Approaches to Enhancing Mucus Production to Counteract Infection

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Para a minha família.

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ABSTRACT

Mucosal surfaces are covered with mucus that protects the underlying epithelium from pathogens and particles. As the primary routes through which pathogens enter the host, mucosal surfaces rely on this mucus layer as a critical first line of defense. Mucins - highly *O*-glycosylated glycoproteins - are synthesized and secreted by mucous and goblet cells, and they play key roles in pathogen binding, clearance, and modulation of microbial growth and virulence. *Helicobacter pylori* is the most common gastric pathogen, it is associated with chronic inflammation of the gastric mucosa, and in some cases progression to gastric adenocarcinoma. While *H. pylori* can attach to gastric epithelial cells, it predominantly resides within the mucus layer where it interacts with mucins. Similarly, *Aeromonas salmonicida* is an opportunistic pathogen responsible for furunculosis in rainbow trout and other fish. Mucins can bind to *A. salmonicida* and regulate its growth.

In this thesis, I investigated the use of compounds known to affect mucin production to enhance and restore mucin biosynthesis and assessed how these changes influence pathogen localization and host inflammation. Mice infected with *H. pylori* were treated with Interleukin-4, (R)-α-methylhistamine, Rebamipide, Roxatidine, or a combination of the latter two compounds. These treatments exhibited gastroprotective effects by reducing inflammation scores. Using metabolic labeling by GalNAz incorporation, we observed increased mucin biosynthesis following treatments. This increase in mucin biosynthesis correlated with a reduction in *H. pylori* colonization in the gastric pits. In

rainbow trout, lipopolysaccharide treatment resulted in an increased mucin production in the stomach and intestine. Furthermore, mucins isolated from rainbow trout regulated *A. salmonicida* virulence and reduced the bacterium's ability to auto-aggregate.

These findings suggest that targeting mucosal surfaces to enhance mucin biosynthesis represents a promising strategy to counteract infections caused by mucosal pathogens such as *H. pylori* and *A. salmonicida*. However, further research is necessary to elucidate the regulatory mechanisms of mucin production and secretion, as well as to better understand the functional characteristics of the secreted mucus.

Keywords: mucosal surfaces, mucus, mucins, host, mice, rainbow trout, pathogen, *Helicobacter pylori*, *Aeromonas salmonicida*

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SAMMANFATTNING PÅ SVENSKA

Slem täcker och skyddar slemhinnornas epitel från patogener och partiklar. Detta viskoelastiska hydrogelskikt utgör en viktig första försvarslinje vid kroppens många exponerade ytor, där muciner – stora *O* glykosylerade glykoproteiner – utgör centrala komponenter i den skyddande barriären. Muciner syntetiseras och utsöndras av bägarceller och andra mukösa epitelceller och spelar en nyckelroll i patogenbindning, eliminering samt i regleringen av mikrobiell tillväxt och virulens.

Helicobacter pylori, den mest förekommande gastriska patogenen, är associerad med kronisk inflammation i magslemhinnan och kan i vissa fall leda till ventrikeladenokarcinom. Även om *H. pylori* kan binda till epitelceller i ventrikeln, förekommer bakterien främst i slemlagret där den interagerar med muciner. På liknande sätt kan muciner binda till *Aeromonas salmonicida* – en opportunistisk patogen som orsakar furunkulos hos bland annat regnbågslax – och påverka dess tillväxt och virulensegenskaper.

I denna avhandling har jag undersökt effekten av flera substanser med känd förmåga att påverka mucinbiosyntesen, i syfte att förstärka slemlagrets skyddande funktion samt studera deras inverkan på patogenlokalisering och slemhinneinflammation. I en musmodell infekterad med *H. pylori* testades interleukin 4, (R)-α-metylhistamin, Rebamipide, Roxatidine samt en kombination av Rebamipide och Roxatidine. Dessa behandlingar uppvisade tydliga gastroprotektiva effekter, med minskade inflammationspoäng som följd. Med hjälp av metabol märkning via GalNAz-inkorporering observerades en ökad mucinbiosyntes efter behandling, vilket korrelerade med minskad kolonisation av *H. pylori* i magsäckens kryptor. Hos regnbågslax inducerade behandling med lipopolysackarid en ökad mucinproduktion i både ventrikeln och tarmen. Vidare visade isolerade muciner från regnbågslax en hämmande effekt på *A. salmonicidas* virulens och minskade dess förmåga till autoaggregering.

Sammanfattningsvis tyder dessa resultat på att riktad stimulering av mucinbiosyntes utgör en lovande strategi för att motverka infektioner orsakade av mukosala patogener såsom *H. pylori* och *A. salmonicida*. Ytterligare studier krävs dock för att klarlägga de molekylära regleringsmekanismerna bakom mucinproduktion och sekretion samt för att fördjupa förståelsen av slemmets biologiska och biokemiska egenskaper.

RESUMO EM PORTUGUÊS

O muco que reveste as superfícies das mucosas desempenha um papel fundamental na proteção do epitélio sujadjacente. A integridade do muco é fundamental enquanto barreira de defesa primária do hospedeiro contra agentes patogénicos e partículas. Mucinas – glicoproteínas altamente *O*-glicosiladas – são sintetizadas e secretadas por células mucosas e caliciformes; têm um papel chave na adesão e eliminação de agentes patogénicos, bem como na modulação da virulência e crescimento microbianos.

Helicobacter pylori é o patogénio gástrico mais comum, e está associado ao desenvolvimento de inflamação crónica da mucosa do estômago, podendo em alguns casos progredir para adenocarcinoma gástrico. *H. pylori* pode ser encontrada ligada a células epitiliais, no entanto a maíoria reside dentro da camada de muco, interagindo diretamente com as mucinas. De forma semelhante, *Aeromonas salmonicida* é um patogénio oportunista que causa furunculose em truta arco-íris e outros peixes. Sabe-se que as mucinas podem ligar *A. Salmonicida* e regular o seu crescimento.

Nesta tese, foi investigado o uso de compostos conhecidos por influenciar a produção de mucinas, de modo a aumentar e restaurar a biossíntese de mucinas e avaliar como estas alterações afectam a localização da bactéria e a inflamação do hospedeiro. Os ratinhos foram infetados com *H. pylori* e tratados com Interleucina 4, (R)-α-metil-histamina, Rebamipide, Roxitadine ou com a combinação dos dois últimos. Estes tratamentos demostraram efeito gastro-protectivo ao reduzir inflamação gástrica. Ao utilizar uma marcação metabólica por incorporação de GalNAz, observamos que a biossíntese de mucinas aumentou após o tratamento. Este aumento em biossíntese de mucinas está relacionado com uma redução da colonização de *H. pylori* nas criptas gástricas. Em trutas arco-íris tratadas com lipopolisacarideo, o tratamento resultou num aumento de produção de mucinas no estômago e no intestino. Verificou-se ainda que mucinas isoladas de truta arco-íris influenciam a virulência de *A. Salmonicida* e reduziram a capacidade de autoagregação desta bactéria.

Tendo em conta os resultados obtidos, podemos concluir que as terapias alvo dirigidas às superfícies mucosas podem constituir uma estratégia promissora no combate às infeções causadas por *H. pylori* e *A. Salmonicida*. Dada a complexidade de funcionamento dos mecanismos regulatórios de produção e secreção de mucinas bem como as características funcionais do muco

secretado, é ainda necessária uma investigação contínua e aprofundada desta temática.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Santos. L, Sharba S, Benktander J, Ojaimi Loibman S, Quintana-Hayashi MP, Erhardsson M, Lindén SK. *Treatment with (R)-α-methylhistamine or IL4 stimulates mucin production and decreases Helicobacter pylori density in the murine stomach*.
Virulence. 2025;16(1).

II. Santos. L, Sharba S, Dolan B, Benktander J, Lindén SK. *Rebamipide and Roxatidine restore mucin biosynthesis and decrease Helicobacter pylori density in the mouse stomach*.
Manuscript.

III. Sharba. S, Sundh H, Sundell K, Benktander J, Santos L, Birchenough G, Lindén SK. *Rainbow trout gastrointestinal mucus, mucin production, mucin glycosylation and response to lipopolysaccharide.*
Fish Shellfish Immunol. 2022;122:181-90.

IV. Ojaimi Loibman S, Quintana-Hayashi MP, Santos L, Lindén SK. *Aeromonas salmonicida AI-1 and AI-2 quorum sensing pathways are differentially regulated by rainbow trout mucins and during in vivo colonization*.
Fish & Shellfish Immunology. 2024;153:109862

CONTENTS

ABBREVIATIONS

NB: Protein names are written in all uppercase letters. Human genes are written in all uppercase letters in *italics*, while mouse genes begin with an uppercase letter followed by lowercase letters in *italics*.

A. salmonicida	*Aeromonas salmonicida*
AB-PAS	Alcian blue-Periodic acid-Schiff
AI	Autoinducer
AlpAB	Adherence-associated lipoproteins A and B
AMPs	Antimicrobial peptides
BabA	Blood group antigen-binding adhesin
cagA	Cytotoxin-associated gene A
cagPAI	cag-pathogenicity island
C. rodentium	*Citrobacter rodentium*
CFU	Colony-forming unit
COXs	Cyclooxygenases
Defb1	Beta-defensin 1 gene
ER	Endoplasmic Reticulum
ETEC	Enterotoxigenic *Escherichia coli*
FISH	Fluorescent *in situ* hybridization
FlaA	Flagellin A
GalNAc	N-acetylgalactosamine

GalNAz	N-azidoacetylgalactosamine
GI	Gastrointestinal
H. pylori	*Helicobacter pylori*
IFN-γ	Interferon-gamma
IL4	Interleukin 4
LabA	LacdiNAc-binding adhesin
LC/MS	Liquid chromatography-electrospray ionization tandem mass spectrometry
Le^b	Lewis b
LPS	Lipopolysaccharide
LTF	Lactoferrin
MALT	Mucosa-associated lymphoid tissue
MAPK	Mitogen-activated protein kinase
MMP-9	Matrix metalloproteinase-9
MUC	Mucin
MyD88	Myeloid differentiation factor 88
NFκB	Nuclear factor κ B
NO	Nitric oxide
OipA	Outer inflammatory protein A
PAS	Periodic acid-Schiff
PGE_2	Prostaglandin E_2

PPI	Proton pump inhibitors
PTGS	Prostaglandin endoperoxide synthase
PTS	Proline, threonine, and serine
QS	Quorum-sensing
Reb	Rebamipide
RebRox	Rebamipide and Roxatidine
Rox	Roxatidine
RαMH	(R)-α-methylhistamine
SabA	Sialic acid-binding adhesin
sLe	Sialyl-Lewis
SLPI	Secretory leukocyte protease inhibitor
SPDEF	SAM pointed domain-containing ETS transcription factor
STAT6	Signal transducer and activator of transcription 6
T4SS	Type IV secretion system
TFF	Trefoil factor family
Th	T helper
TLR4	Toll-like receptor 4
TNFα	Tumor necrosis factor-alpha
UreA	Urease subunit alpha
VacA	Vacuolating cytotoxin A

WT	Wild-type

1 INTRODUCTION

1.1 MUCOSAL SURFACES

Mucosal surfaces such as those of the surface of the eye, respiratory, gastrointestinal (GI), and urogenital tracts are key interface sites between the body and the external environment. As a result, they serve as the most common interaction points for microorganisms and the body. Mucosal surfaces are part of a key component of the innate immune system and are protected by a secreted mucus layer. This mucus layer is important for both normal tissue function and protection of the underlying epithelium against pathogens and other foreign material [1, 2].

1.1.1 MUCUS

Mucus is a gel-like secretion that covers the epithelium of the mucosal surfaces. Mucus is synthesized and secreted by specialized mucous and goblet cells [3, 4]. The secretion rate and properties of mucus are organ-specific and vary according to physiological needs - for example, facilitating nutrient absorption in the small intestine, forming a barrier against microorganisms in the colon, and providing protection from luminal acidity and enzymatic digestion in the stomach [5-7].

A secreted mucus layer covers the mucosal surfaces and protects the underlying epithelium by trapping and removing pathogens and/or particles. It also functions as a barrier, preventing microorganisms from directly accessing epithelial cells [2]. The thickness of the secreted mucus layer varies with anatomical site; 1-7 μm in corneal and conjunctival surfaces [8], 200-300 μm in the stomach, 200–400 μm in the small intestine, up to 800 μm in the colon [9]. In the female reproductive tract, mucus thickness and viscosity vary with the menstrual cycle [10].

There are two types of mucus: non-adherent and adherent. Non-adherent mucus is penetrable and typically found in the airways and small intestine [6, 11, 12]. In the GI tract, it is continuously removed by the movement of luminal contents such as food and fecal matter. This mucus is formed from the adherent one by detachment from the epithelial surface and volume expansion [13]. Adherent mucus, on the other hand, forms a firm layer directly attached to the epithelium and is impenetrable, maintaining a physical separation between

epithelial cells and microorganisms as found in the colon [6]. In the stomach and duodenum, mucus protects the epithelium from luminal acidity (via bicarbonate secretion) and from proteolytic digestion by host proteases such as pepsin [7].

Mucus is composed of approximately 95% water and contains salts, lipids, and proteins such as defensins, immunoglobulins, lysozymes, lactoferrin, growth factors, and trefoil factors, as well [7, 14-17]. Mucins, highly glycosylated glycoproteins, are the major protein element of the mucus and serve as the key structural component of the secreted mucus layer [12, 15]. Mucus secretion occurs either at a low constitutive level or is accelerated in response to external stimuli. The constant production and secretion of mucins maintains the mucus layer [18].

1.1.2 MUCINS

Mucins are high-molecular-weight glycoproteins heavily *O*-glycosylated and are characterized by the presence of at least one mucin domain. Mucins contain a backbone characterized by tandem repeats of the amino acids proline, threonine, and serine (PTS). In the endoplasmic reticulum (ER), the mucin backbone undergoes dimerization at the C-terminal [12]. In the Golgi apparatus, the PTS regions undergo extensive *O*-glycosylation, resulting in the formation of the characteristic mucin domains. The glycans coating the mucin domains give mucins their characteristic “bottlebrush” appearance. Following glycosylation, the molecules are further oligomerized at their N-termini. After oligomerization and *O*-glycosylation, mucins are packed and dehydrated in secretory granules localized in the theca of mucous/goblet cells [19, 20]. Mucins can be classified as transmembrane or secreted [12].

Transmembrane mucins - MUC1, MUC3A/B, MUC4, MUC12, MUC13, MUC15, MUC16, MUC17, MUC21, and MUC22 - are characterized by an extracellular N-terminal mucin domain and a short C-terminal cytoplasmic tail [21]. These mucins are localized to the apical surface of epithelial cells and are a key structural component of the glycocalyx. Some transmembrane mucins exhibit tissue-specific expression; for example, MUC13 and MUC17 are localized in the intestine [22, 23]. In contrast, MUC1 is also expressed in cell types outside of mucosal surfaces and can be differently expressed or overexpressed in pathological conditions [24-27]. As key components of the glycocalyx, transmembrane mucins restrict access to epithelial cells and serve as a barrier against pathogens [2, 28].

Secreted mucins can be further classified into monomeric and polymeric/gel-forming types. MUC7 and MUC20 are the only secreted monomeric mucins identified to date. MUC7 is present in saliva, while MUC20 is found in the kidneys and urinary tract [29, 30]. These are the only secreted mucins that do not contribute to the viscous properties of mucus [12]. There are four gel-forming secreted mucins: MUC2 is found in the intestine; MUC5B in the respiratory tract and saliva; MUC5AC in the respiratory tract and stomach; and MUC6 in the stomach [3, 31-34]. Following secretion, gel-forming mucins become rapidly hydrated and expand 1000-fold. The formation of a functional secreted mucus layer is reliant on further N-terminal oligomerization as seen for MUC2 and MUC5AC, leading to the formation of complex net-like structures, which form a glycoprotein scaffold for the mucus layer [19, 20, 35].

1.1.3 MUCIN-COMMENSAL INTERACTIONS

Commensal microbiota has evolved to colonize the host without causing disease or disrupting the mucus layer. These bacteria rely on mucin-derived nutrients while contributing to host health by synthesizing vitamins and degrading complex carbohydrates from food. They are also relevant to the development of the host's immune system [36]. The gut commensal bacteria possess binding proteins/adhesins. *Lactobacillus reuteri* (lactobacilli found in the GI tract of several animals) produces a cell-surface protein, Mub, which adheres to mucus components [37]. Commensal microbes also produce glycan-degrading enzymes that enable them to cleave and utilize mucin *O*-glycans as an energy source without disrupting the host's mucus layer [36, 38]. For example, *Akkermansia muciniphila* and *Bacteroides thetaiotaomicron* have glycoproteases and glycoside hydrolases that allow them to degrade the protein core of the mucin domains and utilize *O*-glycans, respectively [36].

1.1.4 MUCIN-PATHOGEN INTERACTIONS

Unlike commensal bacteria, pathogens have evolved mechanisms to overcome and/or exploit host defenses such as the mucus barrier. Pathogens express binding proteins that contribute to host cell attachment, invasion, and disease progression [39]. *Campylobacter jejuni*, the most common cause of bacterial diarrhea, binds to intestinal epithelial cells via histo-blood group antigens [40]. *H. pylori* adhesins and interactions with mucins will be discussed in more detail later in this thesis (Section 1.3.2). Pathogens also produce proteases that degrade and dissolve the protective mucus, facilitating direct contact with the underlying epithelium and, in some cases, epithelial invasion [36]. For example, the EatA protease from enterotoxigenic *Escherichia coli* (ETEC) and

its homolog in *Shigella* have been shown to rapidly degrade human MUC2, but not mouse [41, 42]. Similarly, the Pic protease secreted by *Citrobacter rodentium* exhibits glycoprotease activity, breaking down the mucus barrier [43].

Mucins can bind and remove pathogens and are involved in regulating bacterial growth and virulence [2, 44-46]. Mucins also function as a steric barrier and act as releasable decoys that prevent pathogen binding to epithelial cells [28]. In Muc2-deficient mice, bacteria come into direct contact with the intestinal epithelium, leading to heightened inflammation [5]. These mice also exhibit increased susceptibility to *C. rodentium* infection, with rapid colonization and a higher bacterial burden compared to wild-type (WT) mice [47]. These findings indicate that mucins are crucial to host defense and may facilitate the transport of pathogens toward the lumen and away from the epithelial surface.

1.2 COMPOUNDS INDICATED TO AFFECT MUCIN AND/OR MUCUS PRODUCTION

Mucus production and mucin biosynthesis can be affected by several stimuli, including inflammatory and immune response mediators, such as interleukin 4 (IL4), bacterial products like lipopolysaccharide (LPS), and pharmaceutical compounds [48, 49].

The compounds used in Papers I-III will be further discussed below.

1.2.1 INTERLEUKIN 4

IL4, produced by mast cells, T cells, eosinophils and basophils, plays multiple roles in innate and adaptive immunity. During infection, it contributes to increased antibody response [50]. In mouse airways, IL4 increases Alcian blue - Periodic acid-Schiff (AB-PAS) positive staining and *Muc5AC* mRNA expression, indicating increased mucus production [51]. *In vitro,* studies in colorectal cells have shown that IL4 increased the mucus production demonstrated by AB-PAS staining [48]. Similarly, the authors [48] have shown that IL4 enhances mucin production and cellular trafficking using mice metabolically labeled by *N*-azidoacetylgalactosamine (GalNAz) incorporation and detection by Click-IT chemistry. Furthermore, IL4 influences mucus thickness and pathogen localization. IL4 was shown to affect mucus production via the STAT6/SPDEF pathway [48].

1.2.2 (R)-ALPHA-METHYLHISTAMINE

(R)-α-methylhistamine (RαMH) is a selective agonist of the histamine H_3 receptor [52]. In rats, treatment with RαMH has been shown to increase both the number and size of mucous cells in the stomach, as evidenced by AB-PAS staining, and to enhance the thickness of the secreted mucus layer [53]. Immunohistochemical analyses using BrdU incorporation, a thymidine analog, further demonstrated that RαMH promotes gastric epithelial cell proliferation in rats [54, 55]. Additionally, H_3 receptor agonists like RαMH are suggested to contribute to maintaining gastric mucosal integrity, potentially through the modulation of prostaglandin E_2 (PGE_2) production [56].

1.2.3 REBAMIPIDE

Rebamipide (Reb) is commonly used across Southeast Asia, Russia and other countries to treat peptic ulcers [57, 58]. Reb is classified as an anti-ulcer agent that can be used to treat acute and chronic gastritis and peptic ulcers [59]. Clinical studies show that Reb promotes ulcer healing in humans [60], reduces radiation-induced intestinal injury in mice, and lowers levels of inflammatory cytokines [61, 62]. Additionally, Reb has been found to increase *Muc2* mRNA expression, the number of goblet cells in the colons of healthy mice [62]. and to enhance the mucus layer thickness, as observed by PAS staining [63]. Previous studies have shown that Reb upregulates prostaglandin endoperoxide synthase 2 (*Ptgs2*) mRNA expression and enhances the production of PGE_2 [60, 63-65]. PGE_2 likely mediates Reb's effect on increasing mucus production through the upregulation of *Ptgs2* [59, 66]. The mechanisms involving PTGS2, PGE_2, and mucus properties will be discussed later in this thesis (Section 1.6).

In addition, pre-treatment of human gastric carcinoma cells with Reb has been shown to reduce *H. pylori* adhesion [67].

1.2.4 ROXATIDINE

Roxatidine (Rox) is a histamine H_2 receptor antagonist used in the treatment of gastric and duodenal ulcers [68]. It is approved for clinical use in several countries, including South Africa, India, Japan, Korea, and a few European countries [69, 70]. Rox has been reported to reduce the production of proinflammatory cytokines [71]. Treatment with Rox improved the healing of experimentally induced lesions in the rat small intestine [72] and prevented the formation of lesions in the mouse stomach [73]. Moreover, by using the incorporation of radio-labeled glucosamine, several studies have shown that Rox enhances mucin biosynthesis in both the rat intestine [74] and in the mouse

stomach [75, 76]. These effects have been suggested to be mediated via increased production of endogenous nitric oxide (NO) [75].

1.2.5 LIPOPOLYSACCHARIDE

LPS is a component of the gram-negative bacterial cell wall. It is often used as an immune stimulant, activating cells such as monocytes and macrophages, and inducing the production of proinflammatory cytokines [77, 78]. However, LPS can also act directly on the epithelium and the effects of LPS treatment have been linked to stimulation of mucus production [79]. Treating mice with low to moderate doses of LPS results in a higher number of goblet cells and mucin content in the intestine [79]. *In vitro* studies using human colon carcinoma cells and human epidermal stem cell-derived goblet cells show that LPS increases mucin mRNA levels and secretion [80, 81]. In mouse airways, LPS treatment enhances Muc5AC transcriptional and translational levels compared to non-treated mice [82]. LPS increases mRNA mucin production in zebra fish and AB-PAS staining indicated increased mucus in the intestinal tract of common carp (*Cyprinus carpio*) [83, 84].

One study reports that the effect of LPS in enhancing Muc5AC expression is partially mediated by matrix metalloproteinase-9 (MMP-9) [85]. More recent studies also indicate LPS is recognized by Toll-like receptor 4 (TLR4), which activates the nuclear factor κ B (NFκB) and mitogen-activated protein kinase (MAPK) signaling pathways [82, 86].

1.3 *HELICOBACTER PYLORI*

Helicobacter pylori is a gram-negative, flagellated, rod-shaped microaerophilic bacterium. It is the most common gastric pathogen, estimated to infect half of the world's population [87, 88]. The transmission route is often via person-to-person during childhood. Once established, the infection leads to a long-term inflammation of the gastric mucosa [89, 90]. Despite the high prevalence of *H. pylori,* some individuals can remain asymptomatic at times. However, infected people may develop non-ulcer dyspepsia, peptic ulcer disease, mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric adenocarcinoma [89, 91], the fifth most common cause of death due to malignancy [92]. The correlation between *H. pylori* infection and the development of severe gastric disease led the International Agency for Research on Cancer to classify the pathogen as a class 1 carcinogen [93].

To treat *H. pylori* infection, a combination of antibiotics and proton pump inhibitors (PPI) are frequently used. The European *Helicobacter* and Microbiota Study group guidelines of 2022 suggest the use of a quadruple bismuth therapy consisting of PPI, bismuth, tetracycline and metronidazole or a quadruple non-bismuth therapy with PPI, amoxicillin, clarithromycin and metronidazole [90]. The high prevalence of *H. pylori,* together with the bacteria's increasing antibiotic resistance [90], the lack of effective vaccines [94] and the risk of developing gastric cancer after failed eradication therapy [95] contribute to the burden on health care systems, urging the need for alternative therapies.

1.3.1 *H. PYLORI* COLONIZATION FACTORS

H. pylori produces urease, an enzyme that reduces gastric acidity, creating a more appropriate environment for the microorganism in the stomach [96]. The bacteria use their flagella to enter and colonize the mucus layer and migrate towards the epithelial cells [97].

In the stomach, *H. pylori* does not colonize the gastric lumen. The majority of bacteria can be found in the mucus layer and in the neutral environment of the surface epithelium [98]. The attachment to epithelial cells is mediated by *H. pylori*'s adhesins, including the blood group antigen-binding adhesin (BabA), sialic acid-binding adhesin (SabA), LacdiNAc-binding adhesin (LabA), outer membrane proteins HopZ and HopQ, outer inflammatory protein A (OipA), and adherence-associated lipoproteins A and B (AlpAB) [44, 99-106]. This prevents the bacteria from being easily removed from the gastric niche and allows them to obtain nutrients and deliver toxins [107].

1.3.2 *H. PYLORI* INTERACTIONS

H. pylori can adhere to glycan structures present on epithelial cells, such as glycolipids and transmembrane mucins [108-110]. These interactions facilitate bacterial colonization and persistence in the gastric niche, preventing displacement by peristalsis and mucus turnover.

BabA specifically recognizes and binds to H type 1 and Lewis b (Le^b) blood group antigens, as well as other related fucosylated structures [99, 100]. SabA, on the other hand, mediates binding to sialylated glycans, including sialyl-Lewis x (sLe^x) and sialyl-Lewis a (sLe^a) blood group antigens [109]. Mucins such as MUC5AC and MUC1 are known to express Le^b antigens, while sialylated glycan structures are also found on mucins [28, 44, 101].

Additionally, *babA* and *sabA* mutants show lower binding ability to mucins [46, 101]. These observations support the role of BabA and SabA in mediating *H. pylori* adhesion to both secreted and membrane-bound mucins [28, 44, 101].

Host glycosylation is altered during infection, influencing the ability of pathogens to interact with host tissues [111]. In the context of *H. pylori* infection, inflammation leads to an upregulation of sialylated glycan structures, including the *de novo* expression of sLe^a and sLe^x, which are ligands for the bacterial adhesin SabA [101, 112]. A study in experimentally infected rhesus monkeys has also shown an increase in sialylation structures [113]. Collectively, these findings suggest *H. pylori* is uniquely adapted to both the healthy and inflamed stomach, driving persistence and lifelong infection.

Mucins isolated from humans and pigs infected with *Helicobacter suis* exhibit reduced growth-inhibitory activity compared to mucins from non-infected counterparts [111]. Additionally, Navabi and colleagues [114] demonstrated that *H. pylori* infection reduces mucin production in mice, suggesting that the bacterium may create a more stable gastric environment by impairing this defense mechanism.

MUC6, secreted by gastric neck mucous cells, exhibits antimicrobial activity against *H. pylori*, helping to restrict the bacterium to the upper regions of the gastric pits [3, 115]. MUC1, a transmembrane mucin expressed by mucous cells, functions as both a physical barrier (via steric hindrance) and a releasable decoy to prevent *H. pylori* adhesion to epithelial surfaces [28].

Mouse models deficient in Muc1 or Muc5AC exhibit significantly higher bacterial loads following *H. pylori* infection compared to their WT counterparts [116, 117]. Collectively, these findings highlight the crucial role of mucins in host defense by mediating bacterial binding, limiting *H. pylori* colonization, and maintaining the integrity of the gastric epithelium.

Trefoil factor family 1 (TFF1) peptide is co-expressed and secreted with Muc5AC and is thought to contribute to the intracellular assembly of the mucin [17, 118]. Studies have shown that *Tff1*-deficient mice exhibit more severe inflammation and increased expression of the pro-inflammatory cytokine tumor necrosis factor alpha (TNFα) mRNA following *H. pylori* infection, compared to infected WT mice [119]. Furthermore, *H. pylori* LPS has been shown to interact with TFF1 [120, 121]. *H. pylori* binding to the peptide was

reported to reduce the bacteria's motility [122]. This suggests that TFF1 may play a protective role during *H. pylori* infection.

1.3.3 OTHER *H. PYLORI* VIRULENCE FACTORS

H. pylori has a preference for binding to highly differentiated pit cells located in the upper regions of the gastric pits, where it translocates its virulence factors, leading to damage to the host tissue [123]. The vacuolating cytotoxin A (VacA) is a toxin that induces vacuole formation, disrupts cellular membranes through pore formation, and triggers apoptosis in target cells [124]. The cytotoxin-associated gene A (*cagA*), located within the cag-pathogenicity island (cagPAI), encodes both the CagA oncoprotein and a type IV secretion system (T4SS). CagA is cytotoxic to the host cells [125].

1.3.4 *H. PYLORI* SS1 STRAIN

H. pylori SS1 is a mouse-adapted strain commonly used to study infection in animal models since it mimics the effects associated with *H. pylori* infection in humans [126, 127]. The SS1 strain can bind to a wide range of glycans and has several virulence factors, such as *ureA*,*flaA*, *cagA* and *vacA*, that contribute to the colonization of the gastric mucosa [126-130]. *H. pylori* SS1 produces a less active form of the VacA toxin, which is associated with reduced inflammatory responses. However, VacA remains essential for colonization, as strains expressing low-activity VacA colonize the murine stomach more effectively than *vacA*-null mutants [129]. In the SS1 strain, the cagPAI-harbored T4SS is non-functional, largely due to a defective CagY protein. Nevertheless, the strain is still categorized as CagA-positive [126, 131, 132]. Additionally, in C57BL/6 mice, *H. pylori* SS1 infection does not progress from chronic gastritis to gastric carcinoma; it only results in non-malignant epithelial alterations [127].

1.4 MOUSE STOMACH

The mouse's stomach is divided into the forestomach and the glandular stomach. The forestomach is non-glandular, lined with stratified squamous epithelium. The glandular stomach is lined by a single columnar epithelium, forming gastric glands, and is further divided into corpus and antrum. The corpus has parietal cells responsible for producing hydrochloric acid, chief cells that produce pepsinogen, and mucous cells. The antral pits only have mucous cells [7, 133]. There are two types of mucous cells in the stomach: the surface mucous cells that secrete Muc5AC and TFF1, and the neck mucous

cells that produce Muc6 and TFF2 [17, 134, 135]. Additionally, in rodents, the loss of parietal cells is associated with mucous metaplasia [127, 134]. Previous studies have reported that the firmly adherent mucus layer in the mouse stomach is penetrable to fluorescent microbeads and measures approximately 40–45 μm in thickness [6, 136]. This epithelial-adherent mucus functions as a slow diffusion barrier, establishing a pH gradient varying from 1–2 in the lumen to approximately 7 near the epithelial surface [6].

1.5 MUCOSA-ASSOCIATED LYMPHOID TISSUE

Mucosal surfaces are exposed to the external environment, colonized by commensal organisms and used by pathogens as entry points [1, 36]. The MALT is composed of immune cells and organized lymphoid structures, aiming to maintain symbiosis with commensal organisms and respond to pathogens [137].

1.5.1 ANTIMICROBIAL PEPTIDES

Antimicrobial peptides (AMPs), including defensins, are secreted by epithelial cells in the GI tract and protect the mucosal lining from bacteria, viruses, and fungi [14]. *H. pylori*-infected individuals show increased levels of beta-defensins in the stomach and gastric juice [14, 138]. *H. pylori* SS1 infection in mice and MKN7 cells upregulates beta-defensin 1 mRNA (*Defb1*) [138].

Lactoferrin (LTF) is an iron-binding glycoprotein that binds and promotes iron absorption in the GI tract. LTF has been reported to have antimicrobial activity, inhibiting bacteria's growth by depriving them of iron [139, 140]. *H. pylori*-infected patients have higher levels of LTF in the gastric juice [16]. Similarly, in the Mongolian gerbil model, *H. pylori* infection resulted in higher levels of LTF in the gastric mucosa [141].

Secretory leukocyte protease inhibitor (SLPI) is a serine protease inhibitor with diverse biological roles. SLPI has been reported to have bactericidal activity [142]. The levels of SLPI are reduced in gastric biopsies of *H. pylori*-infected patients and infected cell lines' supernatants [143, 144]. However, the latter shows increased *Slpi* mRNA levels, suggesting post-transcriptional regulation [144].

1.5.2 CYTOKINES

Lymphocytes are an important component of the immune defense of the gastric mucosa. Cytokines secreted by T cells within the gastric lamina propria likely play a key role in promoting the infiltration of polymorphonuclear and mononuclear cells, a hallmark of *H. pylori*-associated gastritis. The T helper (Th) 1 type response is characterized by IL2 and interferon-gamma (IFN-γ) linked to cellular immune responses. In contrast, the Th2 type response is characterized by expression of cytokines as IL4 and IL13, promoting antibody-mediated immunity [145].

During *H. pylori* infection in humans, primates, and mice, a predominant Th1-type immune response occurs, characterized by the recruitment of T lymphocytes producing IFN-γ to the stomach, alongside low levels of Th2-type lymphocytes expressing IL4 [145-148]. Additionally, IFN-γ-null mice do not show inflammation even during chronic *H. pylori* infection. This suggests that IFN-γ is needed for the host's inflammatory response against the bacteria [148].

TNFα is a proinflammatory cytokine that may play a role in inflammation-associated carcinogenesis [149]. In humans, TNFα levels are elevated during *H. pylori* infection. However, infection and the levels of TNFα do not correlate with the risk of developing gastric cancer [150]. Similarly, experimental *H. pylori*-infected primates exhibit increased TNFα levels, detectable as early as one week post-infection [145]. All the above may contribute to the development of the gastric disease.

1.6 FACTORS THAT REGULATE MUCUS PRODUCTION

Signaling pathways involving myeloid differentiation factor 88 (MyD88), inflammatory mediators as nuclear factor κ B (NFκB), signal transducer and activator of transcription 6 (STAT6) and PGE2, and growth factor/transcription factors-response signal SAM pointed domain-containing ETS transcription factor (SPDEF) can be involved in maintaining mucosal homeostasis and mucous cell function. [151-154].

MyD88 signaling is critical for proinflammatory pathways; however, it also contributes to the maintenance of mucosal homeostasis following inflammation [151]. Mice deficient in MyD88 are more susceptible to bacterial

infections, such as *C. rodentium,* and are more likely to develop colitis [151, 155, 156]. These mice exhibit reduced expression of Muc2 compared to their WT counterparts following opportunistic infections [151, 155]. Additionally, MyD88-deficient mice display a higher bacterial burden, with microbes located in closer proximity to the intestinal epithelium [151, 156]. Myd88 also serves as a key mediator in the activation of NFκB [157]. The activation of NFκB was initially recognized for its role in the inflammatory response, particularly in promoting the production of pro-inflammatory cytokines. However, its functions have since been expanded to include various signaling pathways and biological processes [157]. NFκB has been shown to regulate mucin gene expression in the airways. Specifically, it plays a role in upregulating *MUC5AC* and *MUC5B* mRNA levels in human airway epithelial cells [158, 159]. In the airways of mice after allergic stimulation, there were more PAS-positive epithelial cells in WT mice than in mice in which NFκB signaling had been deleted [160]. These findings highlight the importance of MyD88 and NFκB in mucosal immune response and the maintenance of mucosal homeostasis.

STAT6 is essential for goblet cell differentiation and mucus secretion in response to allergens in the mouse airway and during intestinal infection [161-163]. In a murine asthma model, the inhibition of STAT6 signaling led to reduced expression of Muc5AC in the airways [152]. Similarly, treatment with a STAT6 inhibitor in mice resulted in a thinner colonic mucus layer, which was penetrable to bacteria-sized beads, allowing the beads to reach the surface epithelium [48]. Pathways involving the activation of STAT6 by IL4 and IL13 upregulate SPDEF, a key transcription factor that drives goblet cell differentiation and mucus production [164].

SPDEF is required for goblet cell differentiation and maturation, as well as for mucus production [153, 165-167]. Loss of *Spdef* in mice results in impaired goblet cell maturation in the intestine and a failure in goblet cell response to allergens in the airways [166, 168]. In the airways of *Spdef*-deficient mice, both mRNA and protein levels of Muc5B and Muc5AC are reduced [165]. In the intestinal epithelium, *Spdef*-deficient mice exhibit lower expression levels of Muc2 compared to WT controls [166]. Additionally, Nyström and colleagues [169] demonstrated that mucus production and secretion by the colonic surface epithelium are decreased in *Spdef*-deficient mice. The STAT6/SPDEF pathway is suggested to contribute to increasing mucus production in the intestine during *C. rodentium* infection [48], indicating that this signaling axis plays a role in mucosal homeostasis.

PTGS1 is constitutively expressed in most tissues, while PTGS2 is typically inducible under conditions of physiological stress. Under normal conditions, PTGS2 expression remains low. PTGS enzymes, also known as cyclooxygenases (COXs), catalyze the synthesis of prostaglandins, including prostaglandin E_2 (PGE_2), by converting the precursor PGH_2 into PGE_2 [170]. Bicarbonate secretion and mucus production protect the gastric epithelium from acidic luminal contents, and both processes are regulated by PGE_2 [154]. PGE_2 has been used to stimulate mucus production in *in vitro* and *ex vivo* models [6, 171-174]. It has been shown to increase mucus thickness and anion secretion in both mouse and human intestinal tissues [173, 175], and also fluid release [172]. Some studies suggest that PGE_2 increases mucus thickness primarily by expanding the existing mucus layer rather than by inducing *de novo* secretion [173, 174]. Additionally, prostaglandins may influence mucus properties by regulating anion secretion [154]. Based on the above, PTGS enzymes contribute to the production of PGE_2, which in turn influences mucus properties.

1.7 AQUACULTURE

Aquaculture is one of the fastest-growing food production industries globally and serves as a major source of protein for human consumption [176]. In Sweden, the primary farmed species include rainbow trout (*Oncorhynchus mykiss*), blue mussel (*Mytilus edulis*), various oyster species, Arctic char (*Salvelinus alpinus*), and Atlantic salmon (*Salmo salar*). The estimated annual production is approximately 12,000 tons, corresponding to an average per capita consumption of 22.46 kg of fishery and aquaculture products [177, 178].

In aquaculture systems, fish are confined to limited spaces, increasing the risk of infectious disease transmission. Rising water temperatures further contribute to the prevalence of certain infectious diseases on fish farms [176]. Due to growing concerns about antibiotic resistance, the use of antibiotics has been prohibited in Europe and is strictly regulated in other regions. As a result, vaccines have become a primary alternative for infection control [179]. However, intraperitoneal vaccination, commonly used in aquaculture, can negatively impact fish welfare, leading to both pathological and behavioral changes, and requires handling of the fish. Although available vaccines can be effective, vaccination programs are often costly and may compromise animal welfare [180]. These limitations highlight the need for alternative strategies to prevent and control infections in aquaculture.

1.7.1 RAINBOW TROUT

In Sweden, rainbow trout is one of the primary aquaculture species farmed for food consumption [178]. The skin of the fish is continuously exposed to the external environment, making it one of the most susceptible organs to pathogen invasion; however, other mucosal surfaces, such as the GI tract and gills, also serve as common entry points for pathogens [181, 182]. Similar to what is observed in mammals, fish mucus and mucins play a crucial role in host defense by preventing pathogens from reaching the epithelium [2, 183].

Several studies have examined mucin expression in species such as pufferfish [184], gilthead seabream [183], Atlantic salmon [185], zebrafish [83] and common carp [186]. However, the assembly, automatic annotation, and identification of mucins in non-mammalian species remain challenging due to the long, repetitive, and poorly conserved sequences within mucin domains [184].

Thomsson and colleagues [187] characterized *O*-glycosylation of mucins isolated from the gills, skin, pyloric ceca, and distal intestine of rainbow trout. Their findings revealed that skin mucins contained the most acidic, shortest, and least branched *O*-glycans among the tissues examined, while gill glycans were comparatively less acidic.

A more detailed understanding of mucus-based defense mechanisms in fish would be beneficial. Enhancing mucosal defenses may offer a promising strategy to limit pathogen spread and control infections in aquaculture, as studies have shown that mucins from the gills and intestine of rainbow trout can bind *Aeromonas salmonicida*, and that infection in gilthead seabream induces goblet cell differentiation and upregulation of mucin mRNA expression [183, 187].

1.8 *AEROMONAS SALMONICIDA*

A. salmonicida is a Gram-negative bacterium primarily recognized as an opportunistic fish pathogen [188]. It is the causative agent of furunculosis, a disease predominantly affecting salmonid species such as Atlantic salmon and rainbow trout [189]. To establish infection, *A. salmonicida* must breach the barrier between the external environment and the host's internal milieu. In rainbow trout, the intestine has been identified as a route of translocation;

however, translocation across the skin and gills has also been proposed [181, 182].

Small extracellular molecules used for communication by microbes such as *A. salmonicida* are referred to as quorum-sensing (QS) signals. QS signaling enables the coordination of gene expression within a population, regulating bacterial density and virulence factors, and the induction of biofilm formation [190, 191]. *A. salmonicida* QS signaling includes autoinducer-1 and 2 (AI-1 and AI-2) [191, 192]. The *luxS* gene encodes an enzyme involved in the synthesis of AI-2 signaling molecules, and when deleted, it stops the production of AI-2 in *A. salmonicida* [192, 193]. In addition, the *A. salmonicida* Δ*luxS* mutant shows lower levels of auto-aggregation [193].

QS signals have been identified as a potential risk factor in aquaculture, and the use of QS inhibitors has been suggested as a strategy to combat the rise of antibiotic resistance in this sector [194]. Previous studies have demonstrated the presence of endogenous QS inhibitors in mucus. For example, mucins from saliva and glycans derived from mucins isolated from the human oral cavity inhibit *Streptococcus* mutans QS, thereby preventing the bacterium from acquiring antimicrobial resistance via natural transformation [195].

Similarly, mucins isolated from the skin, pyloric caeca, and intestines of Atlantic salmon have been shown to reduce the levels of AI-2 signaling molecules in *in vitro* culture media. Enzymatic removal of sialic acid from these mucins diminishes their inhibitory effect [190], suggesting that sialylated structures contribute to the QS-inhibitory properties of fish mucins.

Mucins from the gills and intestines of rainbow trout, as well as intestinal mucins from Atlantic salmon, bind *A. salmonicida* more effectively than skin mucins from either species [45, 187]. Furthermore, intestinal mucins from Atlantic salmon exhibit higher levels of sialylation compared to skin mucins. It has been demonstrated that *A. salmonicida* can bind to sialylated structures even in mammalian mucins [45], suggesting that the bacterium preferentially binds to highly sialylated mucins, such as those found in the intestine.

2 AIM

The hypothesis is that the natural clearance of mucosal infections involves a double mechanism: enhanced mucus production and secretion, combined with the actions of innate and adaptive immunity, working together to remove pathogens from the protected niche of the mucus layer into the lumen. Accordingly, this thesis aimed to investigate the effects of mucus-inducing compounds in the gastrointestinal tract following infection through the following specific aims:

- To test the ability of selected compounds to induce mucin biosynthesis in mucosal tissues.
- To investigate the ability of selected compounds to restore mucin biosynthesis in *H. pylori*-infected mice.
- To evaluate the effects of restoration of mucin biosynthesis on the density of *H. pylori* infection.
- To investigate if rainbow trout's mucins can alter *A. salmonicida*'s aggregation ability.

3 METHODOLOGY

All methods included in this thesis are described in Papers I-IV. In this section, I will discuss the rationale behind the selection of certain experimental approaches. Finally, an ethical reflection on the use of animal models is presented.

3.1 *IN VIVO* LABELING OF MUCIN BIOSYNTHESIS

In this thesis, mucin biosynthesis was investigated using the metabolic label GalNAz.

Mucins are highly *O*-glycosylated, and glycosylation is considered one of the most complex forms of post-translational modification. Consequently, mucin mRNA levels do not necessarily correlate with actual mucin production or mucus thickness. Traditional histological methods, such as AB-PAS staining, which are used to quantify mucin content in mucous and goblet cells, also fail to reflect the thickness of secreted mucus [196]. A reduction in secretion may lead to an accumulation of intracellular mucins, whereas increased secretion may result in a decrease in intracellular mucin content. These dynamics cannot be distinguished using conventional histological techniques. Furthermore, secreted mucus is often dehydrated and prone to artifacts during tissue processing. Other methods, like immunoblotting, allow the semi-quantification of specific mucins and the detection of alterations in mucin expression [197]. However, this method does not allow the detection of histological differences, such as changes in the amount of mucin in certain regions or cell compartments.

Mucin-type *O*-linked glycosylation is initiated by the addition of an N-acetylgalactosamine (GalNAc) residue [198]. GalNAz, an azide-modified analog of GalNAc, is metabolically incorporated into the core regions during *O*-glycosylation [199]. It has been used to study mucin biosynthesis and transport in *in vitro* models [199, 200] as well as *in vivo* in mice [114, 201] and fish [202]. When mice are metabolically labeled with GalNAz, it is possible to visualize newly synthesized mucins in the perinuclear compartment of gastric mucous cells as early as one hour post-intraperitoneal injection. Over the following hours, the mucins are observed moving through the cells toward the apical compartment. By six hours post-intraperitoneal injection, some labeled

mucins remain in the apical compartment; later, little to no labeled mucins are detected within the mucous cells, suggesting secretion into the lumen [114]. To ensure the accurate quantification and localization of intracellularly labeled mucins, mice were injected intraperitoneally with GalNAz, and their stomachs were collected two hours post-injection (Papers I–II).

To our knowledge, GalNAz metabolic labeling has not previously been applied in rainbow trout. Therefore, stomach and proximal intestinal tissues were collected at various time points post-intraperitoneal injection. Based on the outcomes of these preliminary experiments, samples from LPS-stimulated rainbow trout were collected at 4 and 8 hours post-injection (Paper III).

In Papers I–III, labeled mucins were subsequently visualized using Click-IT chemistry, in which a fluorescent alkyne is coupled specifically to the azide-modified molecule [48, 202]. Ten representative mucous cells were selected to determine the location of incorporated GalNAz in the different cell compartments: perinuclear, mid cytoplasm, near apical and at apical cell surface. Each compartment received a score from 0 to 4 based on the quantity of fluorescent label found, as represented in Figure 1.

Lastly, to rule out the possibility that differences in GalNAz incorporation resulted from changes in glycosylation, mucin *O*-glycans were analyzed using liquid chromatography-electrospray ionization tandem mass spectrometry (LC/MS) [203].

3.2 TISSUE WHOLE-MOUNT ANALYSIS OF GASTRIC MUCIN PRODUCTION

In this study (Paper II), we used mCherry-Muc5AC reporter mice [204] to investigate the effects of treatment with a combination of Rebamipide and Roxatidine (RebRox) on the production of Muc5AC in the corpus region of the mouse stomach. Mice expressing fluorescently tagged mucins have been previously used to study mucin expression, localization, and secretion in both live and fixed tissues [204, 205]. GalNAz metabolically labels all mucins, and this approach allowed for a comprehensive overview of the Muc5AC-expressing cells present on the gastric tissue surface. Additionally, analysis of tissue whole-mount allows for the identification of specific regions of the surface epithelium (e.g., pit opening region and/or surface epithelium) which responded to *H. pylori* infection and/or treatment. The sum intensity of the

Muc5AC signal was quantified to map the tissue and to assess differences in expression across distinct tissue regions.

3.3 FLUORESCENT *IN SITU* HYBRIDIZATION

In Papers I–II, *H. pylori* was visualized and quantified using fluorescent *in situ* hybridization (FISH) with a *Helicobacter*-specific probe [206]. During washing (to remove chyme), fixation, and tissue processing, the mucus layer and the bacteria residing within it are usually lost. However, bacteria located within the gastric pits are preserved [111]. Colony-forming unit (CFU) counting is a traditional and widely used method to quantify viable bacteria [207], but does not provide information on the localization of the bacteria within the tissue/pits. The changes induced by *H. pylori* infection are primarily localized to the corpus, where the bacteria preferentially bind to highly differentiated pit cells located in the upper region of the gastric pits [123, 127]. Therefore, using FISH, *H. pylori* density was assessed specifically within the gastric pits of the corpus.

3.4 ANIMAL MODELS

Animal models have contributed to advances in biology and innovation in procedures and treatments. Different animals have been used in experimental research: non-human primates, mice, rats, pigs, gerbils, fish (zebra fish, Atlantic salmon and rainbow trout), and many others [45, 54, 83, 113, 181, 208, 209]. The selection of an animal model must be a deliberate and well-justified process to ensure the generation of relevant, translatable scientific data and to promote the most ethical and effective use of animals in research.

Mice have been widely used to study *H. pylori* infection. Infected mice develop precancerous responses similar to those observed in humans [127]. In our studies, we used C57BL/6 mice (Papers I–II) and mCherry-Muc5AC reporter mice (Paper II) to investigate the infection and the effects of compounds reported to influence mucin production. Male mice were selected due to their higher colonization levels during early infection compared to females [208], which allowed us to better assess the impact of restoring mucin production on *H. pylori* density in the gastric pits. In C57BL/6 mice, *Helicobacter felis* infection can progress from chronic gastritis to gastric carcinoma, whereas *H. pylori* infection typically results only in non-malignant lesions [127]. For our experiments (Papers I–II), we used the mouse-adapted *H. pylori* SS1 strain

[126], and infections were maintained for 14 days. The objective was to evaluate the efficacy of various compounds in restoring mucin biosynthesis. In infection models that cause high gastritis levels with severe epithelial defects, assessing mucous cells is complicated; therefore, a model with moderate gastritis levels was chosen. A 14-day infection period was chosen because *H. pylori* requires approximately 6 days to establish colonization, and by 7 days post-infection, an antibody response is typically detectable [210]. This shorter infection window also enabled us to screen a broader range of compounds compared to long-term infection models. The primary focus was on the corpus and its surface region, as the corpus is the main site of *H. pylori* colonization in mice and the bacteria preferentially bind to pit cells located in the upper region of the gastric pits [123, 127].

Rainbow trout is an important species in Swedish aquaculture and represents one of the major products of fish farming for food consumption [178]. The mucosal surfaces of fish's GI tract, gills, and skin serve as entry points for pathogenic bacteria [181]. These surfaces are covered by mucus; however, the mucus and glycosylation profile of the rainbow trout GI tract remained uncharacterized. Therefore, in Paper III, we characterized the GI mucus, mucin production, and glycosylation in rainbow trout and in response to LPS stimulation [83]. *A. salmonicida* is one of the pathogens that infects rainbow trout, causing furunculosis [188]. It has been shown with other bacterial species that infection can alter glycosylation, and mucins can, in turn, modulate bacterial virulence factors [46, 111, 195]. In Paper IV, we used mucins isolated from rainbow trout infected with *A. salmonicida* to investigate whether these mucins influence the bacterium's virulence factors and auto-aggregation ability. The rainbow trout model enabled the collection of multiple samples and organs from the same fish for histology, mucin isolation, and LC/MS analysis.

3.5 ETHICAL REFLECTION

Over the years, animal models have played a key role in experimental research, contributing to the advancement of knowledge and the search for solutions to biological and biomedical questions. At the same time, growing concern for animal welfare and increased awareness of animal rights have brought ethical considerations to the forefront.

Although significant progress has been made in molecular biology techniques, including cell culture systems, spheroids and organoids, the use of animal

models remains an essential component of biological research, particularly in the development of novel therapeutics and the investigation of host-pathogen interactions.

All experimental procedures in mice were approved by the Gothenburg Animal Ethics Committee (Göteborgs Djurförsöksetiska Nämnd; Ethics Approval No. 52-2021) and conducted following the Swedish Animal Welfare Ordinance (Djurskyddsförordningen DFS 2004:4). C57BL/6 mice and mCherry-Muc5AC reporter mice, aged over six weeks, were used to investigate gastric mucin biosynthesis in response to *H. pylori* infection and compounds reported to affect mucin production. The use of an *in vivo* model was deemed necessary, as other alternative methodologies could not adequately replicate the complex interactions between mucus, infection, and host defense. Furthermore, *in vitro* systems, such as cell cultures, are insufficient substitutes due to the structural and cellular complexity of the gastric mucosa, which comprises multiple distinct cell types and specialized architecture. Lastly, infection studies in organoids lack the host immune response.

The experimental procedures involving fish were approved by the Ethical Committee for Animal Experiments in Gothenburg, Sweden (License No. 5.2.18–8144/2018). As previously noted, *in vivo* models remain the most reliable approach for studying mucin biosynthesis and infection-induced alterations. Accordingly, rainbow trout were used as an experimental model. To reduce the number of animals utilized, several samples (organs, mucus, etc.) were collected from each fish for diverse analyses/experiments.

All experiments were planned and executed respecting the principles of the 3Rs (Replacement, Reduction and Refinement) [211].

4 RESULTS

4.1 COMPOUNDS INDICATED TO AFFECT MUCIN/MUCUS PRODUCTION INCREASED MUCIN BIOSYNTHESIS IN THE GASTROINTESTINAL TRACT (PAPERS I-III)

The host's mucosal surfaces are the primary entry and colonization sites for many pathogens [1]. One such pathogen is the gastric bacterium *H. pylori,* whose infection has been shown to reduce mucin production in mice, suggesting that the bacterium may create a more stable environment in the gastric mucosa by impairing this defense mechanism [114]. Therefore, in Papers I-II, we aimed to restore mucin production in *H. pylori*-infected mice by treating them with compounds indicated to affect mucin production. The compounds used were: IL4, RαMH, Reb, Rox, and a combination of RebRox [48, 54, 63, 75]. The last two compounds were combined due to their synergetic effect. The metabolic label GalNAz was used to evaluate mucin biosynthesis in corpus surface mucous cells 2 hours post-intraperitoneal injection. Metabolically labeled intracellular mucins were visualized using Click-IT chemistry, and cells were scored according to the quantity of label found in each cell compartment (perinuclear, mid cytoplasm, near apical and at apical cell surface).

Papers I-II: In uninfected mice, metabolically labeled mucins were observed throughout the entire mucous cell, with the label appearing to be evenly distributed across all four cellular compartments (Figure 1A). In *H. pylori*-infected vehicle-treated mice, the infection led to an overall reduction in metabolically labeled mucins compared to uninfected controls. This reduction was most pronounced in the apical regions of the cells, whereas the perinuclear region retained similar levels of labeled mucins (Figure 1B).

Paper I: The treatment of *H. pylori*-infected mice with IL-4 restored mucin biosynthesis to levels comparable to those in uninfected mice (Figure 1C). In these mice, labeled mucins were evenly distributed across all cellular compartments and were more abundant than in vehicle-treated infected mice.

Similarly, *H. pylori*-infected mice treated with RαMH (Figure 1D) also exhibited higher levels of labeled mucins compared to vehicle-treated mice.

Paper II: The treatment with Rox restored mucin production to levels similar to uninfected controls, with metabolically labeled mucins evenly distributed across all four cellular compartments (Figure 1F). The combination treatment with RebRox resulted in higher mucin production compared to vehicle-treated mice, with notable increases observed particularly in the mid-cytoplasmic compartments (Figure 1G). Treatment with Reb (Figure 1E) also increased the levels of labeled mucins relative to vehicle controls; however, the effect was less pronounced than that observed with Rox or the combination therapy.

The effect of RebRox was further investigated in Paper II using tissue whole-mount from mCherry-Muc5AC reporter mice [204]. In uninfected mice, Muc5AC signal was primarily localized to the pit region of the gastric corpus. Treatment with RebRox in uninfected mice resulted in increased Muc5AC intensity in both the surface and pit regions. Additionally, GalNAz metabolic labeling confirmed that RebRox enhanced mucin biosynthesis even in the absence of infection.

In mCherry-Muc5AC reporter mice infected with *H. pylori*, Muc5AC signal remained largely restricted to the pit region. However, treatment with RebRox led to increased Muc5AC intensity in both the pit and surface regions. These findings were corroborated by GalNAz metabolic labeling, which demonstrated that RebRox treatment significantly increased mucin biosynthesis in infected mice compared to vehicle-treated controls.

As in Papers I and II, GalNAz metabolic labeling was used in Paper III to investigate mucin production in the stomach and intestine of rainbow trout. Mucins were visualized using Click-IT chemistry, and mucous and goblet cells were scored as previously described. Rainbow trout were maintained at 10 °C, and gastric and intestinal samples were collected at various time points following intraperitoneal injection of GalNAz.

In the stomach, metabolically labeled mucins were mainly observed around the perinuclear region at 4 hours post-injection. By 10 hours post-injection, the label progressed to the mid-cytoplasmic compartments, and by 16 hours, more extensive labeling was observed across all cell compartments. The experiment was extended to 20, 26, and 32 hours post-injection. At these later time points, the distribution of labeled mucins resembled that observed at 16 hours, with a

gradual shift in localization from the basal to the apical region of the mucous cells.

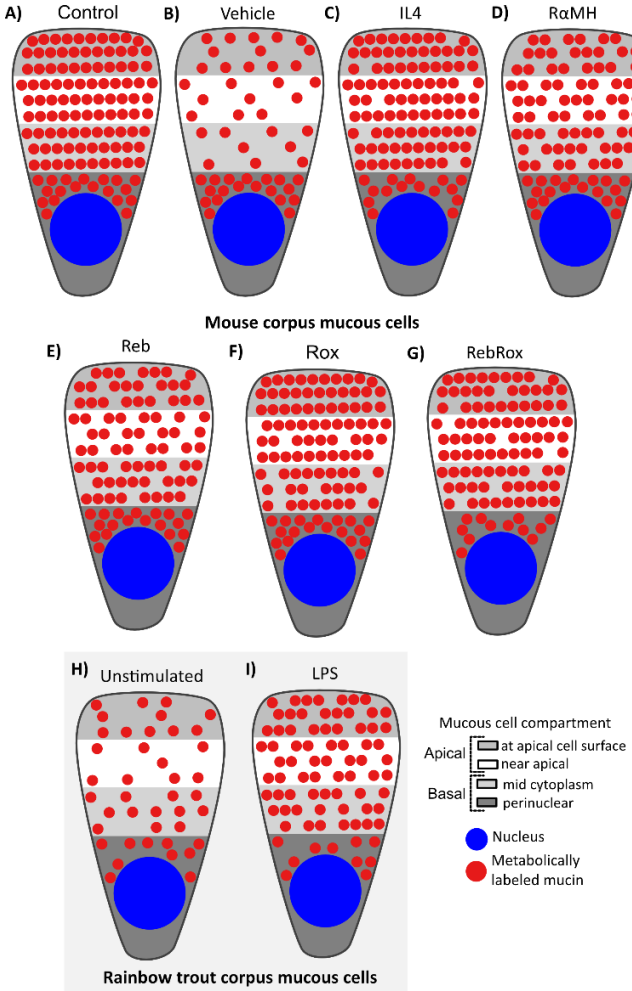

Figure 1. Illustration of the effects of the compounds used to increase mucin biosynthesis on surface mucous cells of the corpus in A-G) mouse 2 hours post-GalNAz injection and H-I) rainbow trout.8 hours post-GalNAz injection The mucous cells were divided into distinct compartments: perinuclear (darkest gray), mid-cytoplasm (lightest gray), near apical (white), and apical cell surface (gray). Metabolically labeled intracellular mucins are represented by red dots, with the number of dots indicating the relative abundance of labeled mucins in each compartment. A higher number of red dots corresponds to a more mucin-rich compartment and thus a higher score, whereas fewer red dots indicate a lower mucin content and score. Nuclei are shown in blue. A) Uninfected mouse. B) H. pylori-infected mouse. C) H. pylori-infected mouse treated with IL4. D) H. pylori-infected mouse treated with RαMH. E) H. pylori-infected mouse treated with Reb. F) H. pylori-infected mouse treated with Rox. G) H. pylori-infected mouse treated with a combination of RebRox. H) Unstimulated rainbow trout. I) Rainbow trout stimulated with LPS.

Mucin biosynthesis in the proximal and distal intestine was assessed at 4, 8, 14, 20, 26, and 32 hours post-GalNAz injection. At 4 hours, most metabolically labeled mucins were localized to the perinuclear region in both the proximal and distal intestine. In the proximal intestine, labeled mucins were predominantly located in the basal region of the cells from 8 to 20 hours post-injection. At 26 and 32 hours, a shift toward the apical cell compartments was observed. In the distal intestine, labeled mucins were distributed throughout all cell compartments from 8 to 32 hours post-injection.

In addition, we examined mucin production in the gastrointestinal tract of rainbow trout in response to LPS stimulation [83]. In the gastric antrum, at 4 hours post-injection, rainbow trout stimulated with LPS exhibited increased levels of labeled mucins in the basal region of the mucous cells compared to unstimulated fish, in which the label was confined to the perinuclear compartment. By 8 hours post-injection, LPS stimulation led to enhanced mucin production in both the gastric corpus (Figure 1I) and antrum when compared to unstimulated fish (Figure 1H). Similarly, at 8 hours post-injection, LPS stimulation also increased mucin production in the intestine. In the proximal intestine, labeled mucins in both stimulated and unstimulated groups were primarily localized to the basal part of the goblet cells. In contrast, in the distal intestine, labeled mucins were distributed throughout all cellular compartments.

Collectively, the findings presented in Papers I–III demonstrate that mucin biosynthesis in the gastrointestinal tract of both uninfected and *H. pylori*-infected mice, as well as in rainbow trout, can be restored or enhanced through treatment with specific compounds.

4.2 MICE TREATED WITH COMPOUNDS THAT RESTORED MUCIN BIOSYNTHESIS HAD LOWER *H. PYLORI* DENSITY IN THEIR GASTRIC PITS (PAPERS I AND II)

H. pylori were visualized and quantified by FISH using a *Helicobacter*-specific probe [206]. Only bacteria residing within the gastric corpus pits were counted [111]. No *H. pylori* were detected in the gastric pits of uninfected mice. In contrast, infected mice exhibited dense colonization of the corpus pits, often filled with large clusters of *H. pylori*.

Treatment with the various compounds led to a noticeable reduction in *H. pylori* density. In treated mice, fewer *H. pylori* were observed, and the bacteria were more often found as single cells dispersed throughout the gastric pits, with only occasional clustering. There was approximately a 50% reduction in *H. pylori* density in mice treated with IL-4, RαMH, Reb, or the combination RebRox. Treatment with Rox alone resulted in a 25% reduction in *H. pylori* density.

As the compounds did not exhibit cytotoxic effects on *H. pylori* and did not significantly alter the expression of virulence factors (*ureA*, *flaA*, *vacA*, and *cagA*), these findings suggest that restoring mucin biosynthesis contributes to the removal of *H. pylori* from the gastric pits of infected mice, as represented in Figure 2.

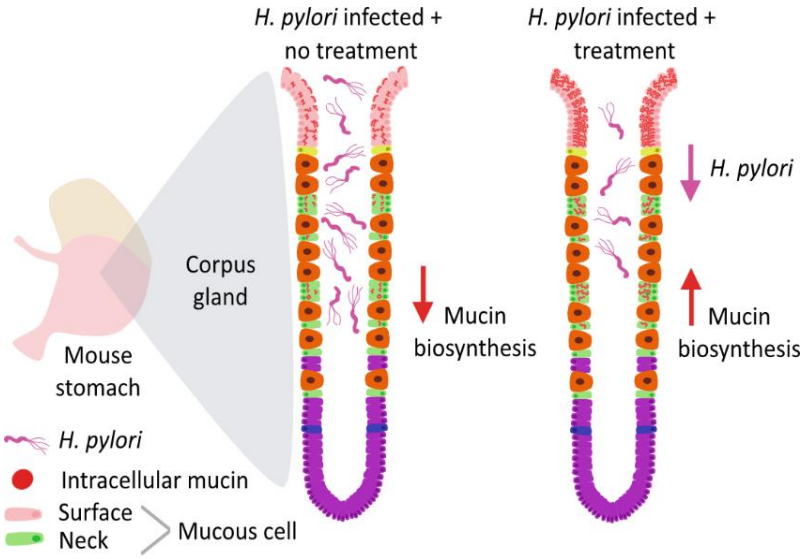

Figure 2. Illustration of mouse gastric pits with and without treatment with compounds that increased mucin biosynthesis. Surface mucous cells in pink, neck mucous cells in green, and H. pylori in magenta. The red dots represent metabolically labeled intracellular mucins. The Figure was adapted from the graphical abstract in Paper I (DOI: 10.1080/21505594.2025.2530173) published under the terms of the Creative Commons CC BY license.

4.3 COMPOUNDS INDICATED TO AFFECT MUCIN PRODUCTION CONFERRED PROTECTIVE EFFECTS AGAINST *H. PYLORI*-INDUCED GASTRIC ALTERATIONS (PAPERS I AND II)

Alterations caused by *H. pylori* SS1in the gastric mucosa are mainly observed in the corpus region of the mouse stomach. These alterations were assessed following the histological activity index (HAI) described by Rogers [127].

Uninfected mice presented an overall healthy gastric mucosa with only occasional presence of leukocytes and epithelial defects. In contrast, *H. pylori*-infected mice showed significantly higher HAI scores. Histological analysis revealed inflammation characterized by the accumulation of polymorphonuclear and mononuclear leukocytes, epithelial defects, and oxyntic atrophy. In a subset of mice, mucous metaplasia was also observed.

All compounds administered to infected mice, IL-4, RαMH, Reb, Rox, and the combination RebRox, have been previously reported to have gastroprotective effects and to promote healing of mucosal surfaces [48, 56, 60, 72]. Consistent with these reports, treatment with any of these compounds resulted in reduced HAI scores compared to infected vehicle mice. The gastroprotective effect was evident by a reduction in inflammation, reduction of oxyntic atrophy, milder epithelial defects, and an absence of mucous metaplasia.

Furthermore, expression of the pro-inflammatory cytokine *Tnf* mRNA [145] was significantly downregulated in mice treated with RαMH and Rox, and showed a decreasing trend in those treated with Reb and RebRox compared to vehicle-infected mice. In addition, the anti-inflammatory cytokine *Il4* mRNA [50] was upregulated following IL4 treatment. These findings suggest that the compounds exert a protective effect against *H. pylori*-induced gastric pathology. Their gastroprotective properties, along with the reduction of *H. pylori* colonization in the gastric pits, contribute to decreased inflammation and the preservation of mucosal integrity.

4.4 MUCINS PREVENTED *A. SALMONICIDA* AUTO-AGGREGATION (PAPER IV)

A. salmonicida WT and Δ*luxS* mutant were cultured at 10 °C and 20 °C to investigate the influence of mucins and AI-2 on bacterial auto-aggregation and were visualized using viability staining. At both temperatures, the WT strain formed large aggregates, whereas the Δ*luxS* mutant produced only small clumps. Skin mucins exhibited a stronger inhibitory effect on *A. salmonicida* aggregation than gill mucins, with the inhibition being more pronounced at 10 °C than at 20 °C. These findings suggest that mucins can inhibit *A. salmonicida* aggregation, similarly to the effect observed following the deletion of *luxS.*

5 DISCUSSION

In this thesis, the use of compounds suggested to affect mucin production was investigated to enhance or restore mucin biosynthesis and to assess how these changes influence pathogen localization and host inflammatory responses. Mice infected with *H. pylori* SS1 were treated with IL4, RαMH, Reb, Rox, or a combination of RebRox. These treatments demonstrated gastroprotective effects, as reflected by reduced histological inflammation scores. Metabolic labeling using GalNAz incorporation revealed increased mucin biosynthesis following treatment, which correlated with reduced *H. pylori* colonization in the gastric pits. In rainbow trout, mucin production in the stomach and intestine was enhanced following LPS stimulation. Furthermore, mucins isolated from rainbow trout were shown to inhibit *A. salmonicida* auto-aggregation.

Mucosal surfaces are directly and indirectly exposed to the outside environment, making them a primary entry point for pathogens [1]. These surfaces are protected by a mucus layer that acts as a barrier to protect the underlying epithelium [2]. However, pathogens such as *H. pylori* have developed strategies, such as impairing mucin production, to create a more stable/favorable environment in the host's stomach [88, 114]. In mice infected with *H. pylori*, mucin biosynthesis is decreased. Treatment with IL4, RαMH, Reb, Rox, or a combination of RebRox successfully enhanced and restored mucin biosynthesis to levels comparable to those observed in uninfected mice. Due to its synergistic effects, the RebRox combination was further investigated using mCherry-Muc5AC reporter mice. Treatment with RebRox led to increased expression of Muc5AC at the epithelial surface and pit openings in both uninfected and *H. pylori*-infected mice.

Sharba and colleagues [48] demonstrated that IL4 enhances mucin biosynthesis in mouse colonic goblet cells via the STAT6/SPDEF pathway. In line with this, we observed that both *Spdef* and *Stat6* mRNA expression levels were upregulated in *H. pylori*-infected mice treated with IL4, suggesting that IL4 increases mucin biosynthesis at least in part through the STAT6/SPDEF pathway in the gastric corpus. Additionally, other regulators of mucus production, such as *Myd88*, *Ptgs1*, and *Ptgs2*, were also upregulated in infected mice treated with IL4, indicating that multiple pathways may contribute to the restoration of mucus production.

Ptgs1 and *Ptgs2* encode the enzymes responsible for converting PGH_2 to PGE_2 [170], a molecule shown to stimulate mucus production in both *in vitro* and *ex*

vivo models [6, 171-174]. Reb has been reported to upregulate *Ptgs2* and enhance PGE_2 production [60, 63-65], suggesting that its mucus-promoting effects are mediated through PGE_2 [59, 66]. In our study, treatment with Reb or the RebRox combination restored *Ptgs2* expression to levels comparable to those of uninfected mice, further implicating *Ptgs2* in mucin biosynthesis. However, further analyses, including more mouse samples are needed to confirm this role. RαMH has been shown to increase PGE_2 production [56] and mucus thickness in the rat stomach [53]. In RαMH-treated mice, *Ptgs2* mRNA levels were elevated, supporting the hypothesis that the observed increase in mucin biosynthesis is mediated by PGE_2 via *Ptgs2*. While some studies suggest that PGE_2 increases mucus primarily through liquid expansion rather than *de novo* secretion [173, 174], other reports that PGE_2 can directly stimulate *de novo* mucin production in human colonic adenocarcinoma cells and the rat colon [212]. Our findings suggest that PGE_2 contributes to increased intracellular mucin biosynthesis in mice treated with RαMH, and possibly in those treated with Reb. Rox has also been reported to enhance mucin biosynthesis [74-76], potentially through the upregulation of endogenous NO [75]. However, in our study, no changes were observed in the expression of *MyD88*, *NfκB*, *Stat6*, *Spdef*, *Ptgs1*, or *Ptgs2* in Rox-treated mice, suggesting that its effect on mucin production does not involve these pathways.

Mucins are known to bind and facilitate the clearance of *H. pylori*, and mice deficient in Muc1 or Muc5AC exhibit higher bacterial densities following infection compared to WT counterparts [28, 44, 116, 117]. In this study, treatment with IL4, RαMH, Reb, Rox, or the combination RebRox successfully restored mucin biosynthesis through distinct mechanisms. This restoration was associated with a reduction in *H. pylori* density, suggesting that enhancing mucin biosynthesis contributes to the displacement and clearance of *H. pylori* from the gastric niche.

TFF1 is co-expressed and co-secreted with Muc5AC and is thought to contribute to the intracellular assembly of this mucin [17, 118, 213]. Additionally, TFF1 has been shown to bind *H. pylori* and slow down its motility within the mucus layer [122]. In this study, *Tff1* expression was upregulated in mice treated with all compounds except RαMH. These findings suggest that TFF1 may contribute to the reduction in *H. pylori* density in the gastric environment both by direct action on the bacteria and as a result of restoration of mucus production.

As shown in Paper II, Rox was among the most effective compounds in restoring mucin biosynthesis to levels comparable to those in uninfected mice. Treatment with Rox also exhibited a gastroprotective effect, as evidenced by reduced histological alterations associated with *H. pylori* infection. However, in mice treated with Rox alone, *H. pylori* density was trended to be reduced by approximately 25% compared to vehicle-treated mice, whereas treatment with IL4, RαMH, Reb, or a combination of RebRox led to reductions of around 50%. In mice treated with IL4, RαMH, Reb, or a combination of RebRox, the increase in mucin biosynthesis correlated with a significant reduction in *H. pylori* in the mouse gastric pits. These findings suggest that, in addition to mucin biosynthesis, other factors such as mucin glycosylation and mucus properties may play critical roles in pathogen clearance. For instance, in mice treated with IL4 and Rox, a reduction in fucosylation per glycan structure was observed. In IL4-treated mice, this reduction was accompanied by an increase in sialylation. While this thesis did not investigate properties such as secreted gastric mucus thickness or penetrability to microbeads [6], it is plausible to hypothesize that mucus properties vary with treatment and that some are more detrimental to *H. pylori* colonization than others.

In fish such as rainbow trout, the mucosal surfaces (including skin and gills) are covered with a mucus layer [182]. Similar to mammals, fish mucus functions as an interface with the external environment, protecting the underlying epithelium [183]. Additionally, fish mucins have been shown to bind pathogens [187]. Based on these similarities, we hypothesized that, as in the mouse–*H. pylori* model, investigating mucus-based defense mechanisms in rainbow trout, would provide valuable insights. To our knowledge, no prior studies have assessed mucin biosynthesis in the GI tract of rainbow trout using a metabolic labeling approach [202]. In our experiments, mucins in gastric mucous cells were first detected in the perinuclear compartment at 4 hours post-GalNAz injection and remained visible in the apical compartment up to 32 hours post-injection. In the intestine, mucin biosynthesis followed a similar pattern but was slower. Mucin secretion was observed only after 16 hours post-GalNAz injection. For comparison, in mice, labeled mucins are secreted approximately 5 hours post-injection in the stomach and 7 hours in the intestine [114, 214]. The slower turnover observed in rainbow trout likely reflects the lower maintenance temperature (10 °C), which may represent a physiological adaptation aimed at conserving resources necessary for mucus production. Moreover, our results confirm that stimulation with LPS enhances mucin biosynthesis in the rainbow trout GI tract. LPS has also been reported to alter glycosylation patterns [84], which may, in turn, influence bacterial adhesion.

Considering the increasing challenges posed by antibiotic resistance and the limitations of current fish vaccination strategies [179, 180], a deeper understanding of the physiological properties of rainbow trout mucus and its inducibility by LPS could be useful for the development of alternative strategies to combat infection.

It has been demonstrated that mucins may interfere with pathogens' virulence and inhibit auto-aggregation [45, 46, 215]. We showed that rainbow trout mucins can inhibit *A. salmonicida* auto-aggregation and cluster formation to levels comparable to those observed in the Δ*luxS* mutant, which lacks one of the key genes involved in QS signaling. Moreover, in *H. pylori*-infected mice, treatment with IL4, RαMH, Reb, Rox, or a combination of RebRox enhanced/restored mucin biosynthesis. In these mice, not only was the density of *H. pylori* reduced, but there were also fewer and smaller bacterial aggregates. Most *H. pylori* detected in the gastric pits of treated mice appeared as single bacteria or small clusters. Given that mucus contains endogenous QS inhibitors [195] and considering that QS inhibitors have been proposed as a strategy to prevent and control infections in aquaculture [194], our findings highlight the role of mucins as part of the defense mechanism against pathogens and as facilitators of host-mediated clearance.

6 CONCLUSION

The results of this thesis suggest that using compounds previously indicated to influence mucus or mucin production can enhance mucin biosynthesis in two distinct animal models, in the presence and absence of infection. Additionally, mucins can affect bacterial auto-aggregation.

In summary, the main findings of this thesis are as follows:

- Treatment with IL4, RαMH, Reb, Rox or a combination of RebRox restored mucin biosynthesis in *H. pylori*-infected mice.
- Restoration of mucin biosynthesis in *H. pylori*-infected mice was associated with a reduced bacterial density in the gastric pits.
- Treatment with RebRox increased Muc5AC intensity in both the pit and surface regions of the corpus, regardless of the presence or absence of *H. pylori* infection.
- LPS stimulation led to an increased mucin production in the GI tract in rainbow trout.
- Mucins isolated from rainbow trout inhibited *A. salmonicida* auto-aggregation.

In conclusion, modulating mucosal properties might be a viable approach to counteracting bacterial infection.

7 FUTURE PERSPECTIVES

Building on the findings of this thesis, several directions for future research can be proposed to further advance our understanding and application of mucin-related host–pathogen interactions:

- Better understand the mechanisms by which different compounds stimulate mucin biosynthesis.
- Investigate the properties of secreted mucus under both infected and uninfected conditions, particularly in response to compounds that modulate mucus and mucin production.
- Combine the treatments that enhance mucus production with various immune stimulation strategies [208, 216] to combat *H. pylori* infection more effectively.
- Explore the effects of the compounds in a long-term/chronic infection model.
- Experimentally infect rainbow trout with *A. salmonicida* to study the host response to infection and LPS stimulation.

ADDITIONAL BIBLIOGRAPHY

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Sharba S, Sundh H, Santos L, Sundell K, Birchenough G, Dalum A, Ojaimi Loibman S, Benktander J. and Lindén S.K. *A. salmonicida infection inhibits rainbow trout gill mucin production.* Submitted.

Erhardsson M, Santos L, Benktander J, Sharba S, Thorell K and Lindén S.K. *The mouse gastric surface epithelial cell and its response to early Helicobacter pylori infection.* Submitted.

USE OF GENERATIVE AI

Portions of the text were reviewed and edited with the assistance of a generative AI language model to improve clarity, grammar, and tone. All content was subsequently reviewed and approved by the author.

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