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DIPLOMARBEIT

Titel der Diplomarbeit

Characterization of putative repressors of the temperate phage ϕCh1
and
analysis of the flagellum operon as a putative receptor of ϕCh1

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Magistra der Naturwissenschaften (Mag. rer.nat.)

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1. Introduction

1.1. *Archaea*

1.1.1. Evolution and phylogeny of *Archaea*

1970 Woese *et al.* proposed the establishment of the three domains of life, consisting of *Eukaryotes, Eubacteria* and *Archaebacteria*, nowadays termed *Eucaryotes, Bacteria* and *Archaea.* By then, the biologists had a 'dichotome' point of view- the primary phylogenetic groups consisted of *Eukaryotes* and *Prokaryotes,* only.

The new conception was the result of the comparative analysis of the 16S (18S) ribosomal RNA. This approach has the advantage that rRNA sequences are universal, readily isolated and its sequences change slowly during evolution (Woese *et al.*, 1977). Based on this approach *Archaea* can be divided into two phyla: the *Euryachaeota* and the *Crenarchaeota* as mentioned in figure 1 (Woese *et al.*, 1990).

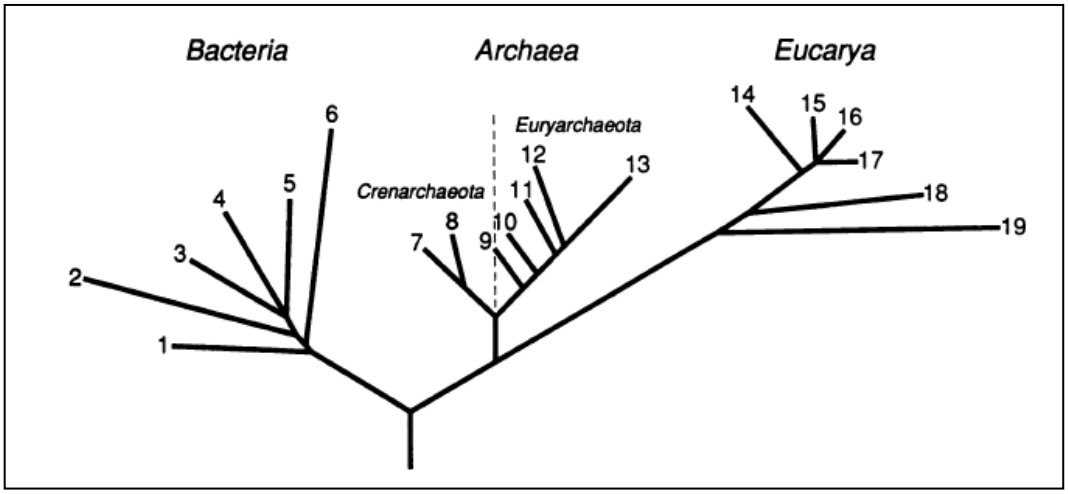


FIGURE 1 | **The tree of life**. This figure shows the three domains of life, based on rRNA analysis in 1990. The points of the roots show the groups of the different phyla (Woese et al., 1990). Today *Archaea* can be subdivided in two additional phyla- The *Nanoarchaeota* and the *Korarchaeota* (Allers and Mevarech, 2005).

In the meanwhile one can distinguish between 4 phyla within the domain of *Archaea*. The most varied phylum is termed *Euryachaeota* which comprise 9 orders, including *Halobacterials*. *Crenoarchaeota* can be subdivided in 3 orders and comprise many hyperthermophylic *Archaea* and psychrophiles. The phylum of *Nanoarchaeota* includes just 1 order, while the phylum of *Korarchaeota* could not be cultivated so far (Allers and Mevarech, 2005).

The phylogeny and reconstruction of the archaeal evolution is extremely difficult because of the presence of lateral gene transfer (LGT) between *Bacteria* and *Archaea* (Wolf *et al.*, 1999). Furthermore, the fact that many archaeal strains could not be cultivated yet, complicates the reproduction of archaeal evolution. Ongoing studies focus on the evolution of the three domains of life, their relation and the last universal cellular ancestor (LUCA) (Forterre *et al.*, 2002). Recent studies proposed that the LUCA was a "*mesophilic eukaryotic before maturing by endosymbiosis into an organism adapted to an atmosphere rich in oxygen*," (Glansdorff *et al.*, 2008).

1.1.2. *Archaea* in comparison to *Bacteria* and *Eucaryotes*

Archaea belong to the group of prokaryotes and therefore lack a nucleus. They can be distinguished from *Bacteria* and *Eukaryotes* by possessing a specific small subunit (SSU) rRNA and a special structure of their membrane. While the membrane lipids of *Bacteria* and *Eukaryote*s contain esters of fatty acids and glycerol, archaeal lipids are ethers of glycerol and isoprenol. Furthermore, all *Archaea* lack murein (Kates, 1993).

Archaea share some features with *Eukaryotes*, like most of the informational genes, especially genes for DNA replication (Edgell and Doolittle, 1997; Forterre, 1999; Leipe *et al.*, 1999). In comparison, most of their operational proteins show similarities with *Bacteria*. Operational genes are involved in housekeeping and get more frequently transferred via LGT than informational genes (Jain *et al.*, 1990).

Concerning their distribution, *Archaea* were long time known as extremophiles, only isolated from extreme environment, such as hot springs or acidic environments. Now it is known that *Archaea* colonise wide sections of the earth, like the oceans (Olsen *et al.*, 1994). Recent estimations imply that 20% of the pico plankton in the oceans consists of *Crenarchaeota* (Kamer *et al.*, 2001).

1.1.3. Halophile lifestyle

A “halophile way of life” requires a large number of adaptations to the high salt environment. The central problem is the water permeability of cell membranes. Because of the osmotic pressure, the cell would lose its water to the environment. One strategy can be to accumulate salt within the cell, adjusted to the environmental salt concentration. In this case the whole cell machinery has to adapt to these conditions. The second strategy to compensate the osmotic pressure, is to acquire organic compatible solutes. Extreme halophilic *Archaea* of the order *Halobacteriales* use the “salt-in” strategy. They accumulate KCL intracellular, while NaCl dominates in the environment, in the majority of cases. Due to these conditions, the cell had to adapt all proteins and cell components. The protein adaptation consists of the presence of a large quantity of acidic amino acids and a small number of hydrophobic amino acids (Oren, 1999).

1.1.4. Alkaliphilic lifestyle

To survive in alkaliphilic conditions (pH values above 9), alkaliphilic microorganisms had to adapt themselves- especially their cell surface. Plasma membranes are not stable at high pH values and they consequently need a specific structure and components to maintain. That was realized with the development of the integration of special glutaminylglycan polymers (Kandler and Konig 1998; Falb *et al.*, 2005) “*as well as characteristic membranes containing C20–C25 in addition to C20–C20 diether core lipids*” (Tindall *et al*. 1984; Falb *et al.*, 2005).

But what adaptations had to happen within the cell? To answer this question, first the pH level had to be determined. To measure the pH level inside the cell, one can estimate the pH optimum for enzyme activity in the cytoplasm. For alkaliphiles, *in vitro* protein synthesis systems, a pH optimum of 8.2- 8.5 was determined. In comparison with this, neutrophilic, like *Bacillus subtilus*, have a 0.5 pH units fewer pH optimum. Taken together, cell wall and cell membrane of alkaliphilic have the important function to separate the high and the low pH level, so that the inner cell does not have to undergo extreme adaptations (Horikoshi, 1999).

1.1.5. Important representatives of *Archaea*

1.1.5.1. *Halobacteriacaea*

All following strains belong to the family of *Halobacteriaceae*, within the phylum of *Euryarchaeota*. *Halobacteriaceae* include 26 genera: *Halobacterium, Haloadaptus, Halalkalicoccus, Haloarcula, Halobaculum, Halobiforma, Halococcus, Haloferax, Halogeometricum, Halomicrobium, Halopiger, Haloplanus, Haloquadratum, Halorhabdus, Halorubrum, Halosimplex, Halostagnicola, Haloterrigena, Halovivax, Natrialba, Natrinema, Natronobacterium, Natronococcus, Natronolimnobius, Natronomonas,* and *Natronorubrum* (Oren *et al*., 2008).

1.1.5.2. *Halobacterium salinarum*

Hbt. salinarum is strictly halophile and requires 4M NaCl and neutral pH values for growth. The rod shaped halophile balances the high salt environment with corresponding amounts of K+ ions. Because it is easy to cultivate, completely sequenced and can be easily altered, *Hbt. salinarum* is an excellent model organism for halophile *Archaea* (Wailap *et al*., 2000).

1.1.5.3. *Haloferax volcanii*

As a further model organism of the halophiles and for *Archaea* in general- *Hfx. volcanii* can be seen. Since it is sequenced, its advantage is the easy handling of cultivation, transformation and its stability concerning its genome. *Hfx. volcanii* grows in 1.7- 2.5 M NaCl and neutral pH values. Except the different salt-optimum*, Hfx. volcanii* is distinguished from *H. salinaurm* by the ability to degrade sugars, whereas *Hbt. salinarum* can grow phototrophic (Hartmann *et al.,* 2010).

In this model organism many protein functions could be verified. Among others, RadB, a protein of the RecA family, which is involved in recombination processes, could be analyzed (Haldenby *et al.,* 2009). Deletions of radB result in UV sensitivity and slow growth (Haldenby, *et al.,* unpublished work). Whereas "*RadB does not catalyse strand exchange. RadB does bind DNA*" only (Guy *et al.,* 2006).

1.1.5.4. *Natronomonas pharaonis*

An important representative of the haloalkaliphilic *Archaea* is *Natronomonas pharaonis*, which was found in habitats with the pH of 11 (optimum pH values of 8.5 for growth). Its salt optimum is at 3,5M NaCl (Falb *et al.*, 2005). *N. pharaonis* was isolated from saline soda lakes in Kenya and Egypt (Soliman *et al.,* 1982 and Tindall *et al.*, 1984).

1.1.6. *Natrialba magadii*

Natrialba magadii, first described as *Natronobacterium magadii* (Tindall *et al.,* 1982), is a rod shaped haloalkiliphilic archaeon, 0.5-0.7µm in size, which requires a high salt concentration and high pH levels. This extremophilic archaeon was isolated from the Lake Magadi, "*a prototype for saline alkaline lakes*" (Eugster, 1980), located in Kenya. Its characteristic reddish colour is the result from carotinoid pigments, stored in the membrane of the microorganisms (Oren, 2002).

When *Natronobacterium magadii* was divided into the genus of *Natronobacteria* in 1982, rRNA analysis was not that common, and therefore the microbes were classified after their phenotype- rod shaped *Natronobacteria* and cocci shaped *Natronococci* (Tindall *et al.,* 1984). In 1997 *Natronobacterim magadii* was assigned to the genus of *Natrialba* as a result of comparative rRNA analysis (Kamerkura *et al.*, 1997).

Due to observations of spontaneous lysis, 1997 a new halophage could be isolated from *Natrialba magadii*. The halophage, called ϕCh1, is a temperate virus, which can integrate into the *Nab. magadii* chromosome as a prophage. This strain, carrying ϕCh1, was termed L11 in comparison to the cured strain L13, which can be used as indicator strain (Witte *et al.,* 1997).

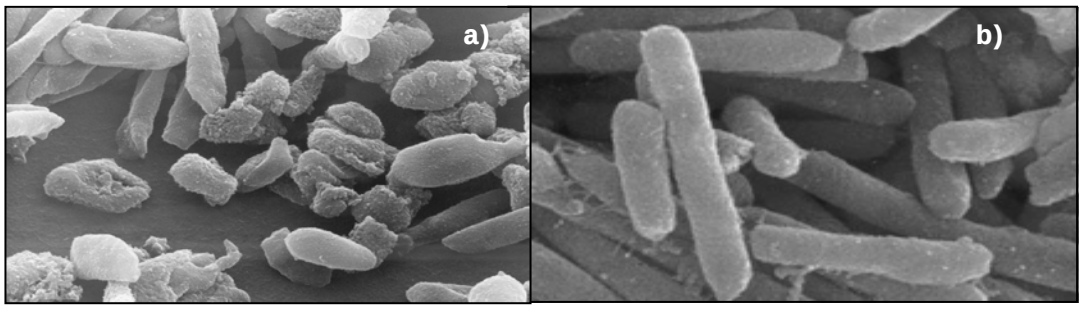


FIGURE 2 | ***Natrialba magadii.*** Electron micrograph of *Nab. magadii* **a|** strain L11, carrying ϕCh1 and **b|** indicatorstrain L13, which is cured from ϕCh1

1.1.7. Working with *Natrialba magadii*

1.1.7.1. Growth conditions

Nab. magadiis optimal growth conditions are 4M NaCl, pH values of 8.5- 10.5, a low Mg^{2+}concentration (below 10mM) and a growth temperature of 37°C- 42°C. *Nab. magadii* is sensitive to salt concentrations below 2M and lower temperatures. Due to cell lysis in solutions below 2M NaCl, *Nab. magadii* is a pleasant tool in the lab, concerning correct disposal of genetic manipulated microorganisms.

1.1.7.2. Generation time

The generation time of *Nab. magadii* is 9 hours in the logarithmic growth phase. In comparison, *E. coli* has a doubling time of 20 minutes.

1.1.7.3. Transformation

An essential component in genetics is to transfer foreign DNA into the microorganism of interest. Till recently, transformation of haloalkaliphilic *Archaea* including *Nab. magadii* remained impossible. Because of that, haloalkaliphilic *Archaea* are not so long-established in genetic research as the easily transformable *Archaea*, like *Hbt. salinarum* or *Hfx. volcanii* (see 1.1.5.2. and 1.1.5.3.). Methods of transforming these halophiles can be referred to the 'Halohandbook' by Michael Dyall- Smith (2009).

Lately, Iro *et al*., developed a method to transform haloalkaliphilic *Archaea* like *Nab. magadii* and *N. pharaonis*. Thereby, the key is to grow the cells in media containing bacitracin, which prevents the glycolysation of the S-Layer. After the enzymatic digestion of peptidbonds by proteinase K of *Tritirachium album*, the spheroblasted cells can be transformed with foreign DNA (Iro *et al.,* in prep).

1.1.7.4. Selection markers

Another prerequisite for transformation was to produce a plasmid with a selectable marker (usually a resistance to antibiotics). Most common antibiotics attack specific parts of *Bacteria*, but since *Archaea* differ in physiology, ordinary antibiotics are not useable. In the case of

Nab. magadii, 2 resistances could be proofed to be useful markers - novobiocin and mevinolin resistence (Iro *et al.*, in prep).

"*Novobiocin is an inhibitor of the bacterial type II DNA topoisomerase (DNA gyrase), an enzyme which introduces negative supercoils into relaxed DNA. The growth of halophilic archaebacteria is inhibited by novobiocin at relatively low concentrations*" (Sioud *et al.,* 1988). The resistance to novobiocin occurs with the expression of a mutated *gyrB* geneproduct (Holmes and Dyall-Smith, 1991).

Mevinolin on the other hand is a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor and thereby terminates the synthesis of the vital isoprenoid lipid side chains. The resistance against this drug is encoded by a mutated *hmg* gene locus and was isolated from *Hfx. volcanii* (Lam and Doolittle, 1989).

1.2. Halophages

1.2.1. Halophages in general

In 1974, Torsvik and Dundas discovered the first halophage (a bacteriophage that can infect halophile *Archaea*)- HS1. That happened eighteen years after discovery of its host *Hbt. salinarum*. The special feature of halophages is to maintain their structure stability and infectivity in high salt environment, as their hosts.

By now 15 different phages are known, which can infect members of the family of *Halobacteriacae* (Dyall- Smith *et al.*, 2003). All *Archaea* infecting phages, which have been cultured till that point of time, have ds DNA. The halophages have several forms, whereas the first isolated halophage looked like a typical member of the family of *Myoviridae*, with a polyhedral head, a contractile tail and ds DNA (Prangishvili *et al.*, 2006). Other common forms are fusiform, bottle-shaped, droplet-shaped, linear, and spherical forms.

Halophages are the predominant organisms in saline and hypersaline environments. In 1997, 10^7 virus like particles were counted per ml in the water of the dead sea (Oren *et al.*, 1997).

In 1982, the first isolation of a halophage succeeded (Schnabel *et al.,* 1982). This head- tail virus is known as ϕH and is the best studied halophage. Since 2010 the linear 59kb large genome is sequenced (Dyall- Smith, personal communication). The phage particles require 3M NaCl or KCL to maintain viability, unless Mg^{2+} is presented (Schnabel *et al.*, 1982). Its ds DNA is 59Kb in size and shows high similarities to the genome of ϕCh1 up to 97%, although it is an halophage of an haloalkaliphilic archaeon and therefore requires a higher pH value for viability (Klein *et al.*, 2002).

1.2.2. ϕCh1

The related halophage ϕCh1 was discovered in 1997, as previously mentioned, by Witte *et al.* and was the first isolated virus of a haloalkaliphilic archaeon. This halophage infects only *Natrialba magadii*. ϕCh1 belongs to the family of *Myoviridaea* and posses their typical structure- with an isoahedrical head, a contractile tail and double stranded DNA. The virus is 200nm in length, whereas the head comprises 70nm and the tail 130nm. The halophage requires a salt concentration of at least 2 M NaCl to maintain infectivity (Witte *et al.,*1997).

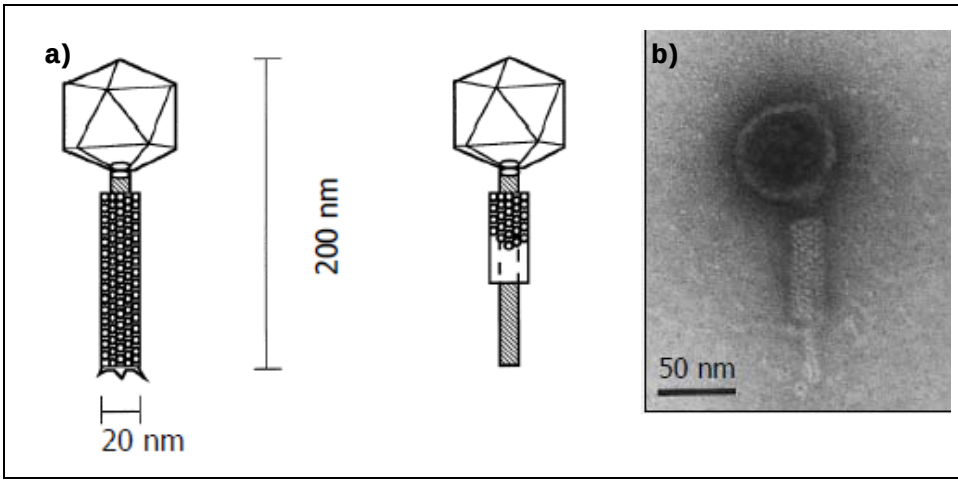


FIGURE 3 | **ϕCh1. a** |scheme of the morphology of ϕCh1 and **b**| electron micrograph of ϕCh1 (Witte *et al.,* 1997)

1.2.3. ϕCh1 Protein constitution

The ϕCh1 capsid possesses at least nine proteins, named in sequence protein A till protein I. They can be subdivided in 4 major proteins (A, E, H, I) and 5 minor proteins (B C, D, F, H) according to their quantity within the phage particle (Witte *et al.*, 1997). The capsid protein E for example is cleaved proteolytical during phage development and partially membrane associated (Klein *et al.*, 2000).

Protein E, as the majority of the ϕCH1 proteins are typical haloalkaliphilic proteins with isoelectric points ranging from pH 3.3 to pH 5.2 (Witte *et al.,* 1997).

1.2.4. Genomic organisation

The ϕCh1 genome has a linear double stranded DNA and a size of 58.498 base pairs. It was sequenced in 2002 by Klein *et al.*. The genome is circular permuted and terminally redundant, like the genome of ϕH1, indicating that the packaging of the viral DNA occurs by the so-called 'headful mechanism' (Klein *et al.,* 2002). Additionally, the phage particles contain 8 different species of RNA, comprising 80- 700 nucleotides. By performing RNA hybridization, it was shown that the RNA is host encoded. The function of the RNA is still unknown (Witte *et al*., 1997).

The G+C content of the DNA is 61,9%, a characteristic value for *Natronobacteria* (Tindall *et al.,* 1984).

Furthermore, it was observed that the DNA of ϕCh1 is partially methylated. This was demonstrated by restriction analysis (Witte *et al*., 1997) and by performing a high pressure liquid chromatography (Baranyi *et al.,* 2000). In the latter case, a fifth base was found, N6-methyladenosine. The responsible enzyme for the methylation, the (N6-adenine) methyltransferase and the corresponding gene was discovered subsequently. This M.ϕCh1-I gene is a late gene and the target sequence of the protein is 5'-GATC-3' and related sequences (Baranyi *et al.,* 2000). A reason for the methylation of DNA could be the stability against endonucleases or host mediated restriction systems (Witte *et al.,* 1997).

Concerning the structure of the linear ϕCh1 genome, it can be subdivided in three parts- the left part, containing structural genes, the central part with genes concerning replication and the right part, harbouring DNA modification genes (see figure 4). Such a subdivision in functional parts is typical for tailed double stranded bacteriophages and may be an indication for an evolutionary mechanism of action (Hendrix *et al.,* 2000).

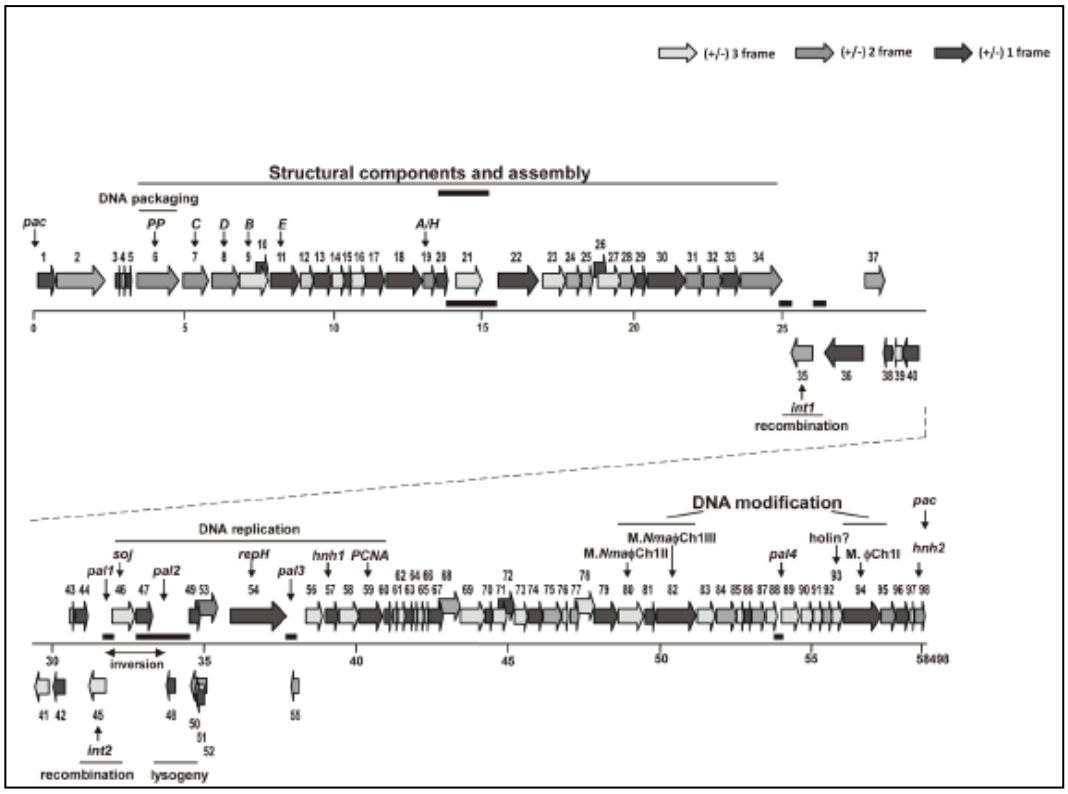


FIGURE 4| **Genomic organisation of ϕCh1.** The ϕCh1 genome is subdivided into three functional parts- the left part for structural genes, the middle part with the genes for DNA replication and the right part, containing genes for DNA modification. (Klein *et al.,* 2002, with modifications)

By searching start codons like ATG and GTGs, with a following length of 30 codons without any stopp codon, 98 ORFs could be predicted to be genes. Actually, most of the genes start with ATG, only ORF3, 41, 79 and ORF 83 start with GTG. The genes are partially overlapping, indicating that they represent transcriptional units.

Another interesting feature could be identified by sequencing: 2 site specific integrases, *int1* (ORF35) and *int2* (ORF45) from the λ integrase type (Klein *et al.,* 2002). Int1 leads to inversion reaction of ORF34 and ORF36, resulting in a 3'- end exchange of the two ORFs (Rössler *et al.,* 2004).

1.2.5. ϕCh1 as a temperate virus

ϕCh1 is a temperate virus, which means, that it can switch between the lysogenic and the lytic life cycle. During the lysogenic cycle, the phage DNA is integrated into the host genome and gets multiplied with each host encoded DNA replication and cell division. The cell lysis of its host occurs when the culture enters the stationary phase. In this lytic state, extrachromosomal DNA of the phage can be observed (Witte *et al.,* 1997). The switch between these two states needs a control mechanism and regulatory elements. In this diploma thesis I will focus on this really interesting topic and clarify previous ideas of regulators.

1.2.6. Putative regulators of lysogeny

As possible regulators of lysogeny, gp48 and gp49 were considered. The encoding ORF's are located next to each other, in a head- to- head constellation. The phage λ shows a similar arrangement with its two repressors Cro and C1 (Ptashne, 1987). Evidences suggest that both gene products are involved in gene regulation of ϕCh1, as described in the following. Another putative regulating element could be gp95, as illustrated afterwards.

1.2.7. ORF48

Gp48 shows similarities with other known repressors, also repressors from the related halophage ϕH, infecting *Hbt. salinarum.* Therefore the gene product was called Rep.

It posses a DNA binding motif, more precisely, it belongs to the winged helix DNA-binding proteins. To verify its repressor activity, expression studies of the reportergene bgaH were performed in the archaeal model organism *Hfx. volcanii*. *BgaH*, obtained from *Hfx. lucentense,* is a reportergene for haloalkaliphilic *Archaea*, similar to the common used reportergene β galactosidase (Pfeifer, 2001; Patenge *et al*., 2000). For the activity assay, transcriptional fusions were constructed, consisting of the intergenic region, between ORF48 and ORF49, fused to *bgaH* instead of ORF49. BgaH activity could be observed in *Hfx. volcanii*, so the intergenic region must contain a promoter region. Following, the same construct was extended with *rep* (ORF48). This construct lead to a decreasing expression of bgaH. If the construct was modified with an AUG deletion of ORF48, the original bgaH activity could be observed again. Taken together, Rep must act as a repressor on protein level, suppressing gene expression by binding the intergenic region (Iro *et al.,* 2006).

1.2.8. ORF49

The original gene controlled by Rep is ORF49, which is also thought to be a repressor. In searching for other elements, influencing lysis behaviour, a mutant strain of *Nab. magadii* was found. This mutant showed an increased plaque formation and was isolated from a single plaque. In liquid culture, *Nab. magadii* L11, carrying these mutated ϕCh1, showed an earlier onset of lysis. Instead of 3-4 days, the strain lysis after 2- 3 days. By performing restriction analysis and DNA sequencing, the mutation could be defined: a duplication of 223 base pairs within the ORF49 had occurred, which led to a gene disruption of ORF49 as mentioned in figure 5 (Iro *et al.,* 2006).

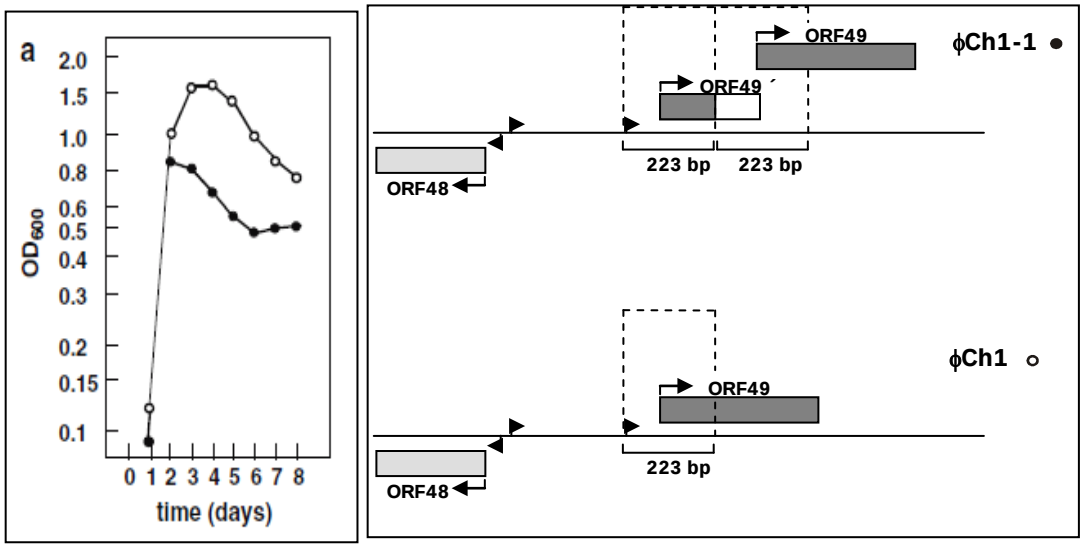


FIGURE 5| **ϕCh1-1- the ORF49 mutant. a**| Growth curve of *Nab. magadii* L11 (◦) , carrying ϕCh1 in comparison to *Nab. magadii* L11-1 , carrying ϕCh1-1(•) . The mutant strain *Nab. magadii* L11-1 shows an earlier onset of lysis. **b**| schematic description of the mutation. At the top, the mutated gene locus is shown. An insertion of the duplicated ORF49 had interrupted the ORF49. Below the wild type gene locus is shown. (Iro *et al.,* 2006)

Furthermore, the protein T4 of the related ϕH, is known to influence lysis and is very similar to ORF49 (Stolt and Zilig, 1994).

For further analysis, the two putative repressors gp48 and gp49 were tested with RT PCR, to verify their gene expression pattern. The experiment showed that ORF48 is constitutively expressed, while ORF49 is expressed in the logarithmic and in the stationary phase (see figure 6). Most of the known repressors for the lytic life cycle are only expressed during the lysogenic life cycle, so there must be an additional mechanism to control ORF49. To identify the exact position of the promotor regions of the two ORF's, primer extension experiments were performed. The mRNA of ORF48 starts at nu. 33944, with an AUG located 20 nu. upstream. The ORF49 mRNA starts at nu. 34385, and the AUG is 95 nu. upstream located.

Between these two ORF's a putative consensus promotor structure is located, 22nu upstream of the ORF49 mRNA start (Iro *et al.*, 2006). This structure has an AT rich composition, typical for haloalkaliphilic *Archaea* (Soppa, 1999).

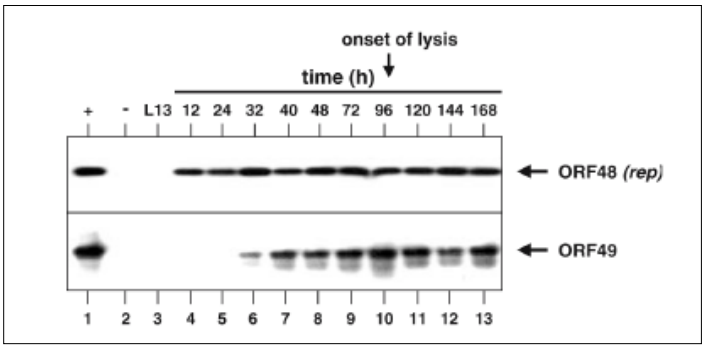


FIGURE 6| **Expression of ORF48 and ORF49.** RT-PCR to verify the time of expression of ORF48 and ORF49. ORF48 is constitutive expressed, while ORF49 is expressed during the logarithmic and the stationary growth phase (Iro *et al.*, 2006).

M. Reiter proved in 2010 the authenticity of the ORF49 as a repressor with further studies. Therefore ORF49 was transformed into *Nab. magadii* L13 and infected with ϕCh1. The presence of ORF49 resulted in a significant reduction of plaque formation as mentioned in figure 7.

With the construction of 3'-end deletion mutants, it could be shown which length of the C-terminus is essential for repressor activity. Therefore the deleted forms of ORF49 were transformed in *Nab. magadii* L13 and infected with ϕCh1. Strains, containing a maximum of 80 codons of gp49, showed still a repressor activity, whereas greater deletions led to a loss of repressor function as mentioned in figure 7 (Reiter, 2010).

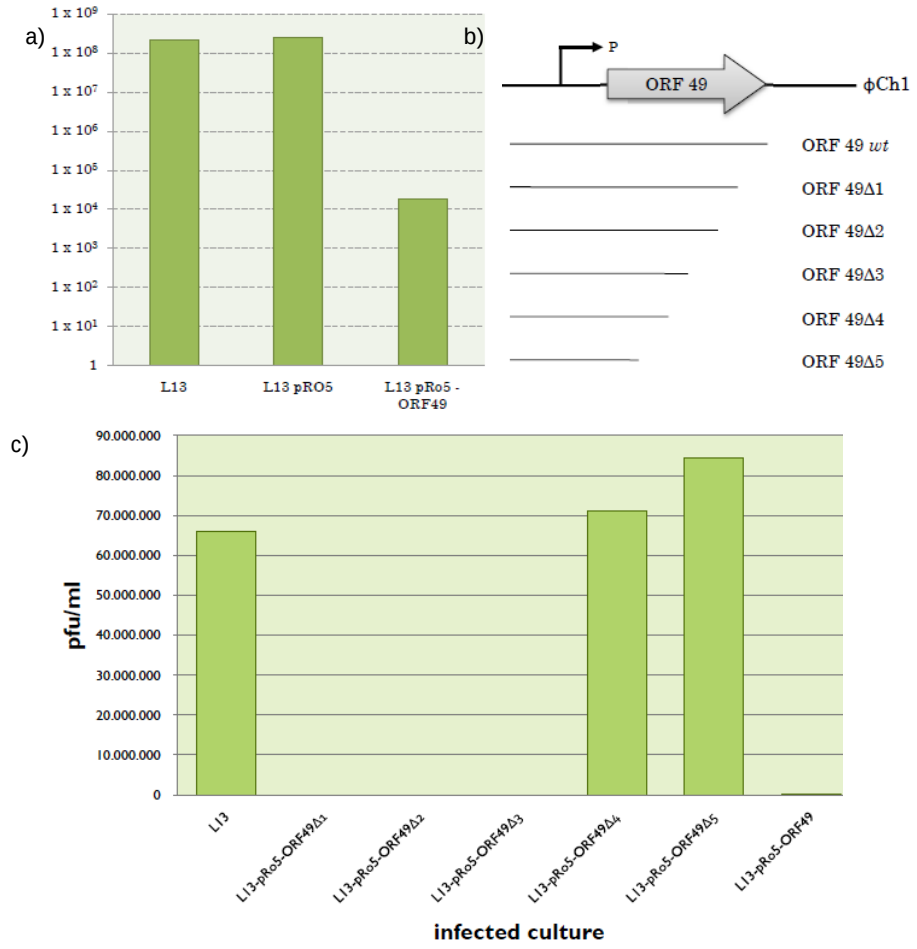


FIGURE 7 |**Repressor activity of ORF49 and c-terminal mutants of ORF49**.

a| Influence of ORF49 on infectivity of ϕCh1 on *Nab magadii* L13. Phage titer and corresponding plaque forming units (left axis) show that *Nab. magadii,* transformed with ORF49 show a decreased lyses behaviour. **b|** Schematic description of the c-terminus mutants. While ORF49 wildtype protein consists of 117aa, the mutants have following number of aminoacids: ORF49Δ1 -105aa, ORF49Δ2-93aa, ORF49Δ3- 81aa, ORF49Δ4-69aa and ORF49Δ5-57aa **c|** Phage titer and corresponding plaque forming units (let axis) show that *Nab. magadii,* transformed with ORF49 and ORF49 Δ1- Δ3 have full repressor activity, while ORF49 Δ4 and Δ5 show no more repressor activity.

(Reiter, with modification, 2010)

Further on it could be proven by performing an EMSA (electrophoretic mobility shift assay) that gpORF49 binds to DNA and that the N-terminus is responsible for DNA binding (Reiter, 2010).

1.2.9. ORF95

Another putative repressor could be ORF95 because it is the only protein in ϕCh1, containing a helix turn helix motif, which is known to be a DNA binding domain.

"The importance of the helix-turn-helix motif in DNA-protein interaction has been supported by a wealth of biochemical and genetic evidence" (Brennan and Matthews, 1989). The helix-turn helix- motif is known from other popular repressors like the Trp repressor from *E. coli* or the repressors from the phages λ and P22. Within the motif, the second helix is called the 'recognition helix', and it interacts with the major groove of the DNA.

With a DNA binding ability, the protein it is likely to be a transcriptional regulation element. With a regulating capability, gp95 maybe have influence on the switch between the lytic and the lysogenic cycle. To complete the search for regulators of lysogeny, ORF95 will be analyzed concerning its repressor activity and DNA binding ability within this diploma thesis.

1.2.10. Phage adsorption

Beside the unsolved question of regulation of lysogeny, it is still unknown, how the phages attach and adsorb to the host cell.

The first step in infection is the recognition of a specific receptor on the host's cell surface. In common cases the binding of the phage is mediated by a tail fiber protein. Here the reaction is irreversible, as the reaction is followed by a conformation change (Trun and Trempy, 2003). The binding of the specific receptor is responsible for the host specificity. ϕCh1's tail structure of consists of an internal shaft, covered by a contractile tail, which ends with a tail fiber protein. This structural protein is encoded by ORF34 and ORF36, respectively (Iro, 2006). The protein shows a conserved glycosylated protein binding domain, which indicates that ϕCh1 binds to any glycosylated structure or receptor. Putative receptors for adsorption are therefore the only 2 glycosylated structures on the cell surface- flagella and S-layer (Gaskell *et al.*, 1995; Eichler *et al.*, 2003). The second topic of this diploma thesis will be to focus on the question if the flagella are the structure, which is responsible for phage attachment and phage adsorption.

1.2.11. Genetic alteration methods of ϕCh1

In 2009, the first deletion mutation of *Nab. magadii* was constructed. The target gene in this experiment was the extracellular protease of the haloalkaliphilic archaeon. The deletion was performed with a disruption cassette, using the mechanisms of homologous recombination (Derntl, 2009).

Based on this, the try to delete genes of its phage ϕCh1, which is located within the *Nab. magadii* L11 genome, could be performed. By then, mutations could not be introduced definable.

In 2010, the first deletion mutation of a ϕCh1 gene was successful (Selb, 2010). Therefore the target gene ORF79, a gene of unknown function (Klein *et al.,* 2000), was displaced by a disrupted version. This disrupted version contained a novobiocin resistance instead of the central part of ORF79, flanked by its surrounding sequence to promote homologous recombination. If the strain carries the disruption and accomplishes lysis, mutated ϕCh1 particles are released.

With the analysis of these phenotypes, a new method to imply the function of ϕCh1 genes could be established.

2. Material and Methods

2.1. Material

2.1.1. Used Strains

Strain	*Genotype / features*	*Source*	*Media*
E. coli XL-1-Blue	endA1, gyrA96, hsdR17 (rk-mK+), lac, recA1, relA1, supE44, thi, (F´, lacIq, lacZΔM15, proAB+, tet)	Stratagene	LB
E.coli Rosetta	F-, ompT, hsdSB (rB-mB-), gal, dcm, lacY1, (DE3), pRARE6, (CmR)	Startagene	LB
Natrialba magadii L11	insertet ϕCh1	Witte *et al.,* 1997	NVM+
Natrialba magadii L13	Cured strain- no insertion of ϕCh1	Witte *et al*., 1997	NVM+
Natronomonas pharaonis	Wild type	DSM 2160	NVM+
Halobacterium salinarum R1	Wild type	Schnabel *et al*., 1982	Halobacteria medium
Haloferax volcanii WFD11	Wild type	Charlebois *et al.,* 1987	MGM 18%

2.1.2. Media

2.1.2.1. NVM (Natrialba rich medium)

Casaminoacids	8.8g
Yeast extract	11.7g
Tri-Na citate	0.8g
KCL	2.35g
NaCl	235g

pH 9,0
add dH2O to the final volume of 934mL

8g/l agar were added for plates
4g/l agar were added for Soft Agar
After autoclaving, the media was completed with
0,57M Na2CO3

1M MgSO4	65mL
20mM FeSO4	1mL
	1mL

2.1.2.2. Halobacteria medium

Casaminoacids	5g
Yeast extract	5g
Na- glutamate	1g
Tri-Na citate	3.4g
KCL	2g
NaCl	200g
$MgSO_4$x 7 H_2O	20g
$FeCl_2$ x 4 H_2O	36mg
$MnCl_2$ x 4H_2O	0.36mg

pH= 7.0- 7.2
add dH_2O to the final volume of 1L and autoclave it
20 g/l agar were added for plates
10g/l were added for softagar

2.1.2.3. LB (rich medium for *E. coli*)

Yeast extract	5g
NaCl	5g
Peptone	10g
add dH2O to the final volume of 1L and autoclave it	
15g/l agar were added for plates	

2.1.2.4. MGM 18%

Yeast extract	1g
Peptone	5g
Salt Water (30% Stock)	600mL
Pure Water	300mL

pH= 7,5

2.1.3. Antibiotics and additives

Additive	*Stockconcentration*	*Endconcentration*	*Remarks*
Ampicillin	20mg/ml	100µg/ml	Dissolved in ddH_2O; filter sterile; stored at 4°C
Tetracyclin	10mg/ml	10µg/ml	Dissolved in 70% Ethanol, stored at -20°C
Novobiocin	3mg/ml	3µg/ml	Dissolved in ddH_2O, filter sterile, stored at -20°C, light protected
Mevinolin	10mg/ml	5µg/ml	Dissolved in 96% Ethanol, stored at -20°C
Bacitracin	7mg/ml	70µg/ml	Dissolved in ddH_2O, filter sterile, stored at 4°C
IPTG	1M	0.5mM	Dissolved in ddH_2O, stored at -20°C

2.1.4. Plasmids

Plasmid	*Features*	*Source*
pQE30	*bla*, ColE1, N-terminal Poly(His)6-tag	Qiagen
pMDS24	*bla*, ColE1, (mevR), DHFR, pHV2 ori	Jolley *et al.*, 1996
pMDS11	*bla*, f1 ori, ColE1 ori, *gyrB*, pHK2 ori	Holmes, Nuttall and Dyall-Smith, 1991
pRSETA	mcs, *bla*, EK, PT7, RBS, His-tag, pUC ori, f1 ori	Invitrogen
pUC19	*bla*, pMB1ori, lacZa, mcs	Yanisch-Perron et al., 1985
pRSET-C	mcs, *bla*, EK, PT7, RBS, His-tag, pUC ori, f1 ori	Invitrogen
pNB102	*bla*, ColE1 ori, *hmg* (mevR), pNB101 ori	Zhou *et al.*, 2004
pRo-5	*bla*, ColE1 ori, *gyrB*, ϕCh1 derived ori	Iro *et al.*, in prep
pKSII+	mcs, *bla*, ColE1 ori, lacZα	Stratagen
pRR007	derived from pKSII+ MevR under 16S promoter control, φCh1 derived ori, MCS	Selb, 2010
pUC19-ORF49Δ1	*bla*, pMB1ori, lacZα, mcs, ϕCh1 ORF49Δ1 (nu. 34321-34795)	Reiter, 2010
pUC19-ORF49Δ2	*bla*, pMB1ori, lacZα, mcs, ϕCh1 ORF49Δ2 (nu. 34321-34760)	Reiter, 2010
pUC19-ORF49Δ3	*bla*, pMB1ori, lacZα, mcs, ϕCh1 ORF49Δ3(nu. 34321-34723)	Reiter, 2010
pUC19-ORF49Δ4	*bla*, pMB1ori, lacZα, mcs, ϕCh1 ORF49Δ4 (nu. 34321-34686)	Reiter, 2010
pUC19-ORF49Δ5	*bla*, pMB1ori, lacZα, mcs, ϕCh1 ORF49Δ5 (nu. 34321-34650)	Reiter, 2010
pUC19-ORF49Δ1-ORF54	*bla*, pMB1ori, lacZα, mcs, ϕCh1 ORF49Δ1(nu. 34321-34795),ORF54 (nu. 35848-37593)	Reiter, 2010
pUC19-ORF49Δ2-ORF54	*bla,* pMB1ori, lacZα, mcs, ϕCh1 ORF49Δ2 (nu. 34321-34760),ORF54 (nu. 35848-37593)	Reiter, 2010
pUC19-ORF49Δ3-ORF54	*bla*, pMB1ori, lacZα, mcs, ϕCh1 ORF49Δ3(nu. 34321-34723),ORF54 (nu. 35848-37593)	Reiter, 2010
pUC19-ORF49Δ4-ORF54	*bla*, pMB1ori, lacZα, mcs, ϕCh1 ORF49Δ4 (nu. 34321-34686),ORF54 (nu. 35848-37593)	Reiter, 2010
pUC19-ORF49Δ5-ORF54	*bla*, pMB1ori, lacZα, mcs , ϕCh1 ORF49Δ5 (nu. 34321-34650),ORF54 (nu. 35848-37593)	Reiter, 2010
pNB102-ORF49Δ1-ORF54	*bla*, ColE1 ori, *hmg* (mevR), pNB101 ori, ϕCh1 ORF49Δ1(nu. 34321-34795),ORF54 (nu. 35848-37593)	this thesis
pNB102-ORF49Δ2-ORF54	*bla*, ColE1 ori, *hmg* (mevR), pNB101 ori, ϕCh1 ORF49Δ2 (nu. 34321-34760),ORF54 (nu. 35848-37593)	this thesis

pNB102-ORF49Δ3-ORF54	*bla*, ColE1 ori, *hmg* (mevR), pNB101 ori, ϕCh1 ORF49Δ3(nu. 34321-34723),ORF54 (nu. 35848-37593)	this thesis
pNB102-ORF49Δ4-ORF54	bla, ColE1 ori, *hmg* (mevR), pNB101 ori, ϕCh1 ORF49Δ4 (nu. 34321-34686),ORF54 (nu. 35848-37593)	this thesis
pNB102-ORF49Δ5-ORF54	*bla,* ColE1 ori, *hmg* (mevR), pNB101 ori , ϕCh1 ORF49Δ5 (nu. 34321-34650),ORF54 (nu. 35848-37593)	this thesis
pNB102-ORF49	*bla,* ColE1 ori, *hmg* (mevR), pNB101 ori , ϕCh1 ORF49 (nu. 34480-34833)	this thesis
pUC19-ORF49Δ1-RadB	*bla,* pMB1ori, lacZα, mcs, ϕCh1 ORF49Δ1 (nu. 34321-34795), RadB (Hfx. volcanii, nu. 772-1498, access. no. AJ704823)	this thesis
pUC19-ORF49Δ2-RadB	*bla*, pMB1ori, lacZα, mcs, ϕCh1 ORF49Δ2 (nu. 34321-34760), RadB (Hfx. volcanii, nu. 772-1498, access. no. AJ704823)	this thesis
pUC19-ORF49Δ3-RadB	*bla*, pMB1ori, lacZα, mcs, ϕCh1 ORF49Δ3(nu. 34321-34723), RadB (Hfx. volcanii, nu. 772-1498, access. no. AJ704823)	this thesis
pUC19-ORF49Δ4-RadB	*bla,* pMB1ori, lacZα, mcs, ϕCh1 ORF49Δ4 (nu. 34321-34686),RadB (Hfx. volcanii, nu. 772-1498, access. no. AJ704823)	this thesis
pUC19-ORF49Δ5-RadB	*bla*, pMB1ori, lacZα, mcs, ϕCh1 ORF49Δ5 (nu. 34321-34650),RadB (Hfx. volcanii, nu. 772-1498, access. no. AJ704823)	this thesis
pRo-5-ORF49Δ1-RadB	*bla,* ColE1 ori, *gyrB,* ϕCh1 derived ori, ϕCh1 ORF49Δ1 (nu. 34321-34795), RadB (Hfx. volcanii, nu. 772-1498, access. no. AJ704823)	this thesis
pRo-5-ORF49Δ2-RadB	*bla,* ColE1 ori, *gyrB*, ϕCh1 derived ori, ϕCh1 ORF49Δ2 (nu. 34321-34760), RadB (Hfx. volcanii, nu. 772-1498, access. no. AJ704823)	this thesis
pRo-5-ORF49Δ3-RadB	*bla,* ColE1 ori, *gyrB*, ϕCh1 derived ori, ϕCh1 ORF49Δ3(nu. 34321-34723), RadB (Hfx. volcanii, nu. 772-1498, access. no. AJ704823)	this thesis
pRo-5-ORF49Δ4-RadB	*bla,* ColE1 ori, *gyrB,* ϕCh1 derived ori, ϕCh1 ORF49Δ4 (nu. 34321-34686),RadB (Hfx. volcanii, nu. 772-1498, access. no. AJ704823)	this thesis
pRo-5-ORF49Δ5-RadB	*bla,* ColE1 ori, *gyrB,* ϕCh1 derived ori, ϕCh1 ORF49Δ5 (nu. 34321-34650),RadB (Hfx. volcanii, nu. 772-1498, access. no. AJ704823)	this thesis
pUC19-ORF49prom	*bla,* pMB1ori, lacZα, mcs, ϕCh1 ORF49promotor (nu.3421- 34479)	this thesis

pUC19-ORF49prom-cterm	*bla*, pMB1ori, lacZα, mcs, ϕCh1 ORF49promotor (nu.3421- 34479) and c-terminus (nu.34635-34817)	this thesis
pRo-5-ORF49prom-cterm	*bla,* ColE1 ori, *gyrB,* ϕCh1 derived ori, ϕCh1 ORF49promotor (nu.3421- 34479) and C-terminus (nu.34635- 34817)	this thesis
pUC19-ORF49prom-cterm-short	*bla,* pMB1ori, lacZα, mcs, ϕCh1 ORF49promotor (nu.3421- 34479) and C-terminus 'short'(nu.34635-34686)	this thesis
pRo-5-ORF49prom-cterm-short	*bla,* ColE1 ori, *gyrB*, ϕCh1 derived ori, ϕCh1 ORF49promotor (nu.3421- 34479) and C-terminus 'short' (nu.34635-34686)	this thesis
pKSII+ORF49-flanking	mcs, *bla*, ColE1 ori, lacZα, Flanking regions of ϕCh1 ORF49(nu.33483-34462 and nu.34841-35954)	this thesis
pKSII+ORF49-deletioncasette	mcs, *bla*, ColE1 ori, lacZα, flanking regions of ϕCh1 ORF49(nu.33483-34462 and nu.34841-35954) + novobiocin resistance	this thesis
pJAS-Fla		this thesis
pRR007-Fla	pKSII+ MevR under 16S promoter control, ϕCh1 derived ori, MCS, Nab magadii flagella operon	this thesis
pUC19-fdx	*bla*, pMB1ori, lacZα, mcs, fdx promotor	Till, 2011
pRo-5-ORF34	*bla*, ColE1 ori, gyrB, ϕCh1 derived ori, ϕCh1 ORF34 (nu. 23246-24626)	this thesis
pUC19-fdx-ORF34	*bla*, pMB1ori, lacZα, mcs, fdx promotor, ϕCh1 ORF34 (nu. 23246-24626)	this thesis
pRo-5-fdx-ORF34	*bla*, ColE1 ori, *gyrB*, ϕCh1 derived ori, fdx promotor, ϕCh1 ORF34 (nu. 23246-24626)	this thesis
pUC19-ORF49prom-ΔAUG	*bla*, pMB1ori, lacZα, mcs, ϕCh1 ORF49 lacking AUG(nu.34321- 34479 and nu. 34483-34818)	this thesis
pRo-5-ORF49prom-ΔAUG	*bla,* ColE1 ori, *gyrB*, ϕCh1 derived ori, ϕCh1ORF49 lacking AUG (nu.34321- 34479 and nu. 34483-34818)	this thesis
pRo-5-ORF95	*bla,* ColE1 ori, *gyrB*, ϕCh1 derived ori, ϕCh1 ORF95 (nu. 57643-57981)	this thesis
pRSET-A-ORF95	mcs, *bla*, EK, PT7, rbs, His-tag, pUC ori, f1 ori, ϕCh1 ORF95 (nu. 57643-57981)	this thesis
pRSET-A-ORF95ΔAUG2-F1+F2	mcs, *bla*, EK, PT7, rbs, His-tag,pUC ori, f1 ori, ϕCh1 ORF95 lacking AUG (nu.56021- 57086 and nu.57095- 57638)	this thesis
pRo-5-A-ORF95ΔAUG2-F1+F2	*bla,* ColE1 ori, *gyrB,* ϕCh1 derived ori, ϕCh1 ORF95 lacking AUG (nu.56021- 57086 and nu.57095- 57638)	this thesis

2.1.5. Primer

Primer	Sequence	Restriction site
FlaB2-1	GACC**GGATCC**ATGTTCACTAACGACACCGA	*BamH1*
FlaB2-2	CAGG**AAGCTT**AGAGTCGGACCGCTTC	*HindIII*
FlaB3-1	GACC**GGATCC**ATGTTCACATCCAATACAGATGA	*BamH1*
FlaB3-2	CAGG**AAGCTT**ATAGCCTCACAGATTCGTCA	*HindIII*
FlaB4-2	CAGG**AAGCTT**AGAGGCGAACTGCTTCG	*HindIII*
Fla-Eco	GACC**GAATTC**GATATCCACCTATCGGCAGTTAG	*EcoRI*
Fla-Hind	GACC**AAGCTT**GATATCCACCTATCGGCAGTTAG	*HindIII*
Fla1-Nco	GAAA**CCATGG**TCGAACAAAACGACGA	*NcoI*
Fla2-Nco	GAAA**CCATGG**TTAGAGGCGAACTGCTTCG	*NcoI*
Fdx-1	GAAT**GGTACC**CTGACGCCGCGGGCAGC	*KpnI*
Fdx-2	GAAT**TCTAGA**CCATGGGCATCACCAGAGTT	*XbaI*
34-5	CAGCAG**AGATCT**ATGAGTAAAATCTGGGAACCGAG	*BglII*
36-3	CAGCAG**AAGCTT**ATTCAGGTTTCATGTCGCTG	*HindIII*
34-XbaI-a	GACG**TCTAGA**CTCCGATGAACACGACACTC	*XbaI*
34-inv1	GAGCGGTGGCGTCGAC	n/a
34-inv2	GTCATCCAGTCGCCGC	n/a
pRo5-95H	GACC**AAGCTT**GGTACGACGGCGACG	*HindIII*
pRo5-95K	GACC**GGTACC**GTGGAAAGAGTCAGTCGTCG	*KpnI*
95-M2-1	GATC**GAATTC**CGGCGTCGCCATTCT	*EcoRI*
95-M2-2	GATC**GAATTC**AGTGATGATATCGAGTACGGCG	*EcoRI*
95-1	GCGAGCGGCGAGGAC	n/a
95-2	GTGGAAAGAGTCAGTCGTCG	n/a
49-Kpn	CAGC**GGTACC**TTGCGTTCAGTTCCG	*KpnI*
49-Cla	GACC**ATCGAT**CGAGGCGTCATCCT	*ClaI*
49-EcoR1-5	CAGC**GAATTC**TTGCGTTCAGTTCCG	*EcoR1*
12-7-5	CAGCAG**AAGCTT**TCATCCTGCGGTTTC	*HindIII*
12-7-3	CAGCAG**AAGCTT** TCATCCTGCGGTTTCG	*HindIII*
12-7-5C	AATT**GGATCC**ATGAGAAAAATCAACGCCG	*BamH1*
49Δ-1	CAGC**AAGCTT**TCAGCCATTGGTCCGCGAGC	*HindIII*
49Δ-2	CAGC**AAGCTT**TCAGCCCGGAAAGGACGACA	*HindIII*
49Δ-3	CAGC**AAGCTT**TCAGCCTCTCACCGAGGCGC	*HindIII*
49Δ-4	CAGC**AAGCTT**TCACAAGAACAGGAGAGTGTCCA	*HindIII*
49Δ-5	CAGC**AAGCTT**TCACCGGCGTTGATTTTTCG	*HindIII*
Δ49-1	GACC**GGATCC**CGATGATCAATCGAAGCG	*BamH1*
Δ49-2	GACC**AAGCTT**CCCGGGTTCCTGGGCCTCTTTGAA	*HindIII*
Δ49-3	GACC**AAGCTT**ACGGGCCTGACGCTTC	*HindIII*
Δ49-4	GACC**GGTACC**CGTAGTCCACGTCGACGC	*KpnI*
49-ΔATG	GACC**GGATCC**AACACCCCCAATAGACACTG	*BamH1*
C-Term2	GACC**GGATCC**ATGCCCCATCGTGATAACGTT	*BamH1*
RadB-1	GAAT**GGATCC**ATGACAGAGTCAGTCTCCACC	*KpnI*
RadB-2	GACC**AAGCTT**CTACACGTCAGTCGCGG	*HindIII*
prom-2	GACGAC**GGATCCT**CCTGGGCCTCTTTG	*BamH1*

56-3X	CAGCA**TCTAGA**TACCGCACTGCAG	*XbaI*
TR-1	AATTGCGGCCGCCGCGTTGAAGGCA	n/a
TR-2	AATTTCTAGATCCTGGGCCTCTTTGAA	n/a
0267-2	GCTGCGTCTTCGATCGT	n/a
0267-1	GCACAGCCACGAATCTTG	n/a
4910-1	GCCGAAGATTCTGCTGC	n/a
4910-2	GGCGGCTTGGAGGTC	n/a
phk2-1	GAAACTCCCCGTCCAGC	n/a
phk2-2	GCGAGCCAACTGCCG	n/a
93-Pst	GATACTGCAGGGTATCTGCGTTCAGTGG	n/a
93-Nae	GATAGCCGGCCAGTATCGAGATCGCGC	n/a
79-Pst	GATACTGCAGCTCTTTGTACCGATGCGTC	n/a
79-Nae	GATAGCCGGCGACTCTCACAAGATCTC	n/a
HV2	GGTGTCGGTTGTAGTCGGGT	n/a
HV1	GACGACCACCGACCGAC	n/a
Nov-6	GGGATCGCAGAGGAGC	n/a
Nov-9	GATGTCGGTCATCGCGG	n/a
38292	CAGCACTGCAGATCTCCCGCTGGCCCTT	n/a

n/a: not available

2.1.6. Enzymes

Enzyme	*Source*	*Remarks*
Restrictionenzymes	Fermentas	the coresponing buffer in case of double digests was determined at following page http://www.fermentas.com/en/tools/doubledigest
Pwo polymerase	Fermentas	complete buffer was used, including Mg^{2+}
Go Taq polymerase	Promega	for analytical PCRs, the green buffer was used.
T4 Ligase	Fermentas	ligase buffer, containing ATP, was aliquoted and not reused after freezing and thawing.
Proteinase K	Roche	
FastAP™ Thermosensitive Alkaline Phosphatase	Fermentas	
Klenow polymerase	Fermentas	

2.1.7. Marker

2.1.7.1. DNA Markers

λ Marker (restricted with *BstE*II) – used for 0.8% agarose gels

Fragments: 8454, 7242, 6369, 5687, 4822, 4324, 3675, 2323, 1929, 1371, 1264, 702 bp

100µg λ DNA were digested with 10µl *Eco*91I (*BstE*II) and Buffer O from Fermentas in a 500µl batch, over night at 37°C. The restriction was diluted with 400µl 5x loading dye and 1100µl ddH_2O.

pUC19 Marker (restricted with *Sau*3a) – used for 6% PAA DNA gels

955 bp, 585 bp, 341 bp, 258 bp, 141 bp, 105 bp, 78/75 bp, 46 bp, 36 bp, 18/17 bp, 12/11 bp, and 8 bp

50µl pUC 19 plasmid preperation (approximately 400ng/µl) were digested with 4µl *Sau*3a in an 80µl batch at 37°C over night. The restriction was checked on gel and afterwards diluted with water and 5x loading dye.

pUC19 Marker (restricted with *Hae*III) – used for 6% PRR DNA gels

Fragments: 587, 458, 434, 298, 257, 174, 102, 80, 18, 11 bp

50µl pUC19 plasmid preparation (approximately 400ng/µl) were digested with3µl *Bsu*R1 *(Hae*III*)* and buffer R from Fermentas in a 120µl batch, over night. The restriction was diluted with 5x loading dye and ddH_2O

2.1.7.2. Protein Markers

PageRuler™ Prestained Protein Ladder (Fermentas # SM0671)

170, 130, 100, 70, 55, 40, 35, 25, 15, 10kDa

Unstained ProteinMolecular Weight Marker (Fermentas; #SM0431)

116, 66.2, 45, 35, 25, 18.4, 14.4 kDa,

2.1.8. Solutions and buffer for DNA methods

2.1.8.1. Electrophoresis

50x TAE

Tris	2 M
Acetic acid	1 M
EDTA, pH 8.2	0.1 M

10x TBE

Tris	108g
Boric acid	60g
0,5M EDTA	40mL
pH8 with Boric acid	

5x loading dye

Tris/ HCL pH 8	50mM
Saccharose	25%
SDS	0.1%
Bromphenolblau	0.05%

6% PAA Gel

30 % PAA (29:1)	1.2ml
1x TBE	4.8ml
10 % APS	60 µl
TEMED	6µl

30% AA Lösung

Acryl amid	29%
N,N”-methylene bisacrylamid	1%

10x Band shift running buffer

Tris-HCL pH8	70mM
NaOAc	30mM
EDTA	20mM

5x GBB

Tris- HCL pH 8	50mM
EDTA	25mM
Glycerol	30%
DTT (freshly added)	5mM

2.1.9. Solutions and buffer for protein methods

2.1.9.1. SDS PAGE

5 mM sodium phosphate buffer pH 6.8

Na H_2PO_4	0.2 M
Na_2HPO_4	0.2M

2x protein sample buffer

Tris-HCl, pH 6.8	0.12 mM
SDS	4%
glycerol	17.4%
β-mercaptoethanol	2%
bromphenol blue	0.02%

10x SDS-PAGE running buffer

Tris	0.25 M
Glycine	1.92 M
SDS	1%

30% AA

Acryl amid	29%
N,N”-methylene bisacrylamid	1%

4x separation gel buffer

Tris- HCL pH 8.8	1.5M
SDS	0.4%

4x stacking gel buffer

Tris- HCL pH 6.8	0.5M
SDS	0.4%

Coomassie staining solution

Methanol	25%
Acetic acid	10%
Coomassie Brilliant Blue R-250	0.15%

Coomassie Destaining solution

Methanol	25%
Acetic Acid	10%

2.1.9.2. Western blot analysis

Ponceau S solution

Ponceau S	0.5%
TCA	3%

10xTBS

Tris- HCL	250mM
NaCl	1.37M
KCl	27mM
Add 0.05% Tween20 for TST-T	

Transblotbuffer

Tris	48mM
Glycine	39mM
SDS	0.037%
Methanol	20%

Primary antibodies

α- ORF36 (serum from rabbit, immunized with ϕCh1ORF36 (1:500 in 1x TBS+ 0.6% BSA)

secondary antibodies

α rabbit (1:5000 in 1x TBS)

antibody conjugated to horseradish peroxidase

2.1.9.3. Protein purification (denaturating)

Lysisbuffer- buffer B

NaH_2PO_4	100mM
Tris-HCL	10mM
Urea	8M
pH= 8.0 with NaOH	

Washbuffer- buffer C

NaH_2PO_4	100mM
Tris-HCL	10mM
Urea	8M
pH= 6.3 with HCL	

Elutionbuffer -buffer E

NaH_2PO_4	100mM
Tris-HCL	10mM
Urea	8M
pH= 4.5 with HCL	

Dialysisbuffer

Urea	4M
NaCl	2M
Tris-HCL	50mM
pH=9.5	

2.1.10. Solutions and buffer for phage isolation

Buffer

NaCl	2M
Tris-HCL	50mM
pH = 8.5- 9	

Lsg 1.1

CsCl	20g
Buffer	200ml

Lsg1.3

CsCl	90g
Buffer	200ml

Lsg1.5

CsCl	135g
Buffer	200ml

Dialisysbuffer

NaCl	4M
Tris-HCL	50mM
pH = 9.5	

2.1.11. Solutions and buffer for *Archaea* methods

High salt buffered spheroplasting solution with Glycerol

NaCl	2M
KCl	27mM
Tris-HCL pH 8.2	50mM
Sucrose	15%
Glycerol	15%

High salt unbuffered spheroplasting solution 60% PEG 600

NaCl	2M	PEG 600, heated	600µl
KCl	27mM	Unbuffered spheroblasting solution	400µl
Tris-HCL pH 8.2	50mM		
Sucrose	15%	Store at -80°C	

2.2. Methods

2.2.1. Methods - Phages

2.2.1.1. Phage isolation

250ml dense culture *Nab. magadii* L11 were transferred into 6l NVM+ (2x3l) and grown at 37°C, shaking. Optical density was measured every day to observe the moment of lysis. When optical density reached values below 0,5, the lysed cell culture was centrifuged at 8krpm, 20 minutes, at room temperature. The supernatant, containing the phages, was mixed up with 10% w/v PEG 6000, to precipitate the phages, stirring, over night.

The phages were centrifuged again, with 8krpm, 20 minutes, at room temperature. The pellet, containing the phage particles were resuspended in 50ml 4M NaCl/50mM Tris-HCL (pH value of 9.5). This suspension was separated with a cesium chlorid density gradient using an ultracentrifuge. Therefore the solution was carefully applied to a Beckman Ultracentrifuge tube, together with 3 layers of CsCl with different densities. Each tube was filled with 2ml solution 1.5- 4ml solution 1.3- 5ml virussuspension and 1 ml solution 1.1. This was centrifuged for 20 hours at 30krpm at room temperature, with a swing bucket rotor (SW40Ti). This procedure resulted in 2 bands- whereas the higher band consists of flagella and the lower one contains the phage particles. These were carefully taken and centrifuged again, this time with a continuous gradient, using solution 1.3, only, under same conditions.

The only band, containing the purified phages, was taken and dialysed against 4M NaCl/ 50mM Tris HCL (pH value of 9.5). To determine the amount of the isolated phages, a phage titer was performed.

2.2.1.2. Phage titer

To verify the quantity of phages/ml, the phage isolation or supernatant had to be plated with an indicator strain (*Nab. magadii* L13) in different dilutions. Therefore the dense indicator strain (400µl) was plated with 5ml softagar (~55°C) and diluted phage suspension on Agarplates. Dilutions reached from 10^{-2} – 10^{-10}, according to the experiment. The phage titer was put in bags at 37°C for 1 week.

2.2.1.3. Isolation of phage DNA

300µl ddH_2O and 200µl phenol chloroform (1:1) were added to 100µl ϕCh1. The mixture was vortexed for 20 seconds and centrifuged at 13.200rpm for 5 minutes. The aqueous phase was transferred into a new eppendorf tube and mixed again with 200µl phenol chloroform (1:1). Following, the last steps were repeated 3 times. The aqueous phase was then mixed with 96% Ethanol, to precipitate the DNA. The mixture was left 15 minutes at -80°C and following centrifuged at 16.400 rpm for 30 minutes. The DNA pellet was washed with 70% ethanol, twice. Therefore 1 ml ethanol was added and following centrifuged for 5 minutes at 13.200 rpm. The pellet was dried and mixed with 25µl ddH_2O.

2.2.1.4. Adsorption assay

Cultures of interest were incubated at 37°C until they were dense. Corresponding amounts of the pre cultures were transferred into 25mL media to reach the OD_{600} of 0.1. This main culture was incubated over night at 37°C, shaking, till an OD_{600} of 0,4 was reached. The culture was divided into two times 10ml. Now a well- defined amount of phages was added. The cultures with phages were incubated at 37°C, NOT shaking. At different points of time, samples were taken. For sample taking, each 1 ml was centrifuged at 10.000rpm for 10 minutes. The supernatant was transferred into a new Eppendorf tube and mixed with 20µl chloroform. The supernatant contains all the phages, which were not adsorbed till this moment. The quantity of these phages was determined with a following page titer analysis. Therefore the supernatant was diluted in 10^{-2}, 10^{-4} and 10^{-6}.

2.2.2. DNA methods

2.2.2.1. Measuring DNA concentration

DNA concentrations were estimated using agarose gel electrophoresis or using the sprectrophotometer NanoDrop ND-1000 from Peqlab.

2.2.2.2. Agarose Gel

The concentration of agarose gels was adapted for size and level of separation. The agarose was solved in 1x TAE and heated in a microwave till it was melted completely. When it was warm to the touch, it was cast in the corresponding apparatus. After polymerization, it was transferred into a running apparatus, filled with the running buffer- 1x TAE. Running conditions and duration were depending on fragment size and size of the running apparatus.

2.2.2.3. PAA Gel

For small fragments (smaller than 700Bp) a 6% PAA gel electrophoresis was used. Therefore PAA was mixed with 1xTBE, 10%APS and TEMED and cast into a Biorad Mini – Protean® apparatus. The polymerized gel was run in the corresponding running apparatus with 20mA/gel for 26minutes.

2.2.2.4. Visualization of DNA

To stain DNA gels, an ethidiumbromid staining bath with the concentration of 10mg/ml ethidiumbromid was used, for approximately 15 minutes followed by short destaining in ddH_2O. The stained gel was visualized with UV light, afterwards.

2.2.2.5. Agarose gel elution

To isolate specific bands from an agarose gel, DNA was load with corresponding amounts of 5x loading dye on a agarose gel with 20µl per slot. The concentration of the agarose gel and run conditions were chosen depending on the required level of separation. After the run, the gel was shortly stained and visualized with 70% UV. The band of interest was cut out and transferred into an eppendorf tube. The following steps of purification were performed with the QIAquick gel extraction kit (Qiagen), following the recommended protocol. The purified DNA was eluted with 40µl- 50µl, depending on the concentration.

2.2.2.6. PAA gel elution

The DNA sample was loaded on 6% PAA gel, mixed up with corresponding amounts of 5x loading dye. The gel was run with 1x TBE and 15mA/ gel. After the run the gel was shortly stained and visualized with UV (70%). The band of interest was cut out and hackled, using a pasteur pipette. The small gel pieces were mixed up with 300µl elutionbuffer (QIAquick PCR purification kit (Qiagen)) and incubated over night at 37°C, shaking. The batch was centrifuged at 13.200 rpm for 5 minutes and the clear supernatant, containing the DNA, was transferred into a new eppendorf tube. Afterwards, it was purified with the QIAquick PCR purification kit (Qiagen). The DNA was eluted with 40µl- 50µldd H_2O, depending on their concentration.

2.2.2.7. Band shift assay

The template DNA concentration was measured with Nano Drop (ND 1000, Peqlab) and cut with the corresponding restriction enzyme. The digested DNA was checked using agarose gel electrophoresis. If the concentration was to less, the DNA was lyophilised, using a speed vac.

The concentration of the protein was estimated with the SDS PAGE. To have an indicator, BSA standards in different concentration were load on the Gel, too.

The ratio of DNA and Protein was calculated. Mostly the ratio was 1:0, 1: 50, 1:200, 1: 400, 1: 800. The batch was composed in the following way:

4µl 5x GBB
2µl BSA (1mg/ml)
xµl protein (x pmol Protein)
xµl DNA (0.02 pmol DNA)
x µl Urea (4M or 8M)
ad 20µl ddH_2O

As controls- 1 batch without protein and 1 batch with BSA as only protein were used.

The mixed batches were incubated 1 h at 37°C and afterward load with 5µl Orange G loading dye on an 0.8% Agarose gel. The Gel was run with 5V/cm. Once an hour, the band shift running buffer was renewed. After the end of the run, the gel was stained with ethidiumbromid and visualized under UV light.

2.2.2.8. PCR

To amplify DNA, the polymerase chain reaction was used. Depending on further purposes, an analytical or a preparative approach was performed. Primers were obtained from VBC (lyophilised) and finally diluted to a concentration of 0,1µg/µl. The template could be obtained by diluting plasmid/genomic ϕCh1 DNA 1:30 or lysing cell culture. Therefore 20µl halophile cell culture were mixed up with 80µl ddH20.

2.2.2.9. Analytical PCR

The analytical approach was used to determine positive clones during cloning process. Therefore a GoTaq® DNA Polymerase (Promega) was used.

The analytical batch consists of:

5µl	2mM dNTPs
10µl	5x GoTaq® buffer
1µl	template
2,5µl	primer reverse (0.1µg/µl)
2,5µl	primer forward (0.1µg/µl)
0,5µl	GoTaq® DNA polymerase
	ddH_2O ad 50µl

PCR program:

1	95°C	5 min
2	95°C	1 min
3	Specific*	1 min
4	72°C	Specific* → 2 # 33
5	72°C	5min

*Annealing temperature depends on composition of the primer binding part of the target DNA. The elongation time depends on the length of the PCR product, whereas GoTaq® DNA polymerase inserts 1000bp/min.

2.2.2.10. Preparative PCR

The preparative approach was used for cloning. The Peqlab *Pwo* polymerase posses a proof reading activity and therefore is less error prone.

The preparative PCR batch consists of:

10µl	2mM dNTPs
10µl	10x Pwo complete Buffer
1µl	template
5µl	primer reverse (0,1µg/µl)
5µl	primer forward (0,1µg/µl)
2µl	Peq *Pwo* DNA polymerase
	ddH_2O ad 100µl

PCR program:

1	95°C	5 min
2	95°C	1 min
3	Specific*	1 min
4	68°C	Specific* → 2 # 33
5	68°C	5min

* Annealing temperature depends on composition of the primer binding part of the target DNA. The elongation time depends on the length of the PCR product, whereas the Peqlab *pwo* polymerase inserts 1000bp/min.

2.2.2.11. PCR purification

PCRs were purified, using the QIAquick PCR purification kit (Qiagen) and following the recommended protocol. The purified PCR was eluted with 40µl- 50µldd H_2O, depending on their concentration.

2.2.2.12. Plasmid prep

To isolate plasmids of *E. coli* cultures, 2 times 1.5ml of the over night culture were pelletised. The cells were lysed and plasmid DNA was isolated and purified using the GeneJET™ plasmid miniprep kit (Fermentas). The purified plasmid DNA was eluted with 40µl- 50µldd H_2O, depending on their concentration.

2.2.2.13. Restrictions

Restrictions were performed with restriction enzymes from Fermentas, following the recommended protocol. Therefore the DNA was measured with the Nano Drop ND-1000 from Peqlab and the batch was calculated according to the DNA concentration. If the restriction was a double digestion, the corresponding buffer was determined at the following homepage http://www.fermentas.com/en/tools/doubledigest.

If the restriction was performed for analytical purposes, the incubation could be shortened (approximately 1 hour) and afterwards loaded on the agarose gel completely.

If the restriction was performed for cloning purposes, the recommended incubation time was adhered. Afterwards, the success of the restriction was analyzed performing an agarose gel electrophoresis- at least in the cases, were differences between restricted and unrestricted DNA could be distinguished. To change the buffer for the next cloning step, the QIAquick PCR purification kit (Qiagen) was used, eluting with water again.

2.2.2.14 Klenow fragment

The Klenow polymerase fills up 5' overhangs and is therefore used to fill in sticky ends. After determining the concentration of the target DNA (Nano Drop ND-1000 from Peqlab) the batch could be calculated, following the protocol from Fermentas. The batch was incubated for 30 minutes at 37°C and inactivated for 15 minutes at 75°C.

2.2.2.15. Alkaline phosphatase

The alkaline phosphatase (Fast AP, Fermentas) was used to prevent vector religation by dephosphorylating the vector DNA. Therefore the restricted vector was measured and the batch was composed as recommended. The batch was incubated 10 minutes at 37°C and afterwards inactivated at 75°C for 5 minutes.

2.2.2.16. Ligation

To ligate insert and vector DNA, the concentration of both had to be estimated by performing a agarose gel electrophoresis. If the restricted vector DNA was too high concentrated, it was diluted 1:10. The ligation batch consists of:

1µl vector DNA
11.5µl fragment
1.5µl Ligase buffer
1µl T4 DNA Ligase (Fermentas)

The batch was incubated over night at 16 °C or 3-4 hours at room temperature.

2.2.3. Transformation

2.2.3.1. *E. coli* transformation

Before transforming of DNA into *E. coli* with the heat shock transformation protocol, the cells had to be prepared to become competent.

Therefore 100ml LB medium with corresponding antibiotic were inoculated with an over night culture of the *E. coli* strain of choice. The OD_{600} should have a value of 0.1 in 100ml medium. The culture was grown at 37°C, shaking till the OD_{600} of 0.6- 0.8 was reached. Then, cells were harvested by centrifuging at 10krpm for 10 minutes at 4°C. The cell pellet was resuspended in 40ml MOPSI and incubated for 10 minutes on ice. Cells were pelletised again at 10krpm for 10 minutes at 4°C. The pellet was resuspended in 40ml cold MOPSII and incubated for 30 minutes on ice. Afterwards, the cells were centrifuged under same conditions and resuspended carefully in 2 ml MopsIIa. The 2 ml were aliquoted in 100µl samples and stored at -80°C till use.

For the actual transformation the competent cells were thawed on ice and DNA was added. In case of transformation of a ligation sample- the complete ligation batch was added, in case of plasmid transformation- 50ng were added.

The batch was incubated for 30 minutes on ice. Afterwards the heat shock occurred for 2 minutes at 42°C. Then, the cells were shortly put back on ice again. To regenerate the cells, 300µl LB, without antibiotic, were added and incubated at 37°C for 30 minutes. Afterwards, the cells were plated on selective plates in 3 equal amounts. Plates were stored at 37°C over night.

2.2.3.2. Screening for positive clones in *E. coli*- quick apply

To screen *E. coli* colonies for carrying the vector WITH the insert, single colonies were picked from the plate in 5ml LB with corresponding antibiotic and incubated over night at 37°C, shaking. 300µl of the culture were pelletised and resuspended in 30µl loading dye. 14µl phenol chloroform (1:1) were added afterwards and vortexed for 30 seconds. The mixture was centrifuged 6 minutes at 13.200rpm. 15µl of the aqueous phase, containing the DNA, were loaded on a 0.8% agarose gel. Vector bands, which run higher were suspected to carry the insert. These vectors were tested with restriction analysis.

2.2.3.3. *Nab magadii* transformation

To make *Nab. magadii* competent, they had to be grown in NVM+ with 70µg/ ml bacitracin. They were grown in buffle flasks till they reached an optical density of 0.6 and harvested at 6krpm for 15 minutes. The cell pellet was resuspended in half of the volume in high salt buffered spheroblast solution with glycerol and proteinase K (20µg/ml). This was incubated at 42°C, shaking over night. Spheroblasting formation was analyzed the next day with a light microscope.

For the transformation, 1.5ml culture were centrifuged at 10.000rpm for 3 minutes. The cell pellet was resuspended in 150µl high salt buffered spheroblasting solution without glycerol and 15µl 0.5 M EDTA were added. The mixture was incubated for 10 minutes at room temperature. Afterwards the DNA had to be added. Because of the osmotic pressure, 10µl DNA were the maximum limit. After 5 minutes incubation, 150µl 60% PEG 600 were added and incubated for 30 minutes. To remove the PEG the cells were centrifuged with 1 ml fresh added medium for 5 minutes at 10.000 rpm. The cell pellet was carefully resuspended in 1ml fresh medium without antibiotics and regenerated over night, shaking at 37°C. Afterwards cells were plated on agar plates, containing antibiotics. The plates were stored in bags at 37°C for about 3 weeks.

2.2.3.4. *N. pharaonis* transformation

Transformation of *N. pharaonis* is similar to the transformation protocol used for *Nab. magadii*, except the fact, that instead of proteinase K- pronase E (0.3mg/ml) was added.

2.2.3.5. *Hbt. salinarum* transformation

Transformation of *Hbt .salinarum* was performed following the protocol of M. Dyall- Smiths “Halohandbook” (2009).

2.2.3.6. Screening for positive clones in *Archaea*

To screen for clones, which carry the plasmid of interest, the single colonies were picked in 1ml medium with the corresponding antibiotic and were grown for approximately 10 days, shaking at 37°C in 1.5ml eppendorf tubes. Because the oxygen in the small tubes is insufficient, they were aired once a day. 20µl of the grown culture were tested with an analytic PCR (see 2.2.2.9.).

2.2.4. Cloning strategies

2.2.4.1. pRo-5-fdx-ORF34

To amplify the ORF34, the primers 34-Xba and 36-3 were used and pBgb52 as template. The purified PCR product was restricted with *Xba*I and *Hind*III, as the pUC19 vector, already carrying the fdx promoter. Vector and fragment were ligated and verified. The fdx-ORF34 fragment was isolated by restriction with *Kpn*I *Hind*III and a following preparative gel electrophoresis. The fusion fragment was ligated in the *Kpn*I *Hind*III restricted pRo-5 and transformed into *Nab. magadii* L13, afterwards.

2.2.4.2. pNB102-ORF49

The ORF49 fragment was constructed by preparative PCR, using the primers 49-Cla and 49-EcoRI, and ϕCh1 as template. The PCR product was purified and restricted with *Cla*I and *Eco*R1, as well as the vector pNB102. Fragment and vector were ligated afterwards and transformed into L13.

2.2.4.3. pNB102-ORF54-ORF49Δ1- Δ 5

The already existing vectors pUC19-ORF54-ORF49 Δ1-5 (Reiter, 2010) were restricted with *Kpn*I and *Aha*III, the fragments were eluted by preparative gel electrophorese. The fragments were cloned into a pNB102, which was digested with *Xba*I and afterwards filled by Klenow Polymerase. To have the same sticky ends, the vector got cut with *Kpn*I. The 5 different vectors were transformed into L13.

2.2.4.4. pRo-5- RadB-ORF49Δ1- Δ 5

For the construction of the fusion protein, RadB was amplified with a preparative PCR, using the primers RadB1 and RadB2, and *Haloferax volcanii* culture as template. The PCR product was purified and cut with *Bam*HI and *Hind*III. The fragment got ligated to the *Bam*HI and *Hind*III cut vector pUC19.

The ORF49Δ1- Δ 5 fragments were cut out of the exsiting vectors pUC19-ORF49 Δ1- Δ5 (Reiter, 2010) with *Kpn*I and *Bam*HI. The pUC19, that already comprises RadB, got cut with *Kpn*I and *Bam*HI and ligated with the 5 different fragments. The vectors, containig the

fusiongenes RadB- ORF49Δ1- Δ 5, were cut with *Hind*III and *Kpn*I, and isolated by preparative gel electrophoresis. The vector pRo-5 got cut with *Kpn*I and *Hind*III, too and afterwards ligated with the fragments. The 5 different constructs were transformed into L13.

2.2.4.5. pRo-5-ORF49prom-cterm

The promotor fragment of ORF49 was constructed with a preparative PCR. As primers, prom2 and 49-KpnI were used, and isolated ϕCh1 as template. The PCR product was purified and restricted with *KpnI* and *BamHI*, like the vector pUC19. Fragment and vector ligated. The second fragment, the 3'end of ORF49 was amplified by using 12-7-5c and 12-7-3 as primers for preparative PCR. The purified PCR product got cut with *BamHI* and *HindIII*, as the pUC19 vector, carrying the ORF49 promotor. Vector and 3' end fragment were ligated. The now existing promoter-c-terminus fusion was isolated from the vector by using *Kpn*I and *Hind*III. The fragment was isolated by preparative gel electrophoresis, and ligated into the *Kpn*I/*Hind*III restricted vector pRo-5. The vector was transformed into *Nab. magadii* L13, afterwards.

2.2.4.6. pRo-5-ORF49prom-cterm-short

The shortened version of the 3' end of ORF49 was produced with a preparative PCR. As primers, 12-7-3 and C-term2 were used and ϕCh1 as template. The purified fragment was restricted with *Bam*HI and *Hind*III and ligated into the *Bam*HI and *Hind*III restricted pUC19, carrying the ORF49 promotor already. The fusion gene 'promotor-3'-short' was isolated from the pUC19, using *Kpn*I and *Hind*III. The fragment was isolated by preparative gel electrophoresis, and ligated into the *Kpn*I/*Hind*III restricted vector. The completed vector was transformed in *Nab. magadii* L13.

2.2.4.7. pRo-5-ORF49prom-ΔAUG

Even for this cloning the already existing vector pUC19-ORF49-prom was used. The fragment, lacking the AUG, was produced with preparative PCR using the primers 49-DATG and 12-7-3. The purified fragment got cut with *Bam*HI and *Hind*III and ligated into the *Bam*HI/ *Hind*III restricted vector. The ORF49 gene fragment, lacking the AUG was isolated by cutting with *Kpn*I/*Hind*III and performing a preparative gel electrophoresis. The fragment was ligated into pRo-5 that has been restricted with *Kpn*I and *Hind*III before. The resulting vector was transformed into *Nab. magadii* L13.

2.2.4.8. pKSII+ORF49-Deletioncasette

In order to create a knock out cassette for ORF49, the flanking regions were cloned and ORF49 was replaced by a novobiocin resistance. The 2 fragments of the ORF49 were amplified with a preparative PCR using the primers Δ49-1/Δ49-2 (fragment 1) and D49-3/D49-4 (fragment2) respectively. The fragments were purified and restricted with *Bam*HI/ *Hind*III (fragment1) and *Hind*III/ *Kpn*I (fragment2). The fragments were ligated in the equivalent restricted vector, successive. The Novobiocin resistance cassette was isolated from the vector pMDS11. The vector was restricted with *Sma*I and *Hind*III and filled in with Klenow polymerase. The 2453 Bp fragment was isolated with a preparative gel electrophoresis. The pKSII+ vector with the 2 fragments was restricted with *Sma*I and ligated with the isolated Novobiocin resistance cassette. As the novobiocin resistance was cloned with blunt ends, the orientation had to be checked. This was performed with an analytic PCR, using the primers Nov6/ Δ49-1 and Nov9/Δ49-1. A PCR product was produced with the primers Δ49-1/ Nov9, thereby the novobiocin resistance cassette is orientated in the other orientation than ORF49. The knock out cassette was transformed into *Nab. magadii* L11.

2.2.4.9. pRR007-Fla

To amplify the flagella operon, a preparative PCR was done with the primers FlaHind and FlaEco and *Nab. magadii* L13 culture as template. The PCR product was purified and restricted with *Hind*III and *Eco*R1, as the vector, pRR007. The construct was transformed into *Halobacterium salinarum* and in *Natronomonas pharaonis,* respectively.

2.2.4.10. pJas- Fla

To amplify the flagella operon with *Nco*I restriction sites, a preparative PCR was performed, using the primers Fla1Nco and Fla2Nco and *Nab. magadii* L13 culture as template. The PCR product was purified and restricted with *Nco*I, as the vector pJas35. Vector and fragment were ligated and later on transformed into *Halobacterium salinarum*.

2.2.4.11. pRo-5-ORF95

The ORF95 gene locus was amplified with a preparative PCR. As primers pRo5-95H and pRo5-95K were used, as template ϕCh1 DNA. The purified PCR fragment was restricted with *Hind*III and *Kpn*I, as the vector pRo-5. Both were ligated and after verification transformed into *Nab. magadii* L13.

2.2.4.12. pRo-5-ORF95 ΔAUG

For amplifying fragment #1, primers 95-m2-1 and 95-Hind were used and ϕCh1 as template. The purified fragment was restricted with *Eco*R1/*Hind*III and ligated in a *Eco*R1/*Hind*III restricted pRSET-A. The fragment #2 was amplified using the primers 95-m2-2 and 95Kpn, and ϕCh1 DNA as template. The fragment was restricted with *Eco*R1/*Kpn*I, as the vector carrying already fragment#1. Both were ligated. The fusion of both fragments was isolated by restricting the pRSET-A with *Kpn*I/*Hind*III and performing a preparative gel electrophoresis. The fragment was ligated into a *Kpn*I/*Hind*III restricted pRo-5 vector and transformed into L13 after verification.

2.2.5. Protein methods

2.2.5.1. SDS PAGE

To separate proteins depending on their size, a sodiumdodecylsulfat polyacrylamid gel electrophoresis was performed. Here, the SDS maskes the native charge of the protein, and therefore the migration in the electric field is only influenced by size with one limitation: proteins of halophiles have another composition of amino acids (see 1.1.3.) and therefore migrate slower than proteins of mesophiles.

Reagent	*Stacking gel (4%)*	*Separation gel (12%)*
PAA (30%)	267µl	2000µl
Stacking gel buffer	500µl	-
Seperation gel buffer	-	1250µl
ddH2O	1233µl	1750µl
APS (10%)	20µl	60µl
TEMED	5µl	10µl

To cast the gel, approximately 4/5 volume of the casting apparatus (Bio Rad Mini Protean 3) was filled with separation gel and overlaid with isopropanol. After polymerization the isopropanol was discarded and the stacking gel was filled in. Before polymerization the comb was inserted. The running chamber was filled with 1x SDS running buffer and the gels were added.

To prepare the samples they were mixed with 2x laemmli protein sample buffer and heated for 10 minutes at 95°C and cooles on ice afterwards. 10µl of these samples were loaded on the gel.

In case of performing a western blot analysis afterwards, 7,5µl marker were loaded. In case of a following coomassie staining, 5µl marker were loaded.

Gels with samples from *E. coli* were run with 15mA for 1hour. Gels with samples from halophiles were run with 40V/ 60V for approximately 4-5 hours.

2.2.5.2. Coomassie staining

If the proteins were stained with coomassie, the gel was transferred into a coomassie bath and stained for approximately 10 minutes. Afterwards it was destained with water for 20 minutes, the first time, and then over night.

2.2.5.3. Western blot analysis

If antibodies were available for the protein of interest, a western blot could be performed. In the case of the α- ϕCh1 antibody and the α-ORF36 antibody, a serum was available from the rabbit. The primary antibody was visualized using an antibody, recognising the constant element of the rabbit antibody. This second antibody was coupled with hourse raddish peroxidise.

In case of analysing proteins from halophiles, the gel had to be washed with water for 10 minutes, in order to remove the salt.

Afterwards the proteins were blotted onto a nitrocellulose membrane (Whatmann). Therefore following components were equilibrated in transblotbuffer and stacked on the semi blotting apparatus: 3 Whatman papers, the protein gel, the nitrocellulose, 3 Whatmann papers. The blot was run with 20V for 20 minutes (20V for 30 minutes for 2 blots). To visualize the protein marker, the nitrocellulose membrane was stained with Ponceau S for 10 minutes. After short destaining with water the bands were marked with a pencil. Afterwards the nitrocellulose was washed with water and blocked over night with TBS and 5% milk powder at 4°C.

The next day, the blocking solution was discarded by washing with 1xTBS for 10 minutes. Afterwards, the primary antibody was added for 1 hour. Then the antibody was removed and the membrane was washed again 3 times each 10 minutes with 1xTBS. The second antibody was added for 1 hour and afterwards removed by washing 3 times, as previously mentioned. For the detection the ECL kit from PIERCE was used, following the protocol. The membrane was transferred into an exposure cassette and exposed to the x ray film. The x-ray was developed after individual exposure time.

2.2.5.4. Protein overexpression and purification

For purification of a recombinant protein, the corresponding gene was cloned in an expression vector (see 2.1.4.) and transformed into an *E. coli* Expression strain (e.g. Rosetta). The strain, carrying the gene of interest was inoculated in 100ml LB with the corresponding antibiotic and grown till an OD_{600} of 0.5. The culture was induced with 0.5mM IPTG and grown for 5 hours. Samples were taken before induction and every hour after induction to follow the expression rate. For sample taking, each 1,5ml culture was centrifuged to collect the cells, which were mixed with 5mM Sodium phosphate buffer and same amount 2x laemmli sample buffer. The amount of added buffer depends on the optical density and is calculated with the formula OD_{600} x 75. The samples were heated for 10 minutes at 95°C and load on SDS PAGE for analysis and stained with coomassie afterwards. With analysis of the resulting growth curve, the adequate incubation time for obtaining maximum of protein amounts could be identified.

When optimal conditions were defined, 1l LB with corresponding antibiotic was inoculated with over night culture of the *E. coli* strain, carrying the gene of interest. The OD_{600} should count 0,1. The culture was incubated till an OD_{600} of 0.5 at 37°C with shaking. Afterwards the culture was induced with 0,5mM IPTG and incubated, also 37°C, shaking. Then, the culture was pelletised at 6krpm, 15 minutes at 4°C. The pellet was left at -20°C over night. The thawed cell pellet was mixed with buffer B (lysis buffer), till it was dissolved completely. The mixture was stored at room temperature for 1hour. To be sure that cells were lysed, they were sonicated afterwards (4x 90 cycles, 2 minutes each fraction) until they were translucent. The lysat was centrifuged at 8krpm for 20minutes at 4°C and the cleared supernatant was transferred into a glass flask. 300µl 50% NI-NTA were added and mixed gently over night. Because the protein contains a His tag (from the expression vector pRSETA- or pQE) it can be purified with the NI-NTA affinity chromatography. Therefore the column was filled with the protein- Ni-NTA mixture and eluted with decreasing pH values. After loading it was washed twice with buffer C (4ml) and 6 times with buffer E (0.5ml). The different fractions, as the fractions from previous steps were collected and analyzed with a SDS PAGE.

3. Results and Discussion

3.1. ORF49

3.1.1. Aim of the studies

Since gp49 is known to have an influence on the lysis behaviour of *Nab. magadii* (see 1.2.5.), the focus was on its mode of action. The aim of the following studies is to find functional domains and essential regions of the repressor and further on the mechanism of action.

3.1.2. Fusion of ORF49 mutants with ORF54

In 2010, M. Reiter showed with C-terminal deletions of different length, which length is essential to maintain repressor activity of gp49. A minimum length of 70- 80 codons could be determined (see 1.2.5.). Within the last 50 codons an essential domain seems to be located. Several evidences indicate that this essential domain could be a dimerization domain.

The first evidence was due to an αHis Western blot against the His- tagged gene product of ORF49. As shown in figure 8, 2 bands of the gp49 could be recognized. The expected band had the size of 13.3 kDa and a second band had a size of approximately 30kDa. The arising question is, if this band contains a residual of the dimerized gp49. Both bands increase simultaneously with ongoing time. Furthermore the PCNA control protein does not show a second band at this level, therefore a general contamination is not estimated (B. Galos, 2001).

Another evidence for dimerization is the comparison with other known repressors, like c1 from the bacteriophage λ and repressors from P22. These repressors bind cooperatively to flanking regions within the genome and therefore dimerizes homologous (Whipple *et al.*, 1994). Whereas "*The C-terminal domain (CTD) residues 132–236, mediates dimerization"* (Bell *et al.*, 2000).

To determine, that the loss of repressor activity depends on the loss of the c-terminal dimerization domain, following concept was realized:

To compensate the loss of the dimerization domain, the c-terminus mutants were fused to another protein with a dimerization domain and tested concerning their repressor activity.

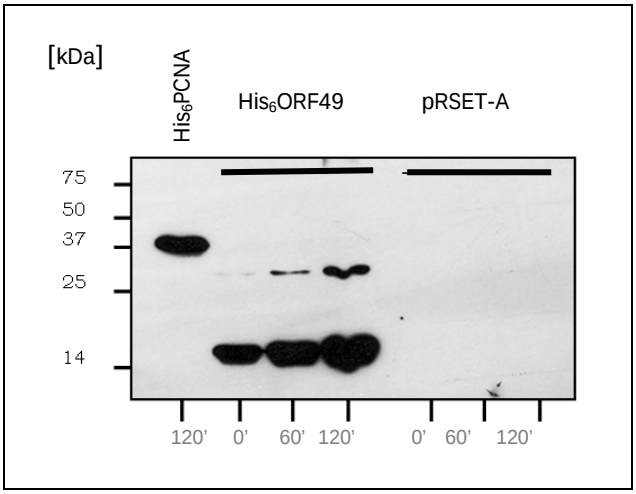


FIGURE 8 | **αHis Western blot for detection of gp49**. αHis western blot with His_6PCNA from ϕCh1 as positive control and the empty vector pRSET-A as negative control. The proteins were expressed in *E. coli.* Left axis shows the different times after IIPTG induction. His_6ORF49 shows the strongest signal 2 hours after induction at ~15kDa and at 30kDa. This could be an evidence for dimerization (B. Galos, 2001)

Due to the default of any known protein in *Natrialba magadii*, which oligomerizes, a protein was chosen, which owns a coiled coil structure, and therefore certainly oligomerizes.

A coiled coil structure is a motif within the protein structure, which consists of several helices, forming a superhelix. "*Coiled coils moved into the mainstream of biology in 1988, when they were identified as the dimerization elements. The homo- or heterooligomeric association of helices into a coiled coil is determined in part by polar interactions between residues flanking the core"* (Lupas, 1996). To predict coiled coil structures, a number of internet programs were developed, as 'ch.EMBnet.org- COILS- Prediction of Coiled Coil Regions in Proteins', for example. This program compares protein sequences of known coiled coil proteins and calculates similarities and predicts with the resulting score, if the protein will form a coiled coil structure (Lupas, 1991).

By screening ϕCh1 proteins with this internet program, one protein showed high scores and therefore contains probably coiled coil structures. This protein, encoded by ORF54, shows high values within the aminoacids 161- 190 as mentioned in figure 9.

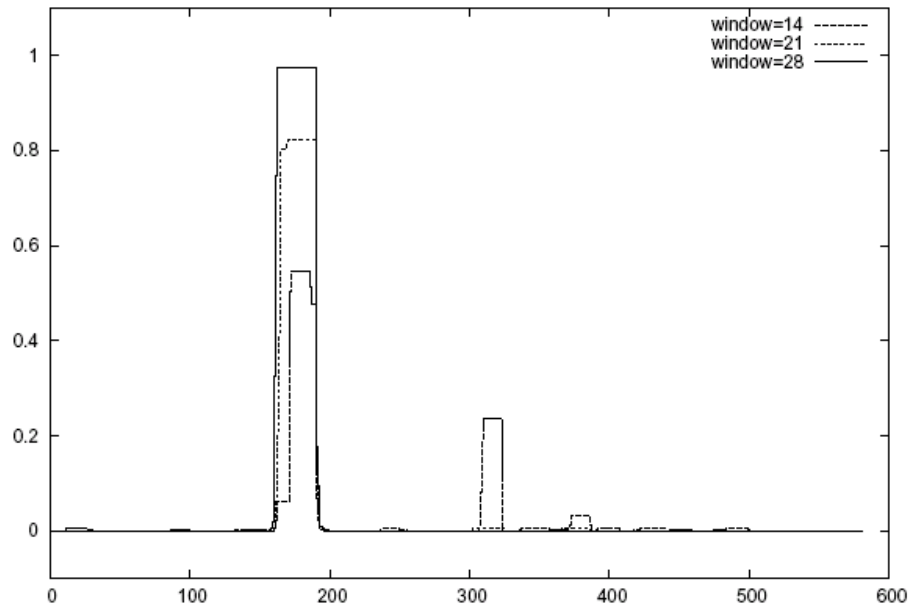


FIGURE 9 | **Coiled coil prediction gp54.** An internet program for prediction of coiled coil regions in proteins was used. High scores were assumed between aminoacids161-190 of gp54. (http://www.ch.embnet.org/software/COILS_form.html)

If the repressor activity could be compensated by a fusion with ORF54 and its dimerization domain, evidence could be performed, that the C-terminus of ORF49 is responsible for oligomerization and that ORF49 acts as an di- or oligomer, respectively.

For the realization, the preexisting mutants, ORF49Δ1-Δ5, were fused to ORF54 (see 2.2.4.2 and 2.2.4.3.) and transformed into *Nab. magadii* L13. These cultures were infected with ϕCh1, to proof their repressor activity (reduction of plaque formation). As a positive control, *Nab. magadii* L13 was transformed with ORF49. As negative control, the empty vector was transformed into *Nab. magadii* L13.

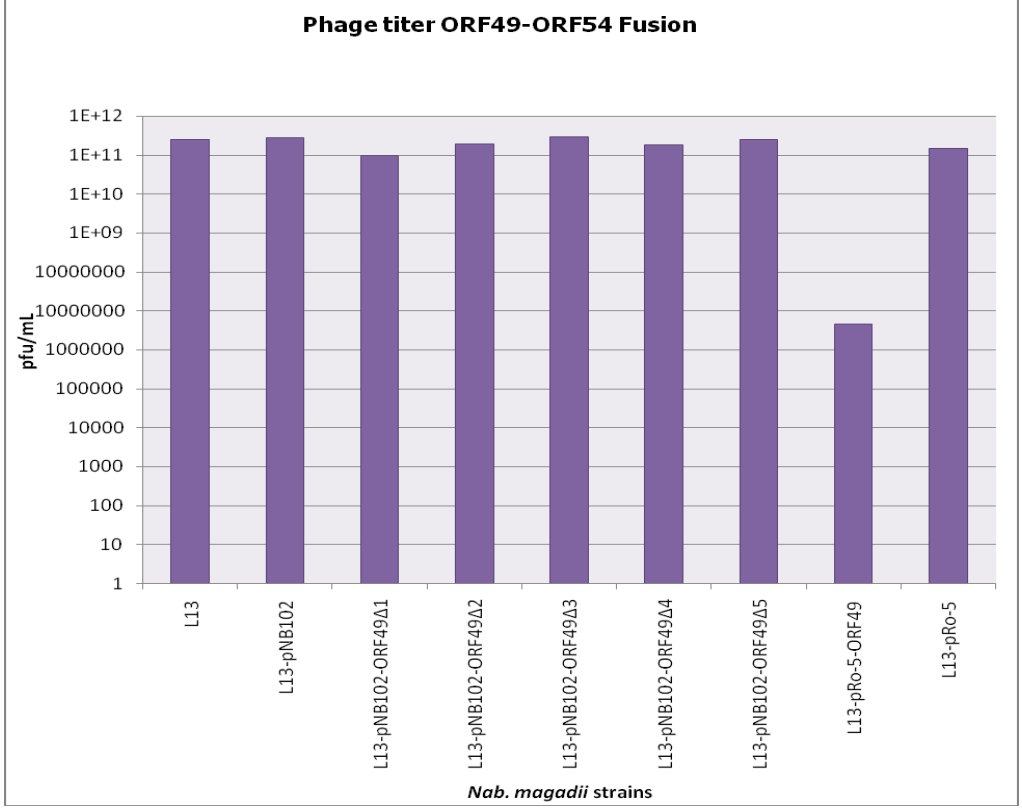


FIGURE 10| **Phage titer analysis ORF49-ORF54 fusionprotein** . 9 different *Nab. magadii* L13 strains were tested with a phage titer analysis concerning their lysis behaviour. *Nab. magadii* L13 represents the normal titer. *Nab. magadii* L13-pNB102 and *Nab. magadii* L13-pRo-5 were used as negative controls. *Nab. magadii* L13, carrying pRo-5-ORF49, serves as positive control for repressor activity. The different ORF49Δ's, fused to ORF54, show no reduction of plaque formation, compared to *Nab. magadii* L13-pRo-5-ORF49. Their plaque forming units are similar to the negative controls. The fusion to ORF54 could not reconstruct the repressor activity; furthermore ORF49Δ1-3 lost their repressor activity through proteinfusion.

In the resulting phage titer none of the strains carrying the fusion of ORF49-ORF54 showed any reduction of plaque formation. Just the positive control, without any ORF54 fusion showed repressor activity as mentioned in figure 10.

3.1.3. Fusion of ORF49 mutants with RadB

Because the completion with ORF54 did not show proper results, another suitable target protein for protein fusion had to be found. As mentioned in 1.1.5.3., RadB is a sufficiently studied protein of *Hfx. volcanii*. The fact that RadB acts as a dimer could be confirmed with SDS PAGE analysis (see figure 11) and further on with mass spectrometry (Guy *et al.,* 2006).

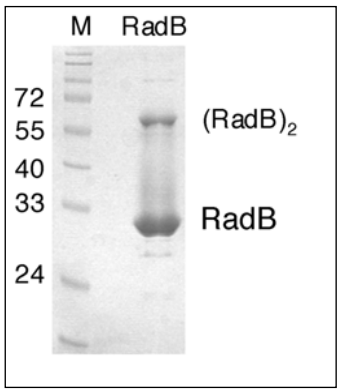


FIGURE 11| **SDS PAGE of RadB.** Purified RadB from *Hfx. volcanii*, loaded on SDS PAGE, without boiling. It appears as a monomer with ~30kDa and as a dimer with ~60 kDa.

(Guy *et al*., 2006)

In order to analyze if a translational fusion with RadB can compensate the mutant phenotype, the encoding gene was fused to the ORF49 3'- deletions and transformed into *Nab magadii* L13. These strains were infected with ϕCh1 and analyzed performing a phage titer. After incubation, no repressor activity could be observed, as mentioned in figure 12.

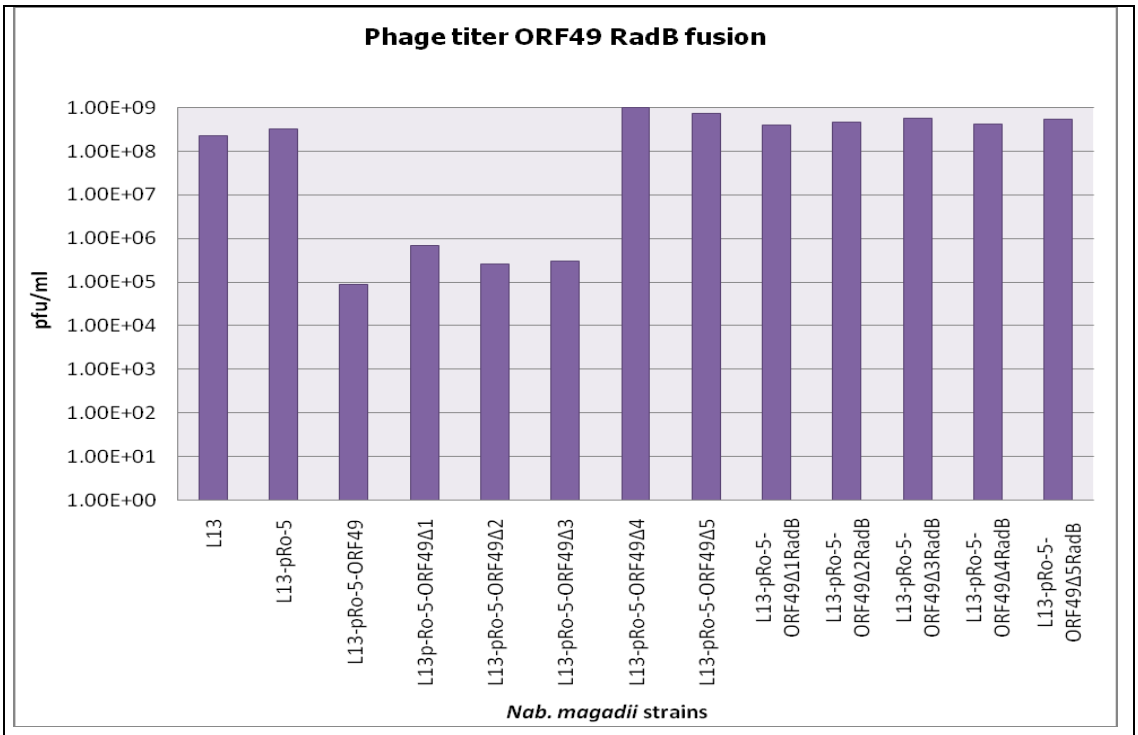


FIGURE 12 | **Phage titer analysis gp49-RadB fusionproteins**. Comparison of plaque forming in different *Nab. magadii* L13 strains. *Nab. magadii* L13 and *Nab. magadii* L13-pRo-5 serve as negative controls. *Nab. magadii* L13-pRo-5-ORF49 serves as a positive control for repressor activity. The different deletions of ORF49 (Δ1-Δ5) (Reiter, 2010) and the deletion mutants fused to RadB, were analysed with this phage titer concerning their repressor activity. *Nab. magadii* L13-pRo-5-ORF49 as *Nab. magadii* L13-pRo5-ORF49Δ1-Δ3 show a reduction of plaque formation, while the negative controls, as the fusionproducts show no reduction, and therefore no repressor activity.

The mutated ORF49Δ1- Δ3 had shown repressor activity before they were fused to ORF54 and RadB, respectively (Figure 7, 1.2.5.). Due to the reduction of plaque formation in case of the positive control (*Nab. magadii* L13-pRo5-ORF49), the test set-up must have worked properly. The only reason for loss of repressor function must be a problem with the protein tag.

One reason for loss of function could be the size of the tags. RadB contains of 229 amino acids (about 30kDa) whereas ORF54 even contains of 581 aa. Common used tags like myc-tag or FLAG-tag size only 1kDa – 1.2kDa. A protein tag, which is comparable in size is the green fluorescent protein, with 238 aa and 26.9 kDa.

GFP is a frequent used protein tag, and belongs to the fluorescent proteins (FP). The protein tag emits green fluorescent light, when it is exposed to blue light and is often used for gene expression studies or for monitoring localization of proteins (Tsien, 1998). In common cases GFP is as big as or even bigger than the target protein. "*The fused FP potentially interferes*

with the function and/or localization of the host protein because of its large size" (Andresen *et al.*, 2004). An example is the completion of null mutants in *Saccharomyces cervisiae*. The C-terminal fusion of GFP to Tub1 and Tub3 can not complement the corresponding null mutants anymore (Carminati and Stearns, 1997). Tub1 and Tub3 consist of 445 and 447 amino acids and therefore size nearly 50kDa.

RadB is comparable in size to GFP, but the target proteins in this study are much shorter than 445 amino acids. They contain between 57 amino acids and 117 amino acids, so they are partially 7 times smaller. If the GFP tag has already influence to 7 times larger proteins, it could likely influence the smaller peptides, as well.

An approach to evade the size depended problem could be to fuse only the core from the coiled- coil part of ORF54 to the ORF49Δs.

Apart from the possible interference depending on the size of the tag, the steric interference could be another reason for loss of function. The 3D structures of the two proteins could have an influence among each other and disturb their function. A number of studies verify the fact, that an introduced linker region can provide functionality because of higher flexibility. An example for this is the fusion of G- vargula from *Straptococcus* and luciferase. The G- protein lost its function due to interference of the two moieties and could be regenerated by introducing of a linker domain. "*These results indicate that spatial separation of the hetero-functional domains of a fusion protein by proper linker peptide might be so effective that the domains work independently"* (Arai *et al.,* 2001).

Taken together, a novel approach could comprise the introduction of a linker region between the ORF49Δ and a shortened version of ORF54.

Nevertheless, to perform a gene fusion is a promising approach to detect if the C-terminal domain contains a domain for oligomerization.

Other common approaches to determine oligomerization, like mass spectrometry are problematically in halophilic organisms. Trials to purify recombinante versions of gp49 in a way that it remains its native structure, failed. The problem in this case was the appearance of insoluble inclusion bodies and the insoluble form of gp49 (Reiter, 2010).

Another frequent approach to identify oligomerization domains is the comparison of homologous regions with well studied organisms. The problem with this is the fact that proteins of halophiles underly special adaptations to maintain functionality in the high saline environment as mentioned in 1.1.3. Therefore motifs of proteins adapted to high salt differ from common motifs of proteins from mesophile organisms.

3.1.4. Deletion of ORF49

Iro *et al*. showed in 2006 the influence of gp49 on the lysis behaviour induced by the mutant phage ϕCh1-1 (1.2.5.). In 2006, it was not possible to delete genes of ϕCh1 completely and it was not sure if this duplication- insertion mutation of ORF49 completely eliminates the gp49 action or if repressor activity remains.

Since the first deletion mutant could be established in 2010 as mentioned in 1.2.11 (Selb, 2010), the complete deletion of ORF49 could be performed, to compare the phenotype with ϕCh1-1.

To create a deletion mutant, a disruption cassette was produced, as mentioned in figure 13. 379 base pairs of the centre of ORF49 were removed and replaced by a novobiocin resistance (NovR) cassette (2.2.4.8.). The resistance is essential to screen for the deletion mutant. The disruption cassette is located on a suicide plasmid, pKSII+, which cannot replicate in *Nab. magadii*.

To carry the disrupted version of ORF49 in the ϕCh1 genome, the mechanism of homological recombination can be used.

Therefore, the disruption cassette on the suicide vector is transformed into a *Nab. magadii* L11 strain, carrying ϕCH1 within its genome. If recombination occurs between the homologous regions of ORF49, the wild type ORF49 is replaced by the disrupted version. In this case, *Nab. magadii* L11 carries a novobiocin resistance within its genome- the cell can survive in media containing novobiocin. If no homologous recombination occurs, the vector got lost with the next cell division, because it cannot replicate autonomously. The cell is not resistant to novobiocin and cannot survive in medium containing this antibiotic.

After a vast number of failed trials to find a clone, which carries the disrupted version of ORF49 within the genome, another approach had to be searched.

Therefore the fact, that an ORF49 deletion may have a detectable phenotype, was used. This expected phenotype is the reduction of lysogenic cycle and therefore a rapid cell lysis, considering detection of ϕCh1-1. After transformation of *Nab. magadii* L11 the cell culture was not plated on agar plates but grown in liquid culture with and without antibiotic, respectively.

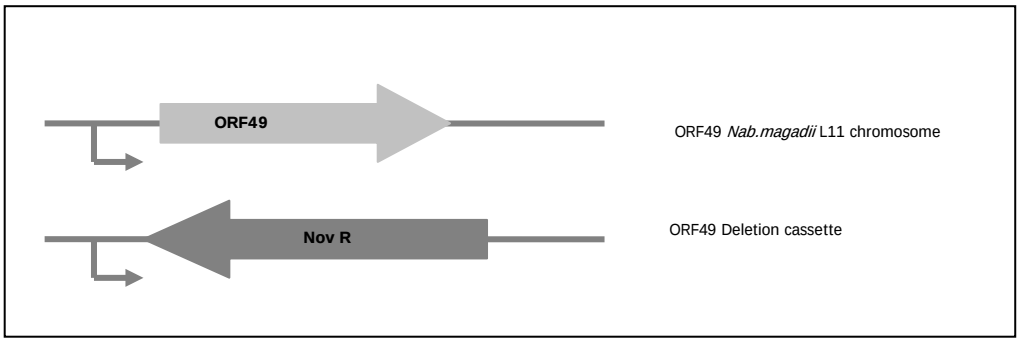


FIGURE 13| **Schematic drawing of ORF49 and the created ORF49 disruptions cassette**. The scheme shows the wild type ORF49 gene locus, located within the *Nab. magadii* L11 genome in comparison to the disrupted version, located on a suicide plasmid (pKSII+). The disrupted version lost the complete ORF49 gene and posses instead of these a novobiocin resistance cassette. The recombination into the *Nab. magadii* L11 genome should occur between the homologous regions down- and upstream of ORF49.

Some of the grown *Nab. magadii* L11 cells were considered to carry the deleted form of ORF49. The problem is that these single cells exist beside cells carrying the wild type ϕCh1. To isolate the phages carrying the deletion, from those in which no homologous recombination had occurred, cells were lysed and the phages were plated with *Nab. magadii* L13. Plaques, which were obviously bigger than others were inoculated and tested concerning their ORF49 gene locus. With this method bigger plaque could be observed that have grown approximately 5 times faster than other ones, as shown in figure 15. These plaques were inoculated and grown in rich media.

To verify the success of the deletion of ORF49, a southern blot analysis was performed. Therefore genomic DNA from the two putative *Nab. magadii* L11::49 strains and the *Nab. magadii* L11 wild type was isolated, restricted with *Nae*I and load on a 0.8% agarose gel. As probe $\Delta 49_{1\text{-}2}$ (5'-probe) was used.

The probe $\Delta 49_{1\text{-}2}$ should hybridize with the wild type band of 2663 Base pairs, while the deleted form should hybridize with a band of 3029 Base pairs.

As the southern blot analysis shows all three strains show the wild type band with a length of 2600 Base pairs (see figure 14). This indicates that the band of the wild type sequence still exists in both putative *Nab. magadii* L11::49 cultures and they are therefore not homozygot, yet.

The ϕCh1Δ49 could be separated from the wild type phages by performing a phage titer with the supernatant of the lysed culture in higher dilutions, to avoid fusion of different plaque.

The southern blot shows furthermore that the 2 putative deletion strains show additional bands, which means, that the target sequence got modified in both strains. The additional bands are 1000 base pairs larger than expected, and a second band arises with 1500 base pairs. This could depend on a modification of the sequence during the cloning process (PCR, amplification in *E. coli*) whereas mutations of the target sequence can lead to a changed recognition of restriction enzymes.

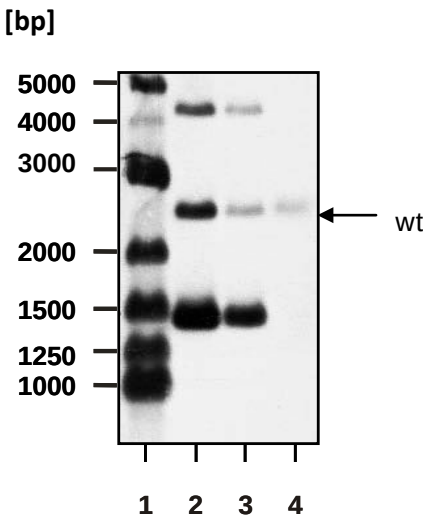
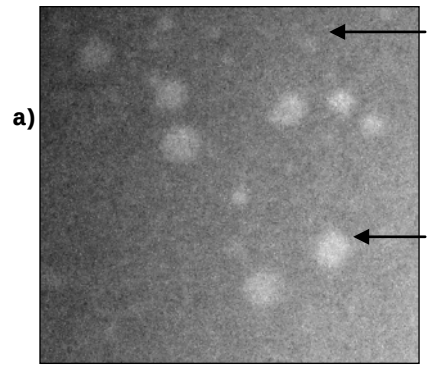


FIGURE 14| **Southern blot analysis of L11::49 with probe $\Delta 49_{1\text{-}2}$.** The marker is located on lane 1, *Nab. magadii* L11 wild type on lane 4 . Lane 2 and 3 represent the two putative *Nab. magadii* L11::49 strains The black arrows show the length of the wild type fragment, which also exist in the two putative *Nab. magadii* L11::49 strains. The *Nab. magadii* L11::49 strains show two additional bands, which indicates that the ORF49 wild type sequence got altered.

Nevertheless, a growth experiment was performed with the 2 different *Nab. magadii* L11::49 cultures, and the *Nab. magadii* L11 wild type. With this, it could be verified that the isolated cells showed an earlier onset of lysis, compared to the wild type strain *Nab. magadii* L11 as mentioned in figure 15. This growth curve shows similarities to the growth curve with the mutant *Nab. magadii* L11-1, whereas *Nab. magadii* L11::49 shows a decreased cell density compared to *Nab. magadii* L11-1. The majority of cell lysis is completed after 3 -4 days in case of *Nab. magadii* L11::49, while L11-1 requires 6 days to stop lysis as shown in figure 15. This is a further demonstration of the influence of ORF49 on the lysis behaviour and shows furthermore that the mutant ϕCh1-1 seems to posses a remaining activity of gp49.



b)

Day	L11-D49	L11
1	0 mm	0 mm
5	3 mm	0.8 mm

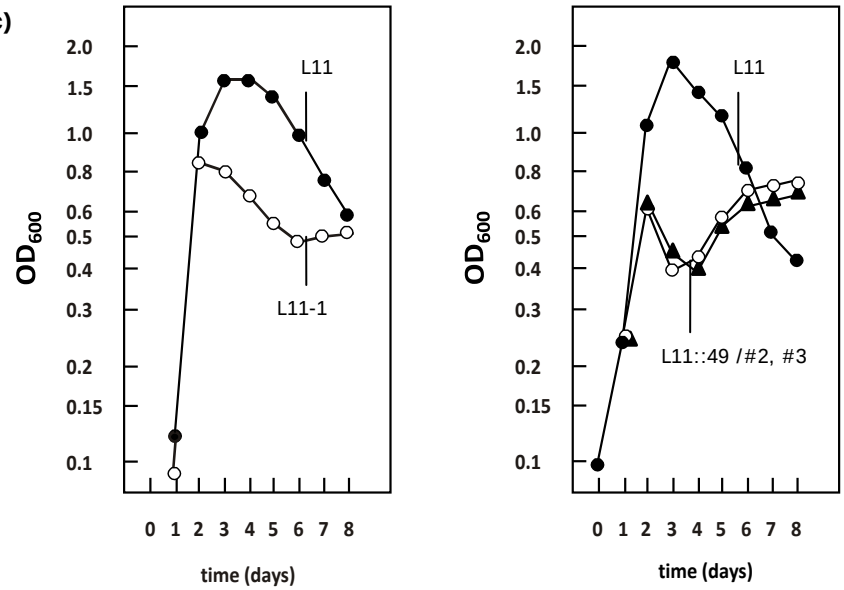


FIGURE 15 | **Phenotype of *Nab. magadii* L11::ORF49**

a| *Nab. magadii* L11 was plated with soft agar technique after transformation of the ORF49 disruption cassette. Different plaque sizes can be observed after 5 days incubation, indicating that the bigger plaques are composed by *Nab. magadii* L11::ORF49.. **b|** plaque sizes measured during incubation of the soft agar plates with the putative *Nab. magadii* L11::ORF49 and the control *Nab. magadii* L11.

c| Growth curve of the inoculated *Nab. magadii* L11::ORF49 (right figure) in comparison to growth curve of *Nab. magadii* L11-1 (left figure). In both experiments *Nab. magadii* L11 (●) was used as a control for lysis behaviour of the wild type ϕCh1. The growth behaviour of *Nab. magadii* L11::ORF49 (o and ▲) and *Nab. magadii* L11-(o)seems to be similar. Comparing the growth curves of the two mutated strains, *Nab. magadii* L11::49 shows an earlier decreasing cell density compared to *Nab. magadii* L11-1.

3.1.4.1 Further prospects

With the establishment of the *Nab. magadii* L11::ORF49 deletion mutant, prospects to characterize mechanisms of gene regulation in ϕCh1 are bright.

The next step will be to perform a phage titer, to get a homozygote L11::49 culture, as mentioned before. With this, the growth experiments can be repeated.

Further prospects could be the analysis of the protein composition within the mutant. Here one could observe a possibly changed protein expression and identify proteins, regulated by ORF49.

Another potential experiment could be the complementation of the deletion mutant. If the phenotype could be compensated by transformation of the cloned ORF49 the evidence that the phenotype depends on deletion of ORF49 only is provided.

3.1.5. N- Terminal deletion of ORF49

With the construction of a 5'- deletion in ORF49 (gp49ΔN) and the performing of an EMSA (electrophoretic mobility shift assay), it was shown, that the DNA- binding domain is located within the N-terminus (see figure 16) (Reiter, 2010).

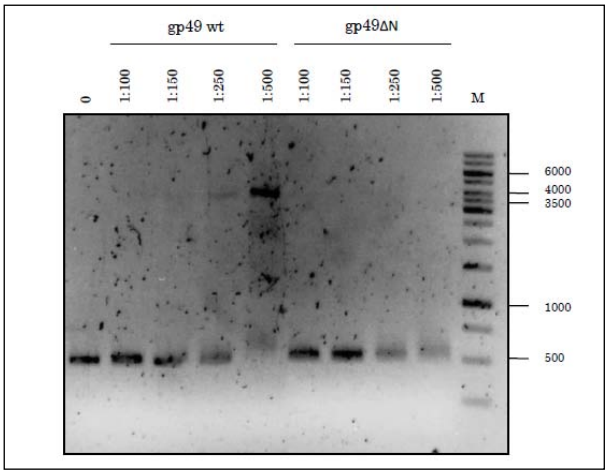


FIGURE 16 |**EMSA with gp49 and gp49ΔN.** The ORF49 wt gp on the left hand shows a band shift with the protein- DNA ratio of 1:500, from 500 to 4000 bp. The N-terminal deletion, containing nu. 34636- 34833, shows no shift anymore. (Reiter, 2010)

Here it should be determined, if a shortened version of the N-terminus shows still repressor activity although it cannot bind DNA anymore.

Therefore two different N-terminal deletions of ORF49 were constructed as mentioned in figure 17 and tested concerning their repressor activity with a phage titer analysis.

The ORF49ΔN1 (nu.34635- 34817 of ORF49) shows a reduced plaque formation, as the wild type version of the ORF49, whereas ORF49ΔN2 (nu.34635-34686) shows no repression, like the negative control (*Nab. magadii* L13 and *Nab. magadii* L13-pRo-5) (see figure 17).

The 43 codons, in which the both deletions differ seems to be essential for repressor activity.

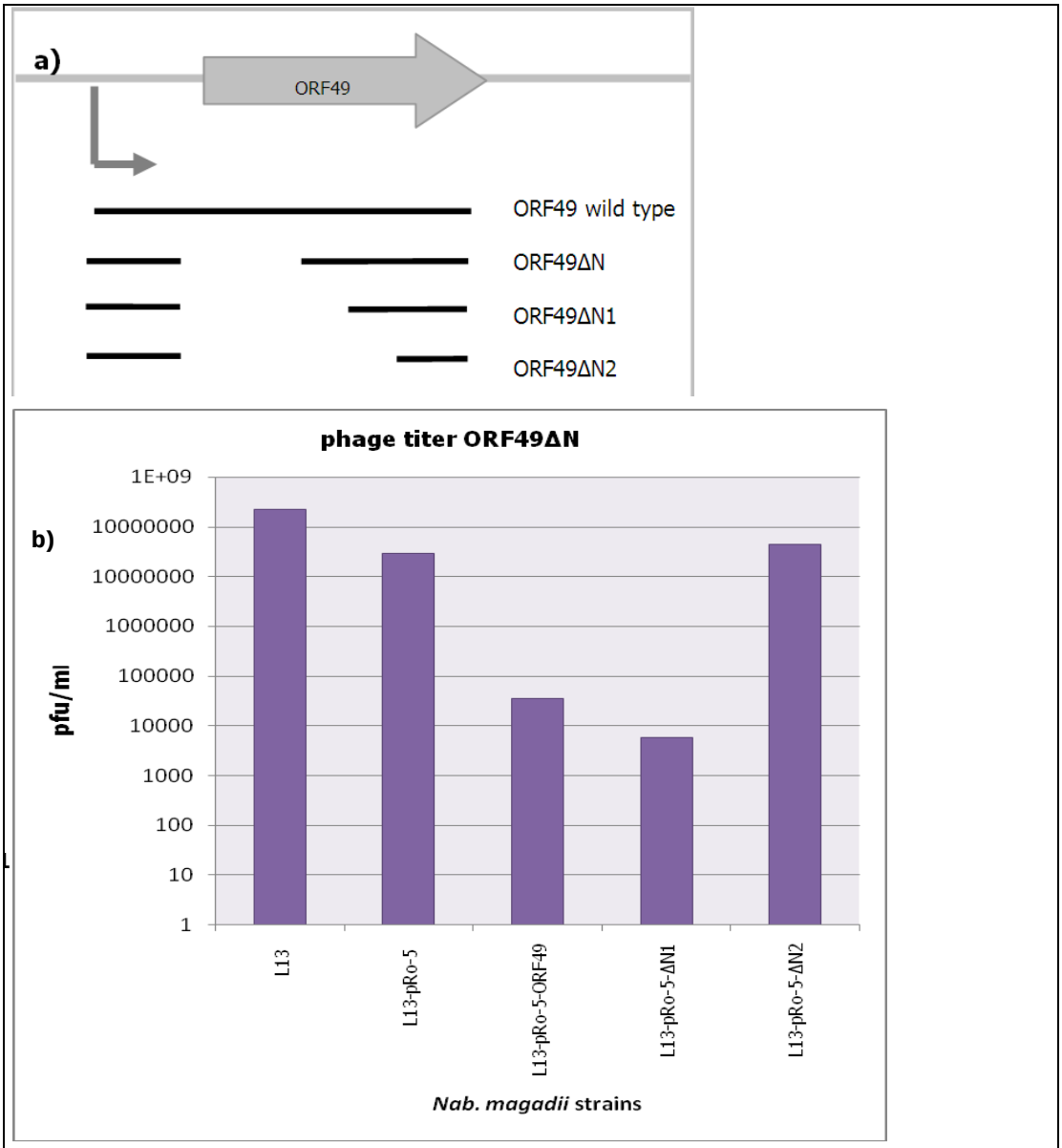
The really interesting finding reveals, comparing results of the EMSA of ΔN (figure 16) with the results of the phage titer (figure 17).

With EMSA it was shown, that the DNA binding domain got lost with the ΔN deletion. The phage titer showed that a shorter version of this deletion, ΔN1, has still repressor activity. Taken together, the repressor shows activity, although the DNA binding domain got lost.

This leads to several conceptions concerning the mechanisms of action. Gp49 could interact with other proteins and the DNA binding domain could be completed by an interaction partner. Another possibility could be an alternative mechanism of action, in which DNA binding is not essential. GP49 could act outside the cell by integrating into the cell membrane,

preventing the phage adsorption for example, or even act on RNA level. These conceptions should be verified in the following experiments.

However, the DNA binding mechanisms seems to be an additional regulatory mechanism, which does not represent the main part of the repressor activity.



a| Schematic image of the n-terminus deletion of ORF49. The deleted forms contain the promoter region nu.3421- 34479 and the c-terminus in different length. b| phage titer with ORF49ΔN1 and ORF49ΔN2. *Nab. magadii* L13 and *Nab. magadii* L13-pRo-5 serve as negative control, whereas *Nab. magadii* L13-pRo-5-ORF49 serves as positive control for repressor activity. *Nab. magadii* L13-pRo-5-ORF49 as *Nab. magadii* L13-pRo-5-ORF49ΔN1 show a reduction of plaque formation and therefore posses repressor activity.

3.1.6. AUG deletion of ORF49

It has been shown, that a large part of gene regulation is mediated via RNA. This is called "riboregulation". A well studied example for this is the 87nt RNA DsrA from *E. coli*., which regulates various parts of the *E. coli* gene expression (Lease *et al*., 1998)

It is still unknown if gp49 acts as a RNA or a protein. To eliminate the possibility that gp49 does act as regulatory RNA, the AUG was removed, in order to avoid translation.

This deletion was performed as previously described (2.2.4.7.), transformed into *Nab. magadii* L13, and tested towards its repressor activity with a phage titer analysis.

The resulting phage titer shows, that the strain, carrying the AUG-deleted ORF49 has no repressor activity, anymore. The pfu/ml are comparable to that of the negative control, L13-pRo-5, whereas the positive control shows a reduction of plaque formation (see figure 18).

These results show that it is necessary to translate the ORF49 mRNA, to receive repressor activity. Therefore, gp49 acts as protein and not as a regulatory RNA.

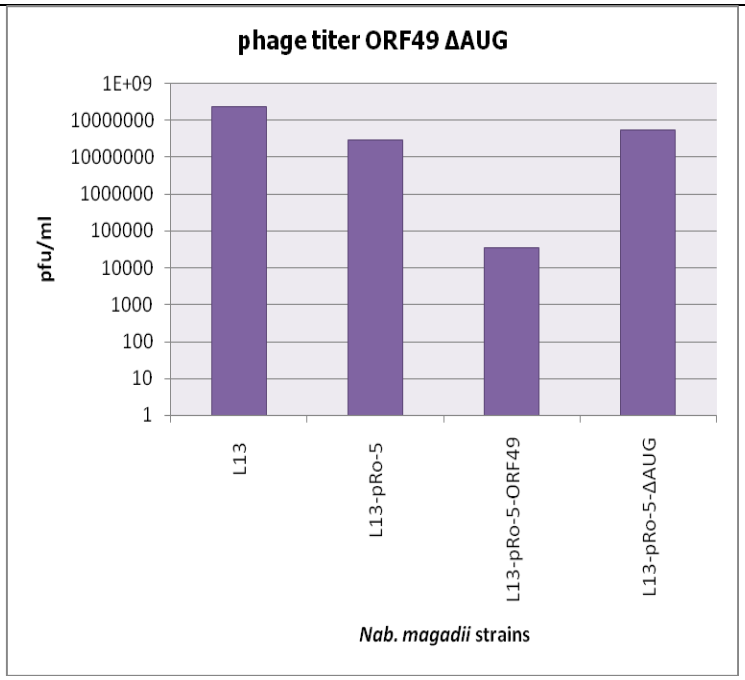


FIGURE 18 **| Phage titer analysis with ORF49ΔAUG.** The page titer was performed with *Nab. magadii* L13 and *Nab. magadii* L13-pRo-5 as negative control and *Nab. magadii* L13-pRo-5-ORF49 as a positive control for repressor activity. The normal titer is represented by *Nab. magadii* L13. *Nab. magadii* L13-pRo-5-ORF49 shows a reduction of plaque formation. L13-pRo-5-ORFΔAUG shows pfu similar to the negative controls and therefore lost its repressor activity.

3.1.7. Adsorption assay

The experiment dealing with gp49 did not give a clear picture towards its function until now. However, the function to repress the infectivity of ϕCh1 is well documented (Reiter, 2010). Therefore the possibility was analysed that gp49 interferes with the adsorption of ϕCh1 and thereby inhibts its infection to *Nab. magadii.* Different strains of *Nab. magadii* with and without gp49 were mixed with ϕCh1 and tested in different time points concerning the adsorption of the phages to the cells. If the phages were adsorbed to the cells, the phage concentration in the surrounding medium decreases.

To determine the phage concentration of the culture, samples were taken and centrifuged. The supernatant, containing the phages from the environment, was analysed by a phage titer analysis (for more details see 2.2.1.4.). Beside *Nab. magadii* L13-pRo-5-ORF49, *Nab. magadii* L11 was tested as a positive control and *Nab. magadii* L13-pRo-5 as well as *Nab. magadii L13* as negative controls.

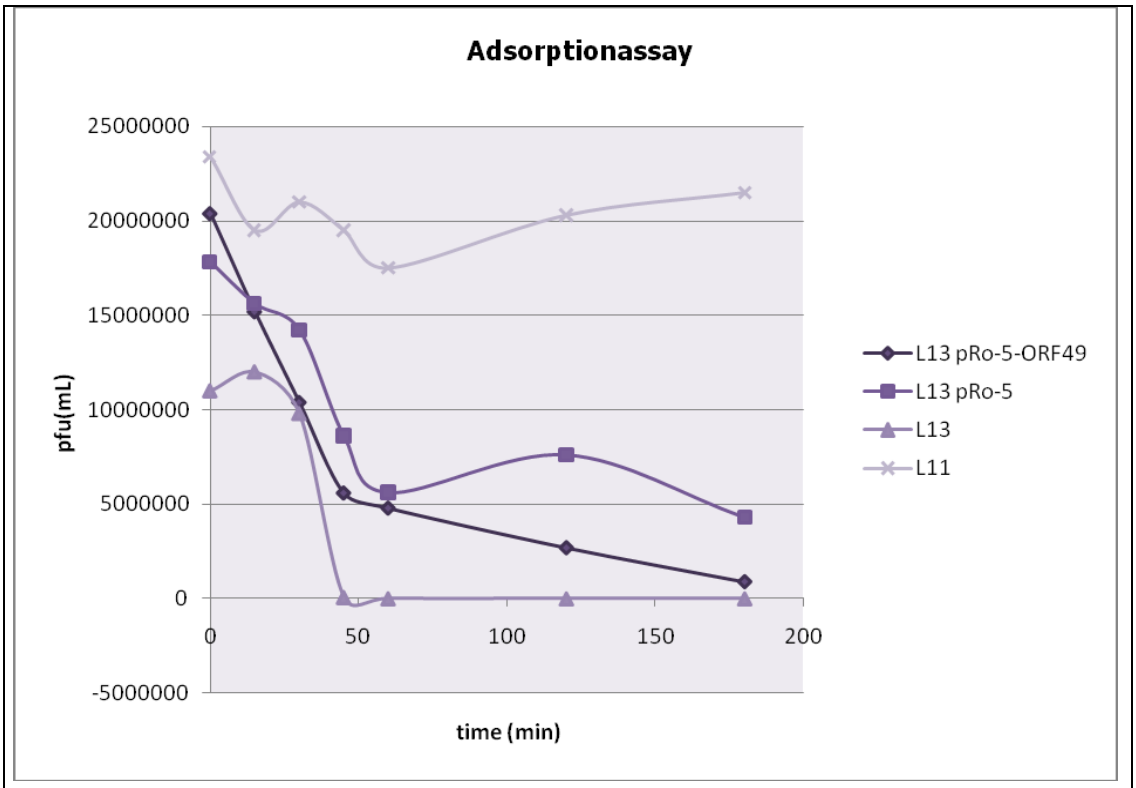


FIGURE 19 | **Adsorption assay**. *Nab. magadii* L13-pRo-5 and *Nab.magadii* L13were used as negative controls. *Nab. magadii L11* serves as positive control, because it harbours ϕCh1 and therefore ORF49. The x-axis shows time after phage addition. The added phages had a titer of 2,3x 10^7pfu/ml. *Nab. magadii* L13-pRo-5-ORF49 shows the same decreasing rate as the negative control. *Nab. magadii* L11 did not show any signs of phage adsorption.

The resulting graph showed adsorption of phages in all of the strains except of *Nab. magadii* L11. Taken together, gp49 does not prevent the adsorption of phages; it must act inside the cell as a repressor.

3.1.7. Discussion

With *Nab. magadii* L11::49 a gp49 deletion mutant could be established. Although the mutation is not homozygote, yet, *Nab. magadii* L11::49 shows a distinct phenotype. The mutated *Nab. magadii* strain showed a larger size in plaque and an earlier lysis depending decreasing cell density, compared to the wild type *Nab. magadii* L11. This is a further evidence for the influence of gp49 on the lysis behaviour of ϕCh1.

To analyze functional domains of the repressor different deletion analysis of gp49 were performed.

The N-terminal located DNA binding domain was deleted in varying magnitude to determine which length of gp49 is essential for repressor activity.

With the analysis of the different N-terminal deletion mutants, following could be determined:

Even if the DNA binding ability got lost with the N-terminal deletion, the protein has still the ability to reduce plaque formation. Therefore the DNA binding domain of gp49 seems not to be essential for repressor activity.

This evidence leads to several ideas concerning the mechanism of repression:

- gp49 acts on other level, than on the DNA level, for example outside the cell
- gp49 acts not as protein but on RNA level, and therefore must not bind DNA
- gp49 has an interaction partner, which compensates its DNA binding domain

The idea that gp49 acts outside the cell was analyzed, performing the adsorption assay. Nevertheless, the result showed that gp49 does not prevent the phage adsorption. So, it must act inside the cell.

The conception that gp49 acts on RNA level, and therefore does not have to bind DNA was analyzed with an AUG deletion. With this kind of deletion, the translation initiation was prevented. Because gp49 lost its repressor function, it could be verified, that ORF49gp acts as a protein.

To prove the consideration concerning an interaction partner, a yeast two hybrid system or a co-immunoprecipitation would be used in common cases. The problem with these methods is again that high-salt proteins are not native folded under low salt conditions, for which these methods are constructed.

In our case the yeast two hybrid system was modified. In the common used system, 2 candidate proteins are fused to a cut transcription factor, and if the two proteins interact, the cut transcription factor is functional again and this leads to the transcription of a reporter gene. In our case, different versions of a protein were fused to an oligomerization domain and if the protein interacts with his equal, the reporter gene activity (repression) could be observed. Unfortunately, this approach showed no suitable results, due to previous mentioned reasons, but with modifications of the protein fusion, this approach can have successful prospects.

3.2. ORF95 as putative repressor

3.2.1 Aim of the studies

The repressor gp49 is able to reduce the plating efficiency by 4 to 6 orders of magnitude (Reiter, 2010). However, gp49 does not block infection of ϕCh1 completely. This is only possible, when strain *Nab. magadii* L11, carrying the complete prophage, is used. Therefore it is assumed that additional elements as the described repressor are responsible for the phenomenon of a complete blocked infection using *Nab. magadii* L11.

As a possible candidate, ORF95 (nu: 57092- 57628) was chosen. A computer analysis using the BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi) revealed similarities to unknown proteins of *Haloferax volcanii* and uncultured viruses, respectively (data not shown).

However, using the interpro- Domian program (http://www.ebi.ac.uk/Tools/pfa/iprscan) revealed a winged helix DNA- binding domain of gp95 as mentioned in figure 20.

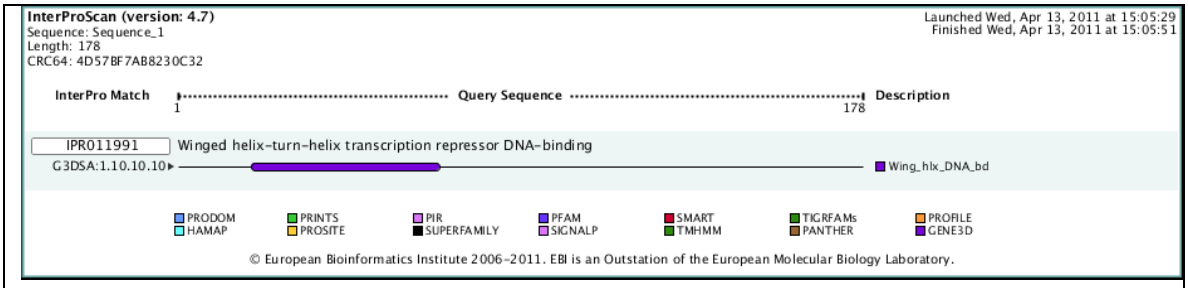


FIGURE 20 | **Result of the interpro Scan of gp 95**. The interpro-domain-program predicts a winged helix-DNA, shown in dark blue, for gp 95

On the basis of these results, a search for helix-turn-helix (HTH) motif was performed

(http://npsa-pbil.ibcp.fr/cgi bin/npsa_automat.pl?page=NPSA/npsa_hth.html).

As shown in figure 21 a HTH motif startinmg with aa43 of gp 95 with a score of 5.75 was observed. In comparison, the HTH score for repressor cI of phage λ is 5.32 (data not shown).

Therefore, ORF95 was tested with two different approaches towards its function as a contemplable repressor.

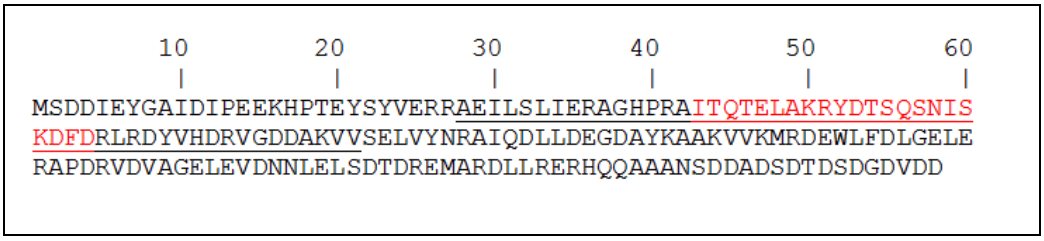


FIGURE 21 | **ORF95 HTH motif analysis**. ORF95 HTH motif 100% (indicated in red). CI like repressor (e- value: 0.65) (underlined). The score is 5.75 at position 43. The sequence at this position is ITQTELAKRYDTSQSNISKDFD. This score suggests an approximately **100% probability** that this protein contains a helix-turn-helix motif.

3.2.2. Study of repressor activity

To proof if gp95 has an influence on the infectivity of ϕCh1 of *Nab.magadii* ORF95 was cloned into the shuttle vector pRo-5 and transformed into *Nab.magadii* L13. The culture was infected with ϕCh1 and tested with a phage titer analysis. This was compared to the plaque formation of *Nab. magadii* L13 and *Nab. magadii* L13-pRo-5 (negative control). Due to the long generation time of *Nab magadiii*, and thereby long transformation procedure, an AUG deletion of ORF95 was cloned accordingly and tested afterwards.

The negative control, as the cultures containing ORF95 and ORF95ΔAUG, showed comparable values of plaque forming units.

Considering these results, gp95 has no influence on the infectivity of ϕCh1.

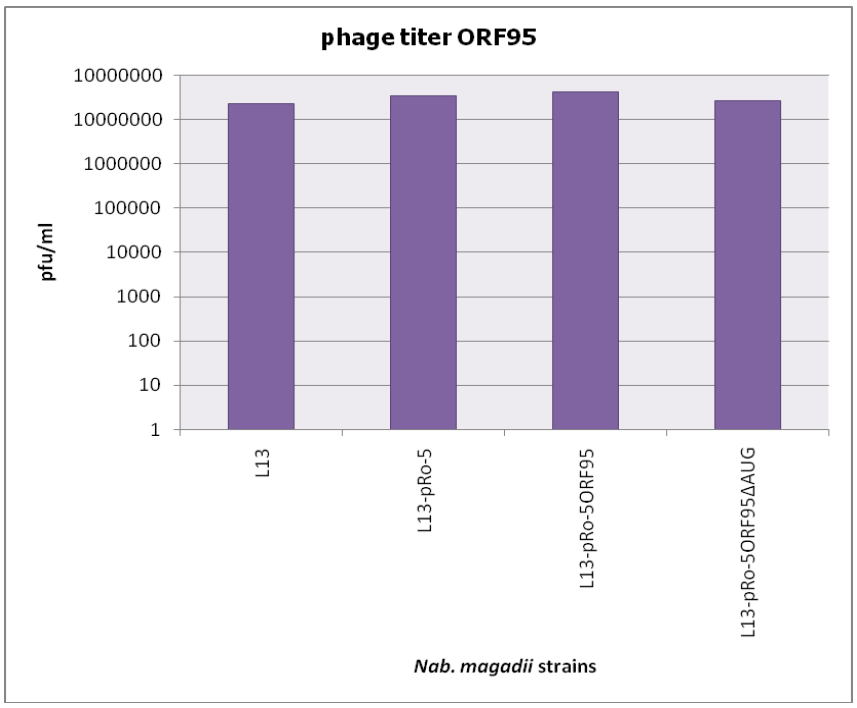


FIGURE 22 |**Phagetiter analysis ORF95**. The phagetiter was performed with 4 different *Nab. magadii* L13 strains. The normal titer is represented by *Nab. magadii* L13 without any vector. The second negative control carries the empty vector (*Nab. magadii* L13-pRo-5). *Nab. magadii* L13-pRo-5-ORF95 and *Nab. magadii* L13-pRo-5-ORF95ΔAUG should be tested concerning their repressor activity. They show no reduced plaque formation, compared with the negative controls. Gp95 seems not to be a repressor for lysogeny.

3.2.3. ORF95 binding studies (EMSA)

Since the helix turn helix motif is a known DNA binding motif and the fact that the majority of the repressors act on DNA, the ability of gp95 to bind to DNA should be verified with an EMSA (electrophoretic mobility shift assay). The principle of EMSA or a bandshift assay, respectively, is a change in migration behaviour within a gel electrophoresis, when a protein is bound to DNA. Therefore, DNA and possible binding partner in different concentrations get incubated and later load on an agarose gel. The protein binding of DNA leads to a slower migration, and if bands shift with increasing concentration of the protein, the evidence for DNA binding is given.

Performing bandshift assays with proteins of halophiles is a balancing act. Because of the specific adaptations to the high saline environment, a decreasing salt concentration often means a loss of function and structure for the specialised protein (Danson and Hough 1997; Vidyasagar *et al.*, 2007). In contrast, an increasing salt concentration within the buffer of the gel electrophoresis means an increasing ionic strength, which leads to a raising conductivity. This on the other hand, results in rising heat, which worsens the resulting separation (HHMI Research Studio, 2007). Because of these two contradictory facts, the bandshift buffer was special composed to compromise high and low salt needs. However, the shifting of the bands is due to this compromise not as strong as in comparable methods, using mesophilic proteins and lower salt systems.

3.2.3.1. Purification of ORF95

In order to perform EMSA, the purified gp95 had to be obtained. Therefore the gene was amplified with PCR, cloned into an expression vector and transformed into an *E. coli* strain. With this strain a time kinetic was performed as described in 2.2.5.4. Three cultures were induced, situated in different optical densities (OD_{600}= 0.3; 0.5; 0.7). The strongest expression was obtained, inducing the culture with an OD_{600} of 0.5.

For purification, 1l of the culture was treated as described in 2.2.5.4., inducing with 0.5mM IPTG, when the culture reached an OD_{600} of 0.5. The success of the purification was analyzed with a SDS PAGE and a following Coomassie staining. The pure gp95 was dialysed against 4M urea. The concentration of gp95 was analyzed with a comperative SDS PAGE. As indicator, BSA standarts with different concentrations were used.

3.2.3.2. Bandshift assay

As target DNA, ϕCh1 DNA was used, isolated, as mentioned in 2.2.1.3.

The DNA was restricted with *Bgl*II to obtain 24 fragments, and lyophilised to reach a final concentration of 0.02pmol/batch. The protein was added with a final concentration of 0pmol, 8pmol and 16pmol. Because the protein was dialysed against urea, the approaches had to be balanced, to prevent precipitation. Therefore the batches were mixed with 8M Urea, to reach 1.6M Urea final concentration. The batches were prepared and incubated as described in 2.2.2.7.

A possible shift at ~11kBp could be observed with 16pmol gp95 as mentioned in figure 23. The corresponding fragment nu.36513- 47654 contains the ORF54- ORF78 (Figure 4).

To verify the shift, another restriction of the target DNA was performed to cleave the area of interest in 5 bands. Therefore ϕCh1 DNA was restricted with *Pst*I and used as template for another EMSA, performed under the same conditions. The EMSA showed a possible shift at nu.41344- 47405. To split the region of interest in smaller pieces, which may show a stronger shift, the target DNA was cut with another restriction enzyme *Nsp*I. The EMSA was performed under same conditions as previously mentioned. This time no possible shift could be observed.

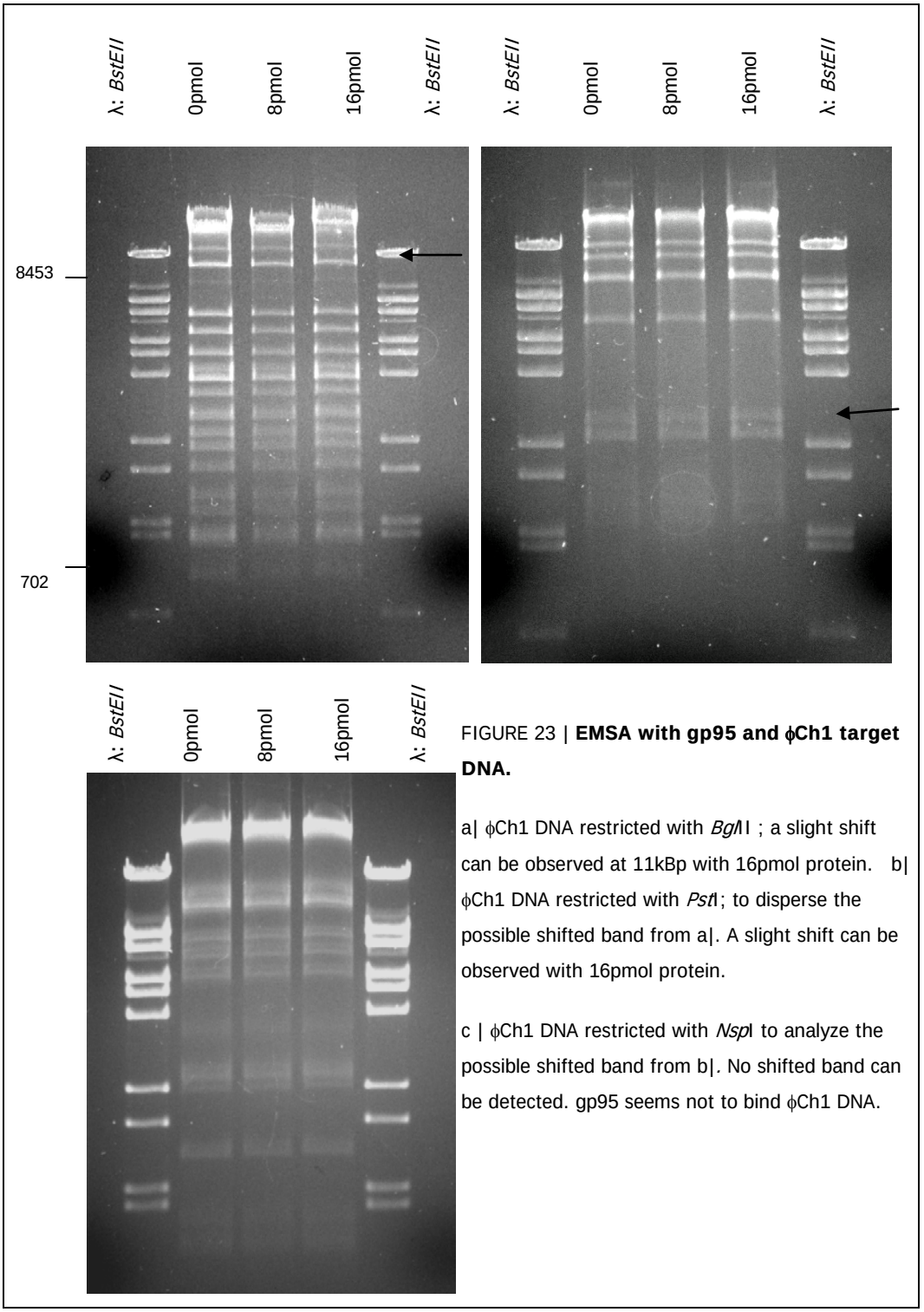


FIGURE 23 | **EMSA with gp95 and ϕCh1 target DNA.**

a| ϕCh1 DNA restricted with *Bgl*I ; a slight shift can be observed at 11kBp with 16pmol protein. b| ϕCh1 DNA restricted with *Pst*I; to disperse the possible shifted band from a|. A slight shift can be observed with 16pmol protein.

c | ϕCh1 DNA restricted with *Nsp*I to analyze the possible shifted band from b|. No shifted band can be detected. gp95 seems not to bind ϕCh1 DNA.

3.2.4. Discussion

The first 2 EMSAs showed possible shifts, which were closer examined with the third EMSA, where no shift could be observed at all. Because the candidate DNA was limited with each

restriction, the shifts would have to exceed. Due to the unchanged conditions, it is likely that no binding to DNA had occurred.

This result disagrees with the prediction, which was made because of the helix- turn- helix motif.

In this way, this experiment demonstrates the difficulty to pre- estimate the function of a halophilic protein based on motif comparison with common (mesophilic) proteins. Halophiles had to develop "…*specific structural elements, to fold into proper native form at high salt concentrations*" (Joo and Kim, 2005). Because of these adaptations, amino acid composition is not comparable to those of mesophile microorganisms. "*Enzymes of halophilic organisms contain unusual peptide motifs that are absent from their mesophilic counterparts"* (Evilia and Hou, 2006).

3.3. Creation of a vector for overexpression in Nab. magadii

3.3.1. Aim of the study

As previously mentioned, transformation of haloalkaliphilic *Archaea* is not well established, and due to this, different tools for genetic manipulation have to be developed by now. One of these tools might be a vector with a strong promoter, to perform overexpression studies. With such gain of function experiments, the function of particular genes can be determined.

To create an archaeal overexpression vector usable in *Nab. magadii*, a strong promoter had to be found and cloned into an established shuttle vector.

Archaeal promoter consist of a TATA Box, 24-28 nt upstream the transcription start site and in some cases the flanking purine rich BRE element for TFB binding (homologous to eucaryal TFIIB). Due to the absence of an *in vitro* transcription system in *Archaea*, all promoter characterising studies had to be done *in vivo*. In course of one of these studies, P_{fdx} could be determined to be the strongest tested promoter in *Hbt. salinarum*. P_{fdx} is the promoter of the ferredoxin gene, which is defined as a housekeeping gene (Gregor and Pfeifer, 2005). By means of this chracterization, P_{fdx} was chosen to be the main element of the planned overexpression vector.

To confirm the expression by P_{fdx}, a reporter gene had to cloned down- stream of P_{fdx}. Since αORF36 antibodies were available to detect gp34 of *Nab. magadii*, ORF34 was cloned down-stream of the fdx promoter without its native promoter. The αORF36 antibodies were obtained from gp36 but also recognize gp34 (Rössler *et al.,* 2003).

To estimate the expression strength, ORF34 was cloned into pRo-5, with its native promoter, too. These 2 vectors were transformed into *Nab. magadii* L13.

The two cultures were grown in buffle flasks, till they reached the stationary phase. After 3 days incubation at 37°C samples were taken and a western blot analysis was performed.

As a positive control *Nab. magadii* L11 was used. *Nab. magadii* L11 contains the integrated ϕCh1 virus and therefore expresses ORF34 in the late logarithmic phase (before onset of lysis). As a negative control L13 was used, which is cured from ϕCh1 and therefore does not express ORF34.

As expected, the signal at 60 kDa could be observed at the positive control and not at the negative control. The signals from the fdx- and the native promoter controlled gene products were equipollent as mentioned in figure 24.

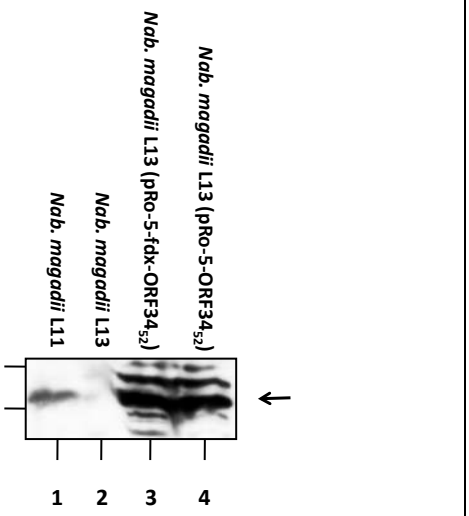


FIGURE 24 | **Western blot with αORF36 antibodies**. *Nab. magadii* L11 was used as a positive control, carrying ϕCh1 and therefore expressing ORF34 in the late stationary phase. *Nab. magadii* L13 serves as negative control. *Nab. magadii* L13 –pRo-5-P_{fdx} ORF34 and *Nab. magadii* L13-pRo-5-ORF34 should be compared with this western blot analysis concerning their strength of expression. Both cultures show a similar signal at ~60kDa. P_{fdx} does not enhance the gene expression in *Nab.magadii.*

3.3.2. Discussion

The reason for the reduced capacity of the fdx promoter in this experiment could be that the fdx promoter is originated from *Hbt. salinarum.* The expression in other organism can be difficult due to the machinery, which recognizes the promoter naturally is not available in *Nab. magadii.* Therefore the next approach to create a vector for overexpression could be to use a strong promoter from *Nab. magadii* itself.

3.4. Flagella as putative receptor for ϕCh1

3.4.1. Aim of the study

Since ϕCh1 is known to adsorb via any unknown glycosylated structure, flagella and the S-layer are the candidate structures for adsorption. Due to the failure of creating *Nab. magadii* without flagella, another approach to determine flagella as a possible receptor for ϕCh1 has to be found. Therefore following consideration was made: if an archaeal strain which normally can not be infected by ϕCh1 should be infected, if it expresses the flagella operon of *Nab. magadii*.

For this experiment two related strains were chosen- *Hbt. salinarum* and *N. pharonis*. Both strains normally can not be infected by ϕCh1 (Witte *et al.*, 1997).

N. pharonis was preferred because of its similar growths conditions, like pH values and salt optimum. It is likely that the expression of foreign flagella structure, as the process of infection by itself, can occur in a naturally way, in a similar environment. *Hbt. salinarum* on the other hand grows at pH values of 7-8. Its advantage is that it can be infected by the related phage ϕH (see 1.1.5.4. *Natronomonas pharaonis*, 1.1.5.2. *Halobacterium salinarum*, 1.2.1.Halophages in general).

To perform this study, initially, the flagella operon of *Nab. magadii* was cloned and transformed into both strains (2.2.4.10. pJas- Fla 2.2.4.9. pRR007-Fla). Afterwards the infectability had to be tested by performing a phage titer analysis. Therefore the strain was plated with ϕCh1 (undiluted and in dilutions from 10^{-2} -10^{-6})

While the infectivity of *N. pharaonis* still has to be confirmed, *Hbt. salinarum* containing the flagella operon of *Nab. magadii,* does not show any plaque formation after infection with ϕCh1. This doesn't mean certainly that flagella are NOT the gateway for ϕCh1.

To proof possible causes of disturbance, the usual procedure of a phage infection has to be considered.

Usually, phages recognize special structures on the cell surface of their hosts. In the case of the temperate phage λ, infecting *E. coli*, LamB, which is important for sugar uptake, gets recognized by the phage. The next step is to eject the head located, tight packed DNA, into the host. Therefore another protein plays a key role to pass the cytoplasm membrane: PstM. After injection, the DNA circularizes, to prevent exonuclease induced degradation. This process is catalyzed by the host encoded DNA ligase. The next step within the process of

infection is to transcribe the phage DNA. Therefore the host encoded RNA polymerase has to recognize the promoter. With the transcription, the decision is made if the lysogenic or the lytic life cycle is being started, depending of expression of possible repressors. If the lytic life cyle is being started, the phage encoded proteins are expressed, like capsid protein etc. The phage gets assembled and the phage encoded protein R and S form pores in the cytoplasm membrane and the peptidoglycan. In this way, the phages can be released out of the cell. (Trun and Trempy, 2003)

Considering all these steps, it is apparent that many host encoded proteins are involved in the process, except for the receptor. Whereas the flagellum is the gateway for ϕCh1, it could be possible that any of the other steps can not take place because of the missing suitable host encoded protein. Phages and their hosts are well matched through evolutionary processes. This adaptation is caused by cycles of defence and counter defence and is called “antagonistic coevolution” (Bucling and Rainey, 2002). If one on these previous mentioned steps can not take place, the phage can not lead to cell lysis and therefore can not be detected by a phage titer analysis.

Another possible reason for absence of lysis can be that the flagella protein could not be expressed in *Hbt. salinarum*. A possible reason could be the different transcription maschinery, another transport mechanism (for example a different signal for transport) or another method for assembly.

Taken together, for aforementioned reasons, the approach has shown that infectivity of ϕCh1 can not be induced by transformation of *Nab. magadii* flagella in the related *Hbt. salinarum* but maybe in *N. pharaonis*.

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5. Appendix

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Acknowledgements

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Abstract

ϕCh1 is a halophage, infecting the archeon *Natrialba magadii*, facing extreme conditions like high salinity and a high pH. The halophage is temperate, which means, that the phage can replicate in 2 different forms- the lytic and the lysogenic cycle.

The aim of this diploma thesis is to identify and analyse regulatory elements, which influence the switch between lysogenic and lytic life cycle. One of these repressors is the gene product of ORF49 (gp49), whose repressor activity has been determined in previous studies. In this work the repressor was analyzed regarding functional domains and mode of action. With the establishment of the deletion mutation of ORF49 the loss of function could be determined. This phenotype shows an increased size of plaque and an earlier onset of lysis of the host *Nab. magadii*. This is a further evidence for the regulatory effect of gp49. With the construction of N-terminal deletions and following analysis it was shown that despite the loss of the N-terminal DNA binding domain the regulatory effect of gp49 maintains. The regulation on DNA level seems not to be essential for repression by gp49. This could indicate that gp49 acts on RNA level or that it acts outside the cell by preventing phage adsorption, for example. To analyse a putative acting on RNA level, an AUG deletion was introduced into the ORF49 to prevent translation. The resulting ORF49ΔAUG was not able to repress the lysis of ϕCh1 anymore. With an adsorption assay the possibility was excepted that gp49 acts outside the cell.

Another putative regulator of lysis is gp95. With the transformation of ORF95 into *Nab. magadii* and following infection studies with ϕCh1 it could be determined that gp95 has no influence on lysis regulation of ϕCh1. Further on it was analysed if gp95 has the ability to bind to DNA. This could not be confirmed.

Another topic of this diploma thesis is to identify the structure, on which the phage binds for identification and adsorption. A possible structure is the flagellum of *Nab. magadii*. To confirm this assumption *Archaea*, which can normally not infected with ϕCh1 like *N. pharaoins* and *Hbt. salinarum* were transformed with the flagella operon of *Nab. magadii* and tried to infect with ϕCh1. No phage dependend lysis could be observed.

Zusammenfassung

ϕCh1 ist ein Halophage, der das Archaeon *Natrialba magadii* befällt, welches unter extremen Bedingungen lebt, wie hohe Salzkonzentrationen und einem hohen pH Wert. Der Halophage ist temperent, was bedeutet, dass er sich in 2 verschiedenen Zyklen vermehren kann, dem lytischen und dem lysogenen Zyklus.

Diese Diplomarbeit hat das Ziel, die Repressoren zu identifizeiren und zu charakterisieren, die für das Verhindern des Eintritts in den lytischen Zyklus verantwortlich sind. Einer dieser Repressoren ist das Genprodukt des ORF49 (gp49). Die Fähigkeit zur Repression der Lyse wurde bereits in vorangegangen Studien verifiziert. In dieser Arbeit wurde der Repressor auf weitere funktionelle Domänen und Wirkmechanismen untersucht. Durch die Etablierung und folgende Analyse einer ORF49 Deletionsmutante konnte der Phänotyp des Funktionsverlusts des gp49 untersucht werden. Dieser zeichnet sich durch stark zunehmende Plaquegröße und schneller voranschreitende Lyse des Wirts *Nab. magadii* aus. Hiermit konnte ein weiterer Beweis für die regulatorische Wirkung des gp49 erbracht werden. Des Weiteren konnte durch Deletions- und folgende Funktionsanalysen gezeigt werden, dass trotz des Verlusts der N-terminalen DNA Bindungsdomäne die regulatorische Wirkung des gp49 erhalten bleibt. Die Regulation auf DNA Ebene scheint also nicht essentiell zu sein für die Repression durch gp49. Dies könnte auf das Agieren auf RNA Ebene hindeuten oder auf das Agieren außerhalb der Zelle, zum Beispiel das Verhindern des Eindringen der Phagen in den Wirt. Um die Möglichkeit der RNA Regulation auszuschließen, wurde eine AUG Deletion in den ORF49 eingefügt, um dessen Translation zu verhindern. Die ORF49ΔAUG Mutante war nun nicht mehr fähig, die Lyse durch ϕCH1 zu repremieren. Mittels eines Adsorptionsassays konnte die Möglichkeit ausgeschlossen werden, dass gp49 das Eindringen der Phagen in den Wirt verhindert.

Einen weiteren möglichen Regulator der Lyse stellt gp95 dar. Dieser wurde im Zuge dieser Arbeit auf seine Repressoraktivität überprüft, indem er in *Nab. magadii* transformiert wurde und der Versuch einer ϕCh1 Infektion unternommen wurde. Es konnte jedoch kein verändertes Lyseverhalten festgestellt werden. gp95 kann also als Repressor ausgeschlossen werden. Des Weiteren wurde die DNA Bindungsfähigkeit des gp95 mittels EMSA überprüft. Diese konnte nicht verifiziert werden.

Ein weiteres Thema dieser Diplomarbeit ist die Identifizierung der Struktur, an die der Phage bindet um in die Wirtszelle aufgenommen zu werden. Eine mögliche Struktur ist hierbei das Flagellum von *Nab. magadii*. Um diese Vermutung zu bestätigen, wurden Archaeen, die normalerweise nicht durch ϕCh1 infizierbar sind (*N. pharaoins* und *Hbt. salinarum*) mit dem Flagellaoperon aus *Nab. magadii* transformiert um anschließend den Versuch zu unternehmen, sie mit ϕCh1 zu infizieren. Es konnte jedoch keine Phagen bedingte Lyse durch das Einbringen der Flagellenstrukturen erzielt werden.

Curriculum Vitae

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