# RANDOM MUTAGENESIS OF LANTIBIOTIC LICHENICIDIN IN *E. COLI*

Tânia Caetano[1], Joana Barbosa[1], João Cruzeiro[1], Roderich D. Suessmuth[2], Sónia Mendo[1]
[1]Department of Biology & CESAM, University of Aveiro, 3810 Aveiro, Portugal
[2]Department of Chemistry, Technical University of Berlin, D-10623 Berlin, Germany

tcaetano@ua.pt

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**Introduction.** Lichenicidin is a two peptide lantibiotic, naturally produced by *Bacillus licheniformis*, which is active against clinically relevant bacteria (e.g. methicillin-resistant *Staphyloccocus aureus* - MRSA)[1]. The bioactivity of two peptide lantibiotics results from the synergistic activity of two structurally different peptides. Lichenicidin was the first lantibiotic produced totally *in vivo* in *E. coli,* using a fosmid containing the complete biosynthetic gene cluster (Fig. 1A)[1]. These antibacterial peptides are more amenable to bioengineering-based strategies, since they are gene-encoded[2]. In this study, a random mutagenesis library of each lichenicidin peptide (Bliα and Bliβ; Fig. 1B) was generated in *E. coli* and screened, taking in consideration improved or lack of activity.

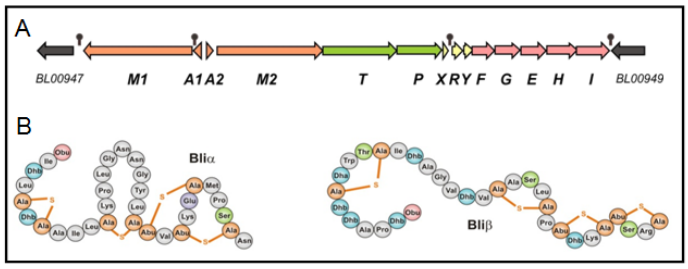


**Figure 1**: Representation of lichenicidin biosynthetic cluster (A) and structure of lichenicidin peptides after posttranslational modifications (B).

**Methods.** Random mutagenesis of each lichenicidin structural gene (*licA1* and *licA2*) was performed with GeneMorph II Random Mutagenesis Kit (Agilent). After amplification, the genes were cloned in the pUC19a vector. Two libraries were obtained by transformation of *E. coli* strains containing the complete *lic* biosynthetic cluster, but lacking either *licA1* (*E. coli* BLic5ΔA1) or *licA2* (*E. coli* BLic5ΔA2) genes. The clones of each library were screened against *M. luteus* indicator strain, using agar medium supplemented with the complementary peptide.

**Results.** About 3000 clones for the Bliα peptide and 2200 for the Bliβ peptide were screened (Fig. 2). Among those, 1625 and 1249 showed reduced or null bioactivity for the α and β peptide, respectively. Among these, 100 clones from each library were selected for sequencing. Some of the identified mutations occurred in essential amino acids of the propeptide (Ser, Thr and Cys). These residues are involved in the formation of lanthionine and methyllanthionine thioether rings, which are essential for the structure and bioactivity of these compounds[1]. Moreover, mutations on the leader sequence were also identified. However, sequencing analysis revealed that more than a single mutation was generally present in each structural gene, making the interpretation of the results more difficult. Further studies of these mutations can contribute for the identification of residues involved in the recognition of the peptide by its correspondent-modifying enzyme (LicM1 and LicM2). Mass spectrometry analysis will help to understand if the absence mutated peptides are inactive compounds or are simply not produced.

In the first screening, only 90 (Bliα) and 73 (Bliβ) mutants showed potentially increased bioactivity (Fig. 2). Presently, these clones are under study, in order to confirm such phenotype.

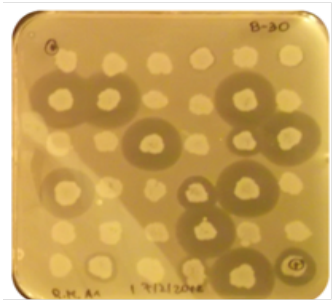

**Figure 2**: Example of a colony-bioassay plate showing mutants with reduced/null and increased activity, when compared with the control (+).

**Conclusions.** Here, we describe a method to generate and screen a library of lantibiotic mutants using the Gram negative host *E. coli*. This strategy resulted in the identification of some variants with improved activity, which are currently under investigation.

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**References.**

1. Caetano T, Krawczyk J, Mösker E, Süssmuth RD, Mendo S. (2011) *Chemistry & Biology*. 18:90-100.
2. Field D, Begley M, O'Connor PM, Daly KM, Hugenholtz F, *et al*. (2012) *PLoS ONE*. 7(10): e46884. doi:10.1371/journal.pone.0046884