# Engineering Improved Variants of Mutacin 1140 by Saturation Mutagenesis

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A. DeFusco[1], A. Fosmoe, II[2], G. Phillips[1], J. Ngoje[1], M. Sivaram[1], F. Anazco[1], W. J. Weiss[3], M. Pulse[3], and **M. Handfield[1]**;

[1] Oragenics, Alachua, FL
[2] AuxThera, Gainesville, FL
[3] University of North Texas Health Science Center, Fort Worth, TX.

**Background**. Mutacin 1140 (MU1140) is a member of a family of ribosomally synthesized peptide bacteriocins called lantibiotics (lanthionine-containing antibiotics) and is produced by *Streptococcus mutans*. MU1140 has been shown to be effective against a broad array of Gram-positive bacteria via a novel mechanism of action termed Lipid-II abduction. Saturation mutagenesis was used in this work to produce single-amino acid variants of MU1140 at all 22 amino acid residues and these were produced and tested in a battery of assays in order to derive useful structure/function data and improve the therapeutic profile of MU1140. **Methods**. A saturated library consisting of over 400 different variants of MU1140 were constructed in collaboration with Intrexon. The addressable library was designed by substituting 19 amino acids codons at each of the 22 amino acid codon positions on a plasmid vector encoding *mutA* or integrated on the chromosome. Plasmids were constructed, transformed into *S. mutans* ΔmutAA', and tested for antimicrobial activity using an *M. luteus* zone of clearing assay. The top 41 variants were selected for future work, reconstructed as chromosomal integrations, and the peptides were purified for characterization. Steps of triage included manufacturability (ability to ferment and purify), MIC (*M. luteus*, *C. difficile*, VREs, *S. pneumonia*, *M. phlei*), solubility, stability by forced degradation and to simulated gastric and intestinal fluids, stability and binding to human serum, CEREP SafetyScreen 44, and HepG2 cytotoxicity. Top performers were used in a Syrian hamster illeal-cecal canulated 21-day efficacy model (cHCDAD) (n=6 per group). **Results**. The top 41 variants (chromosomal integrations) which exhibited greater or equal antimicrobial activity than positive control were subjected to a battery of triage tests, which allowed the culling to 7 variants for animal studies. In the cHCDAD model, the vehicle controls demonstrated 100% mortality by day 9, while Vancomycin-treated animals showed 33% survival. In contrast, the lead compound (OG253) showed 100% survival ($p = 0.0005$ vs vehicle control), following 5 days TID dosing. The other selected peptides displayed varying levels of efficacy ranging from 17% - 67% survival. Post-study analysis of the cecum contents of OG253-treated animals found no detectable *C. difficile* CFUs (≤ 2 Log CFU/g) or Toxin A or B (≤ 0.27 ng/g) compared to appreciably higher levels observed in vehicle controls (4.09 log CFU, 1061 ng/g Toxin A and 848 ng/g B) and in morbid hamsters. **Conclusions**. Saturation mutagenesis of MU1140 allowed us to engineer a novel lantibiotic called OG253 that was efficacious against *C. difficile in vitro* and *in vivo*.