Applied Microbiology and Biotechnology

Supplementary Figures

Smokeless tobacco products harbor diverse bacterial microbiota that differ across products and brands

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Supplementary Figure Legends:

Figure S1: Scatterplot of Good's coverage to sequencing counts per sample. Solid curved red line represents the LOESS fit model and solid black line represents the cutoff point.

Figure S2: Ordination plot derived from nonmetric multidimensional scaling (NMDS) using *Bray*-Curtis distance of bacterial community composition. Colors, and shapes represent the different lots within a product brand. Toombak and the alkalinizing agent do not have different lots.

Figure S3: Abundance of predicted Kyoto Encyclopedia of Genes and Genomes modules in smokeless tobacco product types. Colors designate product type. Bars represent mean relative abundance of predicted modules per product type, and error bars represent the standard error of mean.

Figure S4: Abundance of predicted hydrocarbon degradation pathways across smokeless tobacco product types.

Figure S5: Abundance of predicted antibiotic biosynthesis pathways across smokeless tobacco product types.

Figure S1

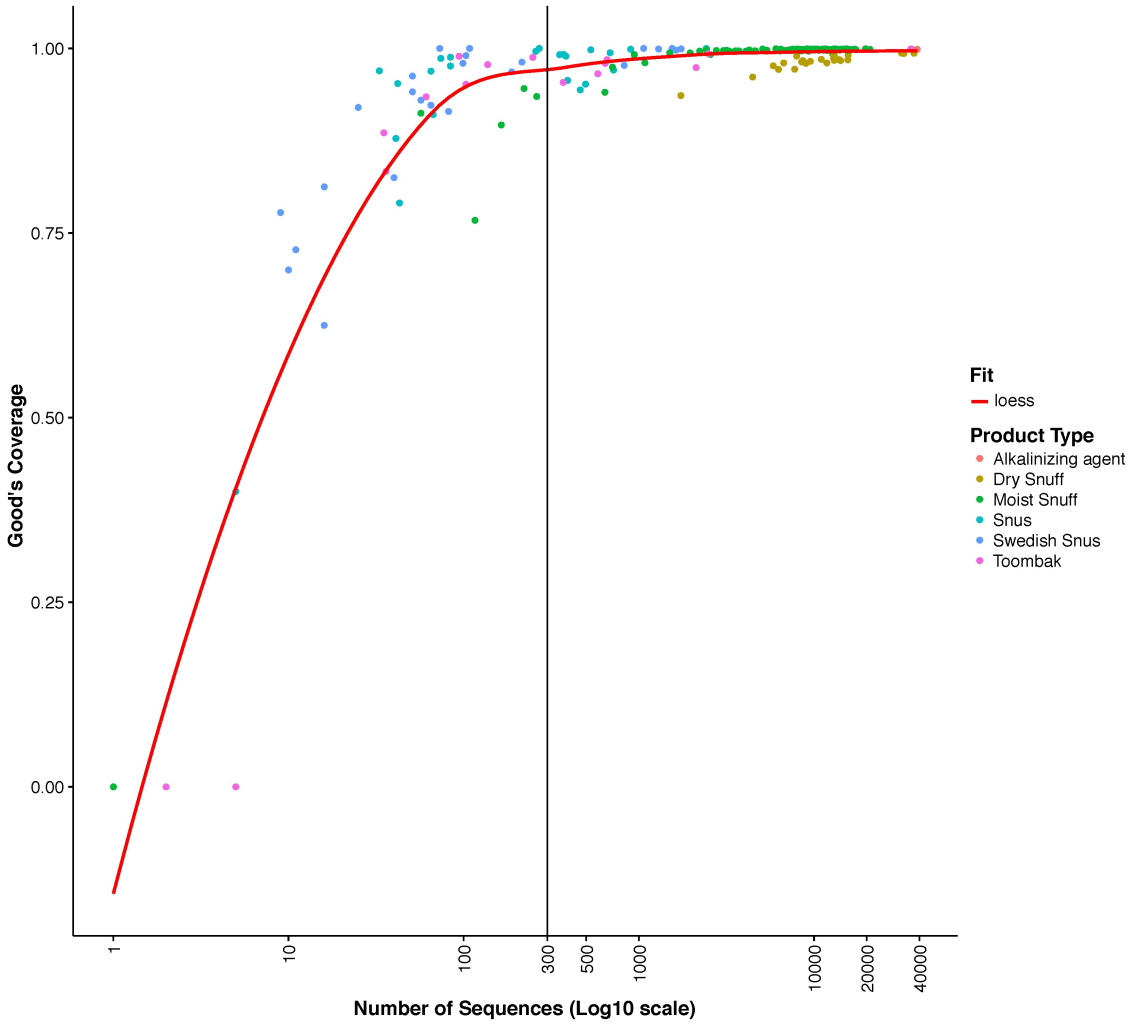

1.00
0.75
0.50
0.25
0.00
Good's Coverage
1
10
100
300
500
1000
10000
20000
40000
Number of Sequences (Log10 scale)
Fit
loess
Product Type
Alkalinizing agent
Dry Snuff
Moist Snuff
Snus
Swedish Snus
Toombak

Figure S2

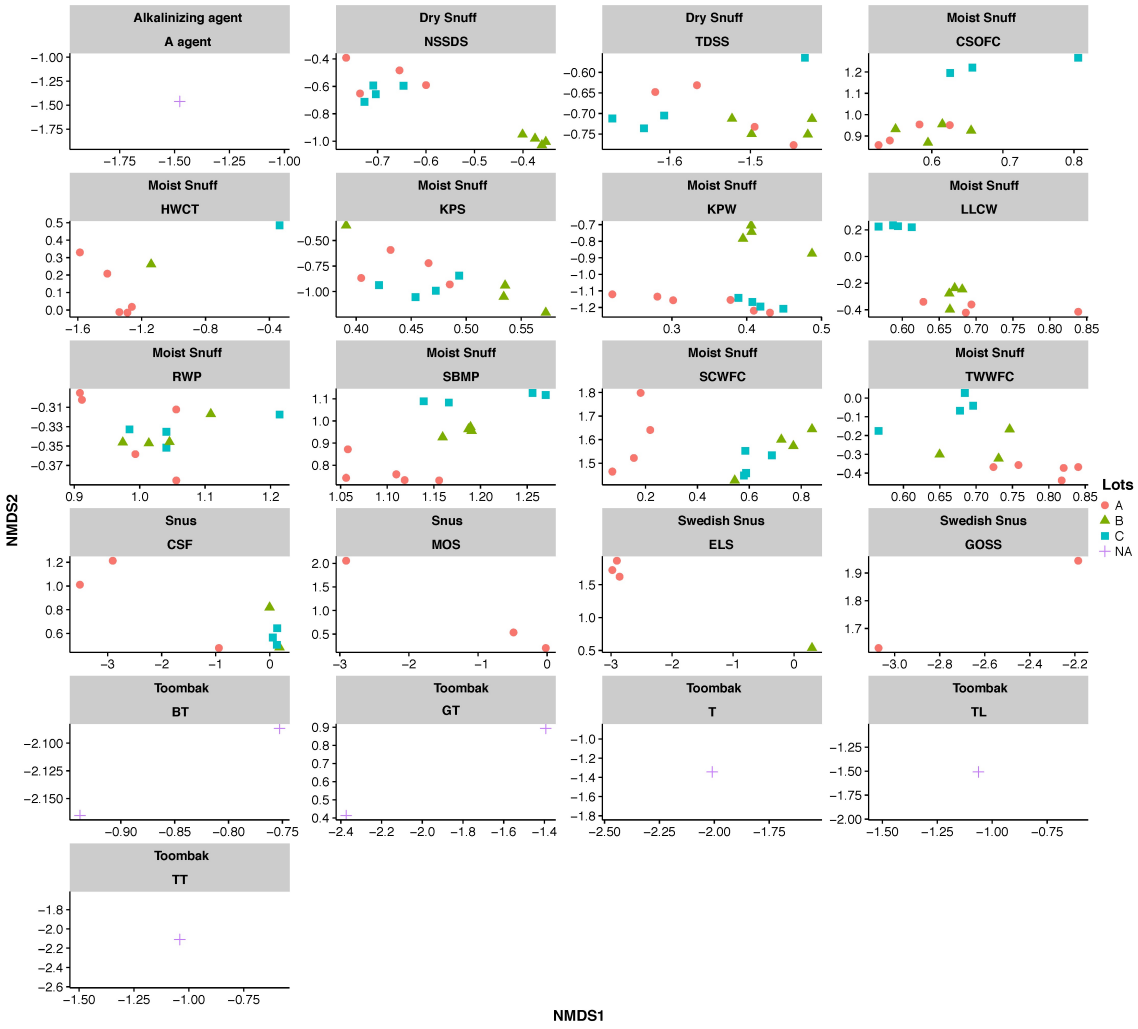

Alkalinizing agent
A agent
Dry Snuff
NSSDS
Dry Snuff
TDSS
Moist Snuff
CSOFC
Moist Snuff
HWCT
Moist Snuff
KPS
Moist Snuff
KPW
Moist Snuff
LLCW
Moist Snuff
RWP
Moist Snuff
SBMP
Moist Snuff
SCWFC
Moist Snuff
TWWFC
Snus
CSF
Snus
MOS
Swedish Snus
ELS
Swedish Snus
GOSS
Toombak
BT
Toombak
GT
Toombak
T
Toombak
TL
Toombak
TT
NMDS1
NMDS2
Lots
A
B
C
NA

Figure S3

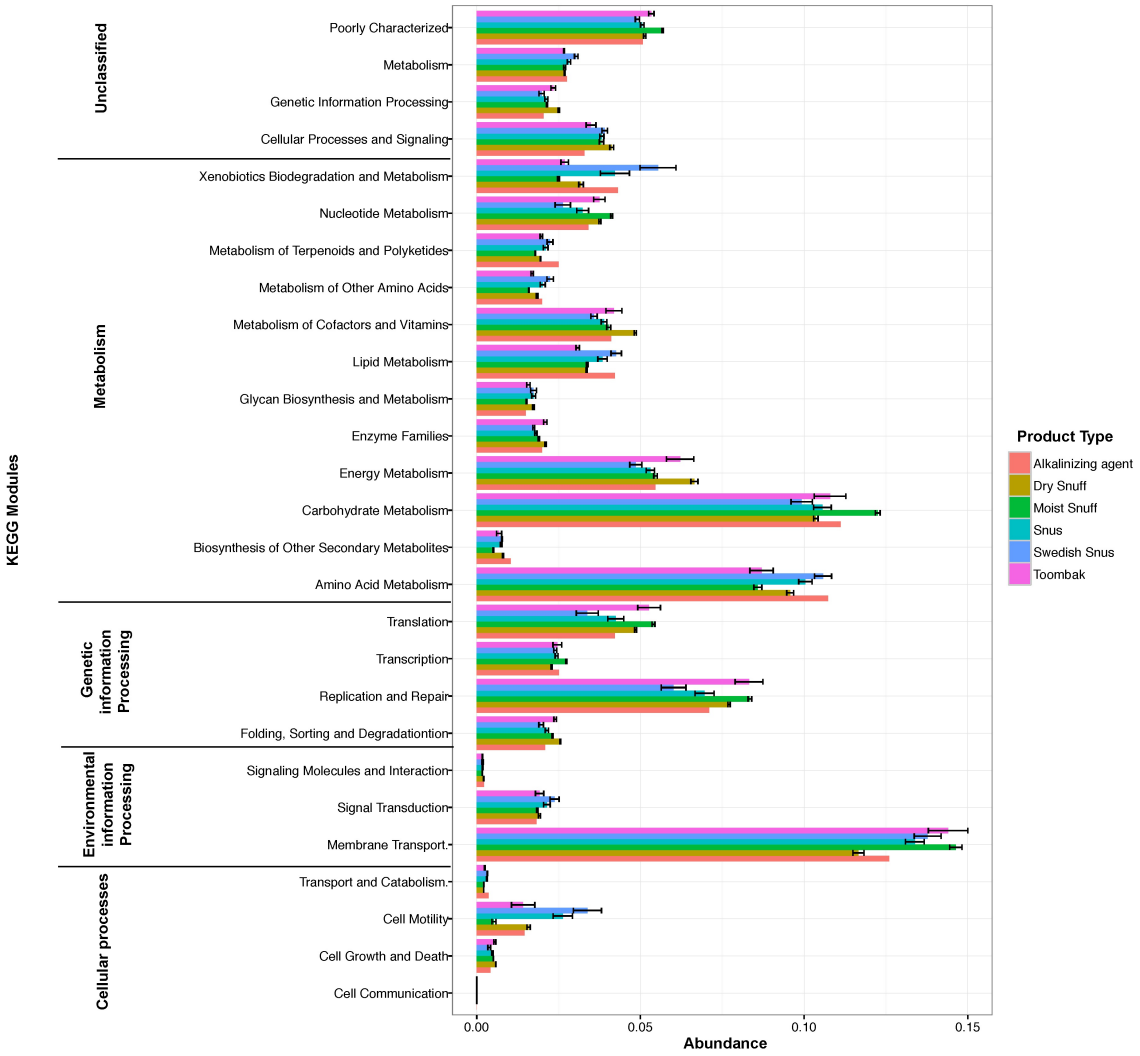

KEGG Modules
Unclassified
Poorly Characterized
Metabolism
Genetic Information Processing
Cellular Processes and Signaling
Metabolism
Xenobiotics Biodegradation and Metabolism
Nucleotide Metabolism
Metabolism of Terpenoids and Polyketides
Metabolism of Other Amino Acids
Metabolism of Cofactors and Vitamins
Lipid Metabolism
Glycan Biosynthesis and Metabolism
Enzyme Families
Energy Metabolism
Carbohydrate Metabolism
Biosynthesis of Other Secondary Metabolites
Amino Acid Metabolism
Genetic information Processing
Translation
Transcription
Replication and Repair
Folding, Sorting and Degradationtion
Environmental information Processing
Signaling Molecules and Interaction
Signal Transduction
Membrane Transport
Cellular processes
Transport and Catabolism
Cell Motility
Cell Growth and Death
Cell Communication
0.00
0.05
0.10
0.15
Abundance
Product Type
Alkalinizing agent
Dry Snuff
Moist Snuff
Snus
Swedish Snus
Toombak

Figure S4

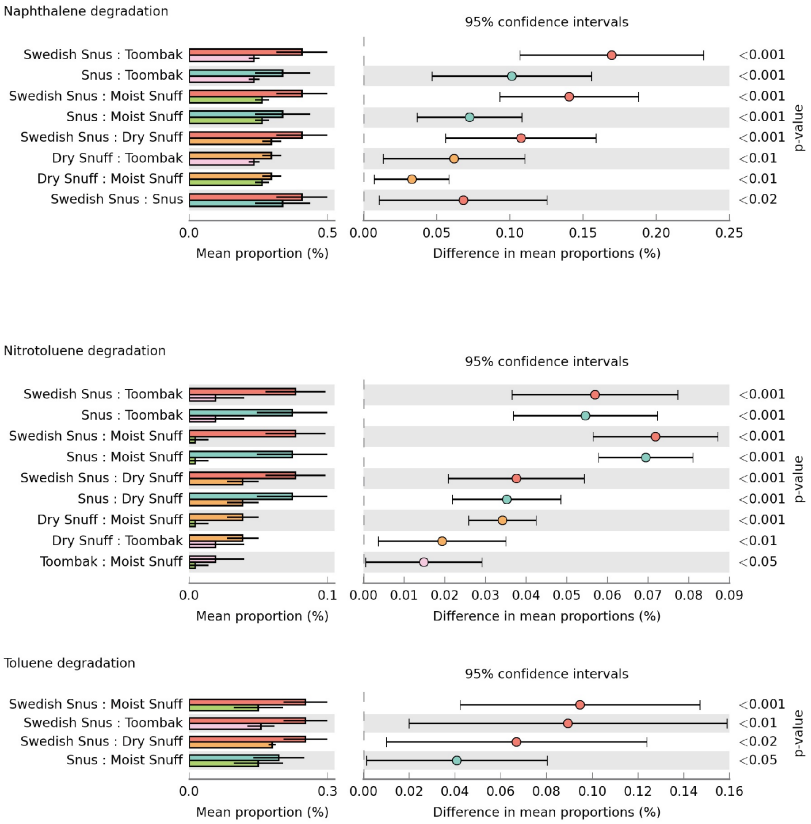
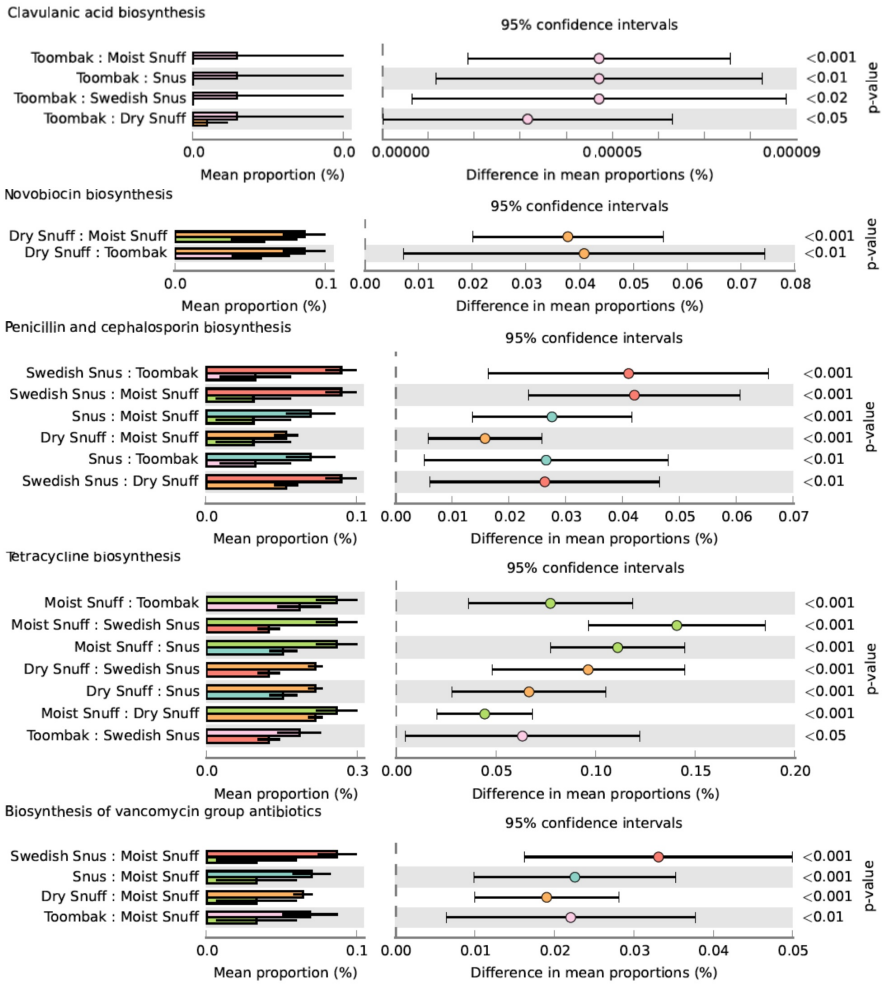

Naphthalene degradation
95% confidence intervals
Swedish Snus : Toombak
Snus : Toombak
Swedish Snus : Moist Snuff
Snus : Moist Snuff
Swedish Snus : Dry Snuff
Dry Snuff : Toombak
Dry Snuff : Moist Snuff
Swedish Snus : Snus
<0.001
<0.001
<0.001
<0.001
<0.001
<0.01
<0.01
<0.02
p-value
0.0
0.5
Mean proportion (%)
0.00
0.05
0.10
0.15
0.20
0.25
Difference in mean proportions (%)
Nitrotoluene degradation
95% confidence intervals
Swedish Snus : Toombak
Snus : Toombak
Swedish Snus : Moist Snuff
Snus : Moist Snuff
Swedish Snus : Dry Snuff
Snus : Dry Snuff
Dry Snuff : Moist Snuff
Dry Snuff : Toombak
Toombak : Moist Snuff
<0.001
<0.001
<0.001
<0.001
<0.001
<0.001
<0.001
<0.01
<0.05
p-value
0.0
0.1
Mean proportion (%)
0.00
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.09
Difference in mean proportions (%)
Toluene degradation
95% confidence intervals
Swedish Snus : Moist Snuff
Swedish Snus : Toombak
Swedish Snus : Dry Snuff
Snus : Moist Snuff
<0.001
<0.01
<0.02
<0.05
p-value
0.0
0.3
Mean proportion (%)
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0.14
0.16
Difference in mean proportions (%)

Figure S5


Clavulanic acid biosynthesis
95% confidence intervals
Toombak : Moist Snuff
Toombak : Snus
Toombak : Swedish Snus
Toombak : Dry Snuff
<0.001
<0.01
<0.02
<0.05
p-value
0.0
0.0
0.00000
0.00005
0.00009
Mean proportion (%)
Difference in mean proportions (%)
Novobiocin biosynthesis
95% confidence intervals
Dry Snuff : Moist Snuff
Dry Snuff : Toombak
<0.001
<0.01
p-value
0.0
0.1
0.00
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
Mean proportion (%)
Difference in mean proportions (%)
Penicillin and cephalosporin biosynthesis
95% confidence intervals
Swedish Snus : Toombak
Swedish Snus : Moist Snuff
Snus : Moist Snuff
Dry Snuff : Moist Snuff
Snus : Toombak
Swedish Snus : Dry Snuff
<0.001
<0.001
<0.001
<0.001
<0.01
<0.01
p-value
0.0
0.1
0.00
0.01
0.02
0.03
0.04
0.05
0.06
0.07
Mean proportion (%)
Difference in mean proportions (%)
Tetracycline biosynthesis
95% confidence intervals
Moist Snuff : Toombak
Moist Snuff : Swedish Snus
Moist Snuff : Snus
Dry Snuff : Swedish Snus
Dry Snuff : Snus
Moist Snuff : Dry Snuff
Toombak : Swedish Snus
<0.001
<0.001
<0.001
<0.001
<0.001
<0.001
<0.05
p-value
0.0
0.3
0.00
0.05
0.10
0.15
0.20
Mean proportion (%)
Difference in mean proportions (%)
Biosynthesis of vancomycin group antibiotics
95% confidence intervals
Swedish Snus : Moist Snuff
Snus : Moist Snuff
Dry Snuff : Moist Snuff
Toombak : Moist Snuff
<0.001
<0.001
<0.001
<0.01
p-value
0.0
0.1
0.00
0.01
0.02
0.03
0.04
0.05
Mean proportion (%)
Difference in mean proportions (%)