Biochemical and Biophysical Research Communications xxx (2018) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Role of acid responsive genes in the susceptibility of *Escherichia coli* to ciclopirox

Jonghoon Shin, Hyejin Cho, Suran Kim, Kwang-sun Kim*

Department of Chemistry and Chemistry Institute for Functional Materials, Pusan National University, Busan 46241, Republic of Korea

A R T I C L E I N F O

Article history:
Received 4 April 2018
Accepted 10 April 2018
Available online xxx

Keywords:
Ciclopirox
Glutamic acid resistance genes
RNA-seq
Motility
Minimum inhibitory concentration
Microscopic analysis

A B S T R A C T

Antibiotic resistance poses a huge threat to the effective treatment of bacterial infections. To circumvent the limitations in developing new antibiotics, researchers are attempting to repurpose pre-developed drugs that are known to be safe. Ciclopirox, an off-patent antifungal agent, inhibits the growth of Gram-negative bacteria, and genes involved in galactose metabolism and lipopolysaccharide (LPS) biosynthesis are plausible antibacterial targets for ciclopirox, since their expression levels partially increase susceptibility at restrictive concentrations. In the present study, to identify new target genes involved in the susceptibility of *Escherichia coli* to ciclopirox, genome-wide mRNA profiling was performed following ciclopirox addition at sublethal concentrations, and glutamate-dependent acid resistance (GDAR) genes were differentially regulated. Additional susceptibility testing, growth analyses and viability assays of GDAR regulatory genes revealed that down-regulation of *evgS* or *hns* strongly enhanced susceptibility to ciclopirox. Further microscopy and phenotypic analyses revealed that down-regulation of these genes increased cell size and decreased motility. Our findings could help to maximise the efficacy of ciclopirox against hard-to-treat Gram-negative pathogens.

© 2018 Elsevier Inc. All rights reserved.

1. Introduction

For decades, a diverse range of antibiotics have been used to combat bacterial infections. The period from the 1950s to the 1970s was considered the golden era for the discovery of novel classes of antibiotics [1]. Although some new classes of antibiotics have since been reported, their excessive and improper use for the treatment of bacterial infections has fuelled a substantial increase in resistant strains [2]. Alarmingly, the spread of resistant bacteria could shift humanity back to a pre-antibiotic era. Thus, new strategies for mitigating resistance to existing and future drugs are desperately required.

Antimicrobial combinations are frequently used in clinical practice for treating mixed bacterial infections in which organisms are not susceptible to a single antibiotic [3,4], to overcome bacterial tolerance [5], to prevent the emergence of drug resistance [6,7] and to minimise cytotoxicity [8]. More recently, certain compounds such as phytochemicals, nutrients and essential oils have been shown to display synergistic effects and an ability to augment the efficacy of currently used antibiotics when co-applied [9–11]. Moreover, repurposed materials often exhibit an excellent safety profile and can enhance antibiotic activity. One example is ciclopirox, a synthetic antifungal agent [12,13] that can likely be effectively repurposed as an antibiotic because fungal resistance has not been identified in over 27 years of clinical use [14]. In addition to its antifungal activity, this agent exhibits anticancer activity, prevents human immunodeficiency virus (HIV) infection [15,16], displays antibacterial activity [14,17] and acts synergistically with the last resort antibiotic polymyxin B in the treatment of Gram-negative pathogens [18]. Therefore, ciclopirox has great potential as a repurposed agent for the treatment of cancer, human diseases and bacterial infections.

Interestingly, the antifungal activity and other more recently discovered activities are generally related to its ability to chelate iron and interfere with related signalling pathways and specific enzymes [16,19,20]. However, the antibacterial targets of ciclopirox have not been thoroughly characterised. Previous studies showed that the *galE* gene product and knockouts of *galU*, *rfaI*, *rfaB*, *rfaC* or *rfaQ* genes either reversed or increased the susceptibility to ciclopirox at relatively low concentrations [17,18]. However, altering the expression levels of these genes did not reduce the minimum inhibitory concentration (MIC) of ciclopirox more than 2-fold [18].

* Corresponding author.
E-mail address: kwangsun.kim@pusan.ac.kr (K.-s. Kim).

https://doi.org/10.1016/j.bbrc.2018.04.063
0006-291X/© 2018 Elsevier Inc. All rights reserved.

Therefore, additional undiscovered target genes or pathways likely serve as novel mechanisms of action for ciclopirox as an antibacterial agent.

In the present study, we performed gene expression profiling to identify genes linked to the antibacterial activity of ciclopirox against *Escherichia coli*. We found that glutamate-dependent acid resistance (GDAR) pathway genes are strongly modulated following ciclopirox treatment, and down-regulation of *evgS* or *hns* enhanced susceptibility to ciclopirox. Down-regulation of *hns* was particularly pronounced and led to a marked reduction in MIC. Furthermore, knockout of *evgS* or *hns* increased cell size and decreased cell motility, demonstrating a novel mechanism by which ciclopirox functions as an antibacterial agent. Our results could help to maximise the efficacy of ciclopirox against hard-to-treat Gram-negative pathogens.

2. Materials and methods

2.1. Strains and growth conditions

All strains, plasmids and oligonucleotides used in this study are listed in Table S1. All Keio collections [21] and ASKA clones were confirmed by PCR before use. Strains were grown in Lennox broth (LB) or on agar plates. The antibiotics chloramphenicol (Cm^R) and Km^R were used at 34 μg/ml and 25 μg/ml, respectively, and isopropyl-β-D-thiogalactopyranoside (IPTG) was used to induce expression of proteins from ASKA clones.

2.2. Analysis of RNA-seq data

Wild-type (WT; BW25113) cells were cultured in LB medium containing ciclopirox at 12.5 μg/ml at 37 °C to the absorbance at 600 nm (A_{600}) of 1.0. Total RNA from cells was extracted using an RNeasy Protect Bacteria Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. All RNA-seq analyses was performed as previously described [23] except the retrieved reference genome (*E. coli* K-12 BW25113) was used. The eggNOG (evolutionary genealogy of genes: Non-supervised Orthologous Groups) program (v.4.5.1) [24] was used to cluster genes into functionally related groups and to analyse metabolic pathways. Data have been deposited in the NCBI Gene Expression database and are accessible through GEO Series accession number GSE110854 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE110854).

2.3. Screening of genes related to ciclopirox susceptibility

The effects of individual genes on ciclopirox susceptibility were screened using either Keio collections [21] or ASKA clones [22] in the BW25113 background in the presence or absence of a sublethal concentration of ciclopirox (12.5 μg/ml). For screening individual genes, strains were cultured in LB broth with (overexpression) or without (knockout) induction by 0.1 mM IPTG at 37 °C for 24 h with vigorous shaking, and growth was measured using a UV/visible spectrophotometer (Ultrospec 2100 pro, GE Healthcare). The A_{600} for cells cultured with or without ciclopirox (0 and 12.5 μg/ml) was measured, and the ratio was calculated as A_{600} (12.5 μg/ml) to A_{600} (0 μg/ml). Finally, the ratio of individual genes relative to control cells (either BW25113 for knockouts or pCA24N for overexpression strains) was calculated and designated as the relative ratio of growth in figures.

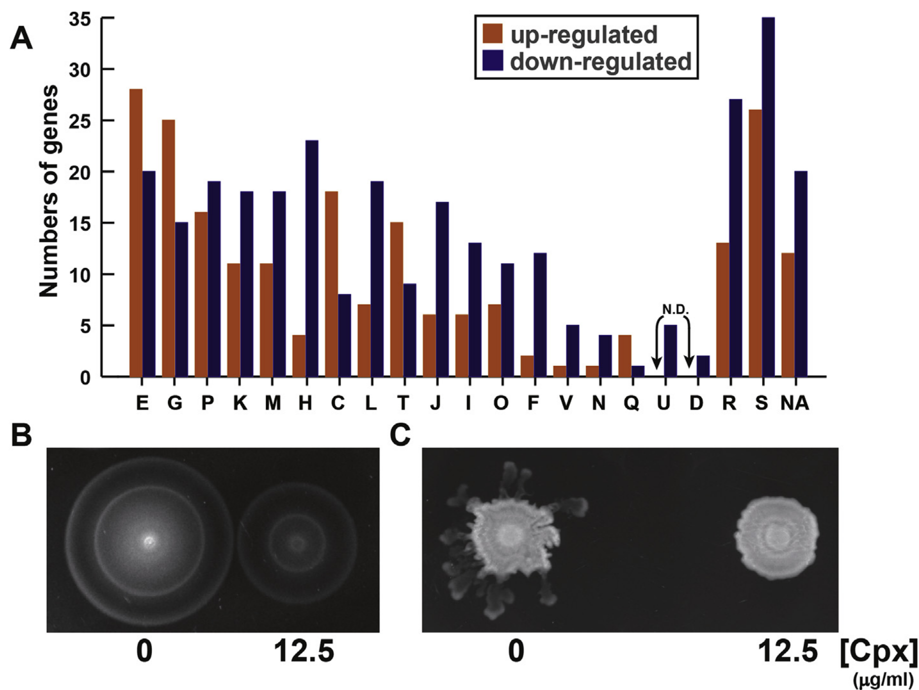

Fig. 1. Global analysis of *Escherichia coli* genes and pathways affected by ciclopirox. (A) Cellular pathways affected by ciclopirox. A total of 514 genes strongly regulated by ciclopirox were categorised into 21 eggNOG terms. The number of genes categorised into individual eggNOG terms is indicated. N.D. indicates that the number of hits is zero. The eggNOG categories are as follows: C, Energy production and conversion; D, Cell cycle control, cell division, chromosome partitioning; E, Amino acid transport; F, Nucleotide transport and mechanism; G, Carbohydrate transport; H, Coenzyme transport; I, Lipid transport; J, Translation, ribosomal structure and biogenesis; K, Transcription; L, Replication, recombination and repair; M, Cell wall/membrane/envelope biogenesis; N, Cell motility; O, Post-translational modification, protein turnover and chaperones; P, Inorganic ion transport; Q, Secondary metabolites biosynthesis, transport and catabolism; R, General function prediction only; S, Function unknown; T, Signal transduction mechanisms; U, Intracellular trafficking, secretion and vesicular transport; V, Defence system; NA, Not available. **(B–C)** Motility assays. Swimming **(B)** and swarming **(C)** motilities were analysed in the presence and absence of ciclopirox at a sublethal concentration. Data from one representative experiment are shown (n = 3).

Please cite this article in press as: J. Shin, et al., Role of acid responsive genes in the susceptibility of *Escherichia coli* to ciclopirox, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.04.063

Download English Version:

https://daneshyari.com/en/article/8292844

Download Persian Version:

https://daneshyari.com/article/8292844

Daneshyari.com