DETECTION AND CHARACTERIZATION OF COMMON MICROORGANISMS FOUND IN BIOPHARMACEUTICAL INDUSTRIES USING MID-INFRARED LASER SPECTROSCOPY AND MULTIVARIATE ANALYSIS

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AGENDA

INTRODUCTION

- Microbial contamination happens because of the existence and proliferation of microorganisms in the environment.
- In the drug manufacturing industry, biological contamination is a medical problem that can lead to drug degradation and sub-potency leading regulatory agencies to require continuous, routine, or regular environmental monitoring of microorganisms to ensure that the environment during production activities is precisely controlled to protect the patient from contact with potential extraneous matter.

INTRODUCTION

- The industry utilizes different methodologies to control the environment in manufacturing room, surfaces, walls using rigorous cleaning processes, ceiling roof HEPA filters to control the air and particulate, equipment to monitor the effectiveness of the cleaning processes and personnel samples to ensure people aseptic technique processes are adequate.
- These techniques take long periods from collect the sample in the area and inoculate it in a culture media about 12 to 24 hours to allow the microorganism's growth.
- Once the incubation period is completed, identification and characterization of bacterial start by inspecting the colony morphology, followed by microscopic analysis of Gram-stained.

PREVIOUS WORK

Year	Investigation Findings
2000	Emad H. Ibrahim, Glenda Sherman, Suzanne Ward, Victoria J. Fraser and Marin H • Influence of inadequate antimicrobial treatment of bloodstream infections on patient outcomes in the ICU • 30-60% higher than in the group that promptly receives appropriate therapy
2000	Naumann, D • Published that the bacterial cells consist of signal contributions of all components in the cells • FT-IR spectroscopy has proven that a wide range of microorganism can be identified using the spectral response
2002	Hvozdara, L., Pennington, N., Kraft, M., Karlowatz, M. and Miziakoff. B., • Published some benefit of QCL with mid Infrared because they operate near room temperature • Offer the possibility of tailoring the emission wavelengths within a broad range of frequencies

PREVIOUS WORK

Year	Investigation Findings
2003	D. Hofstetter and J. Faist • Used mass spectrometry, electrospray ionization, matrix-assisted laser desorption ionization, Fourier Transform Infrared (FT-IR), and Raman spectroscopy • Concluded that vibrational spectroscopy (QCL-GAP) Quantum Cascade Laser Grazing Angle Probe demonstrated is a reagent less/solventless technique
2003	D. Hofstetter and J. Faist. • The system operates at wavelengths in the MIR starting from 3300 to approximately 750 cm-1, which matches well with the fundamental vibrational absorption bands of many biological species
2019	Novais, A., Freitas, A.R., Rodrigues, C., Peixe, L. • Published the uses of Fourier transform infrared spectroscopy prospects for bacterial strain typing • They could discriminate between Gram-positive and Gram-negative bacteria based on sugar coating structures, high relevance for the specificity in patho-gen-host interactions.

PROBLEM AND PROPOSED SOLUTION

Problem

Classical methods of microorganism identification are based on time-consuming and labor-intensive approaches. Screening techniques require the rapid and grouping of bacterial isolates

Proposed Solution

The present research aims to accelerated detection, identification, and discrimination between three bacteria such as Staphylococcus aureus, Staphylococcus epidermidis and Micro-coccus luteus deposited over a stainless-steel substrate-like material used in the biopharmaceutical industry clean rooms using (QCL-GAP) Quantum Cascade Laser Grazing Angle Probe.

EXPERIMENTAL DESIGN APPLICATIONS

General applications

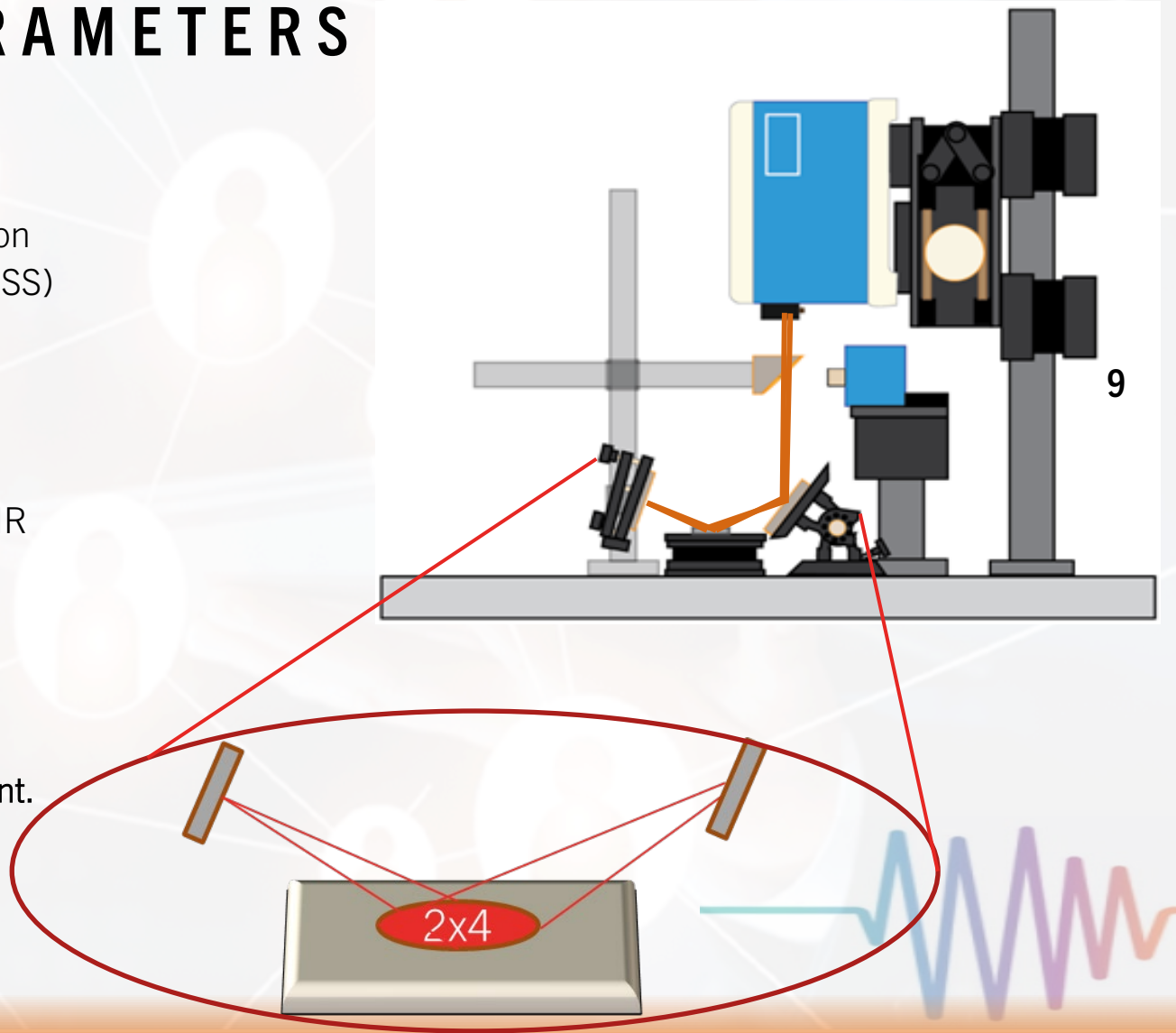
- Thin films containing microorganisms on surfaces.
- Useful for environmental sampling, cleaning validation and biotechnology industries among other
- Detection of chemical and biologic threats agents deposited in surfaces
  - Important for the quick detection, prevention of contaminant at early stages
- Identification of organic compounds
- Identification of impurities in a mixture
- Conformational analysis

Application with Grazing Angles Probe

- Having near grazing of incident becomes the most powerful techniques for surface analysis.
- Study of chemical and biological monolayers including microorganisms
- Improve sensitivity and better S/N ratio.
- Low Limit of Detections (10-50 ng/cm^2) of a single analyte
- API mixture analysis on metallic surface.
- High discrimination of the vibrational signals allowing the isolation of the analyte signals using multivariate chemometrics routine.

INSTRUMENTATION AND PARAMETERS

- QCL-GAP pre-dispersive spectrometer based on QCL technology (LaserScan™, Block En-gineering, LLC, Marlborough, MA, USA)
- Reflection-absorption measurements was used for data acquisition in the MIR: 5.4 – 12.8 μm of the bacterial suspension samples (BSS)
- Vibrational response was collected using a 4 cm-1 spectral resolution.
- Spectrometer was equipped with an internal thermoelectrically Mercury-Cadmium-Tellurium (MCT) detector and a 2 x 4 mm2 MIR laser beam.
- Two adjustable gold mirrors that allow the laser incidence at the grazing angle (~82°)
  - One toward the sample and the second reflects the light from the sample allowing a double-pass reflection-absorption measurement.



MATERIAL/REAGENTS AND PREPARATION/CONDITIONS

Material and Reagents

- Three environmental bacteria isolates (Staphylococcus aureus (Sa), Staphylococcus epidermidis (Se), and Micrococcus luteus (Ml)
- Bacteria certification and identification using MALDI-TOF with supplemental tests as example ID determination with MALDI-TOF confirmation.
- Concentration verification was done by spread plate and incubation conditions required for the bacteria under characterization.
- Lyophilized with a concentration of 50 EU/0.1mL
- Stainless steel (SS) substrate (2in x2in) used for the sample deposition is a grade material like the one used in clean rooms in biopharmaceutical industries

Preparation and conditions

- Bacteria were reconstituted with a reconstitution buffer provided by Microbiologics company.
- 10 mL of the bacteria solution were dispensed in 90mL of Tryptic Soy Broth (TSB)
- The bacteria were incubated at 30 - 35°C for 24 hours.
- Substrates (2X2 in) were cleaned with isopropyl alcohol and left to dry in a chemical hood.
- Aliquots of 20 µL of neat and mixtures bacteria samples were deposited on the 2 X 2-in stainless-steel (SS) plates
- Bacterial samples Sa, Se, and Ml were used to create the following mixtures: Sa/Se, Sa/Ml, and Se/Ml
- Smearing technique using a micropipette tip (~1 mm diameter) was used to deposit the sample onto the substrate

EXPERIMENTAL ANALYSIS

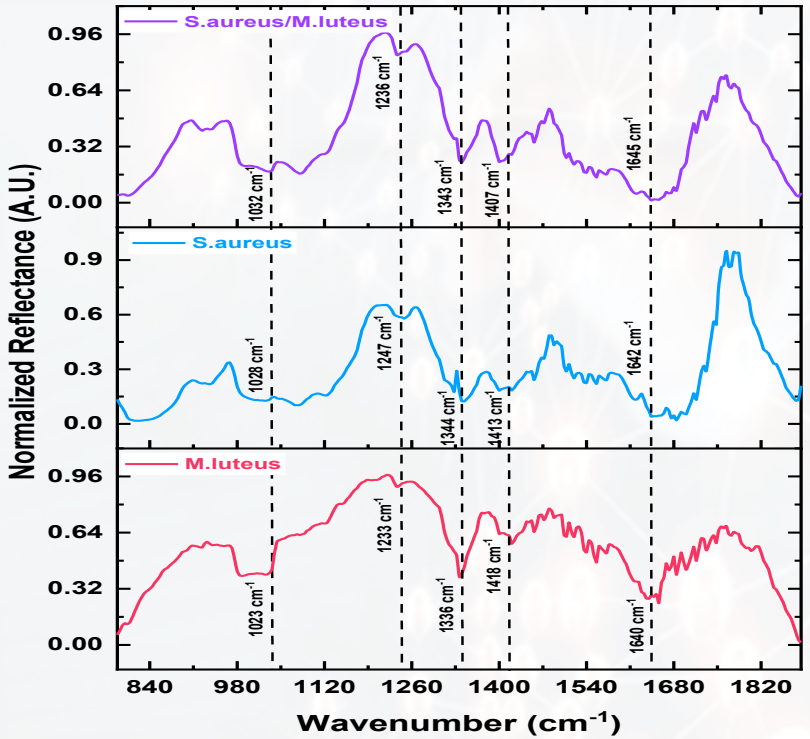
DETECTION AND CHARACTERIZATION OF COMMON MICROORGANISMS FOUND IN BIOPHARMACEUTICAL INDUSTRIES USING MID-INFRARED LASER SPECTROSCOPY AND MULTIVARIATE ANALYSIS

Abstract

We report on the spectroscopic investigation of common bacteria encountered in biopharmaceutical industries with spectroscopic definition and specificity using mid-infrared laser spectroscopy. This study describes the detection of three different bacteria species using quantum cascade laser spectroscopy coupled to a grazing angle probe (QCL-GAP). Stainless steel material, similar to surfaces commonly used in biopharmaceutical industries, was used as support media substrates for the bacterial samples. QCL-GAP spectroscopy was assisted by multivariate analysis (MVA) to assemble a powerful spectroscopic technique with classification, identification, and quantification resources. The bacterial species analyzed: *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Micrococcus luteus*, were used to challenge the technique's capability to discriminate from microorganisms from the same family. Principal component analysis and partial least squares discriminant analysis differentiated between the bacterial species, using (QCL-GAP) as the reference. Spectral differences in the bacterial membrane were used to determine if these microorganisms were present in the samples analyzed. Results herein provided effective discrimination for the bacteria under study with high sensitivity and specificity values.

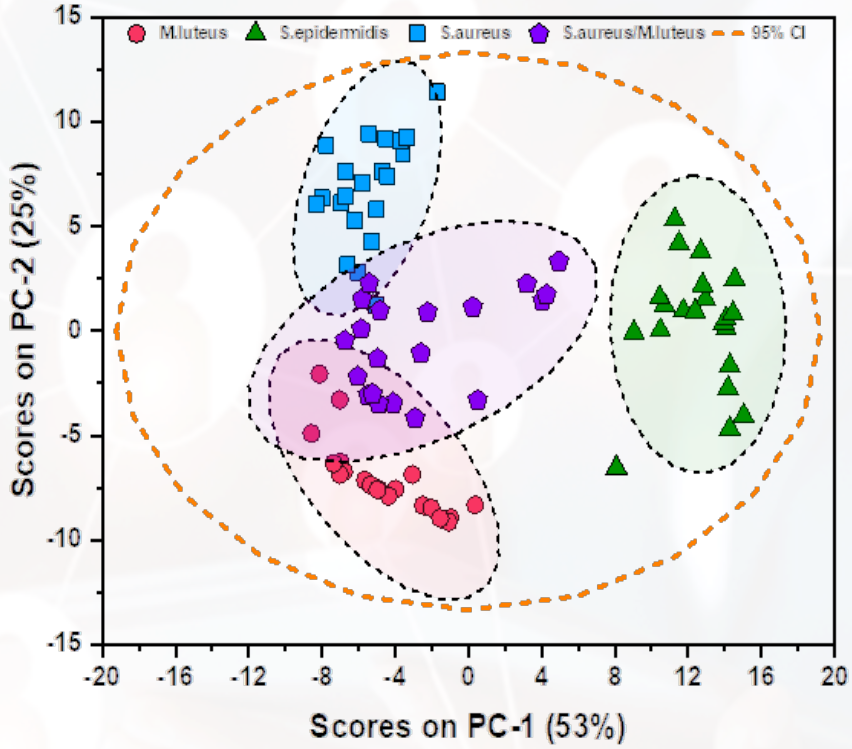
Keywords: quantum cascade laser spectroscopy (QCLS), infrared spectroscopy (IRS), bacteria, stainless steel substrates (SS), principal component analysis (PCA).

S.AUREUS/M.LUTEUSMIXTURES SPECTRUM AND PCA



Spectrum Analysis

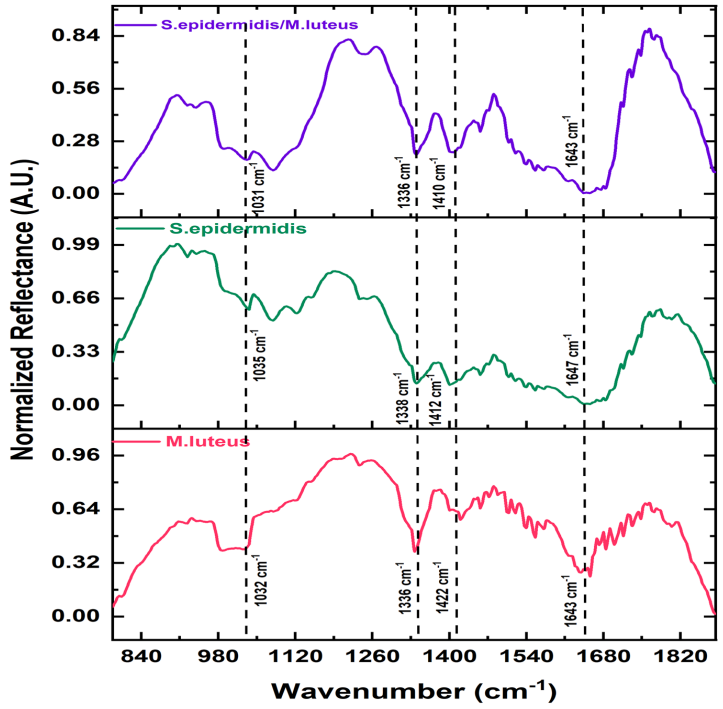
- Normalized reflectance stacked
- Band assignment of Se and MI mix-ture (top) and Se (center) and MI (bottom)
- Comparing the mixture similar bands neat bacteria spectrum deposited in stainless steel substrate
- Characteristic M.luteus band at 990 $cm^{-1}$and in the mixture

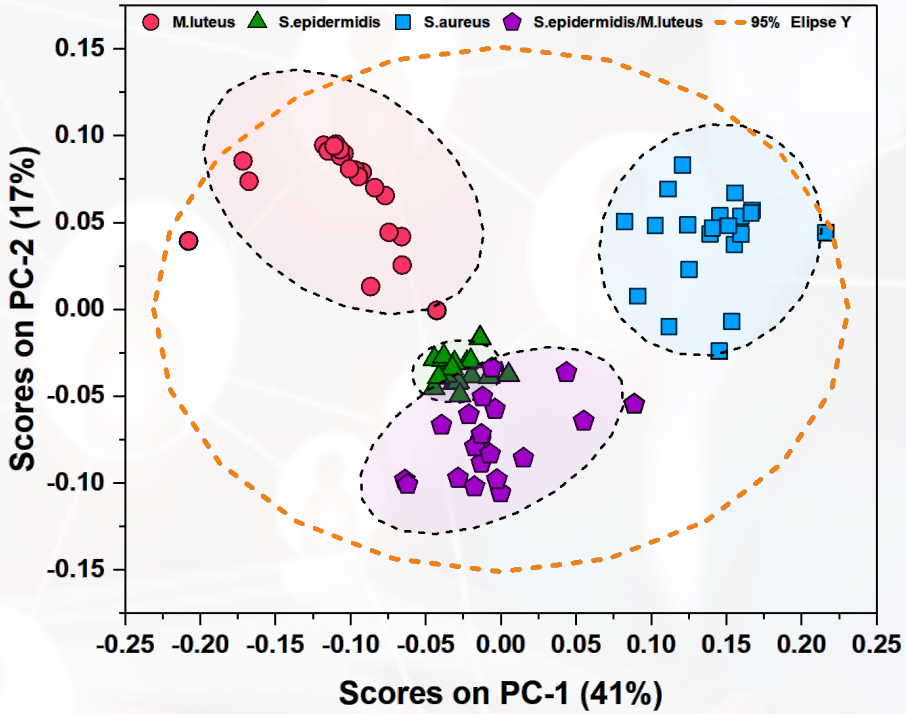


PCA Analysis

- M.luteus/S.aureus mixtures are positioned between M.luteus and S.aureus pure bacteria loads respectively
- S.epidermidis is segregated demonstrating resolution.

S.EPIDERMIDIS/M.LUTEUS MIXTURES SPECTRUM AND PCA





Spectrum Analysis

- Normalized reflectance stacked
- band assignment of Se and MI mixture (top) and Se (center) and MI (bottom)
- Comparing the mixture similar bands neat bacteria spectrum deposited in stainless steel substrate
- Better resolution in the normalized reflectance spectra between the mixture and the S.epidermidis.

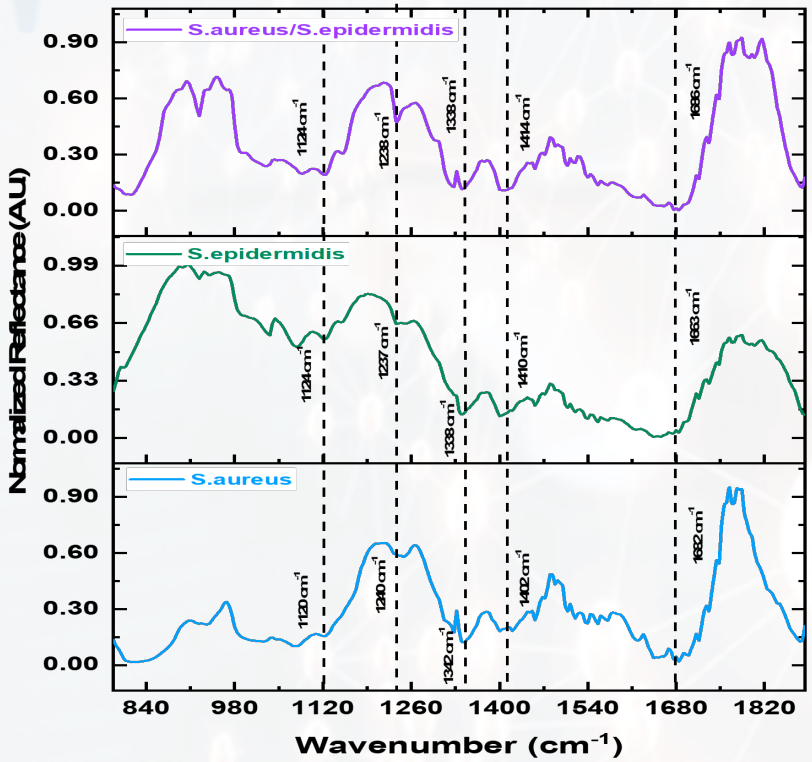
PCA Analysis

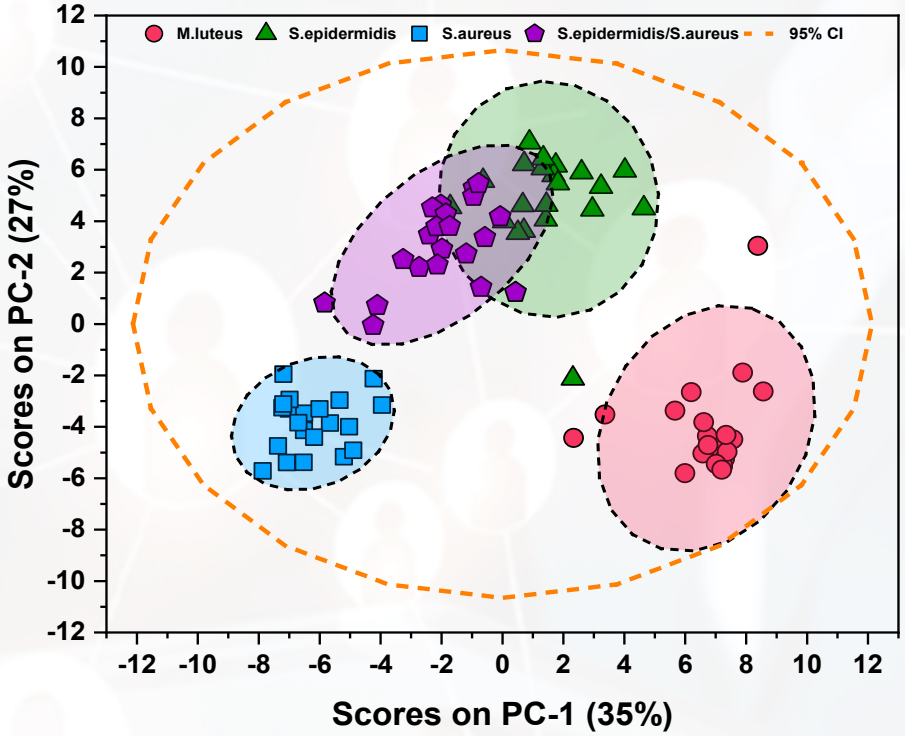
- S.epidermidis/M.luteus mixtures are positioned towards S.epidermidis than M.luteus of pure bacteria loads

Concentration effect

- S.aureus is segregated from the mixtures demonstrating discrimination

S.AUREUS/S.EPIDERMIDIS MIXTURES SPECTRUM AND PCA





Spectrum Analysis

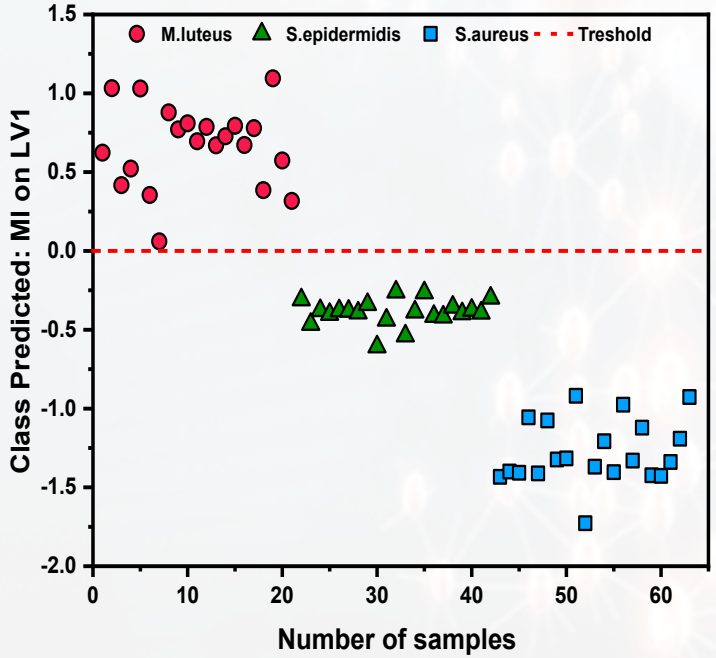
- Normalized reflectance stacked
- Sa (bottom), Se (center), and a mix of Sa:Se (top)
- Comparing the mixture similar bands neat bacteria spectrum deposited in stainless steel substrate
- Better resolution in the normalized reflectance spectra between the mixture and the S.epidermidis.

PCA Analysis

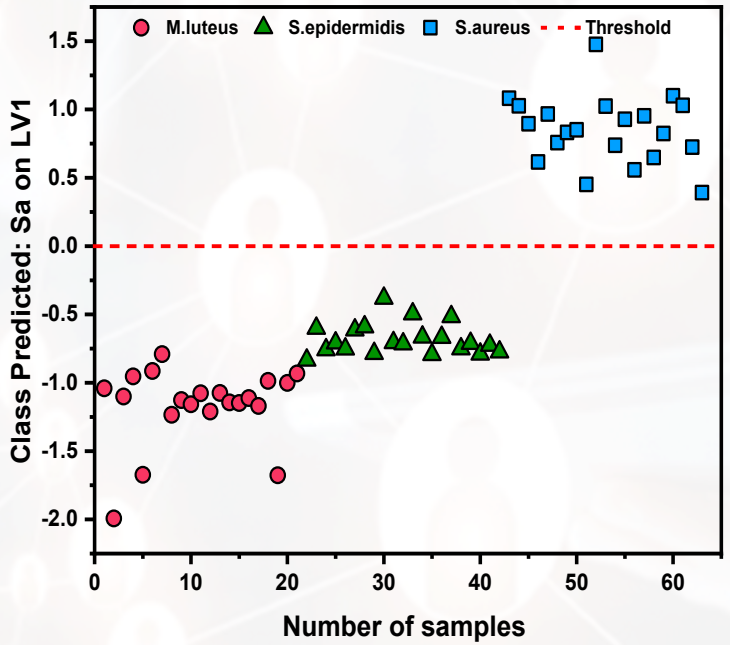
- S.aureus/S.epidermidis mixtures are positioned between S.epidermidis and S.aurerus of pure bacteria loads
- S.luteus is segregated from the mixtures demonstrating discrimination

PLS-DA ANALYSIS

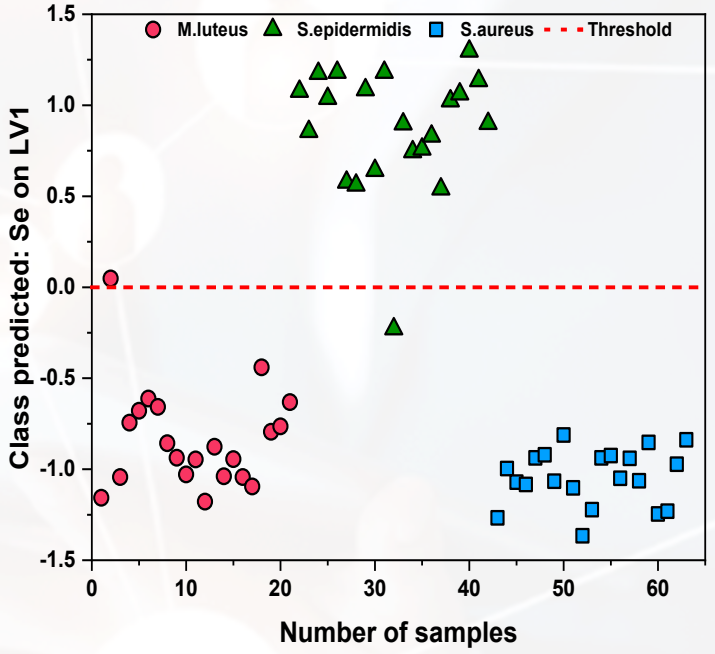
MI with SNV+SG2



Sa with SG1+SNV



Se with SNV+SG2



The optimal results for MI were obtained with SNV+SG2, Se with SG1+SNV, and Sa with SNV+SG2

PLS-DA MODEL EVALUATION

Table 1. PLS-DA model evaluation results for MI, Se, and Sa developed with the optimal preprocessing methods: SNV+SG2 and SG1+SNV.

	M. luteus		*S. epidermidis*		*S. aureus*	
DP	SNV+SG2	SG1+SNV	SNV+SG2	SG1+SNV	SNV+SG2	SG1+SNV
%T	100.0%	100.0%	98.4%	96.8%	100.0%	100.0%
%F	0.0%	0.0%	1.6%	3.2%	0.0%	0.0%
SEN	1.0	1.0	1.0	1.0	1.0	1.0
ξ	1.0	1.0	1.0	1.0	1.0	1.0
MCC	1.0	1.0	1.0	0.9	1.0	1.0

Micrococcus luteus	*Staphylococcus epidermidis*	*Staphylococcus aureus*
Discriminates all samples perfectly for both preprocessing methods	Se appears close to the threshold, indicating slight difficulty The model classifies two samples incorrectly from two different classes	Classifies Sa without difficulty, while the MI samples are close to the threshold value of zero

CONCLUSIONS

- Based on the study it can be concluded that the results obtained demonstrate that the QCL-GAP operating in the range of 788 – 1884 cm^{-1} produced high quality spectral in-formation from the bacterial species studied: Sa, Se, and MI
- Although the bacteria under the investigation belong to the same family, the information provided by the QCL-GAP, had sufficient data to discriminate the bacteria mixtures from the pure bacteria
- PCA with different combinations, the tendency is to observe the mixture ellipse positioned between the pure bacteria combination ellipses
- Developing PLS-DA models to discriminate one microorganism at a time, results show that an average of 99.2% microorganisms were classified correctly
- This study demonstrated the capability of QCL-GAP in combination with PCA to discriminate between bacteria from the same family
- The development of this new methodology, for the analysis of bacteria using QCL-GAP, provided fast and accurate analysis for detecting microorganisms and a great potential to discriminate between similar types of microorganisms

PROPOSED APPLICATION IN CLEAN ROOM

- No Invasive Environmental Sampling
- In Situ Bacteria discrimination
- Cleaning Validation and Verification
- Detection of undesirable chemical or biologic agents deposited in surfaces
- Quick detection, prevention of contaminant at early stages
- Identification of impurities

Questions?