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Review Article

Genetic engineering of bacteria for the production of antibiotics: A review

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Abstract

Due to high demand of antibiotics for treatment of increasing bacterial infections there is an urgent need of engineering bacterial strains to get high concentration and meet industrial demands. Different techniques are being used for this purpose: which include over-expression of a gene in its host strain, engineering of different activators and regulators of antibiotic synthesizing gene cluster and expression of antibiotic gene cluster in heterologous host. The emergence of antibiotic resistant pathogens was a huge problem for existing medications and it urges a need or the development of novel antibiotics with high specificity. These can be produced by combinatorial biosynthesis or awakening of silent genes already present in bacteria. These advancements present a bright future of antibiotic production at industrial level.

Introduction

A wide variety of bioactive secondary metabolites are produced by bacteria which shows strong biological activities such as antibacterial, antitumoral, antiviral, antifungal etc. (Korp et al., 2016; Yin et al., 2017), and have wide applications in the field of agriculture and medicine (Liu et al., 2020). But the efficient improvement in their production is often difficult (Yin et al., 2017). So, different strategies should be adapted to get high yield engineering strains.

Since early 20th century many antibiotics were discovered which had a remarkable impact on human health by treating previously fatal diseases but shortly after their discovery resistance to these antibiotics by different bacteria were also observed (Kealey et al., 2017). Microbial resistance can be defined as presence of acquired or mutated, genetically developed mechanisms of resistance causing the pathogen to be resistant or susceptible to a particular product or metabolite (MacGowan & Macnaughton, 2017). Antibiotic resistance posed by bacteria to almost all antibiotics known yet is a very serious threat for national and global health (Krishnamurthy et al., 2016; Landecker, 2016). Infections caused by antibiotic resistant strains of bacteria are increasing day by day (Fields et al., 2017). Resistance arises due to certain reasons which include non-therapeutic use of antibiotics, integron

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and mobile genetic elements for gene transfer etc. (Jiang et al., 2017; Singh et al., 2019; Sundin & Wang, 2018). This threat highlight the fact that there is an urgent need for the production of new classes of antibiotics with novel mode of action for untreatable diseases (Cochrane & Vederas, 2016). To solve this problem genetic engineering of bacteria plays a major role (MacGowan & Macnaughton, 2017). Genetic engineering of bacteria results in introduction of novel characteristics in bacteria.

Antibiotic production in bacteria is usually governed and controlled by cluster of genes (Kalkreuter & Williams, 2018; Xu et al., 2017). Gene cluster involve some function related genes clustered together with a close position on chromosome. However, the function of each gene is different and the coding product of each gene is also different from other gene. But all the genes in a cluster work together for the synthesis and production of antibiotics (Zou et al., 2018). Genome engineering tools can be used to engineer new biosynthetic pathways for the production of novel antibiotics (Krishnamurthy et al., 2016; Thaker & Wright, 2015). With the advent of new tools promising treatment plans can be devised for bacterial infections.

Due to the sequencing techniques, genetic and biochemical characteristics of these gene clusters are known and can be used for engineering of antibiotics with novel structure and characteristics. Strain improvement of microbes can also be done to get high expression of antibiotics (Liu et al., 2020; Yan & Fong, 2017). We can also get high titre of already known antibiotic by engineering regulation pathways (Zou et al., 2018). For example, genetic engineering can be used for the over-expression of the desired gene or modification of regulators or heterologous expression of the desired antibiotics (Bilyk & Luzhetskyy, 2016). Typically, the best antibiotic yield is obtained by the combination of these different approaches.

Table 1. Summary of the described antibiotics, their producing strains and bio-activities and increase in production level

Antibiotics	Producing strain	Bio-activity	Genetic engineering technique	Production	References
Nikkomycin	*S. ansochromogenes*	Antifungal	Over expression of gene cluster	High production with 20-fold increased	(Du et al., 2015)
Gougerotin	*S. gougerotii* *S. toyocaensis var.*	Antitumor, Antiviral	Over expression of gene cluster	High production with 10-fold increased	(Chen et al., 2017)
Avermectin	*S. avermitilis*	Antihelminthic	Over expression of gene cluster	High production with 1.2-fold increased	(Liu et al., 2020)
Oxytetracycline	*S. rimosus*	Antibacterial	Regulators engineering	High production with 6.5-fold increased	(Sun et al., 2016)
Carbapenem	*S. cattleya*	Antibacterial	Heterologous expression, Regulators engineering	High production with 60-fold increased	(Shomar et al., 2018)
Teicoplanin	*Actinoplanes teichomyceticus*	Antibacterial	Regulators engineering	High production with 40-fold increased	(Horbal et al., 2014)

Continued Table 1

Antibiotics	Producing strain	Bio-activity	Genetic engineering technique	Production	References
Bacteriocin GarKS	*Lactococcus garvieae*	Antimicrobial	Optimizing culture conditions, Increasing gene dose	High production with 2000-fold increased	(Telke et al., 2018)
Haliangicin	*Haliangium ochraceum*	Antifungal	Heterologous expression	High production with 11-fold increased	(Sun et al., 2016)
2-hydroxy-phenazine	*Pseudomonas chlororaphis*	Antifungal	Genetic and metabolic engineering	High production with 99-fold increased	(Liu et al., 2016)

Over expression/amplification of antibiotic producing gene cluster

To get high titre of secondary metabolites in bacteria genetic manipulation of key structural and regulatory genes is done. Genes responsible for an antibiotic production are mostly clustered together so the amplification of entire cluster can enhance the antibiotic production (Chen et al., 2017). Thus controlled multiplication of entire biosynthetic pathway can be used as a tool for engineering bacterial strains to get high antibiotic yield (Bilyk & Luzhetskyy, 2016). For example the duplication of entire nikkomycin gene cluster increased its production by *S. ansochromogenes* (Du et al., 2015). Gougerotin production was enhanced by combination of gene cluster duplication and key structural genes promoter engineering (Niu & Tan, 2015). So, different combinatorial biosynthesis and genetic engineering pathways are designed to create new and hybrid antibiotics.

In many bacteria the metabolite producing gene clusters are controlled by transcriptional factors (TF). The activity of transcriptional factors depends upon the extracellular signal or environmental signal. So the modification of physiological signal can result in activation or over expression of gene cluster. For example, avermectin production by bacteria *S. avermitilis* is enhanced by this method. Its over-expression is controlled and regulated by specific AveT regulatory protein (Guzmán-Trampe et al., 2017). Advances in synthetic biology are helping to construct novel biological systems for new antibiotics with increased bioactivity.

Regulators engineering

Various mechanisms are present in antibiotic producing organisms to protect themselves during antibiotics biosynthesis. Among them the common mechanisms are ribosomal protection and antibiotic efflux. For example, oxytetracycline production in *Streptomyces rimosus* is governed by these protection mechanisms. There are three genes involved in producing resistance to antibiotics. OtrA is ribosomal protection protein and prevent the oxytertacycline binding to its inhibitory sites on ribosomes or remove the bound ones. OtrB is another membrane bound protein involved in efflux of antibiotic, resulting in reduced concentration of oxytetracycline inside cell so protect the ribosome of cell. Third oxytetracycline resistance gene is OtrC. It is a bacterial ABC family transporter and is responsible for ATP dependent efflux of tetracycline. As the antibiotics production is limited due to low tolerance of natural antibiotic producing strain. So the overexpression of these resistance genes resulted in improved production of antibiotic. Over-expression of all three resistance protein genes resulted in 2.78 fold increase in production level (Yin et al., 2017). So increasing the self-resistance novel will enhance the antibiotic production.

Oxytetracycline biosynthesis can also be enhanced by the manipulation of cluster situated pathway specific activator OtcR in *S.rimosus* M4018. Oxytetracycline production level was increased more than 6 times by it (Yin et al., 2015).

Similarly, carbapenem toxicity limits its high density in cell mediums and in turn limits antibiotic titre. Expression of carbapenem is delayed till late exponential phase when sufficient biomass has been produced (Shomar et al., 2018). In this way engineering of strain can be done to increase antibiotic tolerance and biomass by many folds.

Over-expression of positive regulatory genes can also be done to get enhanced production of desired antibiotics (Robertsen et al., 2018; Wang et al., 2013). For example, a clinically important antibiotic teicoplanin gene cluster is regulated by two positive regulators named as tei15 and tei16. Over-expression of both of these regulators in the integrative plasmid resulted in high titre of teicoplanin (Bilyk & Luzhetskyy, 2016). Metabolic engineering is also being approved as a successful strategy in this regard.

Heterologous expression

Heterologous expression of antibiotic synthesizing pathway is required when the natural host is unsuitable for industrial scale production (Bekiesch et al., 2016; Bilal et al., 2017; Bilyk & Luzhetskyy, 2016). It involves cloning of an identified gene cluster into a plasmid and then expressing that plasmid into another organism to get high expression level (Chen et al., 2016; Wohlleben et al., 2016). Different microorganisms can be used for this purpose (Huo et al., 2019). Examples are E. *coli*, and *Streptomyces.*

Actinomycetes are used for heterologous expression of secondary metabolite biosynthetic gene clusters (SMBGC) (Van der Meij et al., 2017; Xu & Wright, 2019). This microorganism is selected on certain bases such as complementary GC content, bioavailibity of precursors for secondary metabolite production etc. Another important factor for selection of heterologous host organism for industrial antibiotic production is genome stability (Weber et al., 2015). Similarly, production level of bacteriocin GarKS was increased 4-fold when a plasmid carrying entire gene cluster was introduced into its native host (Telke et al., 2018). Heterologous gene expression improves the production level effectively.

A marine myxobacteria *Haliangium ochraceum* is responsible for the production of haliangicin but its production level is unsatisfactory. The difficulties in using this bacteria include slow growth rate, low secondary metabolite formation and cell aggregation. So the gene cluster responsible for haliangicin is heterologously expressed in *Myxococcus xanthus*. Yield of antibiotic was tenfold greater and threefold faster in growth speed than the original producer (Sun et al., 2016). Bioactivities can be manipulated by modifying certain biological processes.

2-hydroxyphenazine is an antibiotic produced from *Pseudomonas chlororaphis* GP72 strain (Jin et al., 2015). Its production level was enhanced by over-expression of key genes. Six key genes from the gluconeogenesis, pentose phosphate and shikimate pathways are selected to be over-expressed. When the fusion plasmid pBbB5K containing all six key genes was transformed in the host strain by electroporation, production level was 99-fold higher than in the wild type organism (Bilal et al., 2017; Liu et al., 2016). Over expression of certain genes can be used to get higher level of respective antibiotics.

Novel antibiotic synthesis

The rate at which bacteria are developing resistance against existing antibiotics warrants us for the development of novel antibiotics (Krishnamurthy et al., 2016). So new antibiotics are highly needed to fight bacterial resistance (Shomar et al., 2018). Since the development of new genome analysis techniques in 2000s there has been major advancement in natural products biosynthesis from microorganisms (Onaka, 2017). Different microbial cultures are screened or biological pathway targeting biochemical reaction are performed to discover novel targets for antibiotics synthesis (Chen et al., 2016). Screening of already present compound libraries and modification of existing antibiotics can be used for the development of novel antibiotics (Krishnamurthy et al., 2016; Liu & Myers, 2016). Due to genome sequencing techniques, and transcriptomic and

proteomic approaches high value bacterial targets can be identified and new antibiotics can be developed to inhibit these targets.

Combinatorial biosynthesis

Secondary metabolism genes are interchanged between antibiotic producing bacteria to get 'unnatural' natural products (Baltz, 2018). For example, Telavancin a clinically approved semi synthetic antibiotic differs from its parent molecule by an anionic phosphono group, has significantly improved pharmacodynamics properties (Kealey et al., 2017; Sun et al., 2015). Modern biotechnological approaches can be used to design highly target specific antibiotics.

Awakening of cryptic genes

By genome sequencing technologies it came to know that each bacterial strain contain 30-40 secondary metabolite biosynthetic gene clusters (SMBGC). But under laboratory conditions only some of these are expressed (Okada & Seyedsayamdost, 2017). Several methods have been developed for the expression of these silent gene clusters (Choi et al., 2018; Devine et al., 2017; Weber et al., 2015). Several genome mining approaches can be used in this regard.

Ribosomal engineering: Activation of silent genes is done by introducing mutation in the ribosomal enzymes (d'Aquino et al., 2018; Ochi & Hosaka, 2013). Mutations in the regulatory regions result in enhanced production of antibiotics.

Engineering of growth media: Genetic engineering of metabolic pathways for the enhanced production of antibiotics is restricted by the nutrients present in growth media. For industrial scale bacterial growth and antibiotics production, media is optimized according to needs to get high antibiotic production along with ease of downstream processing (Bhatia et al., 2016). For example, optimization and engineering of media is done for nikkomycin Z production. Its yield was raised upto ~2.3 g/L (Chen et al., 2016). This sets the stage for rational enhancement in antiobiotic production.

Cell to cell interaction: Extracellular environment of bacteria also influence its antibiotics producing ability (Onaka, 2017). This environment may contain specific signals or nutrients responsible for activation of silent genes. For example co-cultivation of *Streptomyces endus* with a mycolic acid containing bacteria resulted in the production of a new antibiotic alchivemycin A (Guzmán-Trampe et al., 2017). It is possible that we may just be visualizing the tip of the iceberg, and these applications will surpass expectations beyond our knowledge.

Future perspective

For antibiotics production enhancement traditional screening methods which are based on bioassay-guided chemical-profiling of metabolites are not suitable with respect to time, effort and cost. Due to the advancement in genetic engineering techniques natural antibiotics can be produced on large scale (Chen et al., 2016). New antibiotics can also be designed to enter the market. Moreover, targeted antibiotics can also be designed (Aparicio et al., 2016). Hopefully the future application of this knowledge at industrial level will meet the demands of commercial antibiotics requirement

Recent advancements in genetic engineering allow rational designing of cell factories for the production of antibiotics (Weber et al., 2015). In this way health problems arising from antibiotic resistance can be overcome. Along with that evolving microbes can also be treated and reduced (Guzmán-Trampe et al., 2017). Even recently (Shen et al., 2017) produced protein and (Ahsan, 2020) fabricated silver Nanoparticle from genetically engineered bacteria *Pseudomonas fluorescens*, to control tobacco mosaic virus. Undoubtedly genetic engineering has a very bright future in antibiotics field.

Conclusion

Different techniques are used for this purpose: which include over-expression of a gene in its host strain, engineering of different activators and regulators of antibiotic synthesizing gene cluster and expression of antibiotic gene cluster in heterologous host. The emergence of antibiotic resistant pathogens is problematic for existing medications and it urge a need for the development of novel antibiotics with high specificity. They can be produced by combinatorial biosynthesis or awakening of silent genes already present in bacteria. These

advancements present a bright future of antibiotic production at industrial level.

Author's declaration and contribution

Authors declare that there is no conflict of interest. TZ search the data and wrote, TA search the data, supervised the writing, proof read, and draft. WY proof read and directed. All authors have read and approved the manuscript and ensure that this is the case.

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