SUMMARY

Persistent or dysregulated lung inflammation is a hallmark of chronic respiratory diseases, such as severe asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF) and bronchiectasis. The role of common pathogens in this overactive inflammatory state has been investigated in depth. Yet, the influence of other microbiota members on inflammation has received limited attention thus far. As current anti-inflammatory treatments often lack efficiency and their usage is limited due to ample side-effects associated with long-term use, investigating novel treatment options is needed. Hence, the overall aim of this dissertation is to explore microbiome-based approaches for treatment of chronic inflammatory respiratory diseases.

In the first part, a screening of multiple bacteria that are commonly isolated from the respiratory tract of patients with chronic lung disease, such as CF, was performed to investigate their role in alveolar and bronchial epithelial inflammatory response to bacterial pathogens (Chapter III part 1). These bacteria included two pathogens commonly isolated from persons with CF (*Pseudomonas aeruginosa* and *Staphylococcus aureus)*, two less frequently recovered CF pathogens (*Streptococcus anginosus* and *Achromobacter xylosoxidans)* and two bacteria that are not commonly considered as CF pathogens, but are often isolated from CF lower airway secretions (*Rothia mucilaginosa* and *Gemella haemolysans*). The ability of these pathogenic and non-pathogenic species to influence *P. aeruginosa*-induced immune response was analyzed, which led to the discovery of anti-inflammatory properties in *R. mucilaginosa*. The anti-inflammatory effect of this commensal of the oral cavity was then further evaluated and confirmed in an *in vivo* mouse model. Moreover, an inverse correlation was observed between *R. mucilaginosa* abundance and pro-inflammatory markers in sputum of patients with chronic airway disease. In addition, *in vitro* analysis of the anti-inflammatory mode of action of *R. mucilaginosa*, using various methods (i.e. qPCR, cytokine ELISA assays, western blotting, and an NF-κB reporter alveolar epithelial cell line) revealed its ability to abolish the induced NF-κB pathway activation.

In the second part, research was conducted to identify the active compound(s) produced by *R. mucilaginosa* (Chapter III, part 2*)*. The anti-inflammatory effect was observed in the bacterial supernatant and the activity was lost over time when storing the supernatant. These insights were exploited to identify possible active compound(s) using a comparative metabolomics approach. Metabolites present in freshly prepared supernatant were compared to metabolites present in the

stored (inactive) supernatant. In addition, the metabolites in the supernatant of *R. mucilaginosa* were compared to metabolites of a closely related species, *Micrococcus luteus* (i.e. belonging to the same family as *Rothia*: Micrococcaceae) that did not show the same anti-inflammatory effect. This comparison led to identification of the siderophore enterobactin as a possible active compound secreted by *R. mucilaginosa* able to reduce LPS-stimulated NF-κB pathway activation.

Finally, a collection of other non-pathogenic bacteria was screened for anti-inflammatory activity, which led to the discovery of novel anti-inflammatory bacteria that are part of the respiratory tract microbiota (Chapter III, part 3). Taking into consideration the interest for locally active probiotic bacteria and the importance to keep the bacterial load in the lungs to a minimum, synergistic anti-inflammatory activity of a multi-strain consortium was explored. The aim hereby was to design a consortium necessitating less bacteria to exert an effect compared to individual species. A consortium of *R. mucilaginosa*, *Rothia dentocariosa* and *Roseomonas gilardii* was able to fulfill this criterium. Finally, possible growth enhancers (prebiotics) for these anti-inflammatory bacteria were investigated as well.

In conclusion, this dissertation shows beneficial properties of non-pathogenic respiratory bacteria, both *in vitro* and *in vivo*, which could lead to important insights on their role in the disease process. Furthermore, these bacterial species may represent novel probiotics to be explored for treatment of chronic inflammatory diseases. Moreover, identification of nutrient sources (i.e. prebiotics) to induce the growth of these beneficial bacteria and identification of their active anti-inflammatory compounds (i.e. postbiotics), can help in the development of microbiome-based therapies using these three 'biotics' approaches or combinations thereof, with the aim of increasing treatment success.