bp upstream promoter regions of LEE5 genes. *C. elegans* conditioned S-medium was added at t = 2.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *C. elegans*, which is defined 1 for each time point (green line). Violet lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.

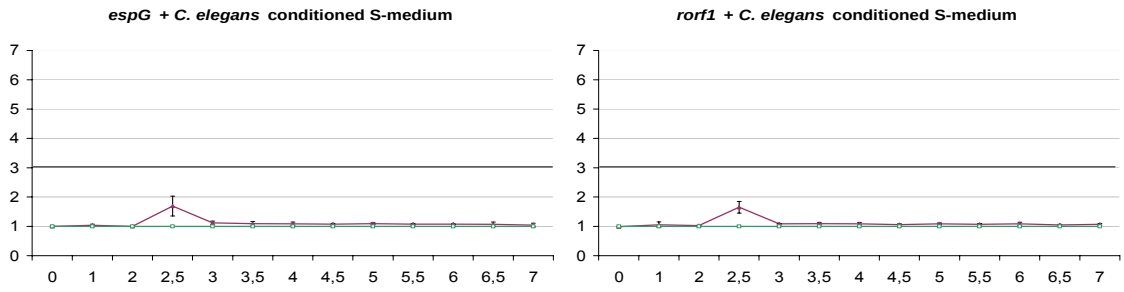


Figure ζ: activation of 100bp upstream promoter regions of espG genes. *C. elegans* conditioned S-medium was added at t = 2.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *C. elegans*, which is defined 1 for each time point (green line). Violet lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.

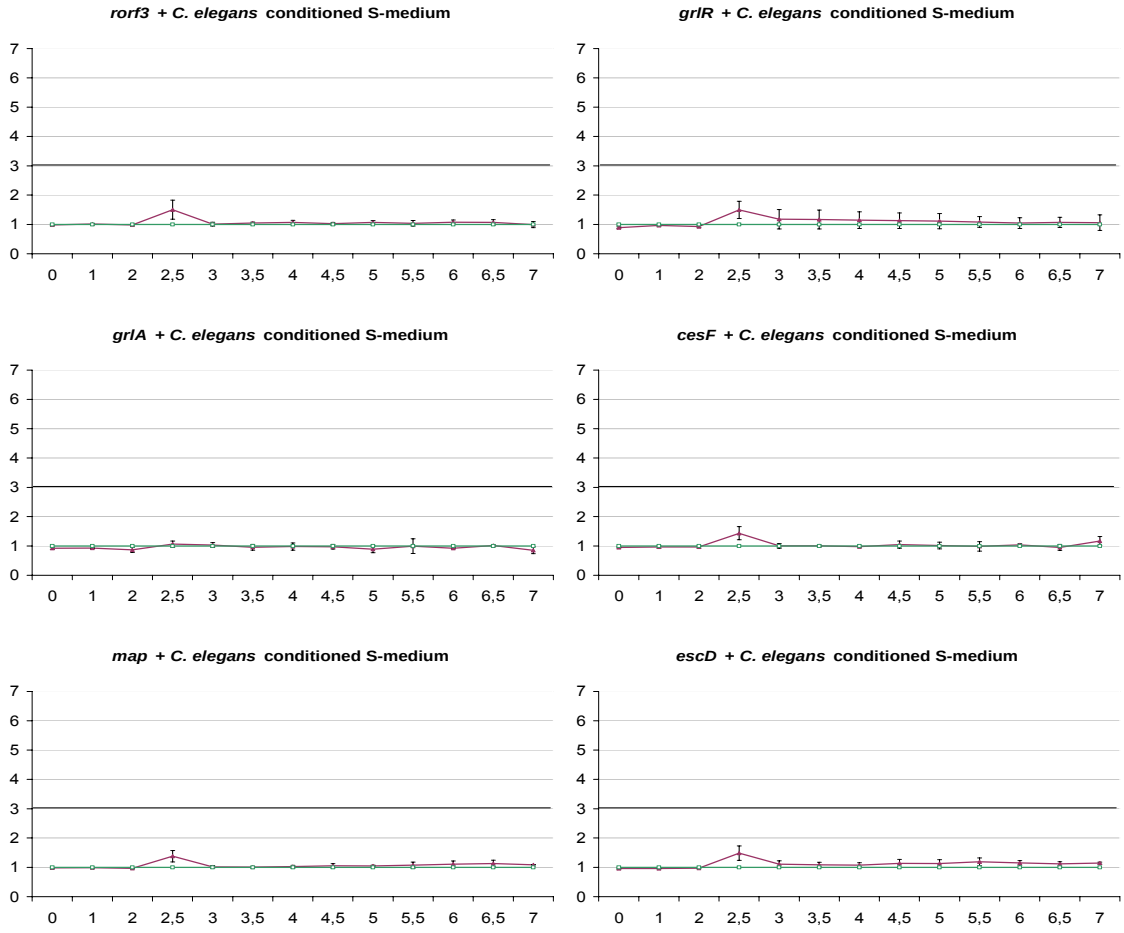


Figure η: activation of 100bp upstream promoter regions of monicistronic LEE genes. *C. elegans* conditioned S-medium was added at t = 2.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *C. elegans*, which is defined 1 for each time point (green line). Violet lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.

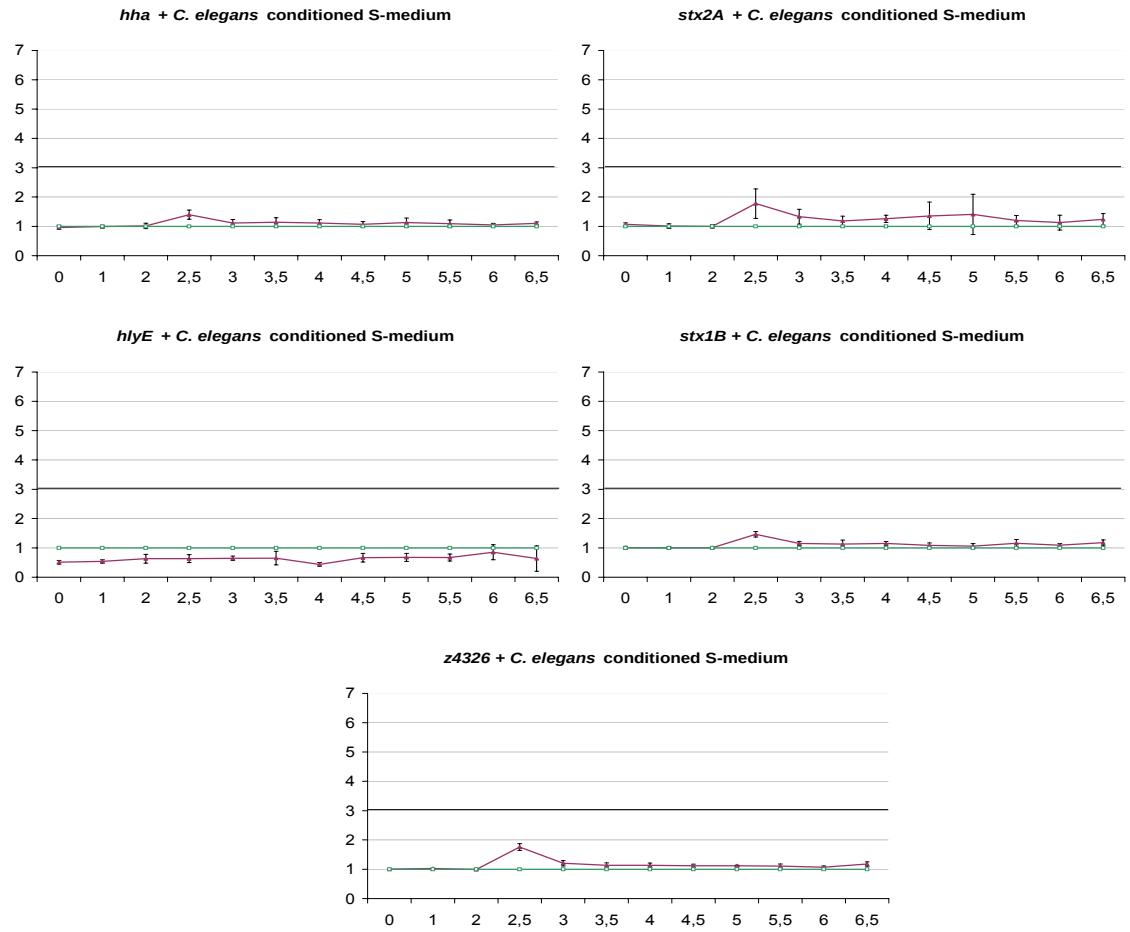
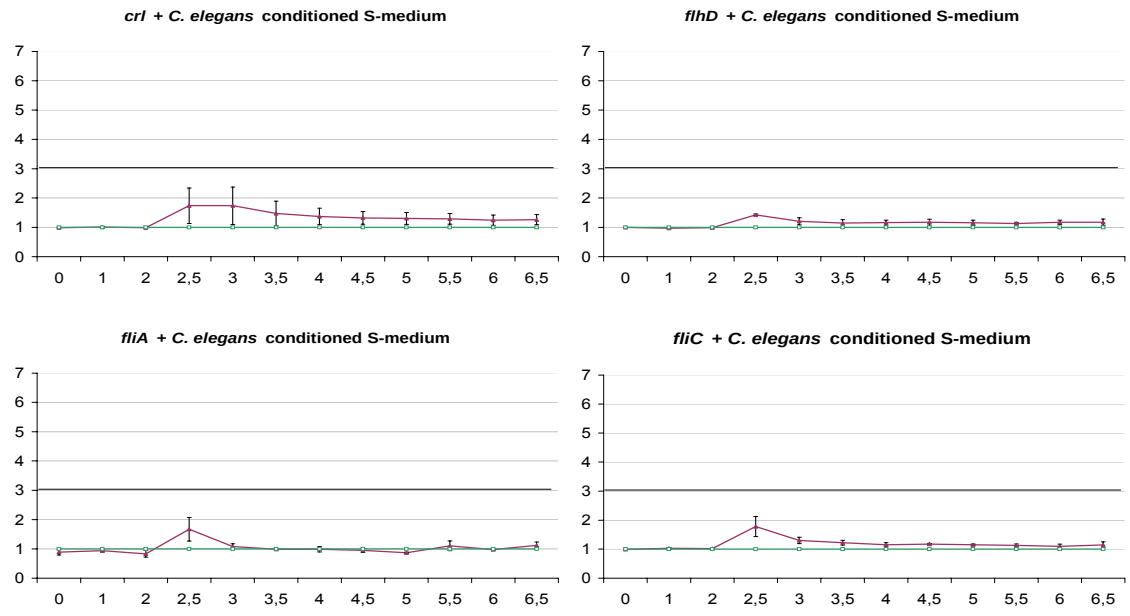


Figure θ: activation of 500bp upstream promoter regions of virulence associated non-LEE genes. *C. elegans* conditioned S-medium was added at t = 2.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *C. elegans*, which is defined 1 for each time point (green line). Violet lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.



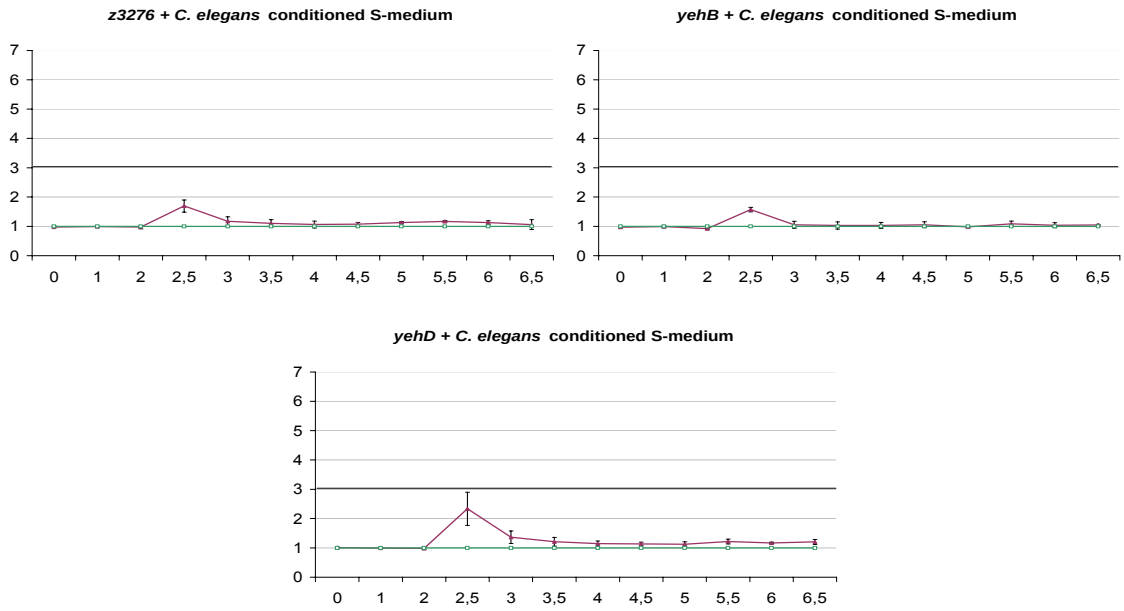
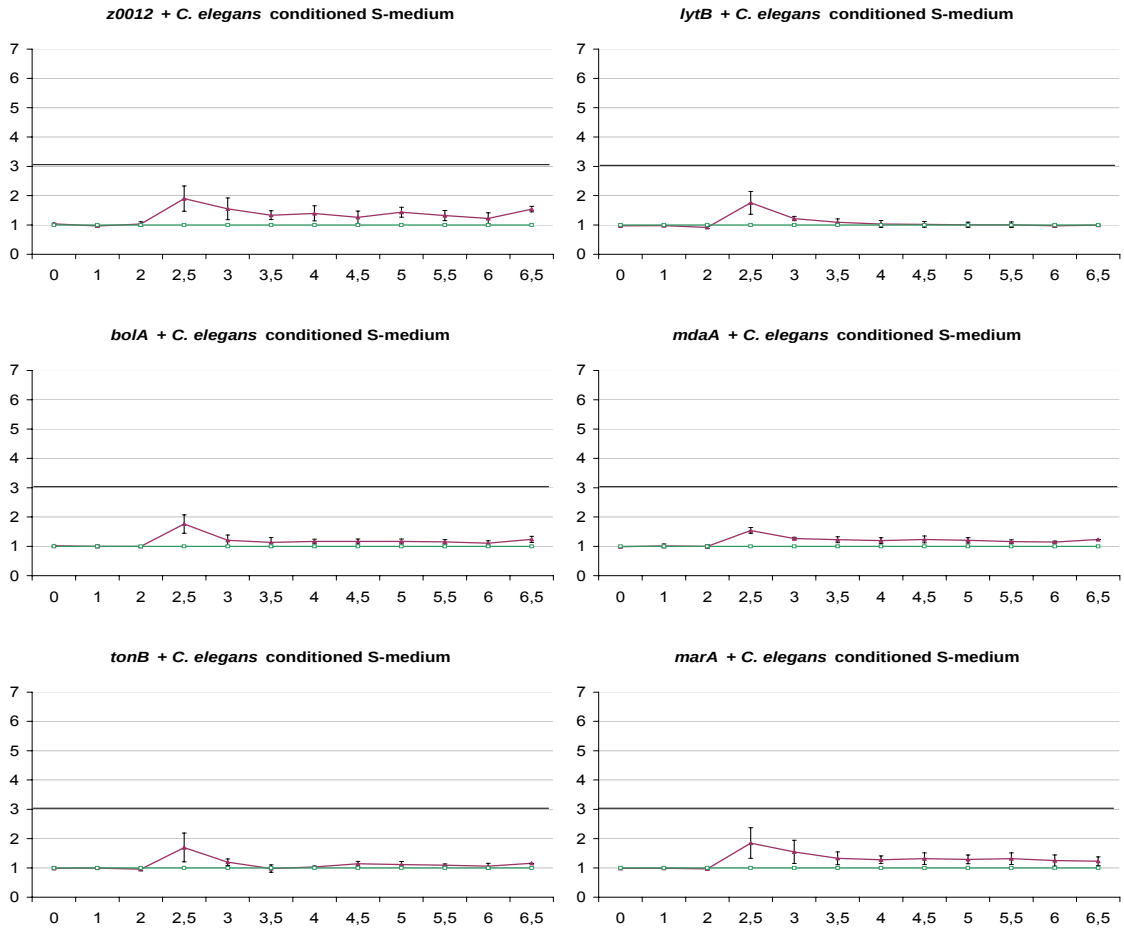


Figure 1: activation of 500bp upstream promoter regions of structure associated non-LEE genes. *C. elegans* conditioned S-medium was added at t = 2.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *C. elegans*, which is defined 1 for each time point (green line). Violet lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.



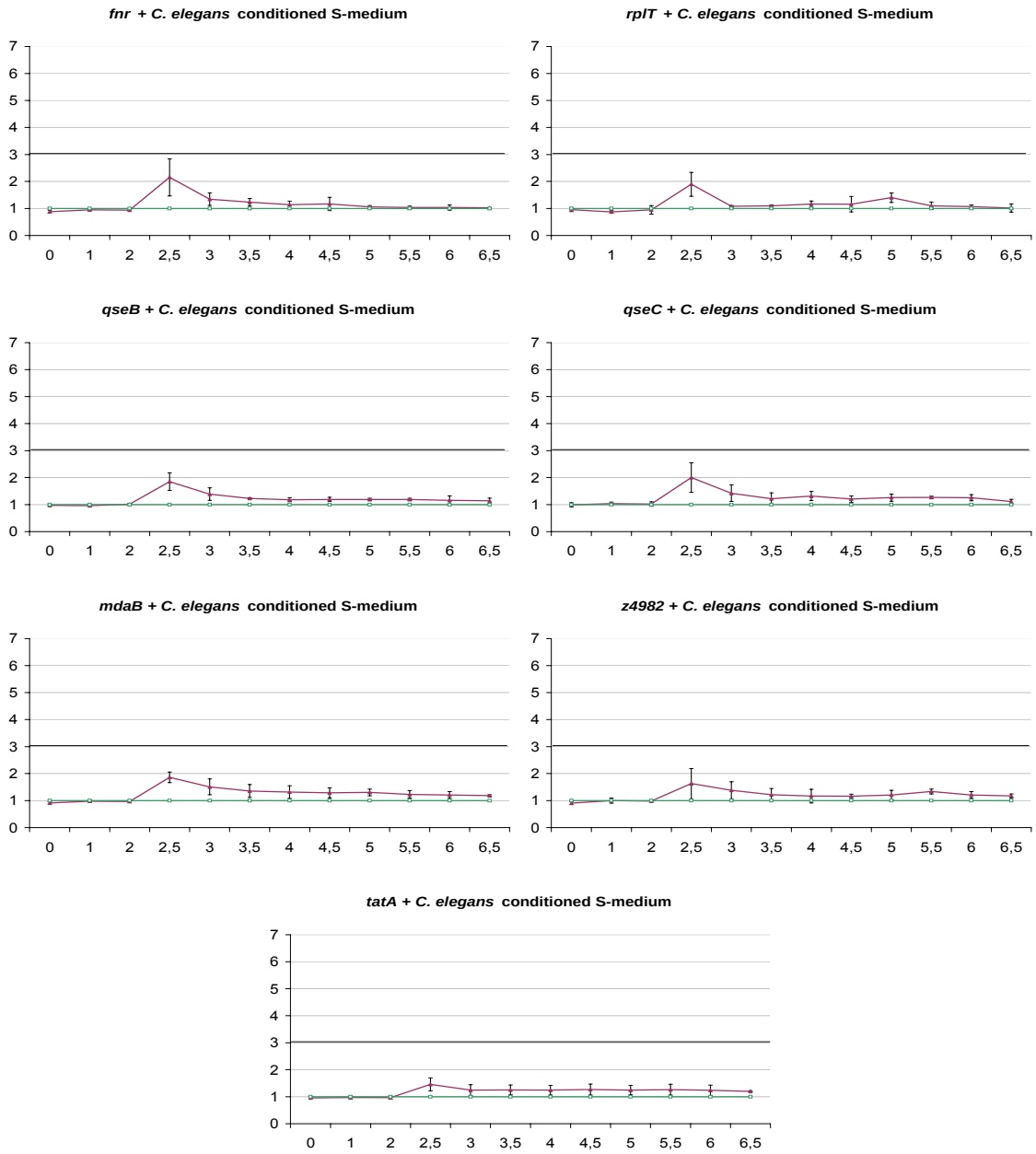


Figure κ: activation of 500bp upstream promoter regions of regulatory non-LEE genes. *C. elegans* conditioned S-medium was added at t = 2.5h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *C. elegans*, which is defined 1 for each time point (green line). Violet lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.

6.1.2 Promoter-*lux* studies with EHEC and conditioned *A. castellanii* medium

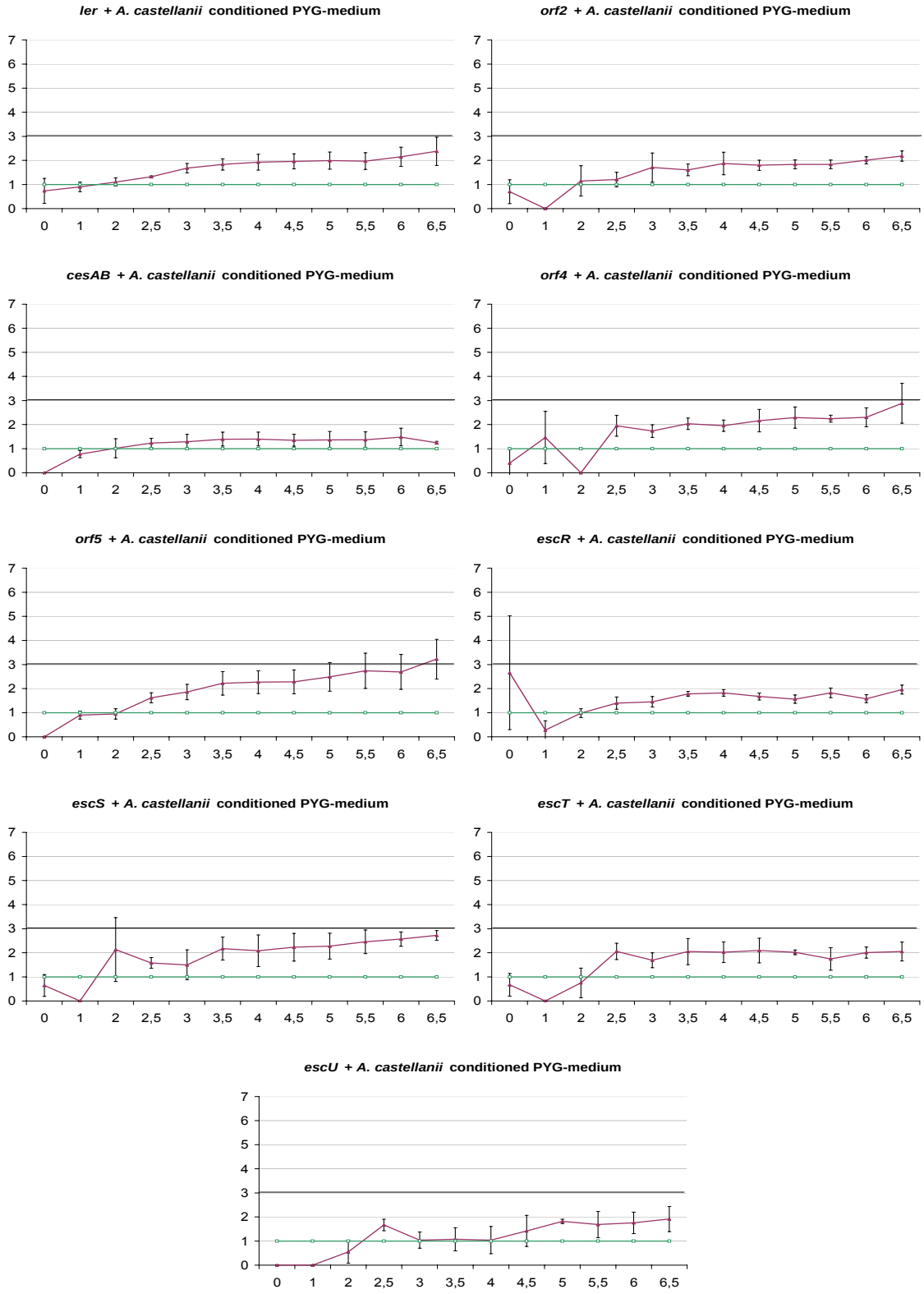


Figure λ: activation of 100bp upstream promoter regions of LEE1 genes. *A. castellanii* conditioned PYG-medium was added at t = 2h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *A. castellanii*, which is defined 1 for each time point (green line). Violet lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.

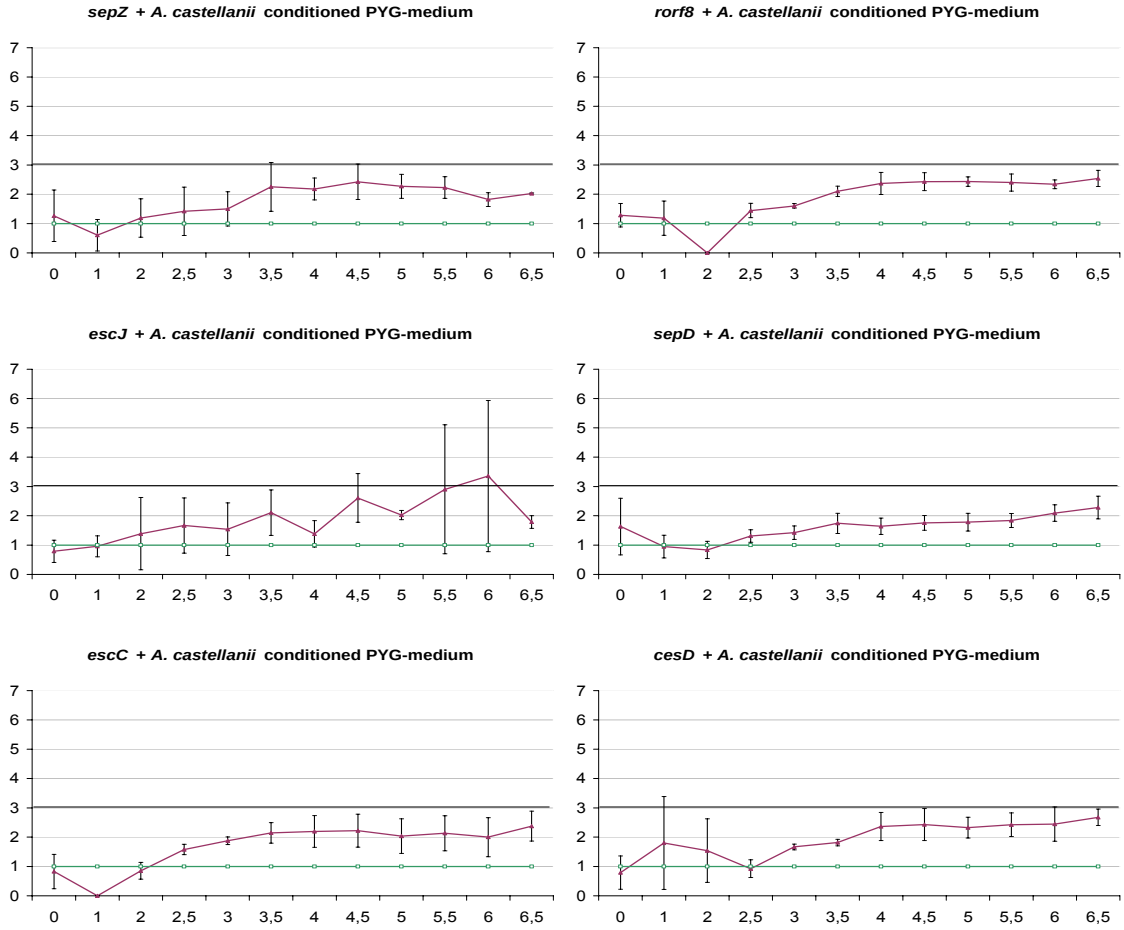
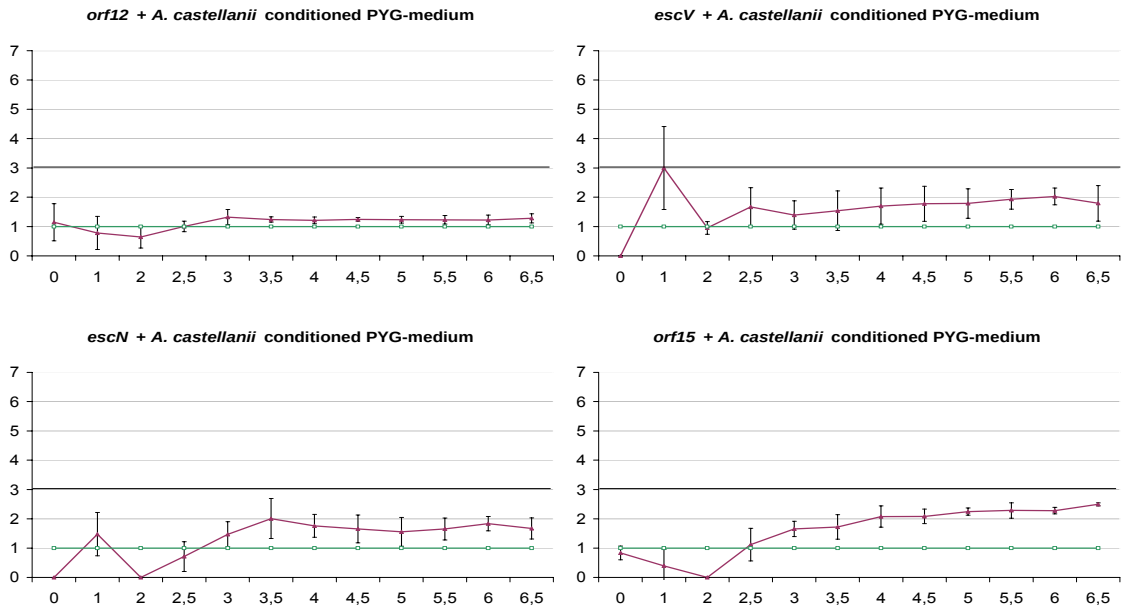


Figure µ: activation of 100bp upstream promoter regions of LEE2 genes. *A. castellanii* conditioned PYG-medium was added at t = 2h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *A. castellanii*, which is defined 1 for each time point (green line). Violet lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.



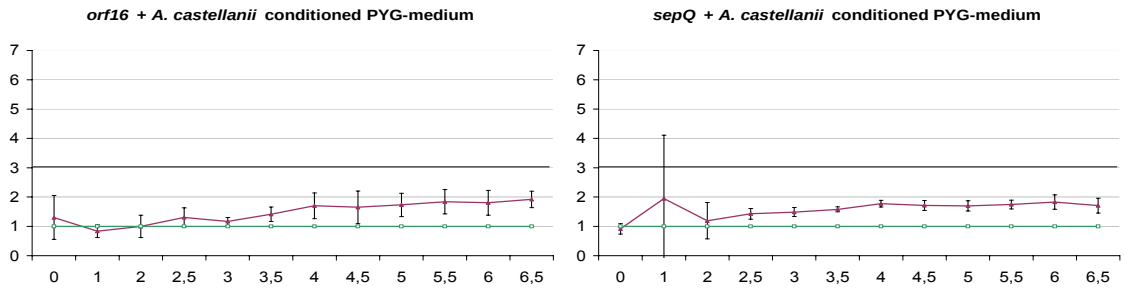
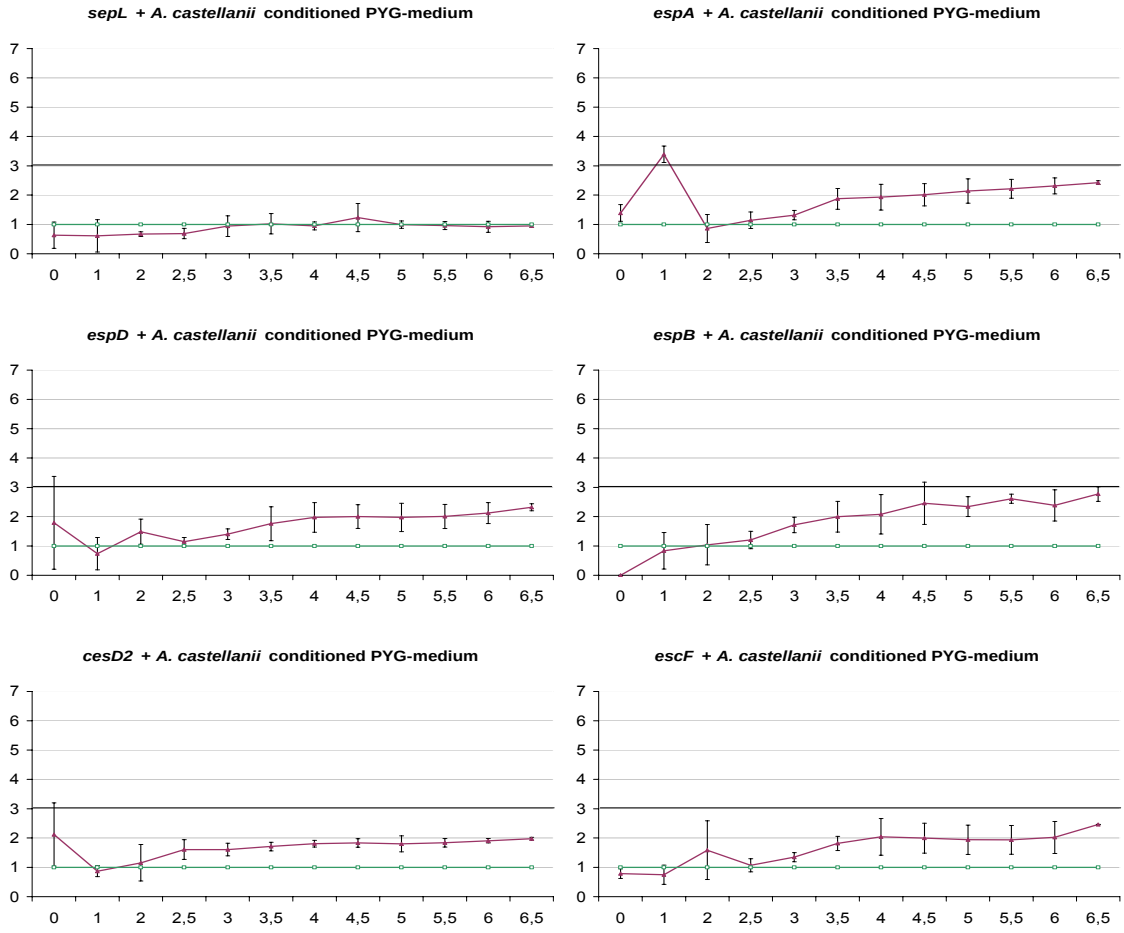


Figure v: activation of 100bp upstream promoter regions of LEE3 genes. *A. castellanii* conditioned PYG-medium was added at t = 2h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *A. castellanii*, which is defined 1 for each time point (green line). Violet lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.



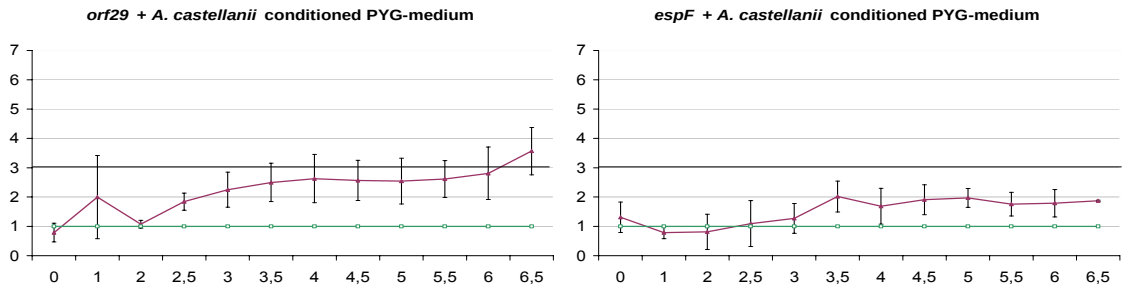


Figure ξ: activation of 100bp upstream promoter regions of LEE4 genes. *A. castellanii* conditioned PYG-medium was added at t = 2h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *A. castellanii*, which is defined 1 for each time point (green line). Violet lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.

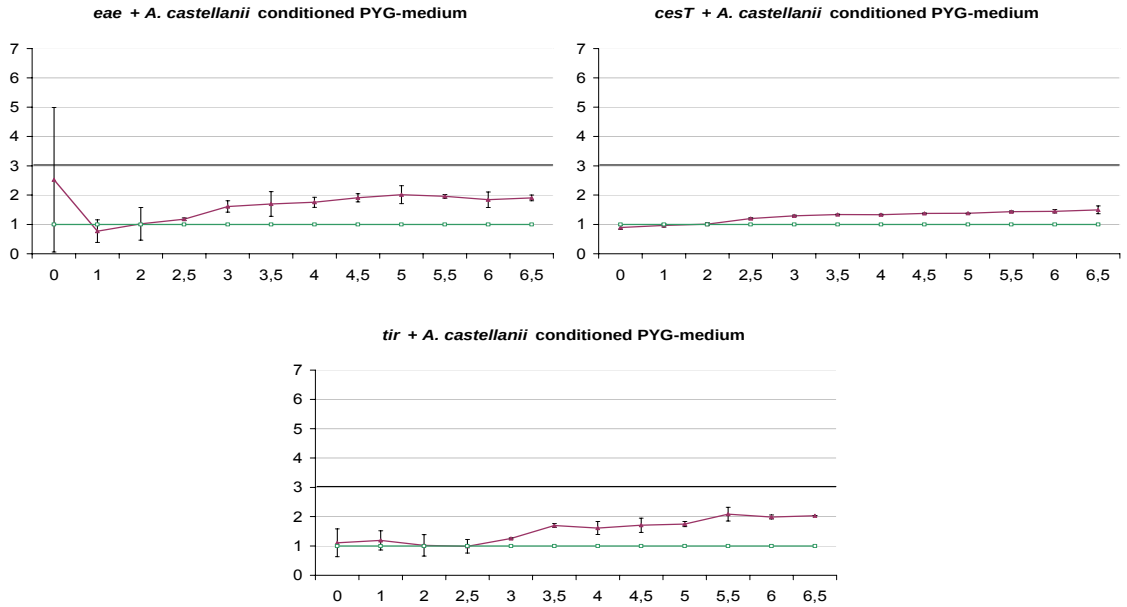


Figure o: activation of 100bp upstream promoter regions of LEE5 genes. *A. castellanii* conditioned PYG-medium was added at t = 2h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *A. castellanii*, which is defined 1 for each time point (green line). Violet lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.

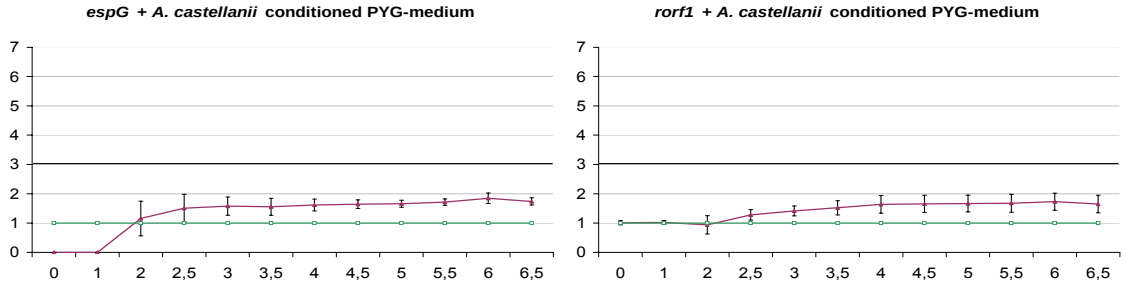


Figure π: activation of 100bp upstream promoter regions of espG genes. *A. castellanii* conditioned PYG-medium was added at t = 2h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *A. castellanii*, which is defined 1 for each time point (green line). Violet lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.

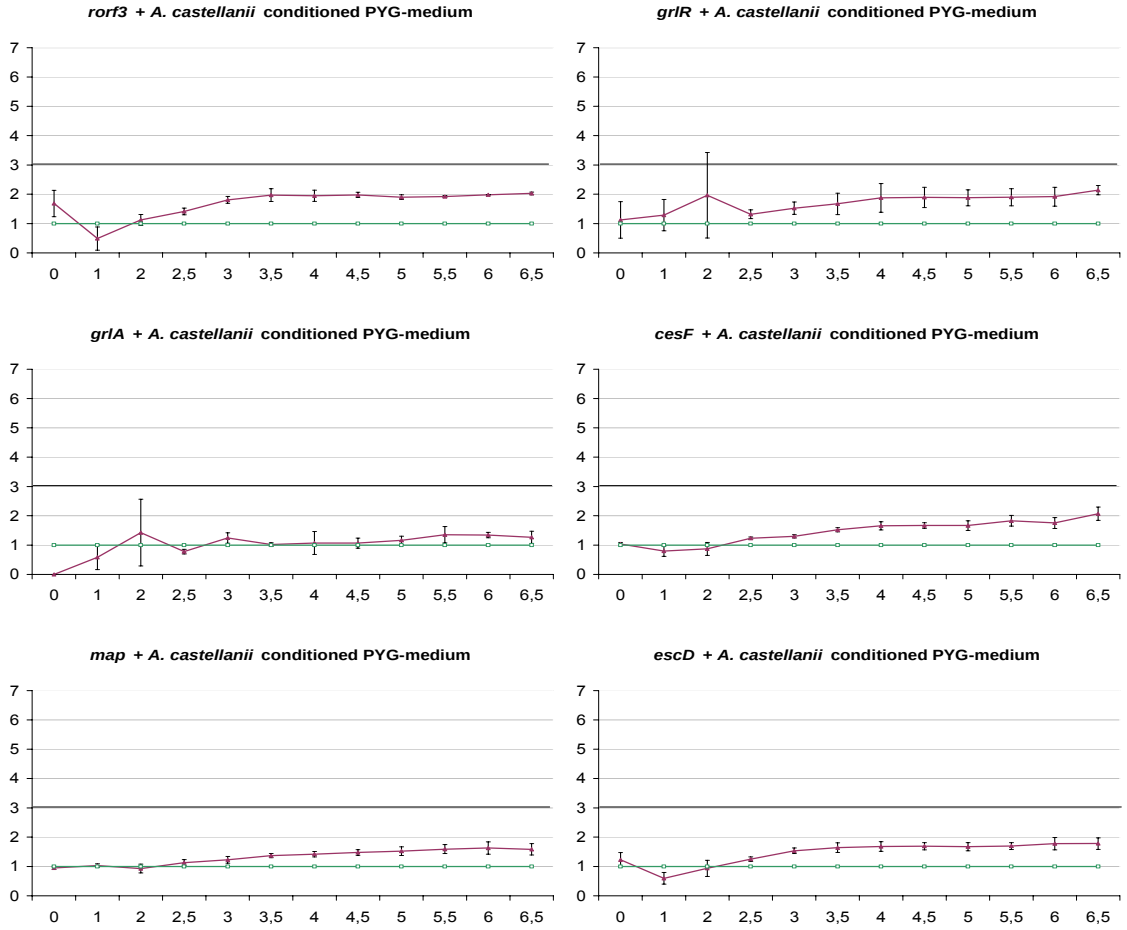
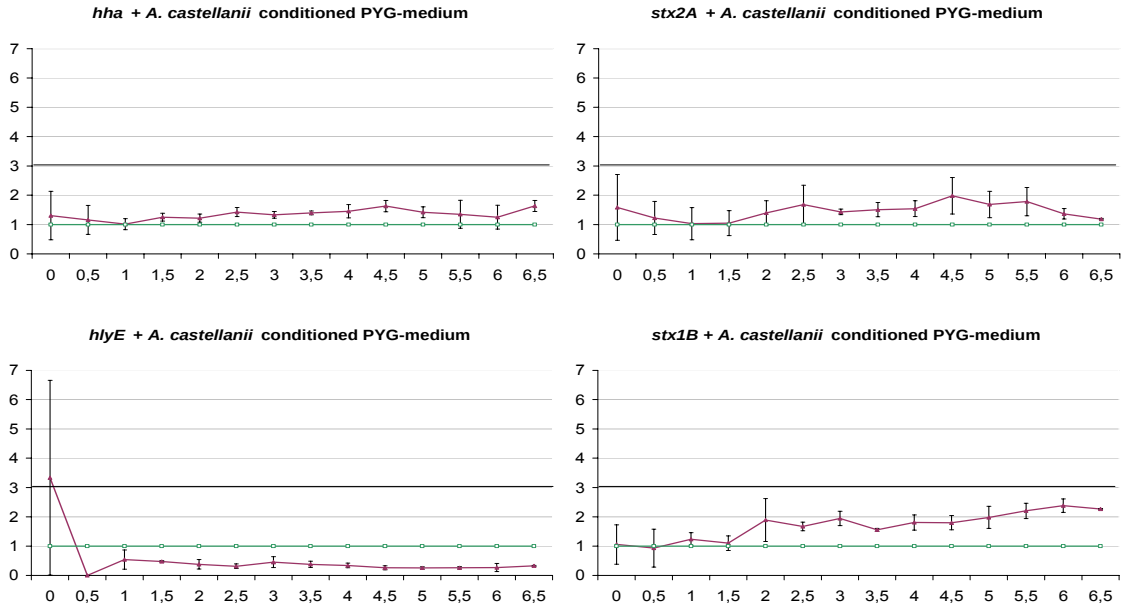


Figure ρ: activation of 100bp upstream promoter regions of monocistronic LEE genes. *A. castellanii* conditioned PYG-medium was added at t = 2h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *A. castellanii*, which is defined 1 for each time point (green line). Violet lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.



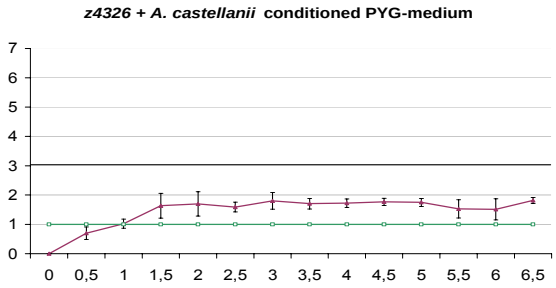
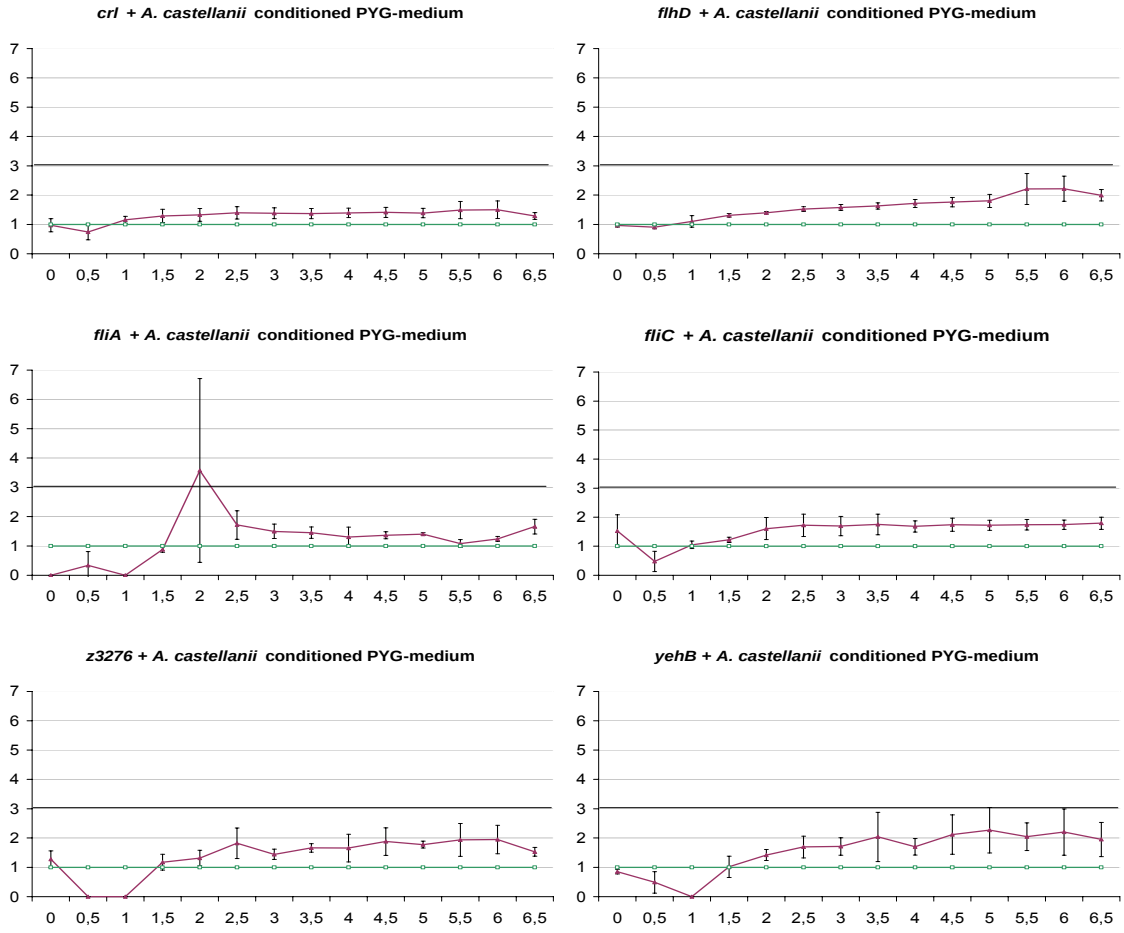


Figure ς: activation of 500bp upstream promoter regions of virulence associated non-LEE genes. *A. castellanii* conditioned PYG-medium was added at t = 2h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *A. castellanii*, which is defined 1 for each time point (green line). Violet lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.



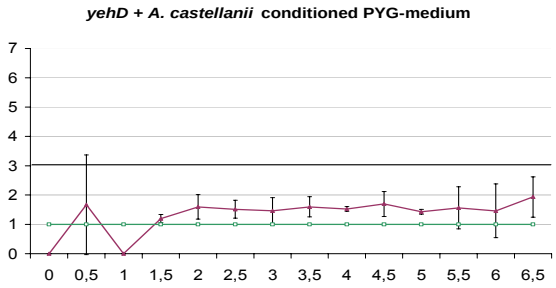
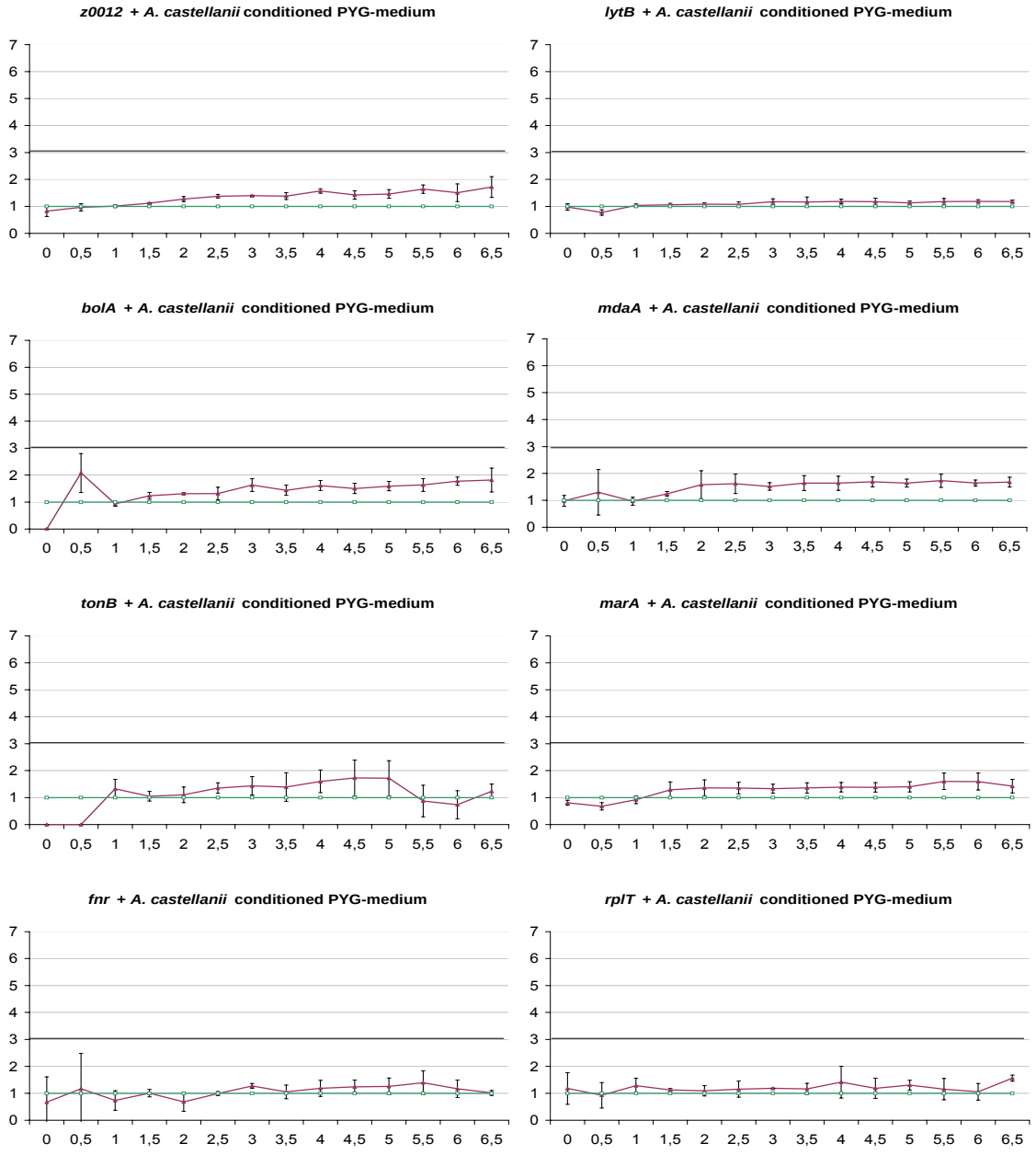


Figure σ: activation of 500bp upstream promoter regions of structure associated non-LEE genes. *A. castellanii* conditioned PYG-medium was added at t = 2h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *A. castellanii*, which is defined 1 for each time point (green line). Violet lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.



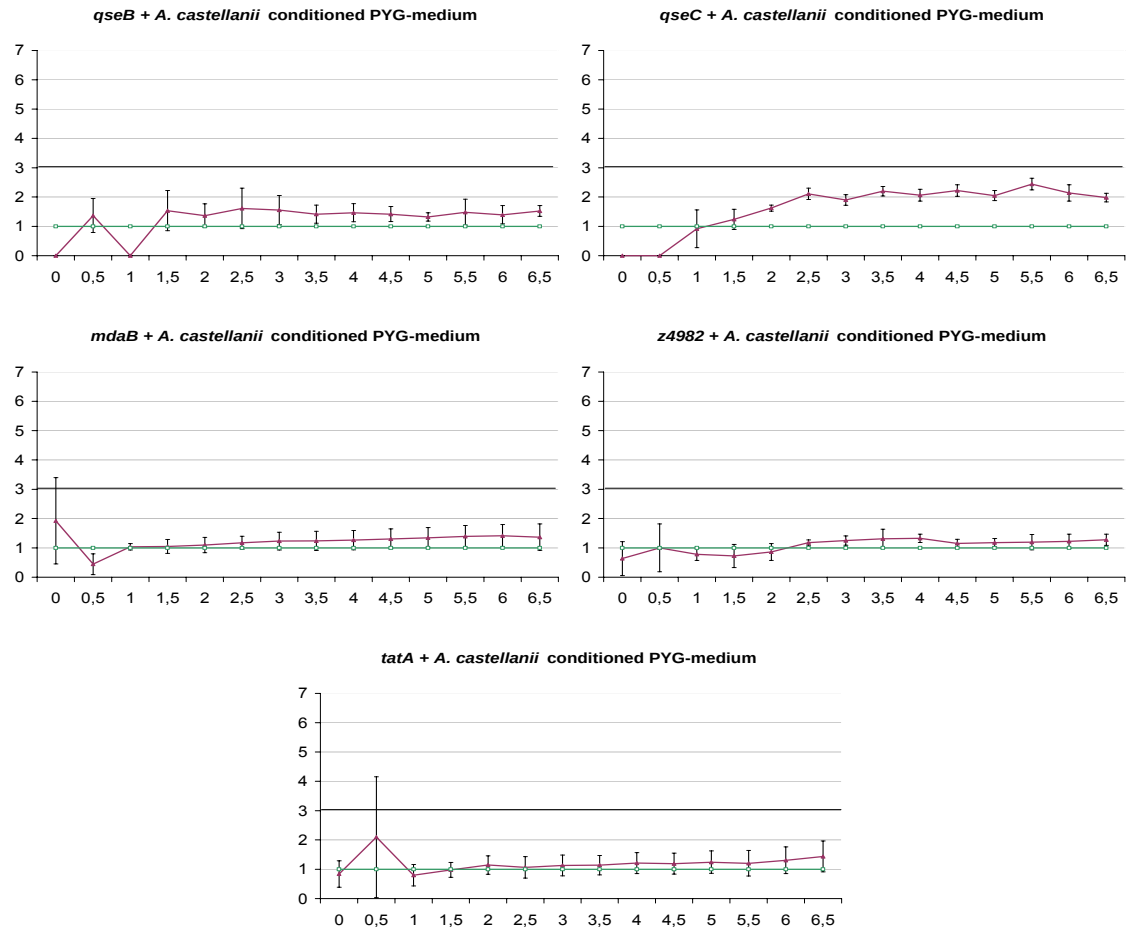


Figure τ: activation of 500bp upstream promoter regions of regulatory non-LEE genes. *A. castellanii* conditioned PYG-medium was added at t = 2h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without *A. castellanii*, which is defined 1 for each time point (green line). Violet lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control. All experiments were carried out in triplicate.

6.1.3 Promoter-*lux* studies with EHEC and conditioned *A. thaliana* medium

The promoter-*lux* studies with EHEC and conditioned MS-medium have been conducted by Dorit Schleinitz and are described in her Master thesis [Vogelsang 2006]. No activation of any tested gene was observed.

6.1.4 Promoter-*lux* studies with LEE-encoded genes and Caco-2 cells

The test assays with Caco-2 cells were conducted several times under different conditions (concentration of cells and bacteria, over night cultures grown in LB or DMEM, addition of 44mM $NaHCO_3$). No induction of any tested promoter region was observed under any conditions I tried. The results shown are from the last optimized measurements, over night cultures were grown in DMEM. As these graphs show the results of only this last assay, no standard deviations are shown.

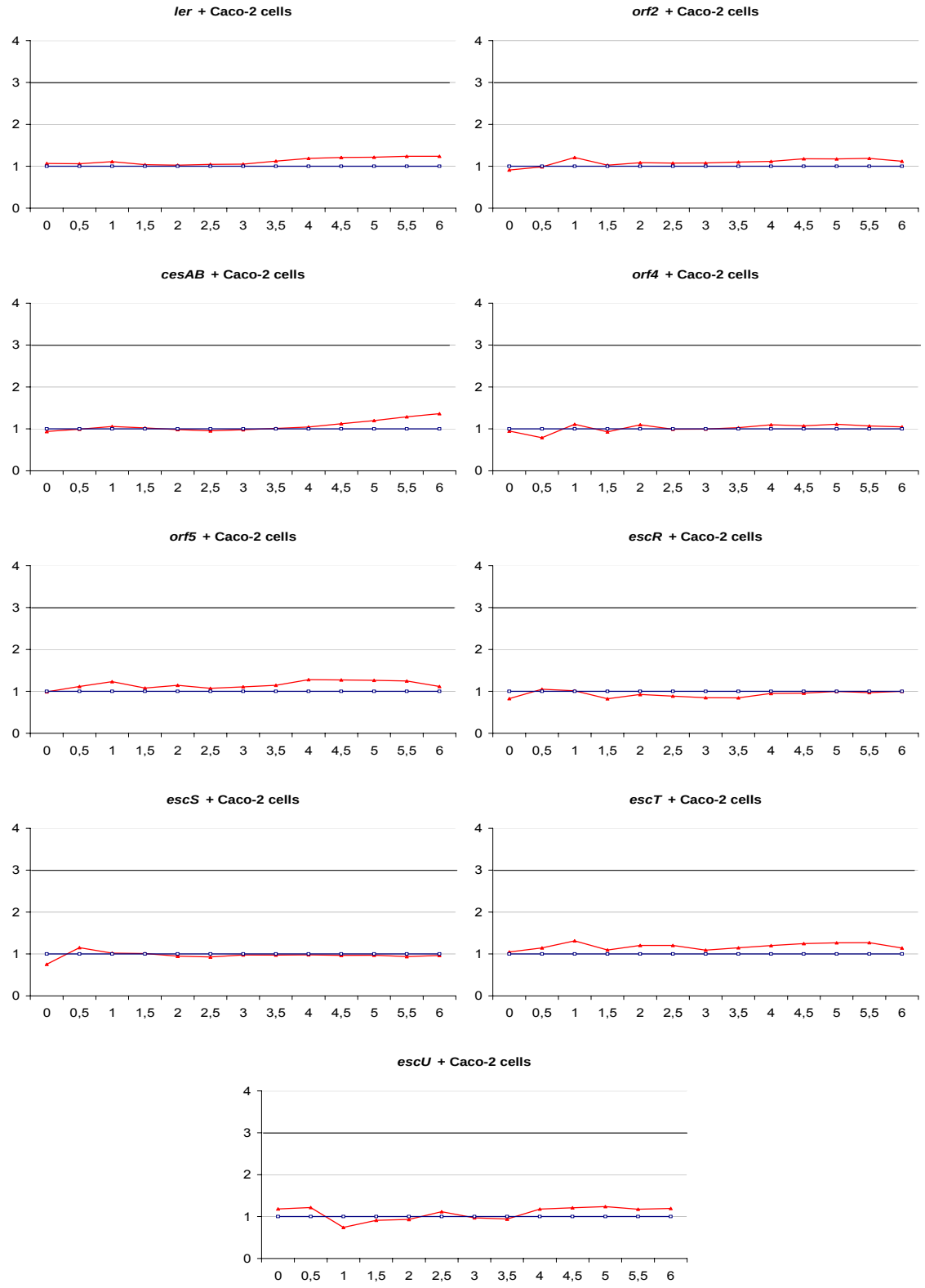


Figure 9: activation of 100bp upstream promoter regions of LEE1 genes. Caco-2 cells were added at t=0h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without Caco-2 cells, which is defined 1 for each time point (blue line). Red lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control.

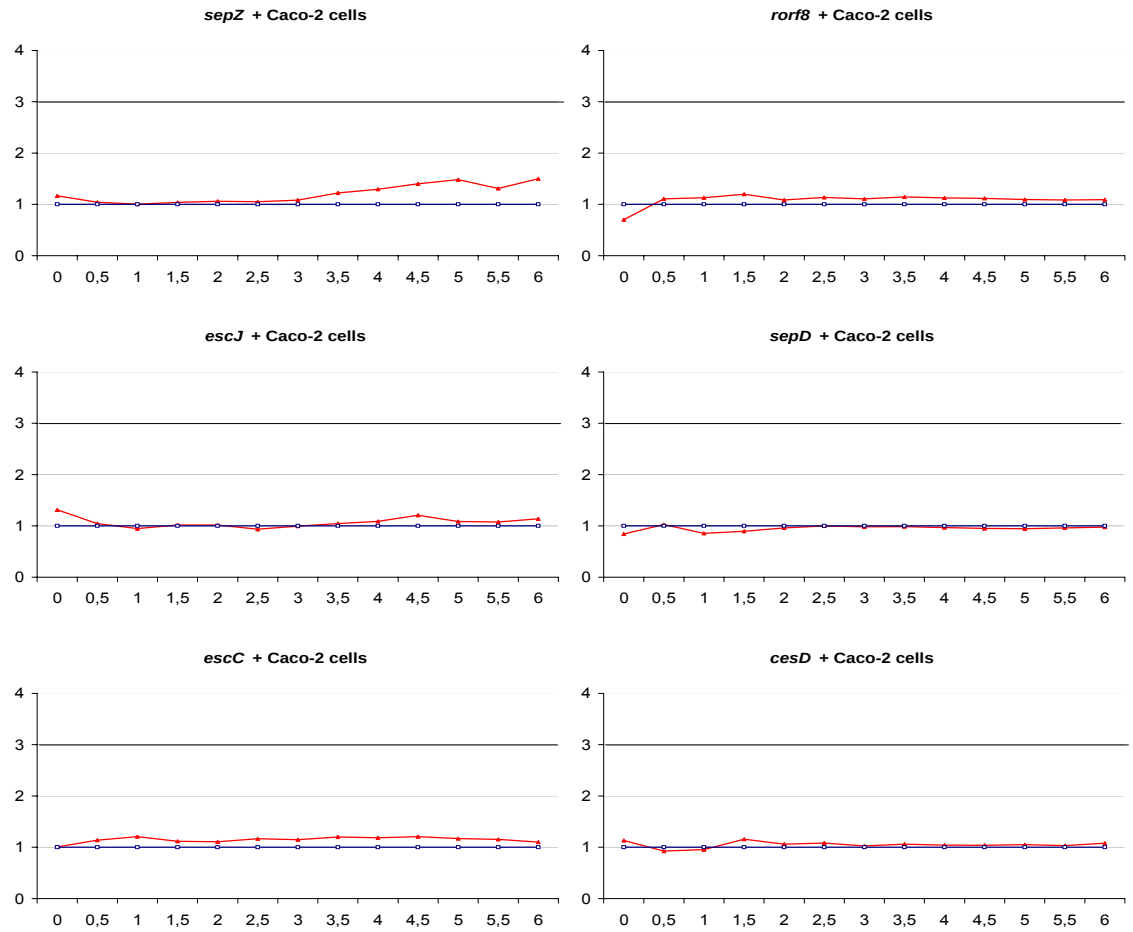
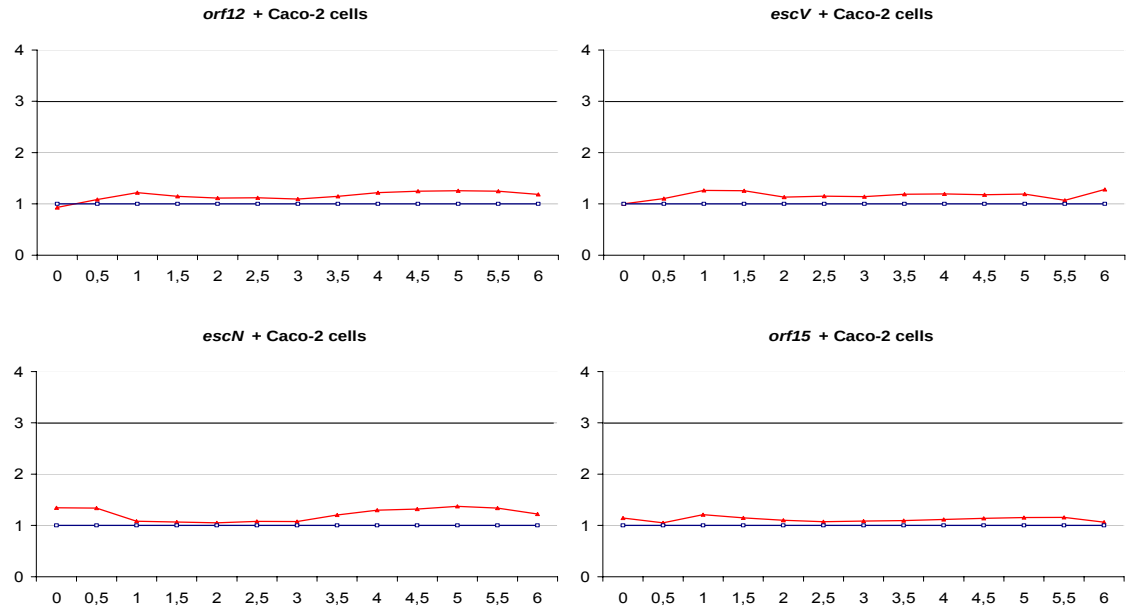


Figure φ: activation of 100bp upstream promoter regions of LEE2 genes. Caco-2 cells were added at t=0h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without Caco-2 cells, which is defined 1 for each time point (blue line). Red lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control.



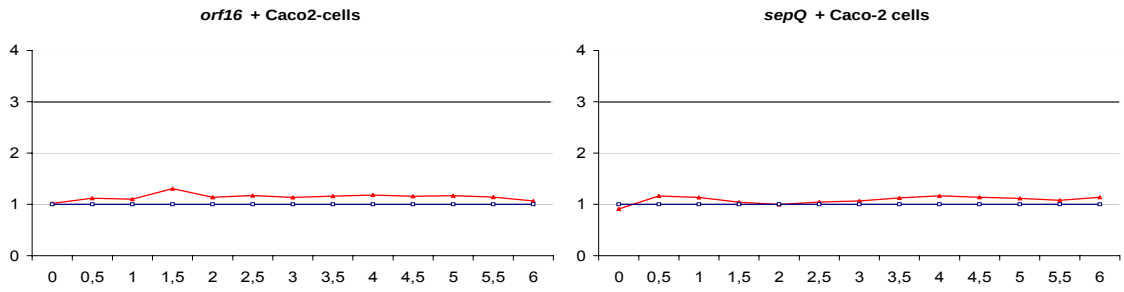
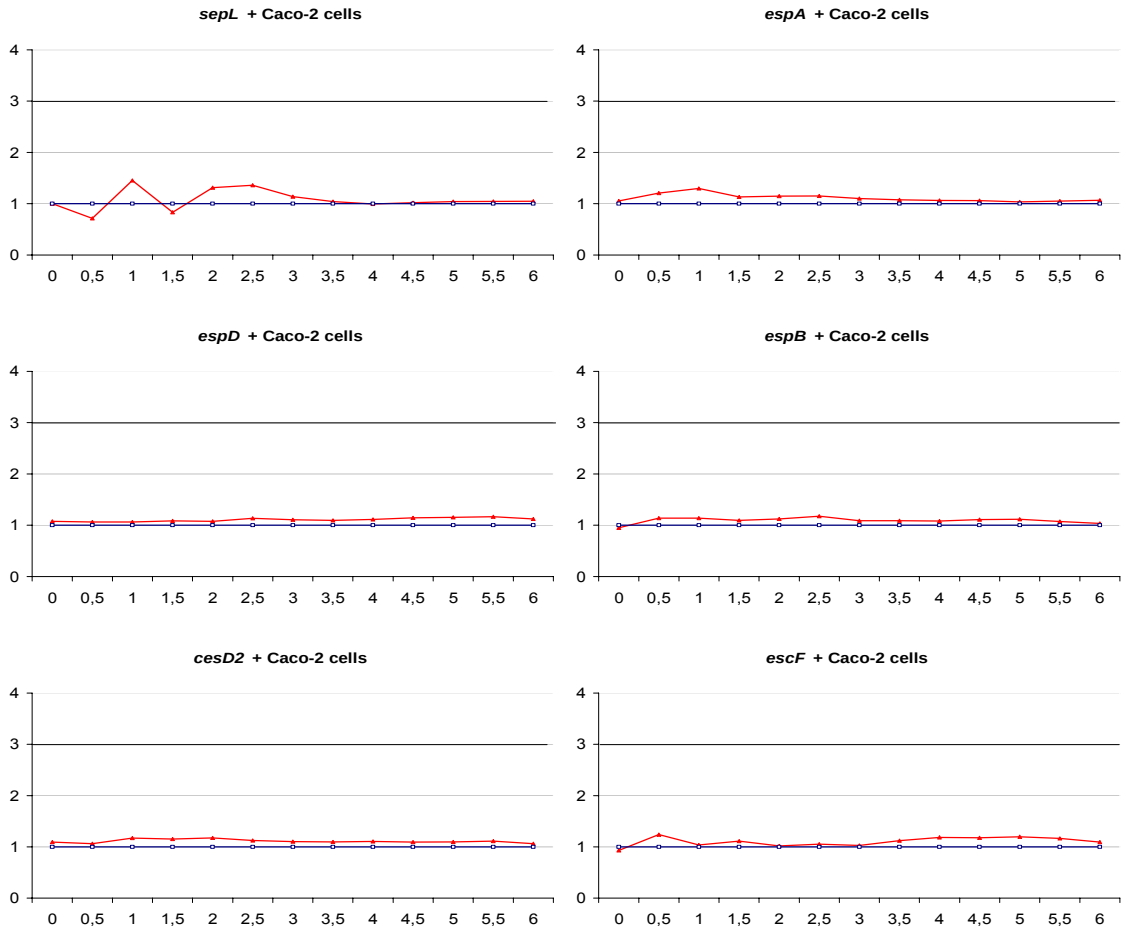


Figure χ: activation of 100bp upstream promoter regions of LEE3 genes. Caco-2 cells were added at t=0h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without Caco-2 cells, which is defined 1 for each time point (blue line). Red lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control.



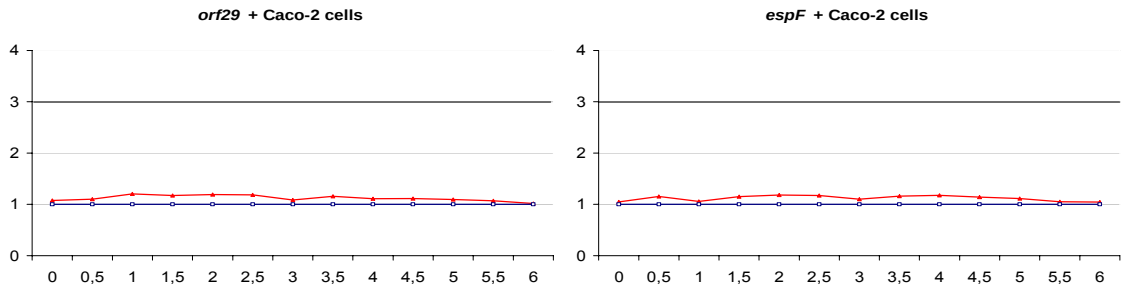


Figure ψ: activation of 100bp upstream promoter regions of LEE4 genes. Caco-2 cells were added at t=0h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without Caco-2 cells, which is defined 1 for each time point (blue line). Red lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control.

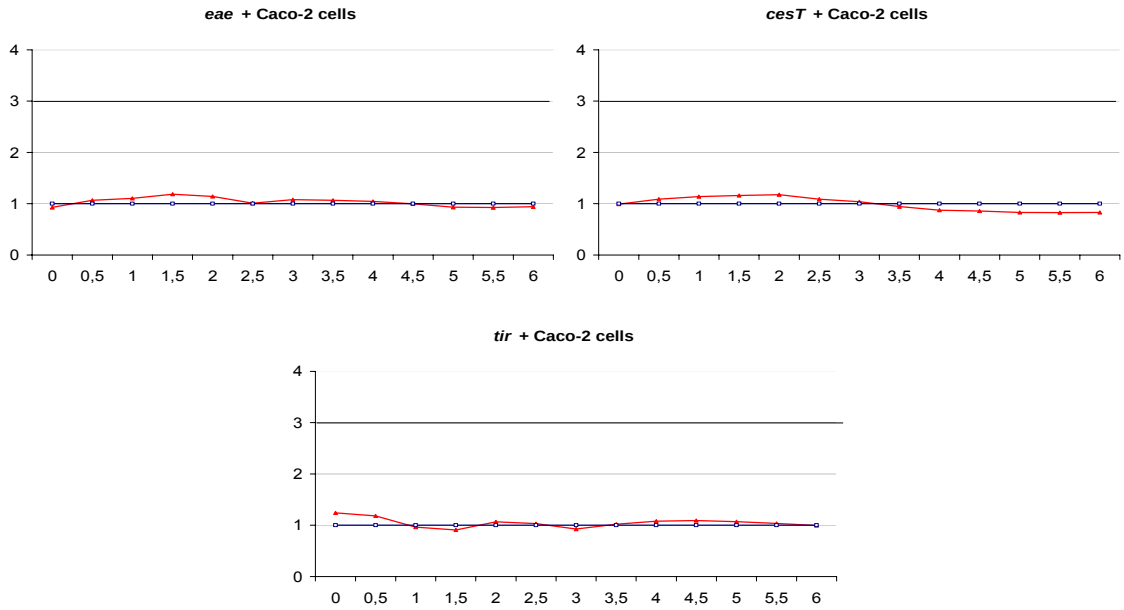


Figure ω: activation of 100bp upstream promoter regions of LEE5 genes. Caco-2 cells were added at t=0h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without Caco-2 cells, which is defined 1 for each time point (blue line). Red lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control.

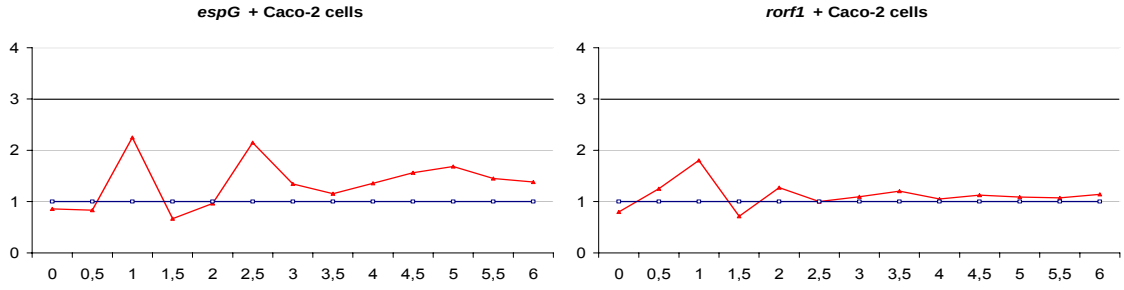


Figure ϊ: activation of 100bp upstream promoter regions of espG genes. Caco-2 cells were added at t=0h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without Caco-2 cells, which is defined 1 for each time point (blue line). Red lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control.

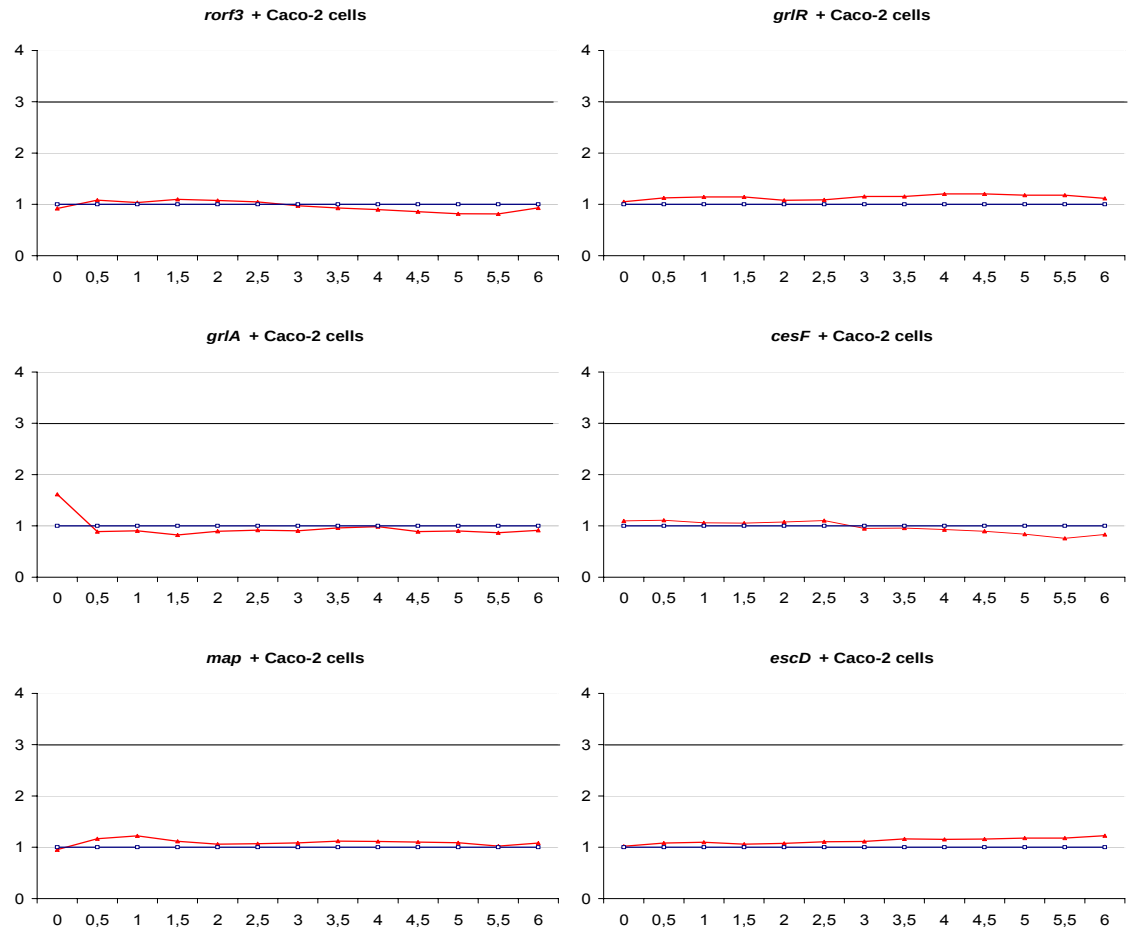
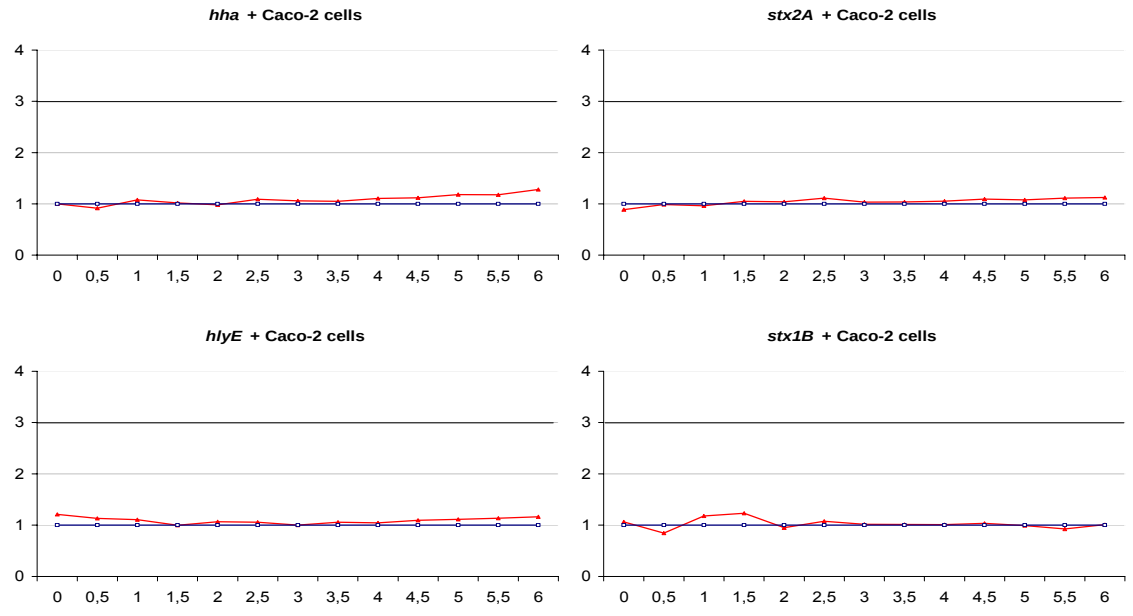


Figure ö: activation of 100bp upstream promoter regions of monocistronic LEE-genes. Caco-2 cells were added at t=0h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without Caco-2 cells, which is defined 1 for each time point (blue line). Red lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control.



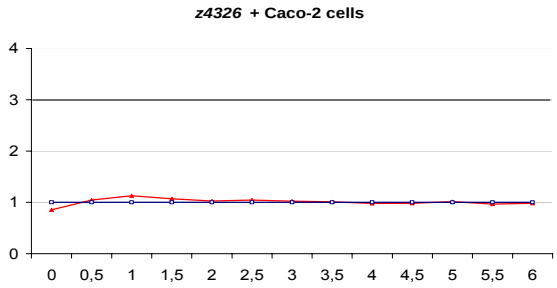
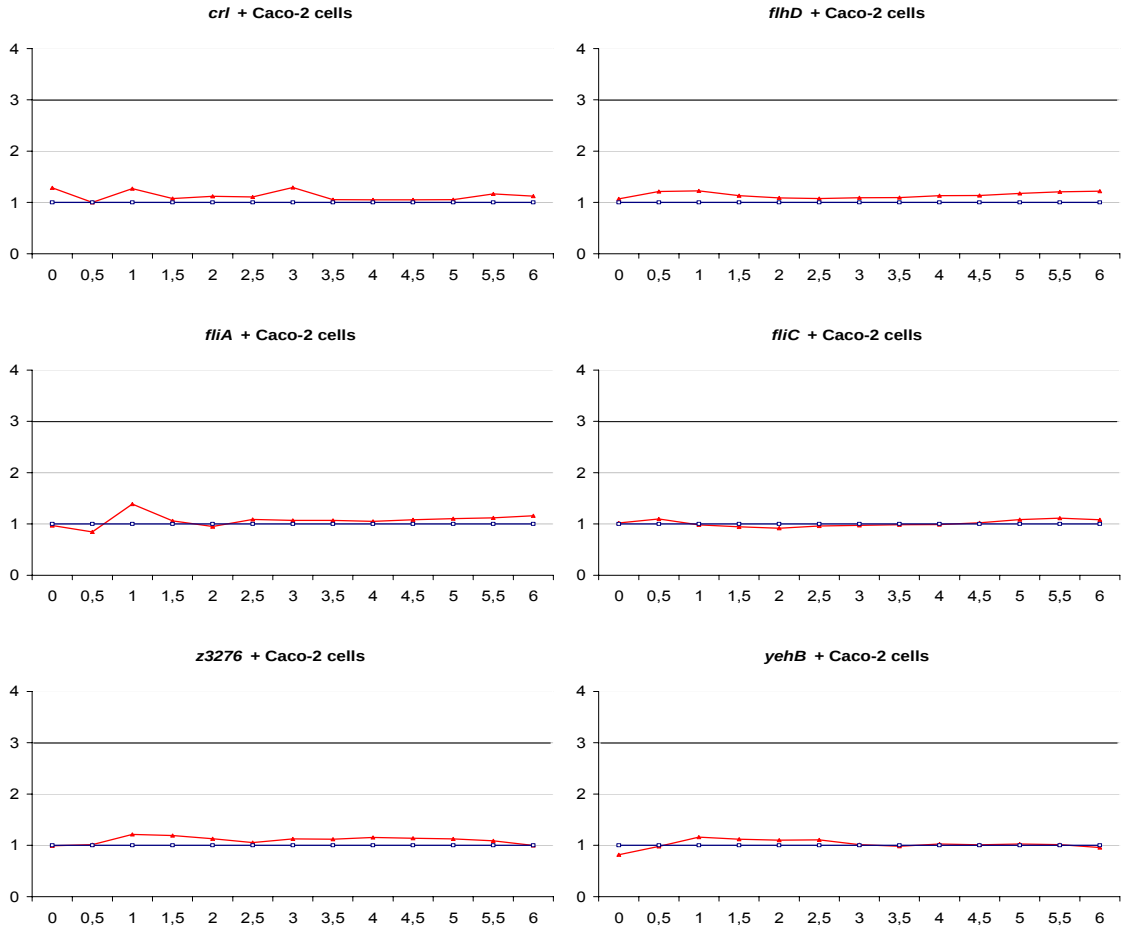


Figure ó: activation of 500bp upstream promoter regions of virulence associated non-LEE-genes. Caco-2 cells were added at t=0h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without Caco-2 cells, which is defined 1 for each time point (blue line). Red lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control.



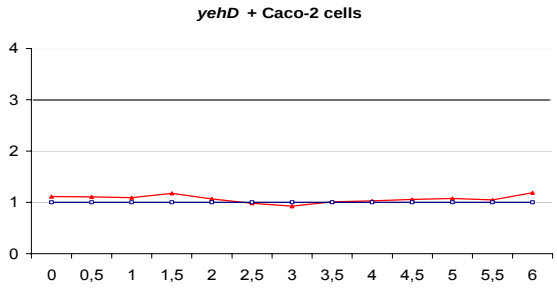
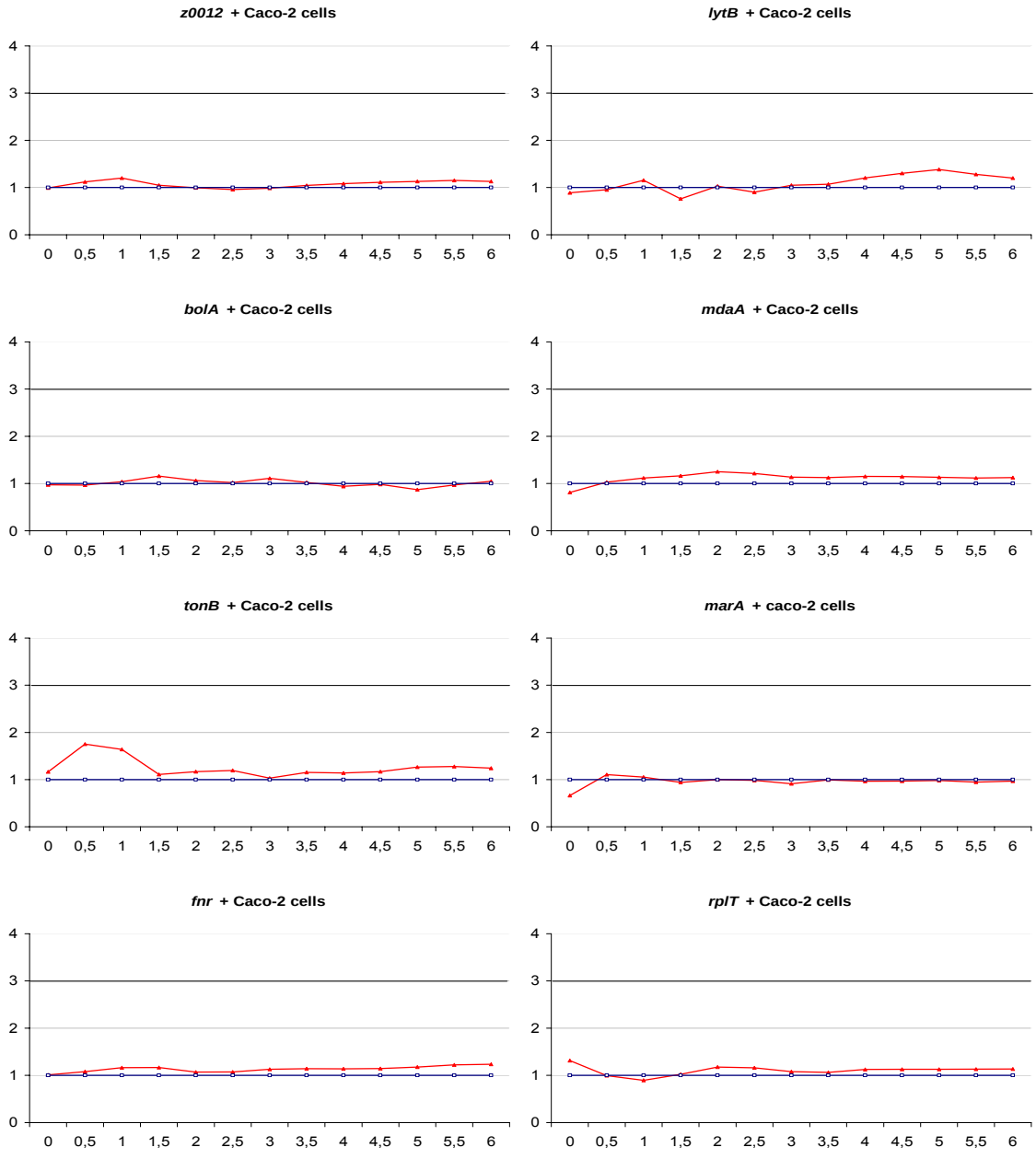


Figure ó: activation of 500bp upstream promoter regions of structural non-LEE-genes. Caco-2 cells were added at t=0h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without Caco-2 cells, which is defined 1 for each time point (blue line). Red lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control.



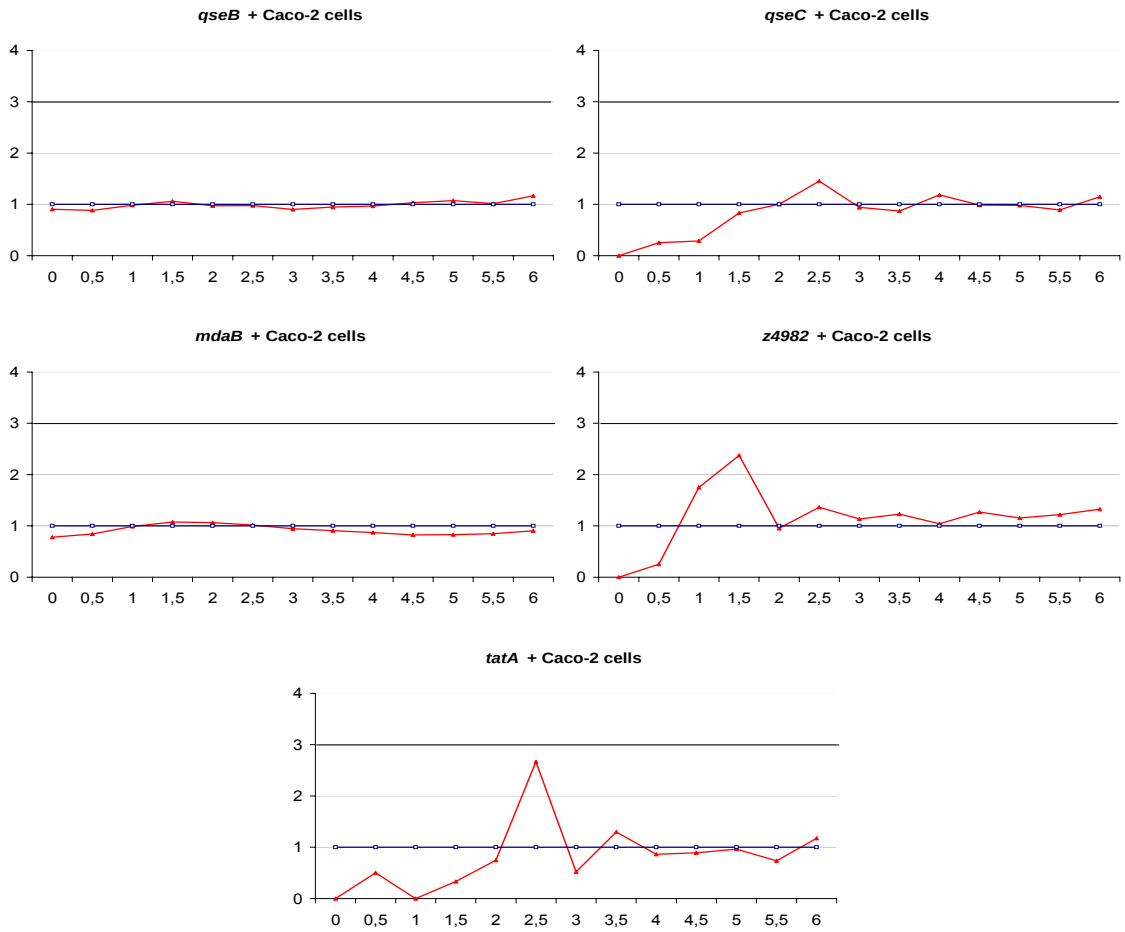
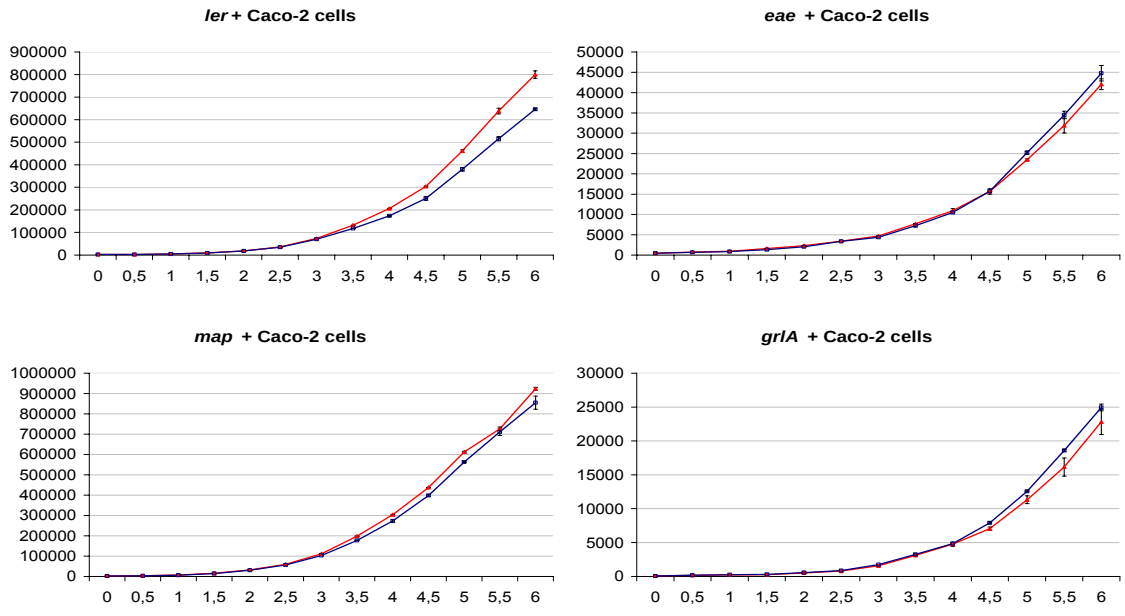


Figure ώ: activation of 500bp upstream promoter regions of regulatory non-LEE-genes. Caco-2 cells were added at t=0h. The x-axis shows the time in h, the y-axis shows the activation in relation to the control activation without Caco-2 cells, which is defined 1 for each time point (blue line). Red lines show the activation of each 100bp promoter region, the black line indicates the threshold line (three-fold activation) compared to control.



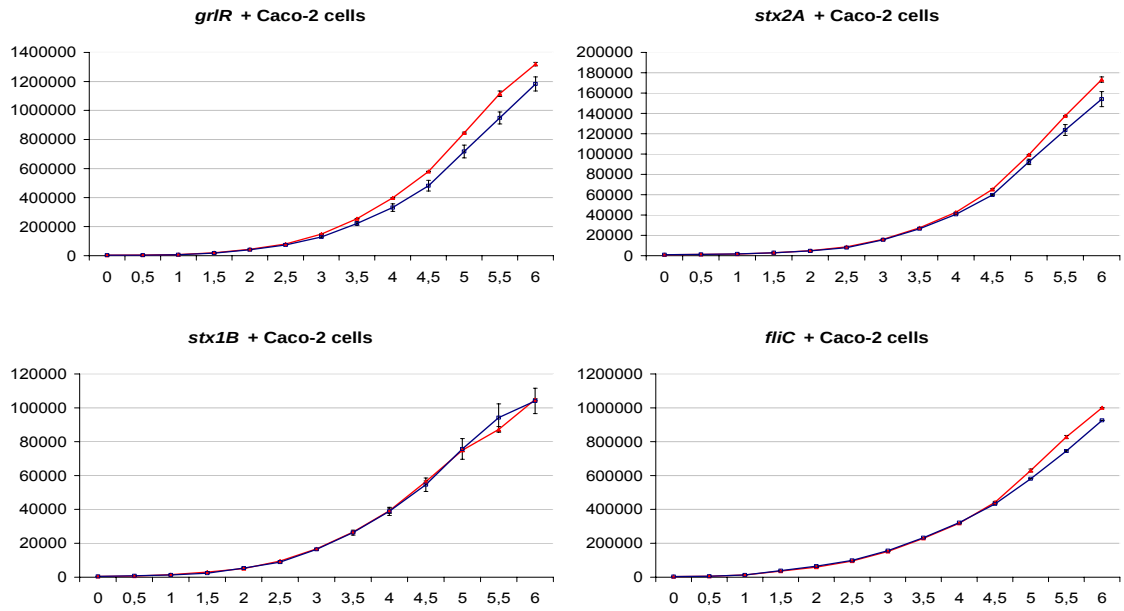


Figure ж: activation of 500bp upstream promoter regions of chosen examples of the tested genes. Caco-2 cells were added at t=0h. The x-axis shows the time in h, the y-axis shows the total amount of luminescence as read by the Victor^3 multilabel counter in defined luminescence units. Blue lines show the luminescence emitted by the control wells without Caco-2 cells, red lines show the emitted luminescence in contact with caco-2 cells. The general luminescence is strongly increased in all experiments, but there is no significant difference between the control and the test wells. So, presumably, the increase of gene transcription is activated by the general environmental conditions given in the cell culture assay which are similar to the conditions in the mammalian colon (37°C, 5%CO^2 rather than by presence of Caco-2 cells.

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Curriculum vitae

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-Biophysics (1.0)
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