Characterization and identification of bacterial sRNAs and their involvement in virulence regulation

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Introduction

- The discovery and characterization of **RNA regulators** (sRNAs) in bacteria has emerged as one of the main post-transcriptional processes (1).
- They belong to two classes: **cis-encoded sRNAs** and **trans-encoded sRNAs.**
- Some trans-encoded sRNAs require a **chaperon** called **Hfq** to perform the hybridization of the sRNA with the mRNA target (2).
- The relevant importance of sRNAs in virulence regulation is only described in *Escherichia coli* and other species related. Since there has been a reduction in the **cost of transcriptomics** more experiments have been reported in other pathogen species to identify novel sRNAs involved in virulence regulation.

Aims

- Describe different strategies used to identify and characterize novel sRNAs.
- Identify the targets of novel sRNAs using computational and experimental methods.
- Characterize different approaches to assign a particular role of a sRNA.

Characterization and identification of sRNAs

Mutants (ΔHfq, TCS component) | Different conditions or stages of growth | Hfq- coIP (coimmunoprecipitation)

→ Extract total RNA. Size selection and rRNA/tRNA depletion

Table 1. Comparison between transcriptomic methods (3)

	Tiling arrays	cDNA seq	RNA-seq
Principle	Hybridization	Sanger sequencing	High-throughput sequencing
Resolution	From several to hundred pb	Single base	Single base
Dynamic range to quantify gene expression	Up-to a few hundred-fold	Not practical	>8000 fold

→ Filtering data (4)

- Run a BLAST **looking for homologs**
- Eliminate all the sequences within known ORF
- Focus on **intergenic regions** or running antisense in known ORF
- **rho-independent** transcription terminator

→ Confirmation of sRNA by Northern blot and RT-PCR

Target validation

Target RNA2/ TargetRNA/ INTARNA (5)

- Regions more conservated in sRNA
- Regions with more accesibility in sRNA or candidate mRNA target
- Energy of hybridization

→ Candidate mRNA targets

→ Pulse overexpression of sRNAs (6)

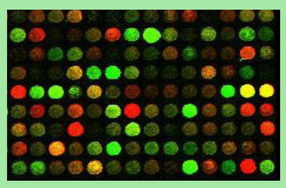
Plasmid with inducible promoter | Plasmid with inducible promoter + sRNA

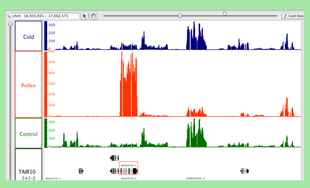
→ Grow to log or early stationary phase

→ Induce sRNA expression for 10 min

→ sRNA recognizes target and induces changes in expression

→ Compare mRNA profile expression





→ GFP reporter system to confirm mRNA target

Virulence implication

During an infection, **monitorization of the levels of sRNA** could reveal the patterns of regulation followed by a microorganism (Figure 1). In different stages of infection it is necessary that some genes quickly increase their expression level, thus considering regulation mediated by sRNAs is a good methodology to detect sRNAs involved in virulence (7).

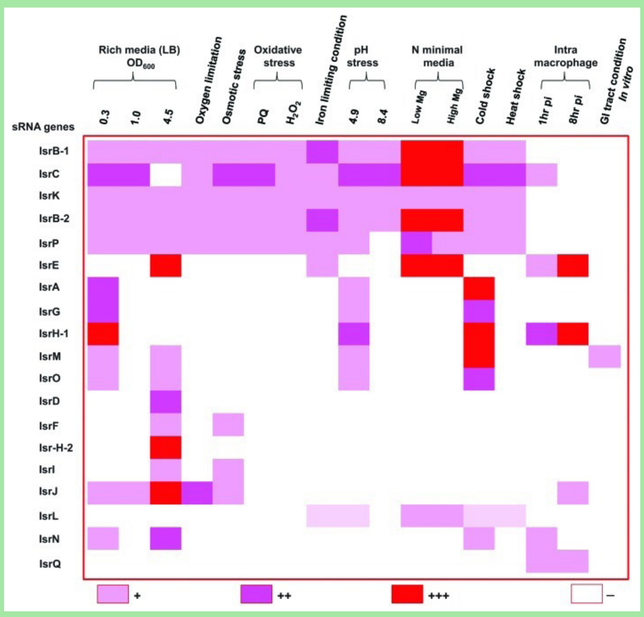


Figure 1. Monitorization of RNA levels in different simulated conditions and media using Northern-blot to quantify RNA expression (7)

Selection of strong sRNA candidates are considered to construct **knock-out mutants** by the red-recombinase method or homologous recombination. **Targeted sRNA deletions** are tested to measure the relative fitness of mutants using different approaches such as **Tn-seq, survival rate, lesion diameter** (Figure 2) or **measure of weight** during the experiment (8).

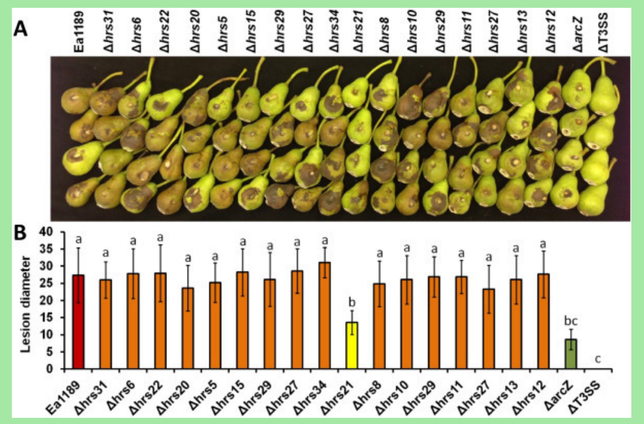


Figure 2. Lesion diameter using wild-type and mutants of *Erwinia amylovora* in immature pear fruits (8)

Conclusions

- **RNA-seq** has become the most useful technique to identify novel sRNAs. It **detects and quantifies** the level of sRNA in complex environments where it is difficult to obtain high levels of RNA.
- During the next years, the pools of sRNAs available in bioinformatics databases as well as novel sRNAs identified through homology to related species will increase exponentially.
- **TargetRNA2** is the most useful computational tool to **integrate the data from RNA-seq** and to reduce false positive rates.
- **Pulse-overexpression of sRNA** is the main approach used to **confirm the pool of targets** identified through computational methods.
- Generating different **knock-out mutants** can confirm an attenuation of **virulence** using different animal or plant models. Even though, it is complicated to observe a clear phenotype if there are other sRNAs involved in the regulation of the same gene.

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