In response to the complicated nitrogen supply environment, bacteria have evolved a complex and elaborate regulatory network to achieve an efficient nitrogen source, which includes transcriptional and post-transcriptional regulation. In recent years, studies have mainly focused on the transcriptional regulatory network of general nitrogen metabolism (Ntr) and biological nitrogen fixation (Nif) in bacteria. It was found that the general nitrogen regulation system, which is involved in nitrogen assimilation and utilization, is widely distributed in bacteria and involves many signal transduction processes and effector proteins, including GlnD, NtrB, NtrC and PII, which are the main proteins. GlnD senses the levels of intracellular glutamine and is the primary receptor for intracellular nitrogen levels, while the PII protein interacts with NtrB to regulate NtrC activity (Shimizu 2016). However, the nitrogen fixation-specific (Nif-specific) regulatory system is only found in a few prokaryotes that can perform nitrogen fixation and is centred on the regulatory protein NifLA, which acts in concert with the Ntr system to regulate the expression of nif genes at the transcriptional level (Dixon and Kahn 2004). In associative nitrogen-fixing Pseudomonas stutzeri A1501, for example, the DNA-binding protein NtrC is central to the regulation
Multiple regulatory mechanisms of bacterial noncoding RNAs

Bacterial noncoding RNAs are widely derived from various bacterial genomes and are mostly located in the noncoding region between two protein-coding genes or sheared from the 5' or 3' noncoding region of mRNAs. Since the length of bacterial noncoding RNAs ranges from 50 nt to 400 nt, these RNA molecules are often referred to as small noncoding RNAs (sRNAs or ncRNAs) (Wagner and Romby 2015).

The regulatory mechanisms of bacterial ncRNAs are divided into two main categories depending on their targets. The first category includes cis-encoded antisense RNAs and trans-encoded antisense RNAs, and the mechanism of these RNAs involves base pairing with target mRNAs. In this category, cis-encoded antisense RNAs are fully complementary to a single target mRNA and form a complete complex. Trans-encoded antisense RNAs, unlike cis-encoded antisense RNAs, are frequently complementary to multiple target mRNAs and form just a partial complex between molecules (Fig. 2). In contrast, the second category includes protein-binding ncRNAs (e.g., 6 S RNA and RmsZ) (Storz et al. 2011). Furthermore, due to limited complementary pairing of trans-encoded antisense RNAs with their target mRNAs, some RNA-binding proteins, such as the RNA chaperone Hfq, may enhance RNA-RNA interactions (Gottesman 2005; Blaxter 2010; Storz et al. 2011). Because trans-encoded antisense RNAs are generally located in the intergenic region, genetic manipulation and functional studies are relatively simple. Compared to cis-encoded antisense RNAs, trans-encoded antisense RNAs have been more extensively studied in bacteria.

Despite the discovery of the first bacterial ncRNA, 6 S RNA, in E. coli in 1967, research on bacterial ncRNAs has been slow due to limitations in the development of sequencing technology. It was not until 1987 that the first chromosomally encoded antisense RNA, MicF, was identified in E. coli, and this RNA inhibits the synthesis of OmpF, an outer membrane protein (Andersen et al. 1987; Ramani et al. 1994). With the advancement of genomic studies and genome sequencing, a large quantity of complicated
genomic sequence data has been produced and revealed that noncoding DNA in prokaryotes ranges from 5 to 50%, and this DNA is incapable of assembling proteins but can be expressed as noncoding RNAs (Rogozin et al. 2002). These RNAs, which have massive numbers, are uniquely positioned and are mechanically distinct, comprise a vast and highly efficient gene regulatory network that plays crucial regulatory roles in many physiological activities. Researchers have been drawn to these findings, and more than 150 ncRNAs have been discovered in *E. coli* to date. By sensing external environmental and internal metabolic cues, these ncRNAs form new regulatory networks to regulate a variety of physiological processes, including iron homeostasis, membrane homeostasis, carbon metabolism, stress resistance and biofilm formation, implying that ncRNAs, as a novel class of post-transcriptional regulators, play important roles in bacterial energy metabolism and environmental adaptation processes.

**Noncoding RNAs involved in nitrogen metabolism regulation in nitrogen-fixing diazotrophs**

In recent years, regulatory ncRNAs have received much interest from microbiologists as an essential class of post-transcriptional regulators in the regulatory network of bacterial metabolism. However, only 15 of the discovered ncRNAs have been demonstrated to be involved in controlling nitrogen metabolism in bacteria. Therefore, a major research hotspot in the study of metabolic regulatory networks in bacteria is the identification and mechanistic

| Table 1 Overview of identified noncoding RNAs in nitrogen-fixing microorganisms |
|---------------------------------------------|
| **Nitrogen-fixing microorganisms**          | **NCrNA** | **Strains** | **Target(s)** | **Mechanism** | **Function(s)** | **Reference(s)** |
| Symbiotic diazotrophs                      | MmgR      | *Sinorhizobium meliloti* | phaP1; phaP2 | Translation inhibition | Carbon metabolism | (Ceizel et al. 2018) |
|                                            | IncA      | *Sinorhizobium meliloti* | repABC       | Translation inhibition | Cell cycle       | (MacLellan et al. 2005) |
|                                            | EcpR1     | *Sinorhizobium meliloti* | gcrA; dnaA   | Translation inhibition | Cell cycle       | (Robledo et al. 2015) |
|                                            | RcsR1     | *Sinorhizobium meliloti* | sinl         | Translation inhibition | Quorum sensing   | (Baumgardt et al. 2016) |
|                                            | AbcR      | *Sinorhizobium meliloti* | livK         | Translation inhibition | Nutrient acquisition | (Sheehan and Caswell 2018) |
|                                            | NfeR1     | *Sinorhizobium meliloti* | ABC transport proteins encoding genes Unkown | Translation inhibition | Osmoadaptation and symbiotic performance | (Robledo et al. 2017) |
| Free-living diazotrophs                    | NsiR1     | *Anabaena sp. PCC* 7120 |               | Unkown | Cell differentiation | (Ionescu et al. 2010; Muro-Pastor 2014) |
|                                            | 6 S RNA   | *Synechocystis sp. PCC* 6803 |               | RNAP encoding gene | Nitrogen metabolism | (Heilmann et al. 2017) |
|                                            | NsiR4     | *Synechocystis sp. PCC* 6803 |               | IF7 (GS-inactivating factor 7) encoding gene | Nitrogen fixation regulation | (Klähn et al. 2015) |
|                                            | Yfr1      | *Synechocystis sp. PCC* 6803 | sbtA         | Translation inhibition | Oxidative and salt stress | (Nakamura et al. 2007) |
|                                            | NsrR1     | *Nostoc sp. PCC* 7120 | nbA          | Translation inhibition | PBS degradation | (Álvarez-Escribano et al. 2018) |
| Associative diazotrophs                    | NfIS      | *Pseudomonas stutzeri* A1501 |               | njK | mRNA stabilization | Nitrogen fixation regulation | (Zhan et al. 2016) |
|                                            | NfIR      | *Pseudomonas stutzeri* A1501 |               | njD | mRNA stabilization | Nitrogen fixation regulation | (Zhan et al. 2019) |
|                                            | RsmZ      | *Pseudomonas stutzeri* A1501 | pslA; sadC   | Translation inhibition | Biofilm formation and nitrogen fixation regulation | (Shang et al. 2021) |
|                                            | ArrF      | *Azotobacter vinelandii* | Unkown       | Unkown | Nitrogen fixation regulation | (Jung and Kwon 2008) |
| Archaea                                    | sRNA<sub>134</sub> | *Methanosarcina mazei* Gől | njH; nra | mRNA stabilization | Nitrogen fixation regulation | (Prasse et al. 2017) |
|                                            |           |                       | glnA1; glnA2 | Translation inhibition | Nitrogen fixation regulation | (Prasse et al. 2017) |
|                                            | sRNA<sub>41</sub> | *Methanosarcina mazei* Gől | ACDS complex encoding gene | Translation inhibition | Amino acids | (Buddeweg et al. 2018) |
elucidation of ncRNAs that control nitrogen metabolism. To date, the importance of ncRNAs in the post-transcriptional regulation of bacterial nitrogen metabolism has been demonstrated in α-proteobacteria (Sinorhizobium meliloti), γ-proteobacteria (Azotobacter vinelandii, Pseudomonas aeruginosa, Pseudomonas stutzeri and Escherichia coli), Cyanobacteria and Archaea (Prasse and Schmitz 2018).

Nitrogen fixation is the very first process in the nitrogen cycle and converts nitrogen from the air into ammonia by nitrogen-fixing microorganisms called diazotrophs, then ammonia is converted into related nitrogenous compounds, such as nitrates and nitrates. Due to the rearrangements of bacterial genomes, diazotrophs are enhanced in more than 100 species, including bacteria and some archaea. Diazotrophs can be classified into symbiotic, free-living and associative diazotrophs according to their characteristics of biological nitrogen fixation (Ju et al. 2007). In recent years, some progress has been made in determining the functions of ncRNAs identified in diazotrophic bacteria. For example, ncRNAs that are involved in nutrient acquisition (AbcR), carbon metabolism (MmgR), cell cycles (IncA, EcpR1), quorum sensing (RcsR1) and symbiotic interaction (NfeR1) have been identified in Rhizobium; ncRNAs involved in heteromorphic cell differentiation (NsiR1), nitrogen metabolism (6 S RNA, NsiR4) and stress response (Yfr1) have been identified in Cyanobacteria; and ncRNAs involved in stress response (NfiS), nitrogen metabolism (NfiS, NfiR) and biofilm formation (RmsZ) have been identified in the associative nitrogen-fixing bacteria (Table 1).

Studies on the ncRNAs involved in the regulation of nitrogen metabolism in nitrogen-fixing bacteria began with the cyanobacterial model strain Anabaena sp. PCC 7120. In 2010, Ionescu and colleagues used transcriptome sequencing to discover the ncRNA NsiR1, which is 60 nt in size and was the first known nitrogen stress-induced ncRNA in Anabaena sp. PCC 7120. Bioinformatics and characterization of its expressions showed that NsiR1 is heteromorphic and cell-specific in different cyanobacteria and that nsiR1 synthesis occurs not only in morphologically-distinct heteromorphic cells but also in potential heteromorphic cells that maintain trophic cell characteristics, implying that NsiR1 may be an early marker of cyanobacterial cell differentiation under nitrogen stress conditions. However, the target and mechanism of action of NsiR1 are unknown (Ionescu et al. 2010; Muro-Pastor 2014). 6 S RNA is the second ncRNA required for the regulation of nitrogen metabolism in nitrogen-fixing bacteria. In addition, 6 S RNA is one of the few well-studied ncRNAs in bacteria and is known to be involved in regulating the activity of the RNA polymerase RNAP. In Synechocystis sp. PCC 6803, the deletion of 6 S RNA reduced the ability of cells to respond to nitrogen starvation stress at physiological and transcriptional levels and improved the ability of RNAP to recruit the sigma factors SigB and SigC. Since SigB and SigC prevent cells from adapting to stressful adverse environments under nitrogen deprivation, it is hypothesized that 6 S RNA is involved in regulating nitrogen metabolism by influencing the recruitment of sigma factors by RNAP (Heilmann et al. 2017). With the development of bioinformatic prediction methods and experimental identification techniques, more highly expressed ncRNAs have been identified under nitrogen starvation conditions in cyanobacteria, but their functions and regulatory mechanisms in nitrogen metabolism remain unknown.

Noncoding RNAs are recognized as important post-transcriptional regulators in the nitrogen fixation process

Nitrogen fixation is a highly energy-consuming process that is tightly regulated within the cell. It has been demonstrated that the expression of nif-related genes is regulated at the transcriptional level by its own specific regulator NifLA and the intracellular nitrogen metabolism regulatory system, such as the PII protein GlnK, the sigma factor RpoN, the binary regulatory system NtrBC and carbon metabolism systems (e.g., CbrAB) (Sadowski et al. 2013; Bueno Batista and Dixon 2019). However, the mechanism of its post-transcriptional regulation is unknown, and it has become a hot topic in research on biological nitrogen fixation.

Currently, five ncRNAs that are directly involved in the regulation of biological nitrogen fixation in nitrogen-fixing microorganisms have been identified. These ncRNAs can affect the expression of glutamine synthetase, nitrogenase, PII protein-encoding genes, or extracellular polysaccharide synthesis genes at the post-transcriptional or translational level, implying that ncRNAs, as a new class of post-transcriptional regulators, play a key role in the regulation of nitrogen-fixing gene networks.

NsiR4 is the first ncRNA that was shown to have a direct role in controlling nitrogen fixation in Synechocystis sp. PCC 6803. In Cyanobacteria, NsiR4 is highly conserved and reacts to nitrogen stress signals in particular. NsiR4 reduces IF7 expression by binding to the 5′ UTR of the gifA gene, which encodes the factor IF7, causing the inactivation of glutamine synthetase (GS) and affecting the response of cyanobacteria to abrupt changes in effective nitrogen sources (Klähn et al. 2015).

Next, the other three ncRNAs were discovered to be directly engaged in the regulation of nitrogen fixation in associative nitrogen-fixing bacteria and archaea. These three ncRNAs alter the post-transcriptional stability of target mRNAs, unlike NsiR4, which represses target gene
As natural ncRNAs play a crucial role in many physiological processes, synthetic ncRNAs are considered a versatile and potent tool for engineering metabolic pathways in bacteria. In recent years, much effort has been dedicated to developing artificial bacterial ncRNA, especially their translation. In associative nitrogen-fixing Pseudomonas stutzeri A1501, NfIS was found to be the first ncRNA that was directly needed for the optimum expression of nitrogenase genes. The nitrogenase-encoding gene nifK recruited the ncRNA regulator NfIS, which sensed stress signals and evolved synergistically over time. NfIS efficiently and finely regulated its mRNA stability or translational activity, and this established a novel regulatory link between the stress response and nitrogen fixation to ensure that nitrogen fixation was efficient (Zhan et al. 2016; Zhang et al. 2019). Furthermore, Zhan et al. (2019) also reported the discovery and characterization of NfIR, a second ncRNA that is inducible under nitrogen fixation conditions, which is directly involved in the regulation of the expression of nitrogenase structural protein NifD in P. stutzeri A1501. NfIR, in collaboration with NfIS, was discovered to optimize nitrogenase at the post-transcriptional level by targeting the mRNAs of the nitrogenase structural genes nifD and nifK, respectively (Zhan et al. 2019). The ncRNA sRNA154 was discovered to be directly involved in the regulation of nitrogenase in Methanobacterium octococci Göl by affecting the stability of the nrgA mRNA that codes the nif-specific activator NrgA, the nitrogenase structural gene nifH mRNA, and the glutamine synthetase encoding genes glnA1 and glnA2 mRNAs. The mechanism by which sRNA154 regulates the translation of nitrogen fixation is complex, as it activates the translation of NifH, GlnA1 and NrgA while inhibiting the translation of GlnA2 (Prasse et al. 2017).

Biofilm and nitrogen fixation are two competitive strategies that are used by many plant-associated bacteria. Nitrogen-fixing bacteria that are associated with the rhizosphere initiate nitrogen fixation reactions by colonizing the host inter-roots and forming biofilms. Until recently, the regulatory ncRNA RsmZ had been experimentally identified in P. stutzeri A1501 as a regulator that is engaged in the formation of coordinated biofilms and regulation of nitrogen fixation. RsmZ was discovered to operate as a signal amplifier to trigger biofilm development by separating the translation repressor protein RsmA from the pslA and sadC mRNAs, the primary genes involved in polysaccharide production and secondary signalling, at the early stage of biofilm formation (Shang et al. 2021).

### Regulation of bacterial nitrogen metabolic engineering by synthetic noncoding RNAs

| Organism                  | mRNA target(s) | ncRNA scaffold | Application                  | Reference(s) |
|---------------------------|----------------|----------------|-------------------------------|---------------|
| Escherichia coli          | ackA, fabI     | A pair-centi-  | Gene silencing                | (Nakashima     |
|                           |                | Em termi-      |                               | et al. 2006)  |
|                           |                | min-termini    |                               |               |
| Escherichia coli          | ompF, fltC     | MicF, Spot42   | Gene silencing                | (Sharma et     |
|                           |                |                |                               | al. 2012)     |
| Escherichia coli          | murE, tyR,     | MicC           | Increase in                   | (Na et al.     |
|                           | tyrA, csrA     |                | cadaverine, phenol and        | 2013; Yoo et  |
|                           |                |                | tyrosine production           | et al. 2013;  |
|                           |                |                |                               | Kim et al.    |
|                           |                |                |                               | 2014)         |
| Escherichia coli          | proB, glnA,    | MicC           | Increase in                   | (Chen et al.   |
|                           | argB           |                | S-adenosyl-                  | 2015)         |
|                           |                |                | methionine (SAM) production   |               |
| Escherichia coli          | pfkA           | MicC           | Increase in 1,3-diamino-      | (Chae et al.   |
|                           |                |                | nopropionate production      | 2015)         |
| Escherichia coli          | rpaA, fadR,    | MicC           | Increase in malonyl-CoA,      | (Yang et al.   |
|                           | nudD, fur,     |                | proline and threonine         | 2018, 2019)   |
|                           | ar0F and etc.  |                | production                   |               |
| Escherichia coli          | ppsA, pta,    | MicC           | Increase in IgG production    | (Zhang et al.  |
|                           | degQ, degS     |                |                               | 2020)         |
| Escherichia coli          | rpoS           | Random         | Gene silencing                | (Jin et al.    |
|                           |                | library        |                               | 2013)         |
| Escherichia coli          | ssrS           | SibC           | Gene silencing                | (Park et al.   |
| Pseudomonas putida        | acnB, sdbB     | MicC           | Gene silencing                | 2013)         |
| Shewanella oneidensis     | mtrA           | MicC           | Gene silencing                | (Apura et al.  |
| Bacillus subtilis         | pfk, glnM      | MicC           | Increase in N-acetyl          | (Cao et al.    |
| Clostridium acetobutylicum| adhE1, pta     | MicC           | glucosamine production        | 2017)         |
| Synechocystis sp. PCC6803 | pyk, ldhA,     | MicC           | Increase in butanol production| (Cho and       |
|                           | odhA           |                |                               | Lee 2017)     |
| Synechocystis sp. PCC6803 | slr1511,      | MicC           | Increase in glutamate         | (Sun et al.    |
|                           | slr11069,      |                | production                   | 2019)         |
|                           | slr1332, glgC  |                |                               |               |
|                           | and etc.       |                | Increase in fatty acid        | (Sun et al.    |
|                           |                |                | biosynthesis                  | 2018)         |
| Synechooccus elongatus    | nblA           | MicC           | Gene silencing                | (Li et al. 2018) |
Conclusions and perspectives

Each class of ncRNA that was involved in the control of nitrogen metabolism underwent identification and functional analysis, revealing a new mechanism for the regulation of bacterial nitrogen and demonstrating that ncRNAs play a key role in bacterial gene expression and regulation. However, despite the discovery of several ncRNAs involved in the regulation of nitrogen metabolism, the regulatory network of nitrogen metabolism in bacteria is complex; thus, the current understanding of the regulatory mechanisms of bacterial nitrogen metabolism, particularly at the post-transcriptional and translational levels, is rather superficial, fragmented and one-sided. The biological functions and regulatory mechanisms of ncRNAs involved in the regulation of bacterial nitrogen metabolism are still under investigation.

Microorganisms are central organisms in the nitrogen cycle because six of the eight known nitrogen cycles are operated by only microorganisms in nature. However, because of the diversity of microbial metabolism, the regulation of nitrogen metabolism-related gene expression is rigorous and complex. By directly base pairing with target mRNAs, regulatory ncRNAs regulate the expression of target genes at the post-transcriptional or translational level with the help of the RNA chaperone Hfq. In contrast to the traditional focus on the optimization of genetic circuits and metabolic pathways, which occur primarily at the transcriptional level, ncRNAs, as novel regulatory factors, have advantages such as rapid response, flexible and precise control, easy recovery and a lack of metabolic burden. As a result, the development of standardized and intelligent artificial ncRNAs can provide new strategies and tools for the construction of efficient nitrogen metabolic circuits and their widespread application in agriculture.

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Declarations

Conflict of interest These authors declare that they have no conflict of interest.

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References

Álvarez-Escribano I, Vioque A, Muro-Pastor AM (2018) NsrR1, a nitrogen stress-repressed sRNA, contributes to the regulation of nblA in *Nostoc* sp. PCC 7120. Front Microbiol 9:2267. https://doi.org/10.3389/fmicb.2018.02267

Andersen J, Delihas N, Ikenaka K et al (1987) The isolation and characterization of RNA coded by the miF gene in *Escherichia coli*. Nucleic Acids Res 15:2089–2101. https://doi.org/10.1093/nar/15.5.2089

Apura P, Saramago M, Peregrina A et al (2020) Tailor-made sRNAs: a plasmid tool to control the expression of target mRNAs in *Pseudomonas putida*. Plasmid 109:102503. https://doi.org/10.1016/j.plasmid.2020.102503

Baumgardt K, Smidová K, Rahn H et al (2016) The stress-related, rhizobial small RNA RsR1 destabilizes the autoinducer synthase encoding mRNA *sin* in *Sinorhizobium meliloti*. RNA Biol 13:486–499. https://doi.org/10.1080/15476286.2015.1110673

Blaxter M (2010) Genetics. Revealing the dark matter of the genome. Science 330:1758–1759. https://doi.org/10.1126/science.1200700

Buddeweg A, Sharma K, Urlaub H, Schmitz RA (2018) sRNA1 affects ribosome binding sites within polycistronic mRNAs in *Methanosarcina mazei* Gs1. Mol Microbiol 107:595–609. https://doi.org/10.1111/mmi.13900

Buono Battista M, Dixon R (2019) Manipulating nitrogen regulation in diazotrophic bacteria for agronomic benefit. Biochem Soc Trans 47:603–614. https://doi.org/10.1042/BST20180342

Cao Y, Li X, Li F, Song H (2017) CRISPRi-sRNA: Transcriptional-translational regulation of extracellular electron transfer in *Shewanella oneidensis*. ACS Synth Biol 6:1679–1690. https://doi.org/10.1021/acssynbio.6b00374

Ceziel Borella G, Lagares A, Valverde C (2018) Expression of the small regulatory RNA gene mmgR is regulated negatively by AniA and positively by Ntc in *Sinorhizobium meliloti* 2011. Microbiol (Reading) 164:88–98. https://doi.org/10.1099/mic.0.000586

Chae TU, Kim WJ, Choi S et al (2015) Metabolic engineering of *Escherichia coli* for the production of 1,3-diaminopropane, a three carbon diamine. Sci Rep 5:13040. https://doi.org/10.1038/srep13040

Chen Y, Lou S, Fan L et al (2015) Control of ATP concentration in *Escherichia coli* using synthetic small regulatory RNAs for enhanced S-adenosylmethionine production. FEMS Microbiol Lett 362:fnv115. https://doi.org/10.1093/femsle/fnv115

Cho C, Lee SY (2017) Efficient gene knockdown in *Clostridium acetobutylicum* by synthetic small regulatory RNAs. Biotechnol Bioeng 114:374–383. https://doi.org/10.1002/bit.26077

Dixon R, Kahn D (2004) Genetic regulation of biological nitrogen fixation. Nat Rev Microbiol 2:621–631. https://doi.org/10.1038/nrmicro954

Gottesman S (2005) Micros for microbes: non-coding regulatory RNAs in bacteria. Trends Genet 21:399–404. https://doi.org/10.1016/j.tig.2005.05.008

Heilmann B, Hakkila K, Georg J et al (2017) 6S RNA plays a role in recovery from nitrogen depletion in *Synechocystis sp.* PCC 6803. BMC Microbiol 17:229. https://doi.org/10.1186/s12866-017-1137-9

Ionescu D, Voss B, Oren A et al (2010) Heterocyst-specific transcription of NsrR1, a non-coding RNA encoded in a tandem array of direct repeats in *cyanobacteria*. J Mol Biol 398:177–188. https://doi.org/10.1016/j.jmb.2010.03.010

Jin Y, Wu J, Li Y et al (2013) Modification of the RpoS network with a synthetic small RNA. Nucleic Acids Res 41:8323–8340. https://doi.org/10.1093/nar/gkt604

Ju X, Zhao L, Sun B (2007) Nitrogen fixation by reductively dechlorinating bacteria. Environ Microbiol 9:1078–1083. https://doi.org/10.1111/j.1462-2920.2006.01199.x

Jung Y-S, Kwon Y-M (2008) Small RNA ArfR regulates the expression of *sodB* and *feSI* genes in *Azotobacter vinelandii*. Curr Microbiol 57:593–597. https://doi.org/10.1007/s00284-008-9248-z

Kang Z, Zhang C, Zhang J et al (2014) Small RNA regulators in bacteria: powerful tools for metabolic engineering and synthetic biology. Appl Microbiol Biotechnol 98:3413–3424. https://doi.org/10.1007/s00253-014-5569-y

Kim B, Park H, Na D, Lee SY (2014) Metabolic engineering of *Escherichia coli* for the production of phenol from glucose. Biotechnol J 9:621–629. https://doi.org/10.1002/biot.201300263

Klihn S, Schaal C, Georg J et al (2015) The sRNA NsrR4 is involved in nitrogen assimilation control in *cyanobacteria* by targeting glutamine synthetase inactivating factor IF7. Proc Natl Acad Sci U S A 112:E6243–6252. https://doi.org/10.1073/pnas.1508412112

Kuypers MMM, Marchant HK, Kartal B (2018) The microbial nitrogen-cycling network. Nat Rev Microbiol 16:263–276. https://doi.org/10.1038/nrmicro.2018.9

Li S, Sun T, Xu C et al (2018) Development and optimization of genetic toolboxes for a fast-growing *cyanobacterium* *Synechococcus elongatus* UTEX 2973. Metab Eng 48:163–174. https://doi.org/10.1016/j.men.2018.06.002

Liu Y, Zhu Y, Li J et al (2014) Modular pathway engineering of *Bacillus subtilis* for improved N-acetylglucosamine production. Metab Eng 23:42–52. https://doi.org/10.1016/j.men.2014.02.005

MacLellan SR, Smallbone LA, Sibley CD, Finan TM (2005) The expression of a novel antisense gene mediates incompatibility within the large repABC family of alpha-proteobacterial plasmids. Mol Microbiol 55:611–623. https://doi.org/10.1111/j.1365-2958.2004.04412.x

Muro-Pastor AM (2014) The heterocyst-specific NsrR1 small RNA is an early marker of cell differentiation in cyanobacterial filaments. mBio 5:e01079–e01014. https://doi.org/10.1128/mBio.01079-14

Na D, Yoo SM, Chung H et al (2013) Metabolic engineering of *Escherichia coli* using synthetic small regulatory RNAs. Nat Biotechnol 31:170–174. https://doi.org/10.1038/nbt.2461

Nakamura T, Naito K, Yokota N et al (2007) A cyanobacterial non-coding RNA, Yfr1, is required for growth under multiple stress conditions. Plant Cell Physiol 48:1309–1318. https://doi.org/10.1093/pcp/pcm098

Nakashima N, Tamura T, Good L (2006) Paired termini stabilize anti-sense RNAs and enhance conditional gene silencing in *Escherichia coli*. Nucleic Acids Res 34:e138. https://doi.org/10.1093/nar/gkl697

Park H, Bak G, Kim SC, Lee Y (2013) Exploring sRNA-mediated gene silencing mechanisms using artificial small RNAs derived from a natural RNA scaffold in *Escherichia coli*. Nucleic Acids Res 41:3787–3804. https://doi.org/10.1093/nar/gkt061

Prasse D, Förstner KU, Jäger D et al (2017) sRNA*34* is a newly identified regulator of nitrogen fixation in *Methanosarcina mazei* strain Gs1. RNA Biol 14:1544–1558. https://doi.org/10.1080/15476286.2017.1306170

Prasse D, Schmitz RA (2018) Small RNAs involved in regulation of nitrogen metabolism. Microbiol Spectr. https://doi.org/10.1128/microbiolspec.RWR-0018-2018.6

Ramanu N, Hedeshian M, Freundlich M (1994) miF antisense RNA has a major role in osmoregulation of OmpF in *Escherichia coli*. J Bacteriol 176:5005–5010. https://doi.org/10.1128/jb.176.15.5005-5010.1994

Robledo M, Flage B, Wright PR, Becker A (2015) A stress-induced small RNA modulates alpha-rhizobial cell cycle progression.
sensor histidine kinase CbrA contributes to free-
for enhanced production of full-length
Springer Nature remains neutral with regard to juris-

Wagner EGH, Romby P (2015) Small RNAs in bacteria and archaea: who they are, what they do, and how they do it. Adv Genet 90:133–208. https://doi.org/10.1016/bs.adgen.2015.05.001

Yan D (2007) Protection of the glutamate pool concentration in enteric bacteria. Proc Natl Acad Sci U S A 104:9475–9480. https://doi.org/10.1073/pnas.0703360104

Yan Y, Yang J, Dou Y et al (2008) Nitrogen fixation island and rhizo-
sphere competence traits in the genome of root-associated Pseudomonas stutzeri A1501. Proc Natl Acad Sci U S A 105:7564–7569. https://doi.org/10.1073/pnas.0801093105

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