

Samples Submitted for Log in 32165

1 cotton-based mask

A blank mask (fresh and not worn) was provided as a control sample.

Methods

Protein Extraction

A 1 cm square was cut from the center region of the mask and placed in an Eppendorf tube. From that 1 cm square, the sample was cut into smaller pieces to increase the surface area. Roughly 5 mm square of each sample was used for further experiments. Each piece of mask was soaked in 100 mL of 0.2% Surfactant Enhancer (Promega, Madison, WI) at 4°C overnight to extract protein.

In Solution Digestion

Total protein was determined on a Qubit and the appropriate volume of each sample was taken to equal 20 µg total protein for digestion. The samples were digested with sequencing grade trypsin/lys C rapid digestion kit from Promega (Madison WI) using manufacture recommended protocol. Three times the sample volume of rapid digestion buffer (provided with the kit) was added to the samples. The sample was incubated at 56°C with 1 µl of dithiothreitol (DTT) solution (0.1 M in 100 mM ammonium bicarbonate) for 30 minutes prior to the addition of 0.54 µL of 55 mM Iodoacetamide in 100 mM ammonium bicarbonate. Iodoacetamide was incubated at room temperature in dark for 30 min. The trypsin/lys C was prepared fresh as 1 µg/µl in the rapid digestion buffer. 1 µl of enzyme was added and the samples were incubated at 70°C for 1 hour. The digestion was stopped with addition of 0.5% TFA. The MS analysis is immediately performed to ensure high quality tryptic peptides with minimal non-specific cleavage.

Q Exactive HF Orbitrap

Nano-liquid chromatography tandem mass spectrometry (Nano-LC/MS/MS) was performed on a Thermo Scientific Q Exactive HF Orbitrap mass spectrometer equipped with a EASY Spray nanospray source (Thermo Scientific) operated in positive ion mode. The LC system was an UltiMate™ 3000 RSLCnano system from Thermo Scientific. The mobile phase A was water containing 0.1% formic acid and the mobile phase B was acetonitrile with 0.1 % formic acid. The mobile phase A for the loading pump was water containing 0.1 % trifluoroacetic acid. 5 µL of sample is injected on to a PharmaFluidics µPAC™ C18 trapping column (C18, 5 µm pillar diameter, 10 mm length, 2.5 µm inter-pillar distance). at 10 µL/ml flow rate. This was held for 3 minutes and washed with 1 %B to desalt and concentrate the peptides. The injector port was switched to inject and the peptides were eluted off of the trap onto the column. PharmaFluidics 50 cm µPAC™ was used for chromatographic separations (C18, 5 µm pillar diameter, 50 cm length, 2.5 µm inter-pillar distance). The column temperature was maintained 40°C. A flowrate of 750 nl/min was used for the first 15 minutes and then the flow was reduced to 300 nl/min. Peptides were eluted directly off the column into the Q Exactive system

using a gradient of 1% B to 20%B over 100 minutes and then to 45%B in 20 minutes for a total run time of 150 minutes:

Time (min)	% B	Flow Rate (nL/min)
0	1	750
3	1	750
15	5	750
15.1	5	300
100	20	300
123	45	300
130	95	300
135	95	300
135.1	1	300
150	1	300

The total run time was 150 minutes. The MS/MS was acquired according to standard conditions established in the lab. The EASY Spray source operated with a spray voltage of 1.5 KV and a capillary temperature of 200°C. The scan sequence of the mass spectrometer was based on the original TopTen™ method; the analysis was programmed for a full scan recorded between 375 – 1575 Da at 60,000 resolution, and a MS/MS scan at resolution 15,000 to generate product ion spectra to determine amino acid sequence in consecutive instrument scans of the fifteen most abundant peaks in the spectrum. The AGC Target ion number was set at 3e6 ions for full scan and 2e5 ions for MS² mode. Maximum ion injection time was set at 50 ms for full scan and 55 ms for MS² mode. Micro scan number was set at 1 for both full scan and MS² scan. The HCD fragmentation energy (N)CE/stepped NCE was set to 28 and an isolation window of 4 *m/z*. Singly charged ions were excluded from MS². Dynamic exclusion was enabled with a repeat count of 1 within 15 seconds and to exclude isotopes. A Siloxane background peak at 445.12003 was used as the internal lock mass.

HeLa protein digest standard is used to evaluate the integrity and the performance of the columns and mass spectrometer. If the number of protein ID's from the HeLa standard falls below 2700, the instrument is cleaned and new columns are installed.

All MS/MS samples were analyzed using Sequest (Thermo Fisher Scientific, San Jose, CA, USA; version IseNode in Proteome Discoverer 2.4.0.305). Sequest was set up to search Full Swiss Prot Database of all species (7/27/2020 475603 sequences) and the SARS2 Covid database (4/14/2021 855 sequences) assuming the digestion enzyme trypsin. Sequest was searched with a fragment ion mass tolerance of 0.020 Da and a parent ion tolerance of 10.0 PPM. Carbamidomethyl of cysteine was specified in Sequest as a fixed modification. Met-loss of methionine, met-loss+Acetyl of methionine, oxidation of methionine and acetyl of the n-terminus were specified in Sequest as variable modifications.

Results

Black and white cotton mask:

Total of 305 proteins identified and listed in the Excel spreadsheet. The most abundant proteins detected are human proteins found in saliva and skin. The following bacteria proteins were detected.

methanothermobacter thermautotrophicus	interesting that this only grows in warm temperatures (55 oC - 65 oC and need carbon dioxide to grow
acinetobacter baumannii	Pathogenic pneumonia, blood stream infections, meningitis, wound and surgical site infections and urinary tract infections Resistant to antibiotics and very difficult to treat.
picophilus torridus	soil dwelling only grows in warm environments
listeria innocua serovar	non-pathogenic version
novosphingobium aromaticivorans	pathogenic gut microbe similar to e. coli
alcelaphine herpesvirus 1	Virus - natural hosts primarily cow but is fatal
streptomyces griseus	soil dwelling used to produce streptomycin, an antibiotic
frankia casuarinae	soil dwelling
saccharomyces cerevisiae	species of yeast - used for baking and making beer
paraburkholderia phytofirmans	found on pie trees - all the pollen in the air
corynebacterium kroppenstedtii	antibiotic resistant pathogen
corynebacterium glutamicum	soil dwelling
streptococcus pyogenes	Strep throat
encephalitozoon cuniculi	Pathogenic in immunocompromised people
prochlorococcus marinus	marine bacteria
streptococcus pneumoniae	
porphyromonas gingivalis	significant human pathogen - major cause pneumonia Pathogenic Found in the oral cavity causing periodontal disease as well as upper gastrointestinal tract, respiratory infections
mycobacterium tuberculosis	Pathogenic Causes Tuberculosis
cupriavidus necator	soil dwelling capable of both aerobic and anaerobic growth
neisseria meningitidis	extremely pathogenic Causes meningitis and life threatening sepsis
staphylococcus aureus	Pathogenic range of illnesses from minor skin infections to life threatening pneumonia, meningitis and sepsis

brucella melitensis
parabacteroides distasonis
geobacillus stearothermophilus
corynebacterium jeikeium

infectious to livestock - mainly sheep
Pathogenic
soil dwelling causes food spoilage
Pathogenic infection in bone marrow transplant patients

polaromonas naphthalenivorans
nitrosomonas europaea
actinobacillus pleuropneumoniae
staphylococcus epidermidis
mycolicibacterium vanbaalenii
saccharomyces cerevisiae

found in water
soil dwelling
Pathogenic to swine
Part of normal skin flora
soil dwelling
species of yeast - used for baking and making beer

lactobacillus gasseri
synechococcus sp
neisseria meningitidis Serogroup C

gastrinointestinal tract bacteria
freshwater bacteria
menigocccal disease. About 1 in 10 people have these bacteria in their nose and throat without being ill. However when it invades the body casues serious disease with fever, headach and stiff neck

staphylococcus suis
Bifidobacterium longum subsp. Intantis
buchnera aphidicola subsp
laribacter hongkongensis
eikenella corrodens
neisseria meningitidis Serogroup B

infectious to swine but can cause severe infection in human
Normal gut bacteria
soil dwelling
anaerobic bacteria potential human pathogen
anaerobic bacteria severe human pathogen
menigocccal disease. About 1 in 10 people have these bacteria in their nose and throat without being ill. However when it invades the body casues serious disease with fever, headach and stiff neck

Corynebacterium efficiens
Rickettsia rickettsii
Corynebacterium diphtheriae

soil dwelling
Pathogenic causes Rocky Mountain Spotted Fever
Causes diptheria - a serious infection - most are vaccinated

Clavibacter michiganensis subsp
chromobacterium violaceum

Pathogenic to tomatos
soil dwelling. Disease to human is rare but mortality is high

Legionella pneumophila
Altermonas mediterranea

Pathogenic causes Legionnaires' disease
Marine bacteria

Acidphilium cryptum	soil dwelling
streptococcus salivarius	Found in the oral cavity - opportunistic pathogen. Harmless unless it enters the bloodstream
cunninghamella elegans	fungus found in soil
shewanella piezotolerans	marine bacteria
Flavobacterium johnsoniae	soil dwelling
Bacteriodes vulgatus	human gut microbiota
Bacteriodes thetaiotaomicron	human gut microbiota
rhodococcus erythropolis	soil dwelling
Nostoc sp	soil dwelling
Bacillus cereus	soil dwelling
Bacteriodes fragilis	human gut microbiota
Sulcisa muelleni	normal insect bacteria
mycoplasma mycoides subsp mycoides SC	Pathogenic to bovine
Corynebacterium aurimucosum	causes UTI
streptococcus agalactiae serotype III	invasive human infections
Paenarthrobacter aurescens	soil dwelling
streptococcus dysgalactiae subsp.	human pathogen antibiotic resistant
Equisimilis	
staphylococcus pyogenes serotype M3	Strep - severe invasive infection
beutenbergia cavernae	soil dwelling
staphylococcus oralis	Found in the oral cavity - opportunistic pathogen. Harmless unless it enters the bloodstream
staphylococcus saprophyticus	common cause of UTI
Dechloromonas aromatica	soil dwelling
Coxiella burnetii	Pathogenic to farm animals like goats, sheep, and bovine
Dichelobacter modosus	Pathogenic to sheep
Acidovorax sp	soil dwelling

Not all bacteria are harmful or pathogenic, and many are a natural part of the human flora on skin, saliva, or in the gut; and natural to the environment in soil and water. However, 21 pathogenic bacteria, were detected and highlighted in yellow. Some are quite dangerous.

Blank mask (Control)

A total of 10 proteins were identified and are all accounted for in the sample preparation steps. For example, trypsin and Lys C enzymes were detected because we add that to the samples digest the proteins. No bacterial proteins were detected.