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Constructing a DNAzyme Delivery and Expression System for *Escherichia coli*: A Prototype for Phage Therapy

Hugo V. C. de Oliveira, Spartaco Astolfi-Filho, Edmar V. Andrade

ABSTRACT

Antisense oligonucleotides exhibit high potential for use as therapeutic agents. '10-23' DNAzymes are antisense molecules with a high chemical stability and catalytic efficiency. In the present study, we developed a phagemid containing a DNAzyme expression system regulated by two promoters. One of these promoters, pA1, promotes constitutive expression of Moloney murine leukemia virus reverse transcriptase (MoMuLV-RT). The other promoter, plac, regulates transcription of the RNA substrate from which MoMuLV-RT produces the DNAzyme by reverse transcription. The *fts*Z DNAzyme was used to validate this expression system in the phagemid, named pDESCP. *fts*Z DNAzyme expression altered the morphological pattern of *Escherichia coli* from a bacillary to filamentous form. In *E. coli* FtsZ is the primary component of the cell division apparatus, forming a structure known as Z-ring, which is the place of division. It is suggested that the DNAzyme *fts*Z is decreasing the translation of this protein. Delivery of pDESCP into F+ strain of *E. coli* cells, using VCSM13, and the possible insertion of other DNAzymes into the cassette makes this phagemid an important prototype for phage therapy.

Keywords: '10-23' DNAzyme, DNAzyme vectors, *Escherichia coli*, phagemid transfection.

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I. Introduction

Antisense oligonucleotides are short segments of DNA or RNA that can inhibit translation or the function of a given gene transcript. Antisenses are organized into three classes: antisense oligodeoxyribonucleotides (AS-ODN), interfering RNAs (iRNA), and nucleic acid enzymes (DNAzymes and ribozymes) [1]-[3]. Although used as potential therapeutic agents, the main problems related to antisense oligonucleotides are that they are easy targets for nucleases, immunogenic, and require appropriate delivery that directs them toward a specific cell type. Recent studies have focused on chemical modifications of these molecules to make them more chemically stable. However, increasing stability almost always causes reduced specificity/affinity for their respective targets [4].

'10-23' DNAzymes, which cleave RNAs, are more chemically stable and exhibit higher catalysis than the other antisense classes [2], [4], [5]. The first plasmid-based DNAzymes expression system was developed to interact with human cells in culture [6]. This system was improved during subsequent years [7]-[10], which also resulted in the development of the first vector for *Escherichia coli* [11]. Intracellular DNAzyme expression becomes necessary because it generates an adequate quantity of these molecules for a sufficient time to obtain a desired biochemical/physiological effect [4]. Plasmid vectors are tools that ensure this expression.

Recently, there has been a renewed interest in the use of phages as they are promising therapeutic agents. They can be used in the treatment of bacterial infections that are super- and multi-resistant to traditional antibiotics [12], [13]. Phage therapy began approximately 20 years before the practical application of penicillin (the first reported antibiotic) in the former Soviet Union and some eastern European countries [12], [14], [15]. However, the antibiotic era ended phage therapy. Recently, the FDA suggested a profile for therapeutic phages: they are obligatorily lytic, "non-transducing" agents that preferably recognize bacterial virulence factors and originate from natural environments [16].

The M13 phage is a non-lytic and non-lysogenic filamentous virus that infects *E. coli* after binding to the sex pilus of this bacteria [12], [17]. Wan and Goddard [17] and Lin *et al.* [18] report that if M13 were a lethal phage, it would be a promising weapon against the horizontal transfer of pathogenicity genes, including antibiotic resistance genes. Genes that confer multidrug resistance are highly disseminated from extraintestinal pathogenic *E. coli* (ExPEC) via conjugation. A modern system for phagemid DNAzyme

expression, which can be delivered by M13, was developed based on these tools.

II. Material and Methods

A. Bacterial Strains

The following bacterial strains were used in this study: *E. coli* BL21(DE3) (F⁻ *omp*T *hsd*S_B (r_B^-, m_B^-) *gal dcm* (DE3)), *E. coli* DH5α (*F⁻ φ80lacZΔM15 Δ(lacZYA-argF)U169 recA1 endA1 hsdR17*(r_K^-, m_K^+) *phoA supE44 λ⁻ thi-1 gyrA96 relA1*), *E. coli* DH5αF'Iq (F⁻ φ80*lac*ZΔM15 Δ(*lac*ZYA-*arg*F)U169 *rec*A1 *end*A1 *hsd*R17 (r_K^-, m_K^+) *pho*A *sup*E44 λ⁻ *thi*-1 *gyr*A96 *rel*A1/F′ [*pro*AB⁺ *lac*I^{q}ZΔM15 *zzf*::*Tn*5 (Km^R)]), E. *coli* DH10b ((F⁻ *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *end*A1 *ara*D139 Δ(*ara-leu*)7697 *gal*U *gal*K λ⁻ *rps*L(Str^R) *nup*G), *E. coli* JM109 (*end*A1, *rec*A1, *gyr*A96, *thi*, *hsd*R17 (r_k^-, m_k^+), *rel*A1, *sup*E44, Δ(*lac-pro*AB), [F′ *tra*D36, *pro*AB, *laq*I^{q}ZΔM15]), *E. coli* K-12 MG1655 (F⁻lambda- *ilv*G- *rfb*-50 *rph*-1), *E. coli* TOPO 10 (F⁻ *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara-leu*)7697 *gal*U *gal*K λ⁻ *rps*L(Str^R) *end*A1 *nup*G).

B. Vectors and Helper Phage

pRT 30-2 is an expression vector for Moloney murine leukemia virus reverse transcriptase (MoMuLV-RT), kindly provided by Dr. Stephen Goff – Columbia University. This vector was used as a basis for constructing the pDESCP phagemid, which contains the *fst*Z DNAzyme expression system developed in the present study. The pGEM®-T Easy vector (PROMEGA) was used to obtain the Ori *f1* gene region. The *fts*Z DNAzyme cassette was chemically synthesized (GenOne, RJ-Brazil) and bound to pUC19 (pUC19-plac-*fts*Z). pRT30-2/Dz-*fts*Z is a vector that contains the *fts*Z DNAzyme expression system without the Ori *f1* region. VCSM13 (Stratagene) was the helper phage used to promote encapsidation of pDESCP and its subsequent delivery into *E. coli*.

C. Construction of pDESCP

Construction of this vector involved integrating two cassettes: one for MoMuLV-RT enzyme expression and the other for RNA transcript production to serve as a substrate for the synthesis of the *fts*Z DNAzyme (developed by Tan *et al.* [11]) by reverse transcription. The cassette for *fts*Z DNAzyme expression was designed and obtained by chemical synthesis (Genome Biotechnologies), and subsequently subcloned into pUC19, thus named pUC19-plac-*fts*Z. This, in 5′→3′ direction, has the following structures: CAP binding region, *lac* promoter, *lac* operator, a terminator of the reverse transcription for MoMuLV-RT, *fts*Z DNAzyme sequence, a binding site for MoMuLV-RT, and a terminator of general transcription. *Hind*III sites were inserted at both ends of the cassette. Other sites for restriction endonucleases (*Eco*RI, *Eco*RV, *Xho*I, *Apa*I, *Xba*I, *Avr*II, *Sal*I) were also strategically inserted throughout this cassette to facilitate future manipulations. The nucleotide sequence of this entire structure is shown in Fig. 1.

Fig. 1. Synthetic *fts*Z DNAzyme expression cassette. The sites for restriction endonucleases, p*lac* promoter (-10 and -35 region), and other relevant regions are highlighted (underlined) with their respective names. The entire cassette contains 476 base pairs.

This cassette was excised from pUC19-plac-*fts*Z and subcloned into pRT30-2, after previously digesting both vectors with HindIII, resulting in the formation of pRT 30-2/Dz-*fts*Z. The Ori *f1* region was amplified from pGEM®-T Easy using the following polymerase chain reaction (PCR) to convert the latter into a phagemid: 1×GoTaq® Flexi buffer; 2.0 mM MgCl2; 0.2 mM dNTP mix; 10 pmoles Orif1-F (5'-ACTCGAGAAATTGTAAACGTTAATATTTTG-3´) and Orif1-R (5'-AGAATTCAATGGACGCGCCCTGTAGC-3'); 1.5 U GoTaq® DNA polymerase and; ~1 ng pGEM®-T Easy Vector. The thermocycling conditions were as follows: 95 °C for 2 min; 30 cycles (95 °C for 40 s; 58 °C for 40 s, 72 °C for 1 min); final extension at 72 °C for 5 min. Orif1-F and Orif1-R were designed containing an adenine and sites for *Xho*I and *Eco*RI at their 5'-ends, respectively. O amplicon Ori *f1* and pRT 30-2/Dz-*fts*Z were both double-digested by *Eco*RI and *Xho*I, and then bound together using 200U T4 DNA ligase. The binding reaction was used to transform electrocompetent DH5αF'Iq *E. coli* cells. The transformants were selected on Luria Bertani (LB) agar medium, containing 100 μg/mL ampicillin, incubated at 37 °C for 16 h. The recombinant vector obtained was independently digested with *Hind*III, *Eco*RI, and double-digested with *Eco*RI-*Xho*I, *Xba*I-*Eco*RI, and *Xba*I-*Xho*I to confirm the inserts, thus named pDESCP (DNAzyme Expression System Carried by Phage) (Fig. 2).

D. Effect of pDESCP in E. coli

The growth curves for five cultures of the DH5αF'Iq strain transformed with the following vectors and cultured under the following conditions, respectively, were constructed to evaluate the effect of pDESCP on growth and/or viability of *E. coli*: pRT 30-2 (1% glucose), pRT 30-2 (1 mM IPTG), pDESCP (1% glucose), and pDESCP (0.1 mM and 1 mM IPTG). All of the cultures were incubated at 37 °C, in LB liquid medium, under agitation at 180 rpm, with absorbance measured every 2 h for 96 h.

E. Analysis of the ftsZ DNAzyme Expression System

DH5αF'Iq/pDESCP *E. coli* clones were grown in both LB liquid and solid culture media, both containing 100 μg/mL ampicillin, in the presence of an inducer (1 mM IPTG) or absence of activation (1% glucose) of *fts*Z DNAzyme expression. Untransformed DH5αF'Iq *E. coli* was used as a negative control. The cells were incubated at three temperatures: 32, 37, and 42 °C for 16 h. Growth in LB liquid medium occurred under continuous agitation at 180 rpm. After the incubation period, microscopic slides of all the cultures were prepared. The slides were heat-fixed, stained with safranin for 45 s, and observed under an optical microscope at 1,000 × magnification to phenotypically

analyze the colonies. *fts*Z DNAzyme expression was determined by analyzing the presence or absence of these molecules together with total RNA, extracted by TRIzol®, obtained from a clone grown in liquid medium containing 1 mM IPTG (modified from Mirochnitchenko *et al.* [19]).

F. Transfection of pDESCP

DH5αF'Iq/pDESCP *E. coli* cells were infected with a VCSM13 helper phage (adapted from Rader *et al.* [20]) to obtain the viral capsids of the M13 phage containing pDESCP. As the VCSM13 genome is strategically defective to packaging, the viral particles obtained were predominantly pDESCP. These particles were used to transfect the DH5αF'Iq and JM109 *E. coli* strains. The DH5α, DH10b, BL21(DE3), and K-12 strains were used as negative controls of the transfection.

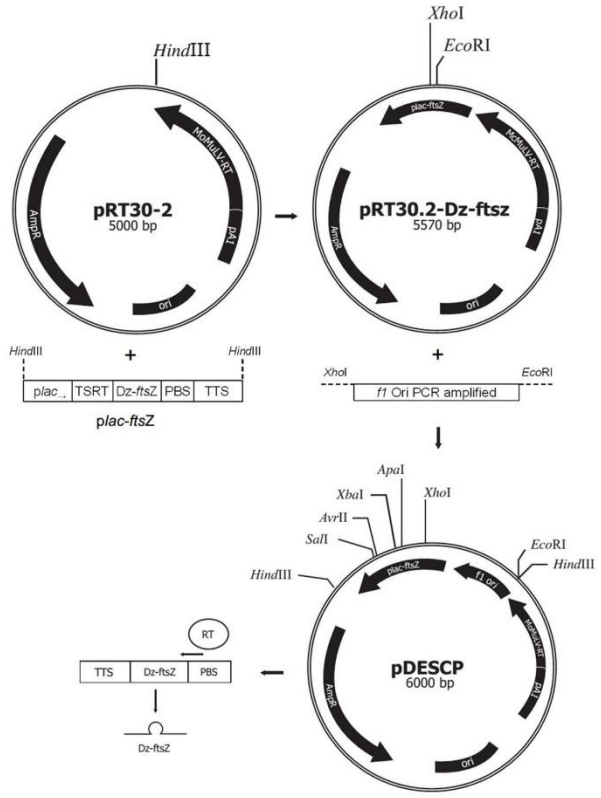

Fig. 2. Construction of pDESCP. The *fts*Z DNAzyme expression cassette (plac-*fts*Z) was inserted into pRT 30-2 at the HindIII site, yielding pRT 30-2/Dz-*fts*Z. MoMuLV-RT is constitutively expressed by the pA1 promoter. The lac promoter regulates transcription of the RNA cassette that will serve as a substrate so that MoMuLV-RT (RT) produces the *fts*Z DNAzyme (Dz-*fts*Z) by reverse transcription.

III. RESULTS

A. Construction of the Phagemid

Construction of the pDESCP vector was confirmed by analyzing the restriction profile (Fig. 3). Lane 3 shows linearization of the pRT30-2 vector by *Hind*III. Digestion of pDESCP with *Hind*III liberated a single 1,048-bp insert (lane 4), as expected. This fragment corresponds to the *fts*Z DNAzyme cassette (576 bp) fused to the Ori *f1* region (472 bp). The Ori *f1* bond within the *fts*Z DNAzyme cassette was also confirmed via double-digestion of the vector with the *Eco*RI and *Xho*I enzymes (lane 5), releasing a 472-bp fragment. These results even corroborate the Ori *f1* amplicon, obtained from pGEM®-T Easy using Orif1-F/Orif1-R (lane 6).

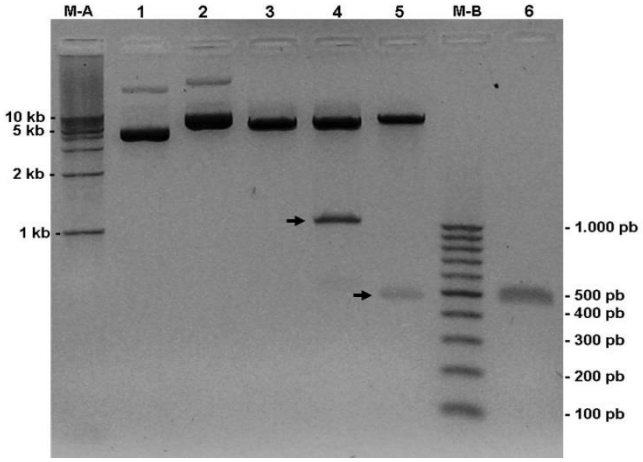

Fig. 3. Construction of pDESCP. M-A: 10-kb molecular marker (GeneRuler™); 1- intact pRT30-2; 2- intact pDESCP; 3- pRT30-2/*Hind*II; 4- pDESCP/*Hind*III; 5- pDESCP/*Eco*RI and *Xho*I; 6- Ori *f1* amplicon. The arrows indicate a 1,048-bp fragment containing the *fts*Z DNAzyme cassette fused to the Ori *f1* region (lane 4) and a 472-bp fragment containing the Ori *f1* region (lanes 5 and 6). M-B: 1-kb molecular marker (GeneRuler™); amplified using pGEM®-T Easy.

B. Analysis of ftsZ DNAzyme induction in E. coli

The growth curve (data not shown) demonstrated that the experimental controls, represented by DH5αF'Iq *E. coli* containing pRT 30-2/1% glucose, pRT 30-2/1 mM IPTG, and pDESCP/1% glucose, respectively, grew exponentially until approximately 4 h. After this time, they enter into a long stationary phase. The cultures containing 0.1 and 1 mM pDESCP/IPTG, respectively, also exhibited exponential growth until 4 h, subsequently entering into a long stationary phase. However, after 4 h there was a significant increase in optical density (OD) of these cultures, compared with the control cultures, indicating higher cell mass.

The *fts*Z DNAzyme expression system induced in DH5αF'Iq *E. coli* resulted in altered morphology of this bacterium, from a bacillary to filamentous form (Fig. 4, boxes 9–12), compared to the control cultures (Fig. 4, boxes 1–4). Throughout each filamentous structure, the contrasting presence of two or more nucleoids was noted when the slides were micrometrically adjusted under the microscope. The cultures grown in solid medium exhibited more expressive filamentation than those grown in liquid medium (images not shown), regardless of the growth temperature analyzed (32, 37, and 42 ºC). The micromorphology of the DH5αF'Iq/pDESCP clones grown in medium containing 1% glucose, and without IPTG, showed cells slightly more elongated than the control cultures (boxes 5–8). In some of these cells, it is also possible to observe the presence of one or more nucleoids.

Even based on pDESCP induced in DH5αF'Iq *E. coli*, it was possible to demonstrate the presence of what appears to possibly be a single-strand DNA through 3% agarose gel (data not shown). These qualitative data generate evidence that the *fts*Z DNAzymes are being properly produced, as all of the extracts obtained for single-strand DNA isolation were exhaustively treated with RNase A.

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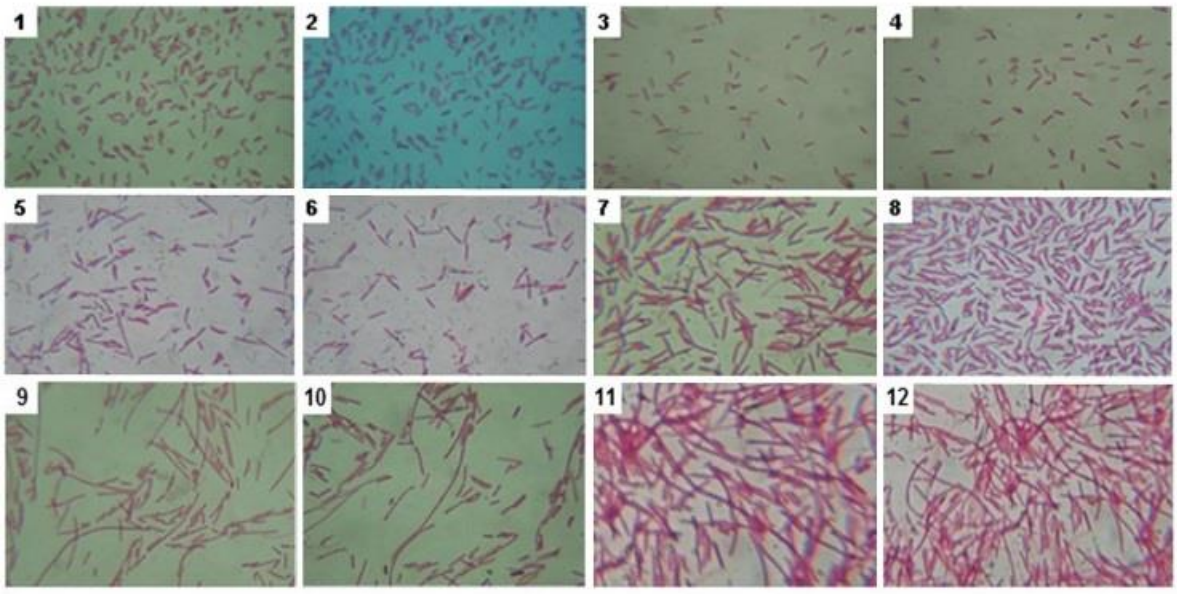

Fig. 4. Effect of pDESCP in DH5αF'Iq *E. coli* grown in LB agar: 1- without vector and with 1% glucose; 2- without vector and with 1 mM IPTG; 3- with pRT 30-2 and 1% glucose; 4- with pRT 30-2 and 1mM IPTG; 5, 6, 7, and 8 - with pDESCP and 1% glucose; 9, 10, 11, and 12- with pDESCP and 1mM IPTG.

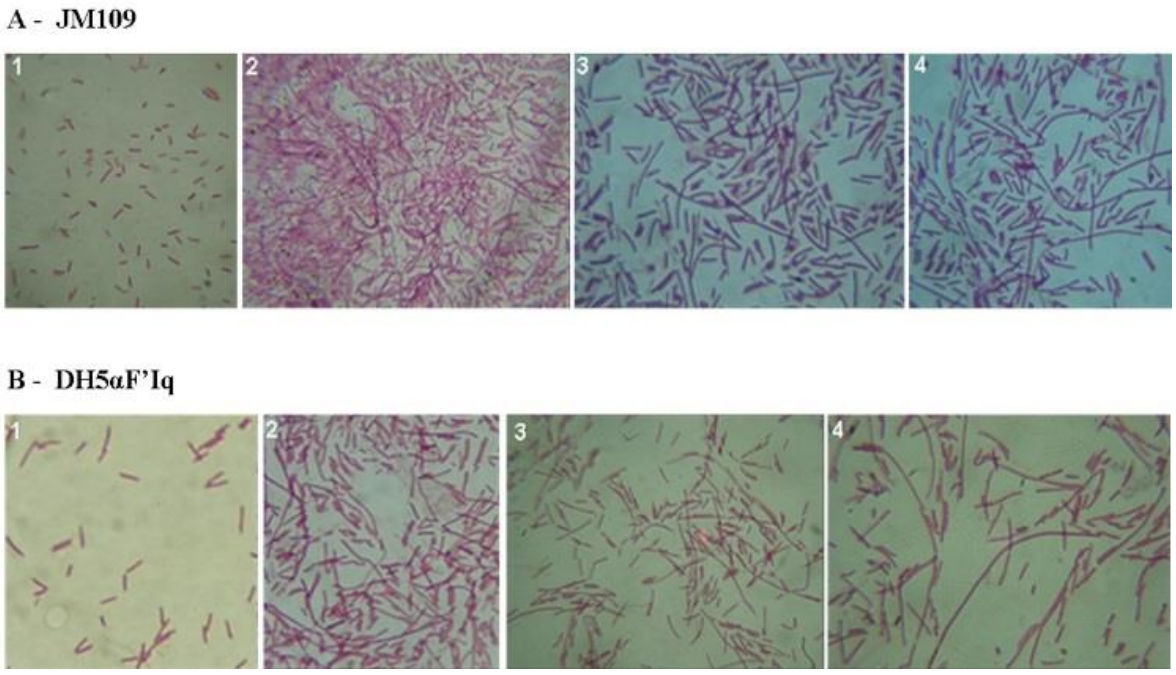

Fig. 5. Transfection in *E. coli*. A- JM109 *E. coli*: 1- untransfected; 2, 3, and 4– transfected. B- DH5αF'Iq *E. coli*: 1- untransfected; 2, 3, and 4– transfected.

C. Transfection of pDESCP

The pDESCP phagemid was properly transfected into JM109 and DH5αF'Iq *E. coli*, both F^+ strains, from VCSM13. Micromorphological analysis of these bacteria confirmed that they became filamentous soon after transfection when they were grown in LB solid medium containing 1 mM IPTG (Fig. 5). Plasmid extraction performed in these cells demonstrated the presence of transfected pDESCP (results not shown). Non-producing strains of the F^+ sex pilus, DH5α, DH10b, BL21(DE3), and K-12 were unable to receive the phagemid and thus did not grow in pDESCP-selective medium (containing ampicillin). These findings demonstrate the efficiency of the delivery system and *fts*Z DNAzyme expression in F^+ *E. coli* strains.

IV. Discussion

pDESCP is an approximately 6-kb phagemid and is based on pRT 30-2. The latter was constructed from pKK 177-3 (Pharmacia, STO-Sweden), where the region comprised between the BamHI and HindIII sites presents the same basic sequence of the *Pvu*II-*Hind*III region of the pKK vectors (the *Bam*HI site was inserted within *Pvu*II – Telesnitsky *et al.* [21]). Analysis of the enzymatic digestions performed with this vector allowed for confirming both insertion of the *fts*Z DNAzyme expression cassette and Ori *f1* (Fig. 3). The latter is positioned between the MoMuLV-RT and *fts*Z DNAzyme cassettes, as shown in Fig. 2.

Construction of pDESCP causes an RNA transcript to be released from the induced lac promoter. This transcript has a

binding site for MoMuLV-RT. For this enzyme to bind to this region, it should initially bind to an endogenous $tRNA^{Val}$ of *E. coli*, which functions as a reverse transcription primer (Tan *et al.* [11]). pDESCP differs from the other DNAzyme expression vectors by exhibiting a more refined system. This is because the *fts*Z DNAzyme exhibits expression independent of the MoMuLV-RT enzyme. Expression of this reverse transcriptase is regulated by pA1, a constitutive expression promoter, derived from the f1 phage (Telesnitsky *et al.* [21]). According to Prof. Stephen Goff (personal communication), pA1 is the most adequate promoter for the expression of this enzyme, as it can express adequate levels, compatible with its efficient catalytic activity. MoMuLV-RT expression by pA1 corresponds to ~ 4% of the total cellular proteins of *E. coli* [21].

pDESCP differs from the main DNAzyme vectors developed: pssXA, pssXB, pssXD, pssXE, and pssXGb. Of these, only pssXGb was constructed to act in *E. coli*, where the others were directed for human cells in culture. Transcription of a single cassette occurs in all of them, which contains both the reverse transcriptase sequence, which will be translated, and the sequence that leads to the DNAzyme of interest. A strong promoter is used in all vectors to ensure a high transcription rate. In 2010, Li *et al.* [5] reported the construction of a modern vector regulated by two promoters, pSDE01–DZt1. This vector was developed to act in human macrophages in culture (It does not work for bacteria). According to the authors, this type of vector is the most adequate model for DNAzyme expression, as it regulates the production of two transcripts that must be produced in different quantities: higher production of the transcript that leads to DNAzyme and lower expression of the reverse transcriptase. MoMuLV-RT has a relatively high catalytic processivity and endogenous RNase H activity that degrades the RNA substrate to the extent that it is retrotranscribed for the respective DNAzyme. Li *et al.* [5] speculated that excessive production of the reverse transcriptase enzyme could be toxic for cells.

The *fts*Z DNAzyme used in this study exhibited a 10-23 catalytic motif and targeted the transcript of the *fts*Z gene. The FtsZ protein is the prokaryotic tubulin homologue in eukaryotes. In *E. coli*, FtsZ is the primary component of the cell division apparatus, forming a structure known as a Z-ring, which is the division site. The Z-ring, associated with other cell division proteins, acts as a contractile ring that performs bacterial division [11], [22], [23].

Tan *et al.* [11] demonstrated in vitro that the *fts*Z DNAzyme they developed, expressed by pssXG, was able to cleave the transcript of the *fts*Z gene at the proposed site. They found, in vivo, that expression of this DNAzyme in DH5α *E. coli* could alter the morphology of this bacteria, from a bacillary to filamentous form, with the presence of two or more nucleoids per cell. Tan *et al.* [11] also reported that these polyploid cells can grow; however, are more sensitive to lysis than normal cells. In the current experiments, induction of pDESCP triggered during the filamentation process of DH5αF'Iq *E. coli* primarily occurred in solid culture medium (Fig. 4). These findings corroborate those described by Tan *et al.* [11]; however, we could not find any possible sensitivity of these cultures to lysis, not even when they were grown in liquid medium. Growth curve analysis did not generate evidence that the DH5αF'Iq/pDESCP *E. coli* cultures, appropriately induced with IPTG, are more sensitive to lysis because of the *fts*Z DNAzyme expression (results not shown). *fts*Z DNAzyme expression based on pDESCP is mainly sustained by the filamentous phenotype observed. The likely presence of single-strand DNA, through 3% agarose gel (not shown), and increased D.O., observed in the growth curve of DH5αF'Iq/pDESCP cultures, also support the hypothesis that *fts*Z DNAzymes are produced. In an important experiment performed by Stricker and Erickson [22], it was found that mutant *fts*Z *E. coli* divided for approximately 180 min, followed by filamentation between 200 and 240 min. During this period, the cells were 6 to 12 times longer than the normal cells and further incubation of these bacteria resulted in slightly increased filamentation, followed by cell lysis.

The filamentous phenotype observed in the DH5αF'Iq (Fig. 4, boxes 9–12; Fig. 5B, boxes 2–4) and JM109 *E. coli* strains (Fig. 5A, boxes 2–4), both transformed and transfected with pDESCP, and suggested some disorganization in the division apparatus of these bacteria. The lack of, or possibly reduced, FtsZ protein at the intracellular level can thus result in the disorganization of the *E. coli* contractile ring, preventing it from dividing correctly. In boxes 5–8, of Fig. 4, some degree of elongation and more than one nucleoid can be seen in some DH5αF'Iq/pDESCP cells that were grown in medium containing 1% glucose. Therefore, because it is the lac promoter, it does not exclude possible residual expression in pDESCP/1% glucose.

An interesting finding described by Charbon *et al.* [24] is the importance of crescentin in the maintenance/stabilization of the elongated cell shape in the bacterium Caulobacter crescentus. Crescentin is a protein with similar functions to the intermediate filaments of eukaryotic cells, i.e., form a two-dimensional mesh under the plasma membrane inside these bacteria, able to support the form and their mechanical stress [24], [25]. According to Charbon *et al.* [24], crescentin extends along the length of the cell. These authors cultured an *E. coli* strain (CJW2209) on a solid surface under conditions in which the *fts*Z gene was not being expressed, whereas the crescentin gene was. It was found that as cells grow, becoming filamentous, the crescentin mesh slowly increased, suggesting that it acts as a mechanical support for the new dimensions of the cell. Elongation of the crescentin structure is bidirectional, occurring at both ends toward the center. When this filamentation process occurred in liquid culture medium, the protein mesh was responsible for occasional bending in the form of the bacteria, concentrating at the points of curvature.

Cabeen *et al.* [25] reported that when crescentin is ectopically synthesized in *E. coli*, a γ-Proteobacterium could form functional filamentous structures throughout the cell membrane, which is responsible for maintaining curvature during this bacteria's elongation. Despite the large evolutionary difference between *E. coli* and C. crescentus, this indicates that the link between crescentin and the cell wall is mediated by genetic components, such as MreB. MreB is a protein found in some bacteria and is considered as an actin homologue. However, crescentin does not bind to bacteria without MreB, and these bacteria should be closer from the evolutionary point of view than *E. coli*. Crescentin, or its equivalent form, has still not been found naturally occurring in *E. coli*. There is still much to be clarified regarding the *E. coli* cytoskeleton.

Because of the FtsZ protein's function and the results

obtained in this study, we suggest that the *fts*Z DNAzyme expression system is in fact functioning. However, we speculate that *E. coli* is creating some mechanism to support the tension imposed by its increased cell surface, to its detriment by reducing the cell division frequency, caused by reduced FtsZ. This does not eliminate that the maintained cell viability of *E. coli* in the filamentous state is possibly supplied by a two-dimensional protein mesh similar to crescentin [], [24]-[28]. Moreover, it is important to note that much remains to be understood about the behavior of the FtsZ protein in *E. coli*, their interactions, and dynamics before and during bacterial division [23], [29], [30].

The efficiency of pDESCP transfection is fundamental as a biopharmaceutical prototype to this phagemid. The possibility of inserting new DNAzymes, via ApaI, XbaI, AvrII, and SalI sites illustrates the probability of developing a more aggressive bactericidal biopharmaceutical. pDESCP has the potential to act as a tool for the reduced dissemination of virulence factors based on pathogenic *E. coli* (ExPEC - extraintestinal pathogenic *E. coli*), as this occurs exclusively via conjugation or horizontal transfer [31].

V. Conclusion

The DNAzyme expression vector operates with two promoters and, from the observed results, it is possible to suggest that the DNAzyme *fts*Z is being expressed and causing a decrease in the translation of this protein, in the E. coli bacterial model. However, for the complete validation of this system, it is necessary to analyze the downregulation of the FtsZ protein. The delivery of pDESCP for F + strain of E. coli cells, using VCSM13, and the possible insertion of other DNAzymes in the cassette makes this phagemid an important prototype for gene silencing using DNAzymes.

Conflicts Of Interest

The authors declare no conflict of interest.

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