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ORIGINAL ARTICLE

In silico prediction and qPCR validation of novel sRNAs in *Propionibacterium acnes* KPA171202

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Abstract *Propionibacterium acnes* is an anaerobic, Gram-positive, opportunistic pathogen known to be involved in a wide variety of diseases ranging from mild acne to prostate cancer. Bacterial small non-coding RNAs are novel regulators of gene expression and are known to be involved in, virulence, pathogenesis, stress tolerance and adaptation to environmental changes in bacteria. The present study was undertaken keeping in view the lack of predicted sRNAs of *P. acnes* KPA171202 in databases. This report represents the first attempt to identify sRNAs in *P. acnes* KPA171202. A total of eight potential candidate sRNAs were predicted using SIPHT, one was found to have a Rfam homolog and seven were novel. Out of these seven predicted sRNAs, five were validated by reverse transcriptase-polymerase chain reaction (RT-PCR) and sequencing. The expression of these sRNAs was quantified in different growth phases by qPCR (quantitative PCR). They were found to be expressed in both exponential and stationary stages of growth but with maximum expression in stationary phase which points to a regulatory role for them. Further investigation of their targets and regulatory functions is in progress.

1. Introduction

Propionibacterium acnes is a Gram-positive, non-spore forming, micro-aerophilic, pleomorphic rod shaped opportunistic pathogen with an optimal growing temperature of 37 °C. The bacterium has been found to be involved in a wide array of diseases ranging from acne [25] to prostate inflammation leading to prostate cancer [1,7]. Other diseases in which the bacterium was isolated from the site of inflammation include, rheumatoid arthritis, endophthalmitis, shunt-associated central nervous system infections, endocarditis, sarcoidosis, osteomyelitis, allergic alveolitis, pulmonary angitis, acne inversa and SAPHO (synovitis, acne, pustulosis, hyperostosis, osteitis) syndrome [5]. Colonization by *P. acnes* in the pilosebaceous follicle is a key factor for inflammatory reaction in acne vulgaris [25]. Acne can manifest as a mild comedonal form to chronic inflammatory cystic acne on the face, chest, and back. The antibiotic therapy involves the use of erythromycin, clindamycin and tetracycline for weeks and months resulting in evolution of resistant strains. It thus becomes necessary to look for alternative therapeutics which do not lend themselves to development of resistance. The sequencing of *P. acnes* KPA171202 genome

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by Bruggemann and co-workers [6], leads to the annotation of genes involved in pathogenesis and virulence [4]. It has also brought up an opportunity to identify small regulatory RNAs among the *P. acnes* genome sequences.

Small RNAs (sRNAs) are small regulatory RNAs, occurring in prokaryotes; in addition to the already known messenger (mRNA), transfer (tRNA) and ribosomal (rRNA). Their sizes range from 30 to 600 nts approximately in length and are usually not translated into proteins. These are encoded by intergenic regions (IGRs) of bacterial chromosomes and are transcribed from their own promoters. Their transcription most often terminates at a strong Rho-independent terminator. The sRNAs might be transcribed in *cis* i.e. encoded on the strand opposite to the gene they regulate, or in *trans* i.e. away from the target genes. The *trans* acting-sRNAs act by partial or imperfect base pairing with the target transcripts while the *cis* acting-sRNAs have a region of perfect complementarity with the target transcript. In some cases, the sRNAs are expressed under highly specific growth conditions [10]. MicF was the first *trans*-encoded antisense RNA found in the *Escherichia coli* genome [22,23]. It showed partial and imperfect sequence complementarity to its target ompF (encoding outer membrane protein F) mRNA near the start codon, leading to strong translation inhibition. Classically, sRNAs were defined as short non-coding transcripts that, together with the RNA chaperone protein, Hfq, act in *trans* to control the translation or stability of target mRNAs. This definition was broadened by Liu and Camilli [16], as many coding, *cis*-acting and Hfq-independent sRNAs were also recognized. RNAIII of *Staphylococcus aureus* is a *cis*-acting regulatory RNA that also encodes a virulence factor δ-hemolysin [3]. SymR of *E. coli* is an example of Hfq-independent, *cis*-acting sRNA that represses the translation of symE which encodes for a toxic protein [13].

Binding of sRNA with its target may lead to translational activation or it may also lead to translational repression. These have been involved in the regulation of metabolism, growth processes, adaptation to stress, and pathogenesis of microorganisms [21]. Their regulatory nature also makes them attractive targets for developing nucleic acid based novel therapeutics. There are many approaches to identify and characterize sRNA molecules, their genes and targets in prokaryotes. These include genome-wide searches based on the bio-computational prediction of sRNA encoding genes. First evidence of sRNAs came in 2001 when three different groups [2,29,34] at the same time developed algorithms and identified 31 new sRNA in *E. coli*. Later on genomes of several organisms were explored for the presence of sRNA candidates. Some authors developed their own in silico approaches for sRNA prediction [33,36], while others used various web based tools singularly [28] or a combination of few tools [35]. Some researchers have used genome tilling microarrays for finding new sRNA transcripts and investigating sRNA expression. However, the microarray results need to be validated by northern blots or qPCR and analyzed further by RACE (rapid amplification of cDNA ends); for end-mapping to discriminate novel sRNA genes from leader sequences of genes. Microarrays were used for sRNA identification in *Caulobacter crescentus* by Landt and co-workers [15] and could verify only 27 out of 300 predicted sRNAs. Various other methods include cDNA library synthesis, next-generation sequencing, northern blotting, RT-PCR analysis of predicted genes and co-purification with proteins like hfq.

Present study systematically identified sRNAs in the *P. acnes* genome beginning with computational approach based on gene localization, intergenic sequence conservation, terminator and secondary structure prediction, followed by validation of predicted sRNAs in experimental approach using qPCR. The results indicate existence of seven and validation of five hitherto unreported sRNAs in *P. acnes* which might play a potential role in regulating gene expression and/or pathogenesis.

2. Materials and methods

2.1. Bacterial strains and culture conditions

P. acnes (strain KPA171202/DSM 16379) was procured form DSMZ Germany (German Collection of Microorganisms and cell cultures). The culture was maintained in Brain Heart Infusion Broth at 37 °C for 48–72 h in anaerobic jar containing 5% CO_2 and on BHI plates supplemented with Vitamin K (10 μg/ml) and Haemin (5 μ/ml) at 37 °C. The growth rate of *P. acnes* in BHI media was monitored spectrophotometrically by observing OD at 600 nm after every 3 h till 78 h. Growth curve was plotted using optical density against time plot (Fig. 1).

2.2. In silico prediction of candidate sRNA genes

Genome sequences of *P. acnes* KPA171202 were downloaded from NCBI server for insilico prediction. Small RNA candidates of *P. acnes* were predicted using the web interface SIPHT (sRNA Identification Protocol using High-throughput Technologies) using default parameters [18]. SIPHT identifies sRNA encoding genes based on intergenic conservation and Rho-independent terminators. Each locus is annotated for many features like sequence conservation in other closely related species, promoter and transcription factor binding site, conserved secondary structure etc that provide information for its potential function. The performance and reliability of the tool was assessed and compared with other algorithms also [20].

2.3. Nomenclature of the sRNAs

Each candidate sRNAs detected by PCR and confirmed by sequencing are indicated by initial "s" (in lowercase), followed by genome identification as used in NCBI database (in uppercase); ending with the number of candidate as predicted by SIPHT. For example: 'sPPAK1' for sRNA of *Propionibacterium acnes* KPA171202 candidate 1.

2.4. Insilico validation of predicted sRNAs

The sequences were scanned for the presence of Shine-Dalgarno (SD) sequence, start codons, stop codons and rho independent terminators. ARNold was used for rho-independent transcription terminator prediction [26]. Secondary structure predictions were carried out using RNAfold program [12] with default parameters. The predicted sRNAs were scanned in Rfam database to check for novelty [11].