SCREENING FOR INHIBITORS OF Hfq-MEDIATED RNA METABOLISM

Ayako Takada[1], Masaaki Wachi[2]; [1]Technical Department, and [2]Department of Bioengineering,
Tokyo Institute of Technology, Yokohama 226-8501, Japan
takada.a.aa@m.titech.ac.jp

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Introduction. In the chemotherapy of bacterial infectious diseases, the emergence of multidrug-resistant strains is becoming a serious problem. One of the strategies to overcome this problem is to develop new antibiotics with a new molecular target.
The Hfq protein is known as an RNA chaperone. An increasing amount of evidence has been accumulated showing that Hfq pleiotropically regulates the expression of many genes by mediating interaction of non-coding small RNAs and mRNA. We previously reported that overproduction of Hfq inhibits cell division by suppressing expression of the cell division protein FtsZ (1). We also reported that Hfq is involved in expression of the acid tolerance GAD system, which plays an important role in virulence of pathogenic *E. coli* strains (2).
In this study, we constructed a new screening system for inhibitors of Hfq-medicated RNA metabolism.

Methods. *E. coli* strain JM109/pHFQ701 (1) was used in this study. pHFQ701 carries the *hfq* gene under control of the IPTG-inducible *lac* promoter. Paper discs (ϕ 8 mm) containing sample solutions were placed on LB agar plates containing JM109/pHFQ701 cells and 0.3 mM IPTG. After incubated at 30 °C, recovery of colony formation around the paper disc was observed (Fig. 1).
Actinomycetes were isolated from soil samples on humic acid-vitamin agar plates.

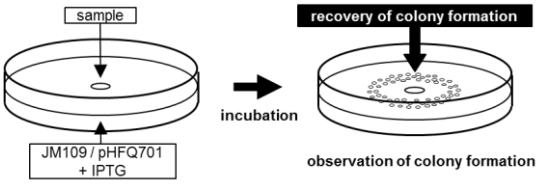

Fig.1 Screening system for inhibitors of Hfq

Results. First we examined the effects of known chemicals and antibiotics in this screening system. Inhibitors for DNA synthesis, protein synthesis and cell wall synthesis did not show any effects. Unexpectedly, colony formation was recovered by RNA synthesis inhibitor, rifampicin (Fig. 2). Recovery was observed at sub-lethal concentrations of rifampicin, i.e., 5 − 10 μg/ml (MIC of rifampicin for *E. coli* is 20 μg/ml).
Then we assayed culture broths of Actinomycetes isolated from soil samples. About 600 samples were assayed by this screening system. Recovery of colony formation was observed for three samples (0.5%). Two of them showed rifampicin-like activity and the other one exhibited a different nature from rifampicin.

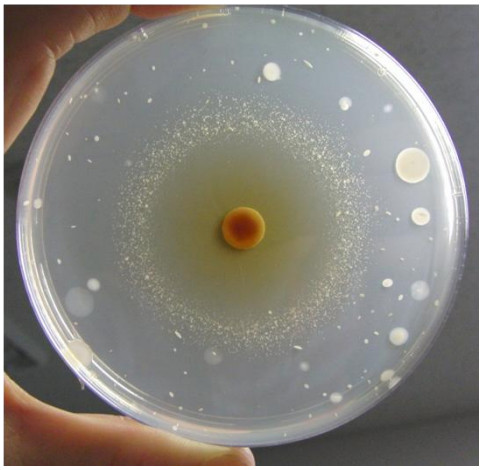

Fig.2 Recovery of colony formation by rifampicin

Conclusions. In this study, we developed a novel screening system for inhibitors of Hfq-mediated RNA metabolism. It was found that RNA synthesis inhibitor, rifampicin, showed positive effect in this assay. Moreover, three screening hits were found from about 600 Actinomycetes samples.

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References.

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