



Regulatory RNAs in the Less Studied Streptococcal Species: from Nomenclature to Identification

Mohamed-Amine Zorgani, Roland Quentin, Marie-Frédérique Lartigue

► To cite this version:

Mohamed-Amine Zorgani, Roland Quentin, Marie-Frédérique Lartigue. Regulatory RNAs in the Less Studied Streptococcal Species: from Nomenclature to Identification. *Frontiers in Microbiology*, 2016, 7, 9 p. <10.3389/fmicb.2016.01161>. <hal-01346398>

HAL Id: hal-01346398

<https://hal.science/hal-01346398v1>

Submitted on 27 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

Regulatory RNAs in the Less Studied Streptococcal Species: from Nomenclature to Identification

Mohamed Amine Zorgani^{1*}, Roland Quentin^{1,2}, Marie-Frédérique Lartigue^{1,2*}

¹ISP, INRA, Université Tours, Equipe "Bactéries et risque materno-foetal", UMR1282, France, ²Service de bactériologie virologie et hygiène hospitalière, Centre Hospitalier Régional Universitaire de Tours, France

Submitted to Journal:
Frontiers in Microbiology

Specialty Section:
Infectious Diseases

ISSN:
1664-302X

Article type:
Mini Review Article

Received on:
02 Jun 2016

Accepted on:
12 Jul 2016

Provisional PDF published on:
12 Jul 2016

Frontiers website link:
www.frontiersin.org

Citation:
Zorgani M, Quentin R and Lartigue M(2016) Regulatory RNAs in the Less Studied Streptococcal Species: from Nomenclature to Identification. *Front. Microbiol.* 7:1161. doi:10.3389/fmicb.2016.01161

Copyright statement:
© 2016 Zorgani, Quentin and Lartigue. This is an open-access article distributed under the terms of the [Creative Commons Attribution License \(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use, distribution and reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

This Provisional PDF corresponds to the article as it appeared upon acceptance, after peer-review. Fully formatted PDF and full text (HTML) versions will be made available soon.

Regulatory RNAs in the Less Studied Streptococcal Species: from Nomenclature to Identification

MA Zorgani^{1,*}, R. Quentin^{1,2}, MF Lartigue^{1,2,*}

¹ ISP, INRA, Université Tours, Equipe "Bactéries et risque materno-foetal", UMR1282, F-37032 Tours, France.

² Centre Hospitalier Régional Universitaire de Tours, Service de bactériologie virologie et hygiène hospitalière, F-27044 Tours, France.

*Corresponding authors: Marie-Frédérique Lartigue, E-mail: marie-frederique.lartigue@univ-tours.fr. Mohamed Amine Zorgani, E-mail: mohamedamine.zorgani@etu.univ-tours.fr.

Université François Rabelais de Tours, Faculté de médecine, UMR1282 Equipe 5 Bactéries et Risque Materno-Fœtal, 10 Boulevard Tonnellé, F-37032 Tours, France.

Running title: Non-coding RNAs in the less studied *Streptococcus* species

Keywords: small RNAs, non-coding RNA, *Streptococcus agalactiae*, *Streptococcus mutans*, *Streptococcus thermophilus*, Regulatory RNA nomenclature

Abstract:

Streptococcal species are Gram-positive bacteria involved in severe and invasive diseases in humans and animals. Although this group includes different pathogenic species involved in life-threatening infections for humans, it also includes beneficial species, such as *Streptococcus thermophilus*, which is used in yogurt production. In bacteria virulence factors are controlled by various regulatory networks including regulatory RNAs. For clearness and to develop logical thinking, we start this review with a revision of regulatory RNAs nomenclature. Previous reviews are mostly dealing with *Streptococcus pyogenes* and *Streptococcus pneumoniae* regulatory RNAs. We especially focused our analysis on regulatory RNAs in *Streptococcus agalactiae*, *Streptococcus mutans*, *Streptococcus thermophilus* and other less studied *Streptococcus* species. Although *S. agalactiae* sRNome remains largely unknown, sRNAs (small RNAs) are supposed to mediate regulation during environmental adaptation and host infection. In the case of *S. mutans*, sRNAs are suggested to be involved in competence regulation, carbohydrate metabolism and Toxin-Antitoxin systems. A new category of miRNA-size small RNAs (msRNAs) was also identified for the first time in this species. The analysis of *S. thermophilus* sRNome shows that many sRNAs are associated to the bacterial immune system known as CRISPR-Cas system. Only few of the other different *Streptococcus* species have been the subject of studies pointed toward the characterization of regulatory RNAs. Finally, understanding bacterial sRNome can constitute one step forward to the elaboration of new strategies in therapy such as substitution of antibiotics in the management of *S. agalactiae* neonatal infections, prevention of *S. mutans* dental caries or use of *S. thermophilus* CRISPR-Cas system in genome editing applications.

Introduction

Gene regulation is fundamental for bacteria to adapt to environmental changes. Therefore, bacteria have developed and evolved strategies to tightly control genetic networks in response to diverse extracellular stimuli (Casadesus and Low, 2006). Bacterial regulatory pathways involving small RNAs (sRNAs) are now being elucidated and their functions are coming to light, particularly in regards to bacterial physiology, adaptation and pathogenesis as well as to plasmid replication control (Cavanagh and Wassarman, 2014; Brantl, 2015; Lopez-Aguilar *et al.*, 2015). Beside transcriptional and translational gene regulation (activation or inhibition) through direct interaction with mRNAs, regulatory RNAs perform a variety of other mechanisms on their targets including: (i) sequestering molecules (*e.g.* binding to proteins) (Babitzke and Romeo, 2007), (ii) affecting the expression of *cis* elements (*e.g.* riboswitches) (Winkler and Breaker, 2005) and (iii) targeting invading cognate DNA (*e.g.* CRISPR RNAs [Clustered Regularly Interspaced Short Palindromic Repeats RNAs]) (Garneau *et al.*, 2010). The importance of sRNAs-mediated regulatory mechanisms depends on a fine-tuning of the expression of an armament of virulence factor genes (Papenfort and Vogel, 2010; Mann *et al.*, 2012). Considering the functional roles of these RNAs, they could be targets for the development of future therapeutic drugs (Lalaouna *et al.*, 2014).

Streptococcal species can colonize and invade humans and animals. They mostly exhibit an asymptomatic interaction within their hosts. However, several of them are well-known pathogenic species responsible for severe and life-threatening infections in humans. Infections can be exclusive to human, as it is the case for *Streptococcus pyogenes* leading to a substantial mortality and morbidity (Carapetis *et al.*, 2005). They can also switch from a benign and commensal stage to an infectious one, like for *Streptococcus pneumoniae* (Gamez and Hammerschmidt, 2011) and the neonatal opportunistic pathogen *Streptococcus agalactiae* (Maisey *et al.*, 2008; Krzysciak *et al.*, 2013). In the last decades, the study of these pathogens has increased intensively due to the spreading of antibiotic resistance. Therefore, researchers are continuously invited to look for novel targets and to develop innovative therapies to combat streptococcal infections. With regard to the current knowledge on regulatory RNAs functions and modes of action, sRNAs are expected to play central roles in the bacterial regulatory networks, physiology, adaptation and virulence. Thus, they are thought-out as alternative types of regulators for adaptive gene regulation and drugs design.

Previous reviews on streptococcal sRNAs were mainly restricted to two streptococcal species: *S. pyogenes* and *S. pneumoniae* (Le Rhun and Charpentier, 2012; Patenge *et al.*, 2013; Brantl and Bruckner, 2014; Miller *et al.*, 2014; Wilton *et al.*, 2015; Bouloc and Repoila, 2016). However, the *Streptococcus* genus includes a large group of commensal and pathogenic Gram-positive bacteria. This review aims to provide more complete and comprehensive state of the art on the hidden part of streptococcal regulatory RNAs. It focuses on *S. agalactiae*, *S. mutans*, *S. thermophilus* sRNAs, as well as other less studied species, and brings in light their sRNome with reference to recent advances in the fast moving area of bacterial sRNAs. The clearness of scientific reports is intimately linked to the simplicity of the nomenclature been used. Thus, we dedicated the first chapter of this review to present and reemphasize the use of bacterial regulatory RNAs nomenclature. Please note that in order to put forward *Streptococci* sRNome, we will not discuss small RNAs from other Gram-positive bacteria (Romby and Charpentier, 2009; Brantl and Bruckner, 2014).

Bacterial regulatory RNAs: terminology issues

Regulatory RNAs are commonly divided into three major groups: the first group includes *cis*-acting elements located on untranslated regions (UTRs) of a translated mRNA. They control the expression of their enclosed gene(s) through the modulation of their secondary structures or stability (Johansson *et al.*, 2002). A second class of sRNAs are antisense RNAs (asRNAs), whose regulatory activity occurs through perfect base-pairing to the mRNA encoded by the opposite DNA strand molecule (Thomason and Storz, 2010). The last group of sRNAs, also known as *trans*-acting sRNAs, includes RNAs that target other RNAs, foreign DNA, or proteins (Gottesman and Storz, 2010). In the case of RNA targets, this group of sRNAs often shares a limited complementarity with their targets; thus, they can bind to multiple mRNAs. This type of interaction requires, often for stabilization issues, the RNA chaperone protein Hfq, which is absent in the *Streptococcus* genus and other Gram-positive bacteria (Brennan and Link, 2007; Nielsen *et al.*, 2009).

As it is the case in proteomics and genomics (Desvaux *et al.*, 2009), regulatory RNAs nomenclature issues raise some confusion in the field of bacterial regulatory networks. For instance, the use of the descriptors “*cis*-acting” or “*cis*-encoded” sRNA to describe an “antisense sRNA”, or the confusion between the use of “*trans*-encoded” and/or “*trans*-acting” sRNA, or even the use of different nomenclatures “Small *trans*-encoded RNA” (Nielsen *et al.*, 2009), “Small regulatory RNA” (Frohlich and Vogel, 2009), “*trans*-encoded sRNA”

(Ross *et al.*, 2013) and “Small non-coding RNAs” (Xia *et al.*, 2012) to cite a regulatory RNA. Thus, it is useful to reemphasize, and to remind the reader on regulatory RNA nomenclature.

In this review section, we propose a simplified and coherent nomenclature, which we believe necessary to develop comprehensible and logical thinking when describing bacterial regulatory RNAs. We propose first to revise the division of regulatory RNAs into two major categories (Table 1). The first category are the *cis*-acting RNAs. The second one, are the *trans*-acting sRNAs. This category comprises the *cis*-encoded sRNAs, known as antisense RNAs, and the *trans*-encoded sRNAs, which we refer to them in this review as “treRNAs”. We propose also the use of other abbreviations for some sRNAs categories. For example, we advise the use of “caRNA” and “traRNA” rather than “*cis*-acting sRNA” and “*trans*-acting sRNA”, respectively. Although few examples are listed for each category of traRNAs (Table 1), the proposed list must be updated continuously since our knowledge on bacterial RNA keep changing. An exception is for the descriptor “ncRNAs” generally used to describe regulatory RNAs that do not encode for proteins, known as “non-coding RNAs”, and which can be applied to all regulatory RNA categories. As shown in Fig. I, II and III regulatory RNAs nomenclature depends on their function and mode of action. The caRNAs can act on transcriptional or translational levels (Fig. I), asRNAs promote transcription attenuation or activation/inhibition of translation (Fig. II). The treRNAs can repress (Fig. IIIa-b), or activate translation (Fig. IIIc-d), sequester proteins (Fig. IIIe) or participate in degrading invading DNA (Fig. IIIf).

***Streptococcus agalactiae*: what is behind the hidden sRNome?**

Apart from its commensal colonization of the gastrointestinal and urogenital tracts, *S. agalactiae*, known also as Group B *Streptococcus* (GBS), has become a predominant cause of invasive infections in neonates and causes pneumonia, septicemia and meningitis (Maisey *et al.*, 2008; Krzysciak *et al.*, 2013). However, the current knowledge on the sRNome encoded by the GBS chromosome remains limited, even though the sRNAs can play an important role in virulence of various Gram-positive bacteria including *Streptococcus* (Patenge *et al.*, 2013). Two *in silico* studies were conducted on the sequenced genome of *S. agalactiae* NEM316. The first study aimed to predict sRNAs associated to the CiaRH two component system; we refer to them as csRNAs (Marx *et al.*, 2010) (Table 1). The CiaRH response regulator in *S. agalactiae* plays an important role in promoting the innate immune system, and intracellular survival (Quach *et al.*, 2009). The search for CiaR-activated promoter (NTTAAG-N5-

TTTAAG) and the transcriptional terminator sequences within the intergenic regions led to the identification of four putative csRNAs (csRNA10, csRNA11, csRNA12 and csRNA13) (Marx *et al.*, 2010). However, no experimental evidence on the expression of *S. agalactiae* csRNAs was reported in this study. The presence of complementarity to translation initiation regions in all the csRNAs suggests that they can bind to mRNAs and block translation initiation by hindering the access of ribosomes. Of note, the expression of csRNA10, csRNA11 and csRNA12 was recently confirmed and their up-regulation by acidic conditions was demonstrated using northern blot analysis in *S. agalactiae* NEM316 strain (Rosinski-Chupin *et al.*, 2015). The control by the CiaRH two-component system of these csRNAs underlines their potential role in *S. agalactiae* acidic stress adaptation in the urogenital tract.

The second *in silico* study was based on prediction of Rho-independent terminators (RITs) as a specific signature of sRNA gene loci. One hundred ninety-seven sRNAs were detected on the genome of *S. agalactiae* NEM316 (Pichon *et al.*, 2011). The expression of 10 out of the 197 sRNAs was confirmed by RT-PCR and northern blot hybridization. One of the sRNAs candidates (SQ517) detected in this study was previously reported as putative csRNA (csRNA12) (Marx *et al.*, 2010). The over-expression of SQ18 asRNA significantly decreased, more than 100-fold, the level of its target *gbs0031* mRNA, which encodes a surface immunogenic protein (Sip). A translational fusion system showed also that the over-expression of SQ18 was associated with 4-fold decrease in the amount of *gbs0031::gfp* mRNA, suggesting that SQ18 acts as a negative post-transcriptional regulator.

A more recent study led to the identification of 125 sRNAs: 39 caRNAs, 39 asRNAs and 47 treRNAs (Rosinski-Chupin *et al.*, 2015). Ten of these sRNAs were differentially expressed in response to acidic stress. The expression of Srn015 (csRNA10), Srn024 (csRNA11), Srn070 (csRNA12), Srn085 (csRNA13), Srn071 and Srn082 was induced by more than 2-fold at pH of 5.2 (natural pH of the genitourinary tract). In contrast, Srn046, Srn056, Srn057 and Srn073 were down regulated in the same experimental condition. All csRNAs identified in this study were previously predicted (Marx *et al.*, 2010), indicating that sRNA *in silico* prediction is a powerful tool for csRNA identification in *Streptococci*.

Other sRNAs were described previously in mobile genetic elements of *S. agalactiae*, like the asRNAs involved in the replication control of the pIP501 and pMV158 plasmids (Brantl and Behnke, 1992; del Solar and Espinosa, 1992; Brantl and Wagner, 1994; Brantl and Wagner, 1996; Brantl, 2015; Lopez-Aguilar *et al.*, 2015). Both pIP501 and pMV158 were originally

isolated from clinical strains of *S. agalactiae* and exhibit a broad range of hosts. The asRNAs RNAIII and RNAII encoded by pIP501 and pMV158, respectively, have been found important for the inhibition of expression of the plasmids essential genes. While pIP501 RNAIII asRNA exhibits unusual stability, the RNAII encoded by pMV158 did not require the formation of a kissing complex for efficient binding to its mRNA target or for inhibition of gene expression. Due to a limited number of available genome sequences, the *S. agalactiae* sRNome remains largely unknown.

***Streptococcus mutans* sRNAs: from very small to a toxic mood**

Proposed to be the new Gram-positive paradigm, *S. mutans* is a host-associated dental pathogen (Lemos *et al.*, 2013). This bacterium is considered to be a cariogenic organism responsible for the formation of dental biofilm matrix and some occasional infective endocarditis. Beside its ability to survive at low pH and to produce large quantities of organic acid, *S. mutans* presents an ease of genetic manipulation. This fluency is due to the presence of a two competence regulatory system ComR/ComS, which makes the bacterium transformable in different culture conditions (Son *et al.*, 2012). In *S. mutans*, sRNAs were first described after high-throughput experiments and a kingdom-wide prediction of bacterial sRNA-encoding genes using SIPHT (sRNA identification protocol using high-throughput technologies) method (Livny *et al.*, 2008). This strategy identifies new treRNAs candidates by co-localizing their intergenic regions and the Rho-independent terminators. Eighteen treRNA genes of *S. mutans* were predicted, however, their functions as those of most *S. mutans* treRNAs remain elusive (Livny *et al.*, 2008). Biocomputational studies predicted that three treRNAs were putatively regulated by CiaRH (csRNA23-1, csRNA23-2, and csRNA24) (Marx *et al.*, 2010). As mentioned earlier, csRNAs should get deeper attention owing to their potential role in *S. mutans* virulence through the interaction with the CiaRH regulon.

A new member of the treRNAs category, baptized “miRNA-size small RNAs” or “msRNAs” was recently described in *S. mutans* (Lee and Hong, 2011) (Table 1). In eukaryotes, microRNAs (miRNAs) are 19-23 nucleotides (nts) non-coding RNAs that act as post-transcriptional regulators leading mainly to gene silencing (Bartel, 2004). However, so far no publication reports sRNAs with comparable size to miRNAs in bacteria. A deep sequencing approach suggested that detected reads corresponding to 15 and 26 nts were putative msRNAs (Lee and Hong, 2011). These RNAs may result from processed RNAs. In addition, northern profiles show discrete band of msRNA-428 suggesting that this msRNA is not produced

197 through a random RNA degradation. Likewise, the expression of seven msRNAs was
198 validated by qRT-PCR. Currently, the role of msRNAs in gene regulation remains unclear.

199 The ability of *S. mutans* to metabolize a multitude of carbohydrate sources and to colonize the
200 tooth surface is intimately linked to its ability to survive at low pH. Xia and collaborators
201 described for the first time in *S. mutans* a pH-dependent sRNA (Xia *et al.*, 2012). This
202 treRNA, L10-leader sRNA, was predicted by combining different bioinformatics approaches.
203 A total number of 334 sRNAs were predicted, however, only the L10-leader was verified by
204 qRT-PCR and northern blot analyses due to its high abundance. The expression level of the
205 L10-leader was growth phase-dependent, and highly affected by pH. In clinical strains, its
206 expression was higher in the most adhering and acidic strains and may be linked to virulence
207 (Xia *et al.*, 2012).

208 Experimental identification or bioinformatics screens alone are not enough to detect all
209 encoded sRNAs, since their expression often depends on environmental conditions (growth
210 phase, stress condition...). Combining *in vivo* and *in silico* approaches is the best way to
211 identify a large number of bacterial sRNAs. Rho-independent terminator-based, transcription
212 initiation signal-based and RNAs prediction methods were coupled to RNA-seq approach to
213 identify the sRNome of *S. mutans* UA159, under carbohydrate repression as well as in the
214 presence/absence of the catabolite control protein CcpA (Zeng *et al.*, 2013). Among the 114
215 sRNAs expressed, five were differentially expressed in *S. mutans* UA159 grown in glucose
216 versus galactose, and two treRNAs were deregulated in the *ccpA* mutant. The fact that these
217 treRNAs are expressed in carbohydrate- and/or *ccpA*-dependent manner highlights that they
218 may be involved in the regulation of the *S. mutans* carbohydrate metabolism.

219 Bacteria have also developed many adaptive systems including Toxin-Antitoxin (TA)
220 modules. These small genetic elements express a stable toxic protein (toxin), and a proximate
221 antitoxin. The nature of the antitoxin, RNA or protein, defines the type of the TA module
222 (Wen and Fozo, 2014). In the type I TA system, the antitoxin is an RNA molecule that
223 controls the mRNA stability and translation of the corresponding toxin molecule. It is
224 proposed that such systems help bacteria to survive to environmental stress, and when
225 exposed to high concentrations of antibiotics through the formation of persister cells (Gerdes
226 *et al.*, 2005). Recently, a functional type I TA system was described for the first time in *S.*
227 *mutans* UA159 (Koyanagi and Levesque, 2013). This TA module is located in a small
228 intergenic region of 318 nts. The toxin (Fst-Sm) is a small hydrophobic peptide and the

antitoxin (srSm) is an asRNA of approximately 70 nts in length. The 5' end mapping of Fst-Sm and srSm transcripts indicates that they are both transcribed through a direct tandem repeat. It was also demonstrated that the expression of srSm represses the expression of the toxin when fused to a GFP gene reporter. Overexpression of the toxin Fst-Sm induced *E. coli* cell death, and was lethal in *S. mutans*. Ectopic expression of the TA module in *S. mutans* was followed with a decrease in persistent cell formation. Altogether, these results suggest that the *S. mutans* TA systems are implicated in infections caused by oral bacteria. *S. mutans* has the ability to cope with complex environments such as oral cavities, being responsible for dental caries and chronic infections. Deciphering the role of sRNAs in *S. mutans* regulatory networks may help providing some clues to design novel therapeutic strategies for dental caries.

***Streptococcus thermophilus* sRNome: Too rich or too poor?**

S. thermophilus is a lactic acid bacterium used in yoghurt manufacturing and also commonly found in fermented milk products. *S. thermophilus* is considered as non-pathogenic for food industries (Blomqvist *et al.*, 2006). In genetics, the name of *S. thermophilus* is intimately linked to the bacterial immune system known as CRISPR-Cas system (Deveau *et al.*, 2008; Sapranaukas *et al.*, 2011; Karvelis *et al.*, 2013; Carte *et al.*, 2014). This sRNA-based immune system protects bacteria from invading DNA, such as viruses and plasmids (Table 1). An important player in this process, as it is the case for the Cas9 protein and the crRNA (CRISPR RNA), is the tracrRNA (trans-activating CRISPR RNA). Northern blot profiling revealed the presence of a tracrRNA located upstream of the *cas9* gene of the CRISPR3-Cas in *S. thermophilus* LMD-9 genome (Karvelis *et al.*, 2013). This tracrRNA is involved in crRNA biogenesis through the pre-crRNA maturation, and is required for the Cas9-mediated interference. In another study, it was demonstrated that this tracrRNA, with the help of the RNase III, can stimulate the cleavage within the CRISPR repeats generating the crRNA units (Carte *et al.*, 2014).

Other sRNAs were reported for *S. thermophilus*. Five csRNAs were predicted on *S. thermophilus* CNRZ1066 genome and one csRNA on the plasmid pSt0 (Marx *et al.*, 2010). The expression of the latter csRNA was verified by northern blot. The *S. thermophilus* CiaRH system is inactivated by the presence of a stop codon interrupting the *ciaR* gene, and a frame shift mutation in *ciaH*. This could be a result of a particular adaptation to the dairy niche leading to loss of the function of virulence genes. Another four tracrRNAs were predicted by

homology comparison to *S. pyogenes* MGAS315 sRNome (Tesorero *et al.*, 2013). The expression of these treRNAs needs to be verified.

***Streptococci* sRNAs: A large species panel**

Currently, 67 different *Streptococcus* species are reported on the NCBI database and their genome sequenced and annotated. Nevertheless, only a few of them have been the subject of studies pointed toward the characterization of sRNAs. Bioinformatics approaches have been widely used to detect sRNAs in bacterial genomes. Tesorero and collaborators reported that among the 67 available genomes, 23 different *Streptococcus* genomes harbor at least one sRNA homolog to the sRNAs detected for *S. pyogenes* MGAS315 strain (Tesorero *et al.*, 2013). Moreover, species as *Streptococcus suis*, *Streptococcus uberis*, *Streptococcus mitis*, *Streptococcus oralis*, *Streptococcus sanguinis*, *Streptococcus dysgalactiae* were also reported to harbor more than two csRNAs (Marx *et al.*, 2010).

For *S. suis*, a pig pathogen, a differential RNA-sequencing approach was conducted on the strain P1/7 grown in rich medium and biological fluids (pig blood and cerebrospinal fluid) and allowed the identification of 29 sRNAs (Wu *et al.*, 2014). Ten of them were predicted to be conserved in 34 pathogenic *Streptococcus* species. The deletion of five treRNAs led to the attenuation of *S. suis* virulence in a zebrafish infection model. *S. suis* mutant strains were more sensitive to killing by pig blood following the deletion of three other treRNAs as compared with the wild-type. This study shows also that genes involved in the synthesis of capsular polysaccharide were differentially regulated in blood and cerebrospinal fluid. The role of *S. suis* sRNAs in virulence, the mechanism of interaction and regulation with their mRNA targets need to be investigated.

Future prospects and concluding remarks

The aim of this focused review was to bring in light the sRNomes of *Streptococcus* species. As cited earlier, understanding *S. agalactiae* sRNome can constitute one step forward in providing new strategies for the substitution of antibiotics in the treatment of neonatal infections. A major cause of dental caries, *S. mutans*, harbors a significant number of sRNAs that can play an important role in its virulence. *S. thermophilus* is the only *Streptococcus* species used in food industry. The use of CRISPR-Cas system in genome editing through genetic engineering is a very promising tool in bacterial genome new function design. The future challenges in the study of sRNAs in bacteria are the identification of their targets and

292 the understanding of their regulatory mechanism. In *Streptococci* cited in this review, there is
293 a lack between the identification of sRNAs and their functional characterization. Future works
294 should be done on the mechanistic level; target identification and mechanism of regulation, in
295 order to better appreciate these regulatory networks.

296 **Acknowledgement:**

297 We are grateful to Philippe Bouloc and Tatiana Rochat for critical reading of the manuscript
298 and helpful discussion. We thank the reviewers for critical reading of the manuscript.
299 Mohamed Amine Zorgani received a PhD fellowship from the french Ministère de l'Éducation
300 et de l'Enseignement Supérieur.

Provisional

301 **References**

- 302 Babitzke, P., and Romeo, T. (2007). CsrB sRNA family: sequestration of RNA-binding regulatory
303 proteins. *Curr. Opin. Microbiol.* 10,156-163. doi: 10.1016/j.mib.2007.03.007
- 304 Bartel, D.P. (2004). MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell.* 116, 281-297.
305 doi: S0092867404000455
- 306 Blomqvist, T., Steinmoen, H., and Havarstein, L.S. (2006). Natural genetic transformation: A novel
307 tool for efficient genetic engineering of the dairy bacterium *Streptococcus thermophilus*. *Appl.*
308 *Environ. Microbiol.* 72, 6751-6756. doi: 10.1128/AEM.01156-06
- 309 Bouloc, P., and Repoila, F. (2016). Fresh layers of RNA-mediated regulation in Gram-positive
310 bacteria. *Curr. Opin. Microbiol.* 30, 30-35. doi: 10.1016/j.mib.2015.12.008
- 311 Brantl, S. (2009). Bacterial chromosome-encoded small regulatory RNAs. *Future. Microbiol.* 4,85-
312 103. doi: 10.2217/17460913.4.1.85
- 313 Brantl, S. (2015). Antisense-RNA mediated control of plasmid replication - pIP501 revisited. *Plasmid.*
314 78, 4-16. doi: 10.1016/j.plasmid.2014.07.004
- 315 Brantl, S., and Behnke, D. (1992). Copy number control of the streptococcal plasmid pIP501 occurs at
316 three levels. *Nucleic Acids Res.* 20, 395-400. doi: 10.1093/nar/20.3.395
- 317 Brantl, S., and Bruckner, R. (2014). Small regulatory RNAs from low-GC Gram-positive bacteria.
318 *RNA Biol.* 11, 443-456. doi: 10.4161/rna.28036
- 319 Brantl, S., and Wagner, E.G. (1994). Antisense RNA-mediated transcriptional attenuation occurs
320 faster than stable antisense/target RNA pairing: an *in vitro* study of plasmid pIP501. *EMBO J.*
321 13, 3599-3607.
- 322 Brantl, S., and Wagner, E.G. (1996). An unusually long-lived antisense RNA in plasmid copy number
323 control: *in vivo* RNAs encoded by the streptococcal plasmid pIP501. *J. Mol. Biol.* 255, 275-
324 288. doi: 10.1006/jmbi.1996.0023
- 325 Brennan, R.G., and Link, T.M. (2007). Hfq structure, function and ligand binding. *Curr. Opin.*
326 *Microbiol.* 10, 125-133. doi: 10.1016/j.mib.2007.03.015
- 327 Carapetis, J.R., Steer, A.C., Mulholland, E.K., and Weber, M. (2005). The global burden of group A
328 streptococcal diseases. *Lancet Infect Dis.* 5, 685-694. doi: 10.1016/S1473-3099(05)70267-X
- 329 Carte, J., Christopher, R.T., Smith, J.T., Olson, S., Barrangou, R., Moineau, S., et al. (2014). The three
330 major types of CRISPR-Cas systems function independently in CRISPR RNA biogenesis in
331 *Streptococcus thermophilus*. *Mol. Microbiol.* 93, 98-112. doi: 10.1111/mmi.12644
- 332 Casadesus, J., and Low, D. (2006). Epigenetic gene regulation in the bacterial world. *Microbiol. Mol.*
333 *Biol. Rev.* 70, 830-856. doi: 10.1128/MMBR.00016-06
- 334 Cavanagh, A.T., and Wassarman, K.M. (2014). 6S RNA, a global regulator of transcription in
335 *Escherichia coli*, *Bacillus subtilis*, and beyond. *Annu. Rev. Microbiol.* 68, 45-60. doi:
336 10.1146/annurev-micro-092611-150135
- 337 Chao, Y., Papenfort, K., Reinhardt, R., Sharma, C.M., and Vogel, J. (2012). An atlas of Hfq-bound
338 transcripts reveals 3' UTRs as a genomic reservoir of regulatory small RNAs. *EMBO J.* 31,
339 4005-4019. doi: 10.1038/emboj.2012.229
- 340 Cho, K. H., and Kim, J. H. (2015). "Cis-encoded non-coding antisense RNAs in *streptococci* and other
341 low GC Gram (+) bacterial pathogens." *Front. Genet.* 6: 110. doi: 10.3389/fgene.2015.00110
- 342 del Solar, G., and Espinosa, M. (1992). The copy number of plasmid pLS1 is regulated by two trans-
343 acting plasmid products: the antisense RNA II and the repressor protein, RepA. *Mol.*
344 *Microbiol.* 6, 83-94. doi: 10.1111/j.1365-2958.1992.tb00840.x
- 345 Desvaux, M., Hebraud, M., Talon, R., and Henderson, I.R. (2009). Secretion and subcellular
346 localizations of bacterial proteins: a semantic awareness issue. *Trends Microbiol.* 17, 139-45.
347 doi: 10.1016/j.tim.2009.01.004
- 348 Deveau, H., Barrangou, R., Garneau, J.E., Labonte, J., Fremaux, C., Boyaval, P. et al. (2008). Phage
349 response to CRISPR-encoded resistance in *Streptococcus thermophilus*. *J. Bacteriol.* 190,
350 1390-1400. doi: 10.1128/JB.01412-07

- Frohlich, K.S., and Vogel, J. (2009). Activation of gene expression by small RNA. *Curr. Opin. Microbiol.* 12, 674-682. doi: 10.1016/j.mib.2009.09.009
- Gamez, G., and Hammerschmidt, S. (2011). Combat pneumococcal infections: adhesins as candidates for protein-based vaccine development. *Curr. Drug. Targets.* 13, 323-337. doi: 10.2174/138945012799424697
- Garneau, J.E., Dupuis, M.E., Villion, M., Romero, D.A., Barrangou, R., Boyaval, P., et al. (2010). The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA. *Nature.* 468, 67-71. doi: 10.1038/nature09523
- Gerdes, K., Christensen, S.K. and Lobner-Olesen, A. (2005) Prokaryotic toxin-antitoxin stress response loci. *Nat. Rev. Microbiol.* 3, 371-382. doi: 10.1038/nrmicro1147
- Gossringer, M., and Hartmann, R.K. (2012). 3'-UTRs as a source of regulatory RNAs in bacteria. *EMBO J.* 31, 3958-3960. doi: 10.1038/emboj.2012.269
- Gottesman, S., and Storz, G. (2010). Bacterial small RNA regulators: versatile roles and rapidly evolving variations. *Cold. Spring. Harb. Perspect. Biol.* 3:12. doi: 10.1101/cshperspect.a003798
- Johansson, J., Mandin, P., Renzoni, A., Chiaruttini, C., Springer, M., and Cossart, P. (2002). An RNA thermosensor controls expression of virulence genes in *Listeria monocytogenes*. *Cell.* 110, p. 551-561. doi: S0092867402009054
- Kang, S.M., Choi, J.W., Lee, Y., Hong, S.H., and Lee, H.J. (2013). Identification of microRNA-size, small RNAs in *Escherichia coli*. *Curr. Microbiol.* 67, 609-613. doi: 10.1007/s00284-013-0411-9
- Karvelis, T., Gasiunas, G., Miksys, A., Barrangou, R., Horvath, P., and Siksnys, V. (2013). crRNA and tracrRNA guide Cas9-mediated DNA interference in *Streptococcus thermophilus*. *RNA Biol.* 10, 841-851. doi: 10.4161/rna.24203
- Koyanagi, S., and Levesque, C.M. (2013). Characterization of a *Streptococcus mutans* intergenic region containing a small toxic peptide and its cis-encoded antisense small RNA antitoxin. *PLoS One.* 8: e54291. doi: 10.1371/journal.pone.0054291
- Kreikemeyer, B., Boyle, M.D., Buttaro, B.A., Heinemann, M., and Podbielski, A. (2001). Group A streptococcal growth phase-associated virulence factor regulation by a novel operon (Fas) with homologies to two-component-type regulators requires a small RNA molecule. *Mol. Microbiol.* 39, 392-406. doi: 10.1046/j.1365-2958.2001.02226.x
- Krzysciak, W., Pluskwa, K.K., Jurczak, A., and Koscielniak, D. (2013). The pathogenicity of the *Streptococcus* genus. *Eur. J. Clin. Microbiol. Infect. Dis.* 32, 1361-76. doi: 10.1007/s10096-013-1914-9
- Kumar, R., Shah, P., Swiatlo, E., Burgess, S.C., Lawrence, M.L., and Nanduri, B. (2010). Identification of novel non-coding small RNAs from *Streptococcus pneumoniae* TIGR4 using high-resolution genome tiling arrays. *BMC Genomics.* 11: 350. doi: 10.1186/1471-2164-11-350
- Lalaouna, D., Eyraud, A., Chabelskaya, S., Felden, B., and Masse, E. (2014). Regulatory RNAs involved in bacterial antibiotic resistance. *PLoS Pathog.* 10:e1004299. doi: 10.1371/journal.ppat.1004299
- Le Rhun, A., and Charpentier, E. (2012). Small RNAs in *streptococci*. *RNA Biol.* 9, 414-426. doi: 10.4161/rna.20104
- Lee, H.J., and Hong, S.H. (2011). Analysis of microRNA-size, small RNAs in *Streptococcus mutans* by deep sequencing. *FEMS Microbiol. Lett.* 326, 131-136. doi: 10.1111/j.1574-6968.2011.02441.x
- Lemos, J.A., Quivey, R.G. Jr., Koo, H., and Abranches, J. (2013). *Streptococcus mutans*: a new Gram-positive paradigm? *Microbiology.* 159:436-445. doi: 10.1099/mic.0.066134-0
- Livny, J., Teonadi, H., Livny, M., and Waldor, M.K. (2008). High-throughput, kingdom-wide prediction and annotation of bacterial non-coding RNAs. *PLoS One.* 3:e3197. doi: 10.1371/journal.pone.0003197
- Loh, E., Dussurget, O., Gripenland, J., Vaitkevicius, K., Tiensuu, T., Mandin, P., et al. (2009). A trans-acting riboswitch controls expression of the virulence regulator PrfA in *Listeria monocytogenes*. *Cell.* 139, 770-779. doi: 10.1016/j.cell.2009.08.046

- Lopez-Aguilar, C., Romero-Lopez, C., Espinosa, M., Berzal-Herranz, A., and Del Solar, G. (2015). The 5'-tail of antisense RNAII of pMV158 plays a critical role in binding to the target mRNA and in translation inhibition of repB. *Front. Genet.* 6:225. doi: 10.3389/fgene.2015.00225
- Maisey, H.C., Doran, K.S., and Nizet, V. (2008). Recent advances in understanding the molecular basis of group B *Streptococcus* virulence. *Expert Rev. Mol. Med.* 10: 27. doi: 10.1017/S1462399408000811
- Mann, B., van Opijnen, T., Wang, J., Obert, C., Wang, Y.D., Carter, R., et al. (2012). Control of virulence by small RNAs in *Streptococcus pneumoniae*. *PLoS Pathog.* 8: e1002788. doi: 10.1371/journal.ppat.1002788
- Marx, P., Nuhn, M., Kovacs, M., Hakenbeck, R., and Bruckner, R. (2010). Identification of genes for small non-coding RNAs that belong to the regulon of the two-component regulatory system CiaRH in *Streptococcus*. *BMC Genomics.* 11:661. doi: 10.1186/1471-2164-11-661
- Miller, E.W., Cao, T.N., Pflughoeft, K.J., and Sumby, P. (2014). RNA-mediated regulation in Gram-positive pathogens: an overview punctuated with examples from the group A *Streptococcus*. *Mol. Microbiol.* 94, 9-20. doi: 10.1111/mmi.12742
- Nielsen, J.S., Lei, L.K., Ebersbach, T., Olsen, A.S., Klitgaard, J.K., Valentin-Hansen, P., et al. (2009). Defining a role for Hfq in Gram-positive bacteria: evidence for Hfq-dependent antisense regulation in *Listeria monocytogenes*. *Nucleic Acids Res.* 38, 907-919. doi: 10.1093/nar/gkp1081
- Opdyke, J.A., Kang, J.G., and Storz, G. (2004). GadY, a small-RNA regulator of acid response genes in *Escherichia coli*. *J. Bacteriol.* 186: 6698-6705. doi: 10.1128/JB.186.20.6698-6705.2004
- Padalon-Brauch, G., Hershberg, R., Elgrably-Weiss, M., Baruch, K., Rosenshine, I., Margalit, H., et al. (2008). Small RNAs encoded within genetic islands of *Salmonella typhimurium* show host-induced expression and role in virulence. *Nucleic Acids Res.* 36:1913-1927. doi: 10.1093/nar/gkn050
- Papenfort, K., and Vogel, J. (2010). Regulatory RNA in bacterial pathogens. *Cell. Host. Microbe.* 8, 116-127. doi: 10.1016/j.chom.2010.06.008
- Patenge, N., Fiedler, T., and Kreikemeyer, B. (2013). Common regulators of virulence in streptococci. *Curr. Top. Microbiol. Immunol.* 368, 111-153. doi: 10.1007/82_2012_295
- Pichon, C., du Merle, L., Caliot, M.E., Trieu-Cuot, P., and Le Bouguenec, C. (2011). An in silico model for identification of small RNAs in whole bacterial genomes: characterization of antisense RNAs in pathogenic *Escherichia coli* and *Streptococcus agalactiae* strains. *Nucleic Acids Res.* 40, 2846-2861. doi: 10.1093/nar/gkr1141
- Quach, D., van Sorge, N.M., Kristian, S.A., Bryan, J.D., Shelver, D.W., and Doran, K.S. (2009). The CiaR response regulator in group B *Streptococcus* promotes intracellular survival and resistance to innate immune defenses. *J. Bacteriol.* 191, 2023-2032. doi: 10.1128/JB.01216-08
- Romby, P., and Charpentier, E. (2009). An overview of RNAs with regulatory functions in Gram-positive bacteria. *Cell. Mol. Life. Sci.* 67, 217-237. doi: 10.1007/s00018-009-0162-8
- Rosinski-Chupin, I., Sauvage, E., Sismeiro, O., Villain, A., Da Cunha, V., Caliot, M.E., et al. (2010). Single nucleotide resolution RNA-seq uncovers new regulatory mechanisms in the opportunistic pathogen *Streptococcus agalactiae*. *BMC Genomics.* 16:419. doi: 10.1186/s12864-015-1583-4
- Ross, J.A., Ellis, M.J., Hossain, S., and Haniford, D.B. (2013). Hfq restructures RNA-IN and RNA-OUT and facilitates antisense pairing in the Tn10/IS10 system. *RNA.* 19, 670-684. doi: 10.1261/rna.037747.112
- Ruiz de los Mozos, I., Vergara-Irigaray, M., Segura, V., Villanueva, M., Bitarte, N., Saramago, M., et al. (2013). Base pairing interaction between 5'- and 3'-UTRs controls icaR mRNA translation in *Staphylococcus aureus*. *PLoS Genet.* 9:e1004001. doi: 10.1371/journal.pgen.1004001
- Sapranaukas, R., Gasiunas, G., Fremaux, C., Barrangou, R., Horvath, P., and Siksnys, V. (2011). The *Streptococcus thermophilus* CRISPR/Cas system provides immunity in *Escherichia coli*. *Nucleic Acids Res.* 39, 9275-9282. doi: 10.1093/nar/gkr606
- Son, M., Ahn, S.J., Guo, Q., Burne, R.A., and Hagen, S.J. (2012). Microfluidic study of competence regulation in *Streptococcus mutans*: environmental inputs modulate bimodal and unimodal expression of comX. *Mol. Microbiol.* 86, 258-272. doi: 10.1111/j.1365-2958.2012.08187.x

- Stazic, D., Lindell, D., and Steglich, C. (2011). Antisense RNA protects mRNA from RNaseE degradation by RNA-RNA duplex formation during phage infection. *Nucleic Acids Res.* 39, 4890–4899. doi:10.1093/nar/gkr037
- Storz, G., Vogel, J., and Wassarman, K.M. (2011). Regulation by small RNAs in bacteria: expanding frontiers. *Mol. Cell.* 43, 880-891. doi: 10.1016/j.molcel.2011.08.022
- Tesorero, R.A., Yu, N., Wright, J.O., Svencionis, J.P., Cheng, Q., Kim, J.H., et al. (2013). Novel regulatory small RNAs in *Streptococcus pyogenes*. *PLoS One.* 8:e64021. doi: 10.1371/journal.pone.0064021
- Thomason, M.K., and Storz, G. (2010). Bacterial antisense RNAs: how many are there, and what are they doing? *Annu. Rev. Genet.* 44:167-188. doi: 10.1146/annurev-genet-102209-163523
- Vadaie, N., and Morris, K.V. (2013) Long antisense non-coding RNAs and the epigenetic regulation of gene expression. *Biomol. Concepts.* 4, 411-415. doi: 10.1515/bmc-2013-0014
- Wen, J., and Fozo, E.M. (2012). sRNA antitoxins: more than one way to repress a toxin. *Toxins.* 6, 2310-2335. doi: 10.3390/toxins6082310
- Wilton, J., Acebo, P., Herranz, C., Gomez, A., and Amblar, M. (2015). Small regulatory RNAs in *Streptococcus pneumoniae*: discovery and biological functions. *Front. Genet.* 6:126. doi: 10.3389/fgene.2015.00126
- Winkler, W.C., and Breaker, R.R. (2005) Regulation of bacterial gene expression by riboswitches. *Annu. Rev. Microbiol.* 59: 487-517. doi: 10.1146/annurev.micro.59.030804.121336
- Wu, Z., Wu, C., Shao, J., Zhu, Z., Wang, W., Zhang, W., et al. (2014). The *Streptococcus suis* transcriptional landscape reveals adaptation mechanisms in pig blood and cerebrospinal fluid. *RNA.* 20, 882-898. doi: 10.1261/rna.041822.113
- Xia, L., Xia, W., Li, S., Li, W., Liu, J., Ding, H., et al. (2012). Identification and expression of small non-coding RNA, L10-Leader, in different growth phases of *Streptococcus mutans*. *Nucleic Acid. Ther.* 22, 177-186. doi: 10.1089/nat.2011.0339
- Zeng, L., Choi, S.C., Danko, C.G., Siepel, A., Stanhope, M.J., and Burne, R.A. (2013). Gene regulation by CcpA and catabolite repression explored by RNA-Seq in *Streptococcus mutans*. *PLoS One.* 8:e60465. doi: 10.1371/journal.pone.0060465

487 Figure legend

488 **Figure: (I)** *cis*-acting sRNAs (caRNAs) and their mechanism of action. **(a)** 5'UTR-mediated
489 transcriptional regulation. (Left panel) In the absence of ligand, transcription is initiated
490 through a permissive stem-loop region, an aptamer region in the 5'UTR (blue) of an open
491 reading frame (ORF, red). In the presence of ligand, the aptamer region assumes a
492 conformational change and is stabilized by the direct recognition of ligand. The complex
493 (5'UTR/Ligand) leads to the formation of a U-rich hairpin that acts as a transcriptional
494 terminator. (Right panel) In the absence of ligand, the transcription is initially attenuated. The
495 recognition of the ligand by the U-rich hairpin leads to the disruption of the stem-loop and
496 thus to the transcription anti-termination or activation. **(b)** 5'UTR-mediated translational
497 regulation. (Left panel) In the absence of ligand, the aptamer region forms an anti-terminator
498 hairpin. The ribosome binding site (RBS, green) is available, and mRNA translation is
499 initiated. Upon ligand binding, the access to the RBS is hindered, preventing translation.
500 (Right panel) In the absence of ligand, translation is inhibited due to a sequestered RBS. The
501 recognition of the ligand by the stem-loop releases the RBS and translation can be initiated. In
502 other cases, the ligand can be replaced by a temperature-dependent mechanism, where the
503 mRNA adopts a secondary structure that hinders the access to the RBS [*Listeria*
504 *monocytogenes*]. **(II)** Mechanisms of mRNA regulation by antisense RNAs (asRNA). (Left
505 upper panel) The binding of asRNA (blue) to the TIR (translation initiation region) leads to
506 translation repression. The RNA duplex (asRNA-sense RNA) can be degraded by RNase III, a
507 double strand specific endoribonuclease conserved in all the three kingdoms (Cho and Kim,
508 2015); or by RNase E which belong to the Gram-negative bacteria degradosome (Stazic *et al.*,
509 2011). (Left lower panel) The asRNA can bind to the 5'end of the target mRNA and causes
510 changes in the target RNA structure leading to transcription termination. (Right panel). The
511 complex asRNA-Hfq can also stabilize target mRNA for translation activation. **(III)** *trans*-
512 encoded sRNAs (treRNAs)-based regulatory mechanisms of mRNA expression. treRNAs act
513 through an imperfect basepairing to mRNA targets. The treRNA/mRNA duplex is stabilized
514 by Hfq and can either repress **(a-b)**, **(c)** activate or **(d)** stabilize mRNA translation. **(e)** It was
515 also demonstrated that treRNAs can bind to proteins to inhibit and/or modify their activity. **(f)**
516 tracrRNAs are a treRNA involved in the maturation of crRNAs through the interaction with
517 the complex CRISPR-Cas9, pre-crRNA and RNase III. The processed crRNAs target the
518 invading DNA.

Table 1: Bacterial regulatory RNAs nomenclature and mode of action

Bacterial regulatory RNAs			
Mode of action	Nomenclature	Abbreviations	Description
<i>cis</i> -acting RNAs	<i>cis</i> -acting RNA	caRNA	The descriptor " <i>cis</i> -acting RNA" is used to describe regulatory RNAs that act (Ruiz de los Mozos <i>et al.</i> , 2013), or are located at the untranslated regions (UTRs) of mRNA molecules (Gossringer and Hartmann, 2012).
	UnTranslated Region	UTR	UTRs are <i>cis</i> -acting RNAs located on untranslated regions of a coding transcript. They can activate/inhibit translation by releasing/hindering the RBS, respectively. They can also participate in the mRNA transcript stabilization (Gossringer and Hartmann, 2012). The base pairing between 5'- and 3'-UTRs can also control mRNA translation (Ruiz de los Mozos <i>et al.</i> , 2013).
	5' UnTranslated Region	5' UTR	5' UTRs are RNAs that act on the 5'-end of the mRNA transcript. They modulate the expression of the downstream genes by controlling the access of the ribosome to the translation initiation region (TIR). This class of UTRs includes Riboswitches, non-transcribed RNA, and independent transcripts. They play an important role in bacterial metabolism control. The modulation of the expression of the 5'UTR-downstream genes occurs through a metabolite-induced alteration of the RNA secondary structure, such as tRNAs (Loh <i>et al.</i> , 2009).
	3' UnTranslated Region	3' UTR	3' UTRs are RNAs that act on the 3'-end of the mRNA transcript. They constitute the region of the mRNA that immediately follows the translation termination codon (TTC) (Chao <i>et al.</i> , 2012).
<i>trans</i> -acting RNAs (traRNAs)	<i>cis</i> -encoded antisense RNA	asRNA	asRNAs are <i>cis</i> -encoded RNAs that act on the mRNA molecule encoded by the opposite DNA strand. They present a perfect base-pairing with their mRNA target (Thomason and Storz, 2010). We also distinguish toxin-antitoxin systems (Lee and Hong, 2011) and asRNAs encoded by the streptococcal pIP501 plasmid (Brantl and Wagner, 1996), or long asRNAs described in eukaryotes (Vadaie and Morris, 2013).
		5' asRNA	5' asRNAs are asRNAs that act on the 5'-end of the sense mRNA target. They activate or inhibit the translation of the mRNA transcript (Thomason and Storz, 2010). 5' asRNAs can also act by transcription attenuation, which is frequently found on plasmids (Thomason and Storz, 2010).
		3' asRNA	3' asRNAs are asRNAs that act on the 3'-end of the sense mRNA target. The role of the 3' asRNAs is not yet well deciphered. However, it was suggested that they participate in the transcript stabilization (Opdyke <i>et al.</i> , 2004; Padalon-Brauch <i>et al.</i> , 2008).
	<i>trans</i> -encoded RNA	treRNA	treRNAs are RNAs that target distant and/or different RNAs, eliminate invading cognate DNA, or form ribonucleo-protein complexes (Storz <i>et al.</i> , 2011). We can also distinguish in this class: housekeeping RNAs such as 6S RNA, RNase P RNA (Brantl, 2009), overlapping ncRNA (Kumar <i>et al.</i> , 2010), and operon RNAs (Kreikemeyer <i>et al.</i> , 2001).
	<i>cis</i> -dependent small RNA	csRNA	csRNAs are treRNAs that are transcribed from the promoters associated to CiaRH two-component system. They present a complementarity to the Shine-Dalgarno sequence and the translation initiation codon (AUG) (Marx <i>et al.</i> , 2010).
	microRNA-size sRNA	msRNA	msRNAs are a very small RNAs (15 to 26 nts) that are first described in <i>S. mutans</i> . The role of these microRNA-like sRNAs in bacterial gene regulation is not yet understood (Lee and Hong, 2011; Kang <i>et al.</i> , 2013).
	CRISPR RNA	crRNA	As a part of the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeat) system, the crRNAs act as treRNAs targeting and processing foreign DNA (Karvelis <i>et al.</i> , 2013).
	<i>trans</i> -activating CRISPR RNA	tracrRNA	In the CRISPR/Cas system, tracrRNAs are in charge of the activation of the pre-crRNAs (Karvelis <i>et al.</i> , 2013).

Provisional

Figure 01.TIF

