

**Grant No. IBD-0198R**

**Project Title: Non-invasive biosensors for the diagnosis and management of Crohn's disease**

**Principal Investigator: Dr. Sylvia Daunert**

## **Final Technical Progress Report**

### Summary of project aims

The aims of this project are to clarify that:

- quorum sensing signaling molecules (QSMs) are biomarkers to measure intestinal inflammatory activity;
- genetically engineered bacterial whole-cell sensing systems provide a useful tool to assess the QSM levels in body fluids, such as saliva and stool, thus allowing the non-invasive monitoring of inflammatory activity in Crohn's disease (CD).

### Accomplishments towards meeting those aims

We demonstrated that genetically engineered whole-cell biosensing systems can successfully be employed for quantitative detection of QSMs in biological samples, such as, saliva, stool and bowel secretion from, both, healthy individuals and subjects affected by gastrointestinal disorders, including, inflammatory bowel disease (IBD) and necrotizing enterocolitis (NEC).

Preliminary analysis of bowel secretion samples showed a statistically significant increase in QSM presence in IBD patients when compared to controls. Additionally, statistically significant lower levels of QSMs were detected in the stools of low birth weight infants who developed NEC with respect to comparable infant population who did not develop the disease. This evidence suggests the viability of employing QSMs as biomarkers of gut inflammatory activity in various clinical settings.

### Significant results

Inflammatory bowel disease patients and healthy controls were enrolled in the study. Bowel secretion samples were obtained from enrolled subjects presenting to endoscopy for procedures associated with their IBD or for screening colonoscopy. A total of 40 bowel secretion samples were analyzed directly, without sample pretreatment, for the presence of *N*-acyl homoserine lactones (AHLs), a family of quorum sensing signaling molecules produced by Gram-negative bacteria. For that, we employed two whole-cell

sensing systems, which are based on bioluminescent genetically engineered bacterial cells bearing plasmid pSB406 or pSB1075, and allow for detection of short and long side chain AHLs, respectively.

Twenty-eight out of 40 samples were proven to contain detectable levels of short chain AHLs, with concentrations ranging  $1 \times 10^{-8}$  –  $1 \times 10^{-6}$  M. Thirty-three out of 40 samples

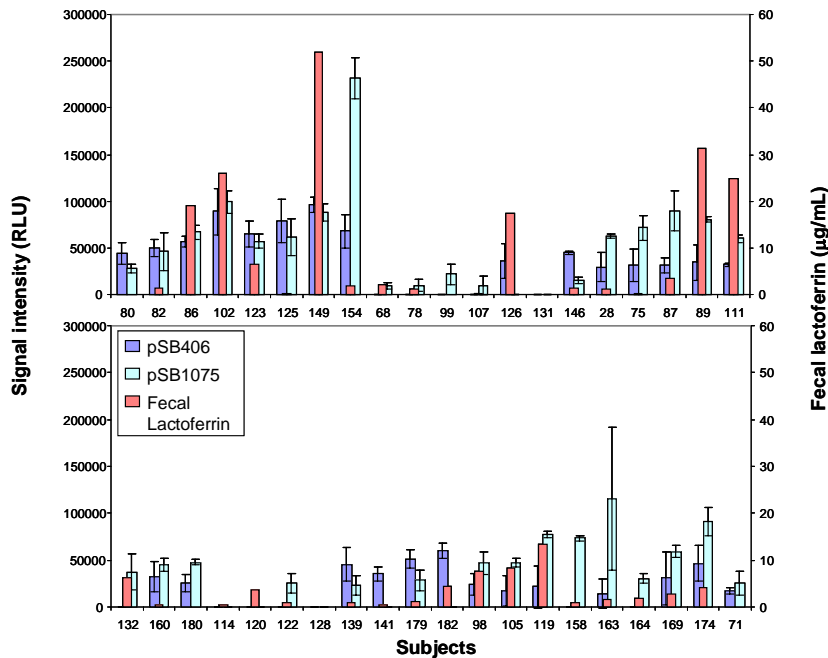


Figure 1. Short and long chain AHLs and fecal lactoferrin detected in bowel secretion samples from IBD patients.

were shown to contain detectable levels of long chain AHLs, with concentrations also ranging  $1 \times 10^{-8}$  –  $1 \times 10^{-6}$  M. Samples were considered to be positive for AHLs when their signals were equal to or higher than the average signal of the blank plus 3 standard deviations. The bowel secretion

samples were also analyzed for fecal lactoferrin, a molecule that is suggested to be a non-invasive, sensitive and specific biomarker representing intestinal inflammation in patients with IBD. For that, a commercially available immunoassay kit was employed. The AHL and fecal lactoferrin levels detected in bowel secretions are shown in Figure 1.

Although a limited number of control subjects (3) were enrolled, a statistically significant difference in the levels of long chain AHLs was observed between IBD patients and controls (Figure 2). An unpaired, two tailed t-test with Welch’s correction was performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, [www.graphpad.com](http://www.graphpad.com)). Samples from further healthy controls should be analyzed to confirm the preliminary data obtained.

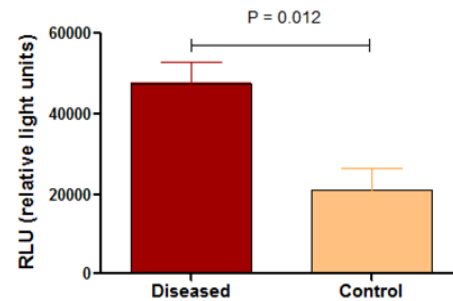


Figure 2. Comparison between long chain AHL levels in diseased and control subjects.

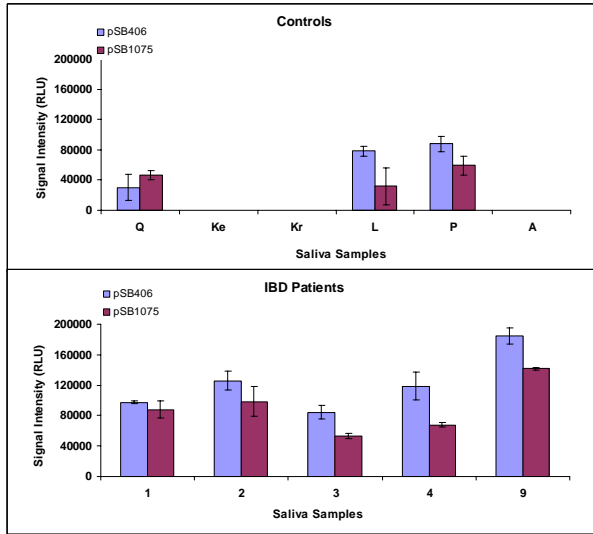


Figure 3. Short and long chain AHLs detected in saliva samples from IBD patients and healthy controls.

Saliva samples were also collected from IBD patients and healthy controls. Short and long chain AHLs were detected in all of the patient samples analyzed, while both types of AHLs were detected in 3 out of 6 control samples (Figure 3).

In parallel to this study, which was carried out on saliva and stool samples from IBD patients and healthy controls, another study was performed that

involved detection of AHLs in stools from newborns admitted to the Neonatal Intensive Care Unit (NICU). It is known that the NICU provides a diverse community of infants at an increased risk of inflammatory or infectious diseases. Bowel inflammation (necrotizing enterocolitis) and bacterial sepsis are typical examples of such illnesses

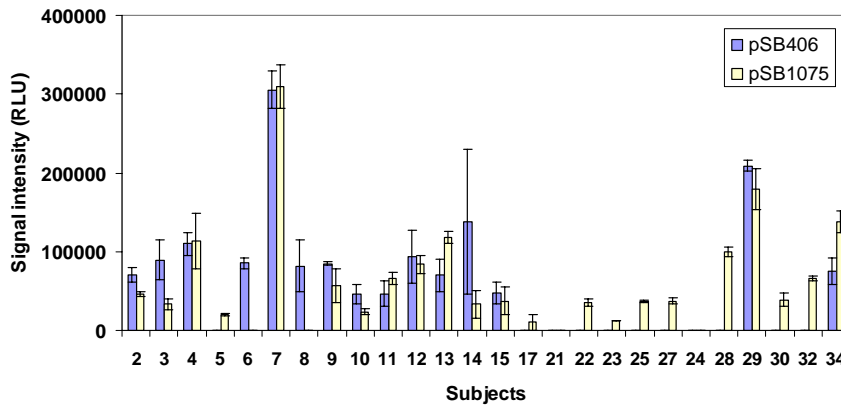


Figure 4. Short and long chain AHLs detected in stool samples from NICU infants.

affecting neonates. Thirty-one newborns with birth weights less than 1500 g were enrolled and monitored for a period of time, with stools collected once or twice a week. Two hundred and twenty five stool samples were analyzed. In Figure 4 we report the results obtained from the analysis of the samples collected from each infant upon admission/enrollment in the study. It is interesting to note that the presence of AHLs was detected in the stool of the majority of neonates, suggesting the presence of Gram-negative bacteria in their gut. This observation points toward establishment of microflora early on in these neonates. Six of the 31 infants had a diagnosis of NEC at some time during their hospitalization. pSB1075 sensor results were significantly negatively associated with infants who ever had NEC in their course, with a Pearson's coefficient of -0.225 and a p value of 0.001. Decreased levels of long

chain AHLs in infants with NEC may reflect lower levels of commensal Gram-negative microorganisms and less biodiversity in affected infants.

#### Lay summary of the progress report

Studies in animals and humans have established that the many bacterial species present in the bowel are essential for, both, its health and disease. Altered bacterial balance in the gut may lead to inflammation such as that occurring in inflammatory bowel disease (IBD), especially Crohn's disease (CD). Such imbalance may not only initiate unrelenting inflammation, but also translate to periodic exacerbations experienced by patients with IBD. Hence, bacterial load and interactions may play an important role in IBD. Bacterial behavior and colonization depend upon communication between bacteria, of the same or of different species. This 'conversation' between bacteria is based on a phenomenon known as quorum sensing (QS). Quorum sensing enables bacteria to communicate with each other and control expression of certain specialized genes by producing and responding to extracellular chemical signals in proportion to cell density. Given the interplay of bacterial flora and the status of luminal inflammation, we believe that measurement of these quorum sensing signaling molecules (QSMs) is an attractive tool in the assessment of the degree of inflammation. Varying levels of quorum sensing molecules may thus be an early predictor of an exacerbation. In that regard, *the overall goal of this project is to clarify that quorum sensing signaling molecules can function as biomarkers to measure intestinal inflammatory activity, and biosensors provide a useful tool to assess the QSM levels in body fluids, such as saliva and stool, thus allowing the non-invasive monitoring of inflammatory activity in the gut.*

In this work we showed that genetically engineered bacteria can successfully be employed as whole-cell biosensing systems for simple, fast, and quantitative detection of QSMs in biological samples from, both, healthy individuals and subjects affected by gastrointestinal disorders. Specifically, we evaluated saliva and bowel secretion samples from subjects with IBD as well as stool samples from low birth weight infants at risk of developing bowel inflammation (necrotizing enterocolitis, NEC). Preliminary analysis of bowel secretion samples showed a statistically significant increase in QSM presence in IBD patients when compared to controls. Additionally, statistically significant lower levels of QSMs were detected in the stools of neonates who developed NEC with respect to comparable neonate population who did not develop the disease. This evidence suggests the potential of QSMs for being employed as biomarkers of gut

inflammatory activity in various clinical settings. Importantly, when saliva and stool are analyzed, sample collection is non-invasive, thus reducing the burden for the patient.