

ehp

**ENVIRONMENTAL
HEALTH
PERSPECTIVES**

ehponline.org

Human Pathogens Abundant in the Bacterial Metagenome of Cigarettes

Amy R. Sapkota, Sibel Berger, and Timothy M. Vogel

doi: 10.1289/ehp.0901201 (available at <http://dx.doi.org/>)

Online 22 October 2009



NIEHS

National Institute of
Environmental Health Sciences

National Institutes of Health
U.S. Department of Health and Human Services

Human Pathogens Abundant in the Bacterial Metagenome of Cigarettes

Amy R. Sapkota^{1,2}, Sibel Berger,² Timothy M. Vogel²

¹ Maryland Institute for Applied Environmental Health, University of Maryland College Park School of Public Health, College Park, Maryland 20742, USA

² Environmental Microbial Genomics Group, Laboratoire Ampère, UMR CNRS 5005, Ecole Centrale de Lyon, 69134 Ecully cedex, France

Corresponding Author:

Amy R. Sapkota, PhD, MPH

University of Maryland College Park, School of Public Health

Maryland Institute for Applied Environmental Health

Room 2308, SPH Building

College Park, MD 20742

Phone: 301-405-1772

Fax: 301-405-8397

Email: ars@umd.edu

Running Title: Bacterial Pathogens Present in Cigarettes

Key Words: bacteria, bacterial metagenome, cigarettes, pathogens, smoking, tobacco

Article descriptor: Respiratory Disease

Acknowledgements and Grant Information: Thank you to the Rhone-Alpes Region, which supported, in part, the metagenomic and microarray work performed in this study. We also would like to acknowledge Amir Sapkota, Sam W. Joseph and Pamela I. Clark for their insightful comments and suggestions.

Disclaimers/Competing Interests Declaration

All of the authors have no potential competing financial interests. The authors “freedom to design, conduct, interpret, and publish [this] research [was] not compromised by any controlling sponsor.”

List of Abbreviations and Definitions:

C = Camel

DNA = deoxyribonucleic acid

dNTPs = deoxynucleotide triphosphates

FOI = frequency of incorporation

K = Kool Filter Kings

L = Lucky Strike Original Red

M = Marlboro Red

PCA = principal components analysis

PCR = polymerase chain reaction

RNA = ribonucleic acid

spp. = species

Outline of Section Headers:

Abstract

Introduction

Materials and Methods

 Sample collection

 DNA extraction

 PCR, cloning and sequencing

 Labeling and sample preparation

 Microarray hybridization and scanning

 Filtration, normalization and data analysis

Results

Discussion

Conclusions

References

Tables

Figure Legends

Figures

Abstract

Background: Many studies have evaluated chemical, heavy metal and other abiotic substances present in cigarettes and their roles in the development of lung cancers and other diseases. Yet, no studies have comprehensively evaluated bacterial diversity of cigarettes and the possible impacts of these microbes on respiratory illnesses in smokers and exposed non-smokers.

Objectives: Thus, the goal of this study was to explore the bacterial metagenomes of commercially-available cigarettes.

Methods: A 16S rRNA-based taxonomic microarray approach and cloning and sequencing were used to evaluate total bacterial diversity of four brands of cigarettes. Normalized microarray data were compared using principal components analysis and hierarchical cluster analysis to evaluate potential differences in microbial diversity across cigarette brands.

Results: Fifteen different classes of bacteria and a broad range of potentially pathogenic organisms were detected in all cigarette samples. Most notably, we detected *Acinetobacter*, *Bacillus*, *Burkholderia*, *Clostridium*, *Klebsiella*, *Pseudomonas aeruginosa*, and *Serratia* in $\geq 90\%$ of all cigarette samples. Other pathogenic bacteria detected included *Campylobacter*, *Enterococcus*, *Proteus*, and *Staphylococcus*. No significant variability in bacterial diversity was observed across the four different cigarette brands.

Conclusions: Previous studies have shown that smoking is associated with colonization by pathogenic bacteria and an increased risk of lung infections. However, this is the first study to show that cigarettes themselves could be the direct source of exposure to a wide

array of potentially pathogenic microbes among smokers and other people exposed to secondhand smoke. The overall public health implications of these findings are unclear at this time, and future studies are necessary to determine whether or not bacteria in cigarettes could play important roles in the development of both infectious and chronic respiratory diseases.