Development of gut-restricted antibiofilm peptides to target gastrointestinal biofilms

https://doi.org/10.17952/37EPS.2024.P1134

Bernhard Jandl[1,2,3,4], Michaela Lang[4], Athanasios Makristathis[5], Christoph Gasche[4,6] and Markus Muttenthaler[1,3]

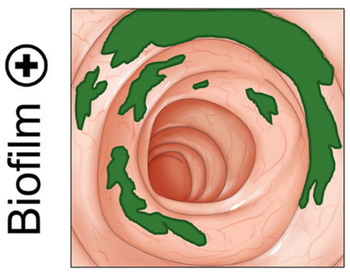
[1]Institute of Biological Chemistry, Faculty of Chemistry, University of Vienna, 1090 Vienna, Austria.
[2]University of Vienna, Vienna Doctoral School in Chemistry (DoSChem), Währinger Str. 42, 1090 Vienna, Austria.
[3]Institute for Molecular Bioscience, The University of Queensland, 4072 Brisbane, Queensland, Australia.
[4]Division of Gastroenterology and Hepatology, Department of Internal Medicine 3, Medical University of Vienna, 1090 Vienna, Austria.
[5]Division of Clinical Microbiology, Department of Laboratory Medicine, Medical University of Vienna, 1090 Vienna, Austria.
[6]Loha for Life, Center for Gastroenterology and Iron Deficiency, 1190 Vienna, Austria.

Email: bernhard.jandl@univie.ac.at

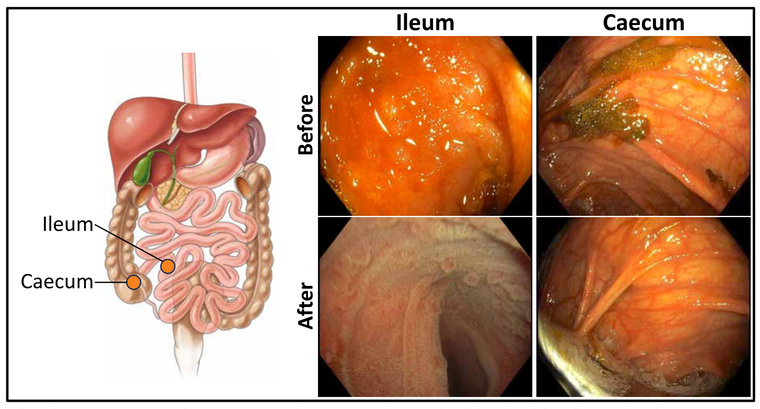
Introduction

Inflammatory bowel diseases (IBD) and **irritable bowel syndrome (IBS)** are gastrointestinal (GI) disorders that together **affect 10–15%** of the Western population. A recent study identified **mucosal biofilms** in **57% of IBS, 34% of ulcerative colitis (UC)** and **22% of Crohn's disease (CD) patients** compared with **6%** in the **control group**.[1] No drug is on the market that selectively targets biofilms and conventional antibiotics are mostly ineffective, leaving jet-washing during endoscopy the only way to remove gut biofilms.

This work explores the potential of antimicrobial peptides (AMP) as antibiofilm agents and investigates chemical strategies to improve potency and gut-stability. Gut-stable antibiofilm peptides are promising therapeutic candidates to target mucosal biofilms in patients with GI disorders, as their large size prevents systemic uptake and reduces side effects by keeping them gut-restricted when orally administered.

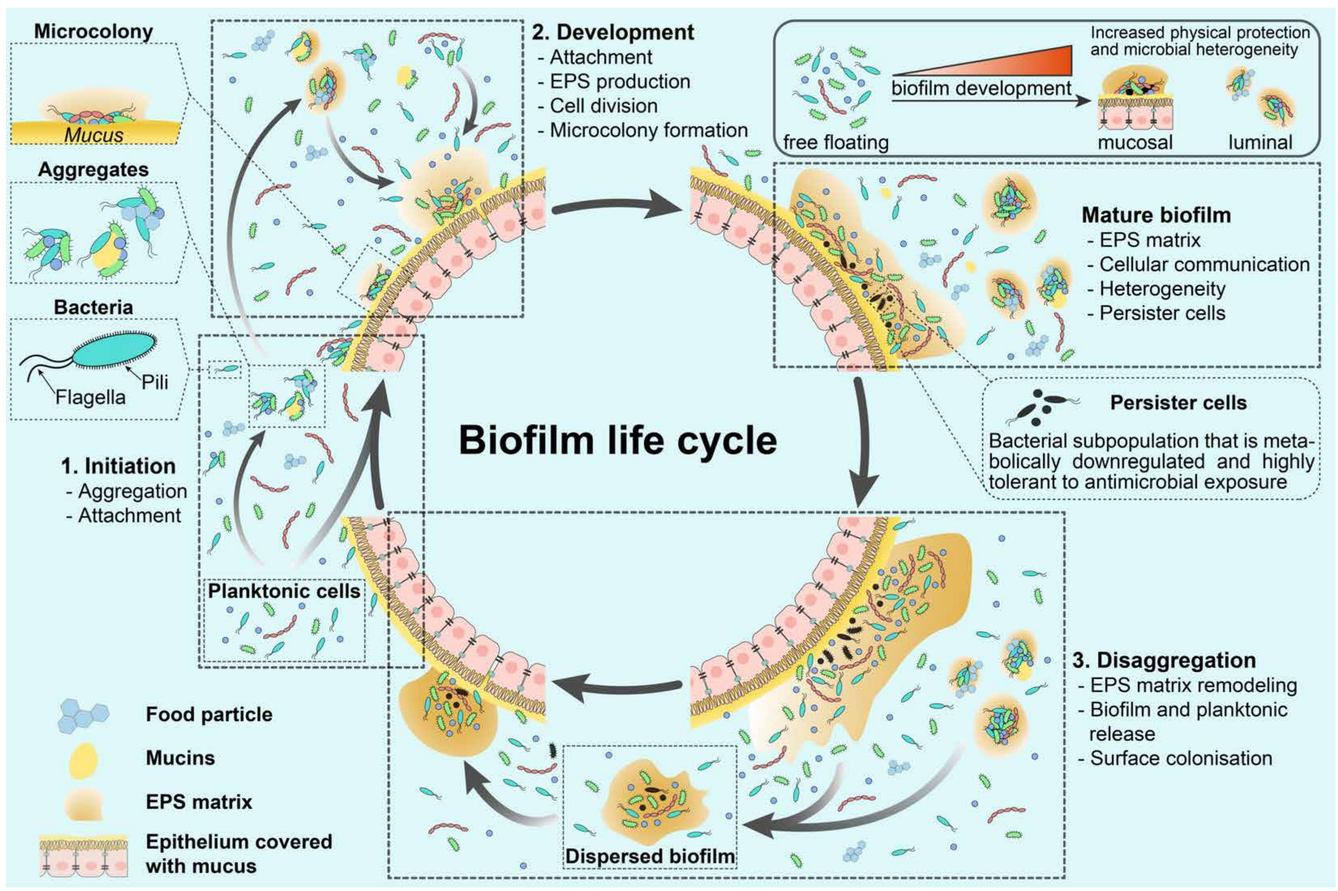


57% of IBS
34% of UC
22% of CD
6% of healthy patients



Colonoscopy images of caecal and ileal biofilm before and after jet-washing.

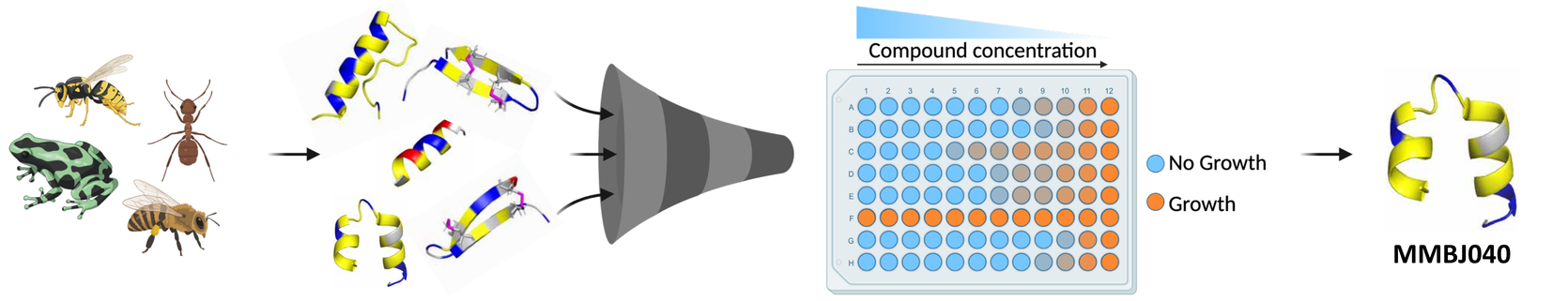
Therapeutic target



The biofilm life cycle. The four stages of the biofilm life cycle are: initial adhesion, early development, biofilm maturation and biofilm dispersal. **Therapeutic approaches** focus on these stages of the biofilm life cycle, aiming to **(i) inhibit bacterial surface adhesion**, **(ii) inhibit biofilm formation**, and **(iii) eradicate mature biofilms**.

Approach

We have chemically synthesized a medium-size AMP compound library (>40 peptides), including peptides produced by ants, bees, frogs, and wasps. By screening of our library we identified 16 hits with promising antibiofilm activity. Out of these hits, we selected **MMBJ040** to conduct a systematic **structure-activity relationship (SAR)** using diverse medicinal chemistry approaches.



1) Synthesis of an AMP library **2) Antibiofilm screening using clinical isolates** **3) Hit to lead**

Study design

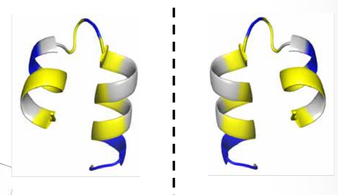
We applied Chemical strategies such as truncation, lipidation and the establishment of mirror images and evaluated the **minimum inhibition concentration (MIC)**, **minimum biofilm inhibition concentration (MBIC)**, and **minimum biofilm eradication concentration (MBEC)** using two clinical isolates from biofilm-positive patients (*Streptococcus parasanguinis* (Gram-positive (G^+)) and *Escherichia coli* (Gram-negative (G^-))) and two biofilm-forming type strains (*Staphylococcus aureus* (G^+) and *Pseudomonas aeruginosa* (G^-)). Further, we determined the gut stability of our best-performing candidates using **simulated gastric fluid (SGF)** and **simulated intestinal fluid (SIF)** assays.

Truncation

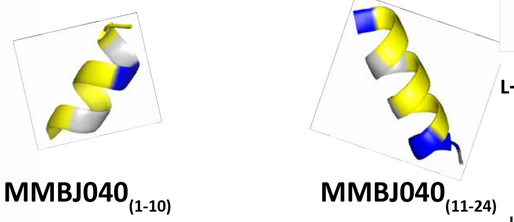
	Net charge	Hydrophobicity
MMBJ040	+5	58%
$MMBJ040_{(1\text{-}10)}$	+1	70%
$MMBJ040_{(11\text{-}24)}$	+4	50%

Mirror images

MMBJ040

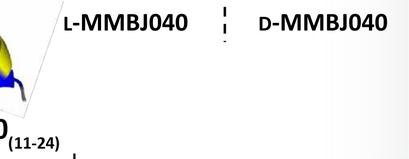


L-MMBJ040 **D-MMBJ040**



$MMBJ040_{(1\text{-}10)}$ **$MMBJ040_{(11\text{-}24)}$**

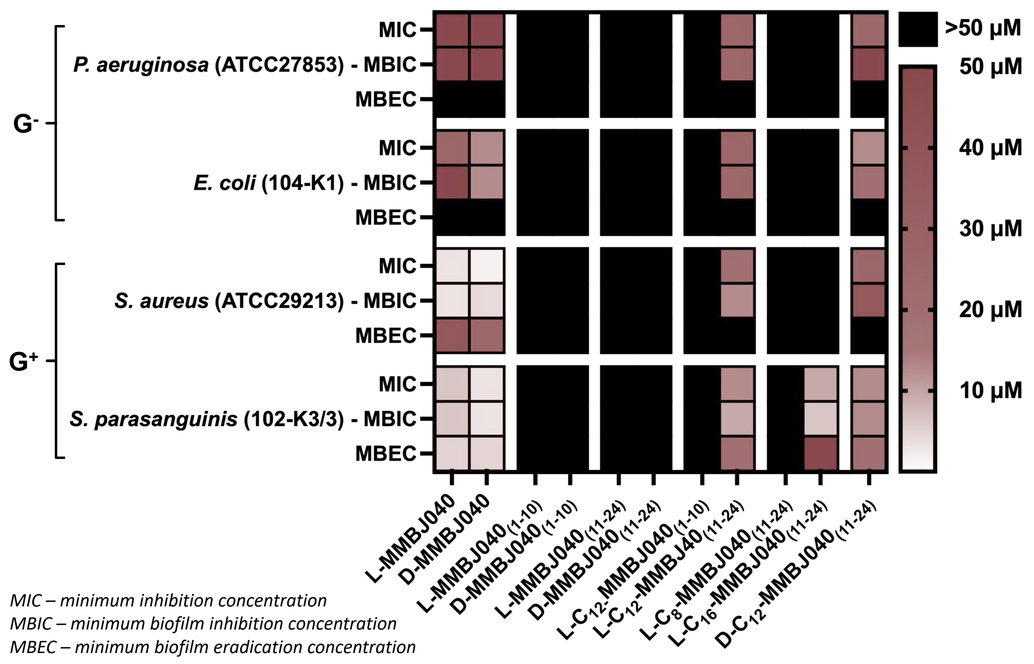
Lipidation



blue – positively charged residues, red – negatively charged residues and yellow – hydrophobic residues

Results

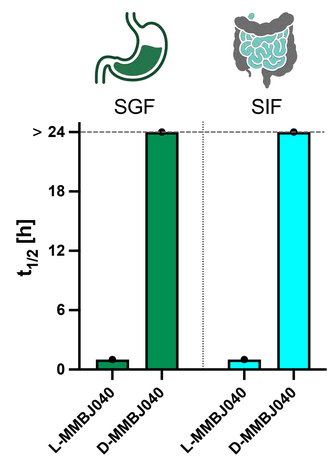
Antibiofilm activity



MIC – minimum inhibition concentration
MBIC – minimum biofilm inhibition concentration
MBEC – minimum biofilm eradication concentration

Gut stability

Half-life ($t_{1/2}$) of **L-MMBJ040 [<1 min]** and **mirror image D-MMBJ040 [>24 h]** in **simulated gastric fluid (SGF)** and **simulated intestinal fluid (SIF)**.



Truncation: Our MMBJ040 SAR exploration revealed that the two truncated α-helical domains $MMBJ040_{(1\text{-}10)}$ and $MMBJ040_{(11\text{-}24)}$ had no antimicrobial or antibiofilm activity. **Lipidation:** The lipidation of $MMBJ040_{(11\text{-}24)}$ reestablished antimicrobial activity but did not increase the antibiofilm activity, and C_{12} fatty acid conjugation led to the best result; lipidation of $MMBJ40_{(1\text{-}10)}$ did not display antimicrobial activity. **Mirror images:** All-D versions of active all-L parent peptides were also active. **D-MMBJ040** has the most potent antibiofilm activity.

In conclusion, we developed the gut-stable peptide **D-MMBJ040** with potent antibiofilm activity against G^+ biofilm-forming bacteria. Moreover, we identified that fatty acid substitution of hydrophobic domains in antimicrobial peptides could serve as an attractive approach to lower the production costs of antimicrobials.

Acknowledgement

We gratefully acknowledge financial support by the Vienna Science and Technology Fund (WWTF) – funding: LS18-053

Reference [1] Baumgartner, M. *et al*, Mucosal Biofilms Are an Endoscopic Feature of Irritable Bowel Syndrome and Ulcerative Colitis. *Gastroenterology* **2021**, *161* (4), 1245-1256.e20.