

# The Gut Microbiota and Seleno-Compound as Emerging Risk Factors for Acute Myocardial Infarction

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## Research Article

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# Abstract

## Background

It has been seemed that the gut microbiome alterations might be proposed as the metabolic disorder. However, the relationship between the microbiome and AMI has not been well validated.

## Methods

The feces of the 44 subjects (AMI: 19; control: 25) were collected for fecal genomic DNAs extraction. The variable region V3–V4 of the 16S rRNA gene was sequencing by the platform of Illumina Miseq. The abundances of metabolites were analyzed by the Kyoto Encyclopedia of Genes and Genomes (KEGG) metabolic pathways.

## Results

The abundance of bacteria was more enriched in the AMI group both in the observed operational taxonomic units (OTUs) and faith phylogenetic diversity (PD) ( $p$ -value=0.01 and <0.001 with 95% CI, individually). *Selenomonadales* was less enriched in the AMI group at the Family, Genus, and Species levels [all linear discriminant analysis (LDA) scores >2]. The seleno-compound was more abundant in the AMI group at the Family, Genus, and Species levels (all LDA scores >2).

## Conclusions

This is the first study to demonstrate *Selenomonadales* and seleno-compound to be associated with the occurrence of AMI. Our findings provide an opportunity for a novel prevention and treatment of AMI.

## Introduction

Acute myocardial infarction (AMI) is a growing epidemic in the developing countries, and the leading cause of death in the industrialized societies. The episodes of AMI are related to transmural myocardial ischemia, and result in myocardial injury or necrosis.<sup>1</sup> The pathophysiology of AMI is initiated by the erosion, fissuring, dissection or rupture of the atherosclerotic plaques of the coronary arteries. The onset of the plaque rupture results in the cascade of platelet adhesion, activation, aggregation, and atherothrombosis formation.<sup>1</sup> Therefore, the blood flow of the coronary artery is disrupted abruptly, and occluded by the thrombus formation. The traditional risk factors of AMI have been known as hypertension, diabetes mellitus, dyslipidemia, family history, smoking, age, and post-menopause.<sup>1</sup>

Nowadays, the microbiota, which is a dynamic ecosystem shaped by a few factors such as microbiome, genetics, diet, and environment has been investigated for its crucial function for the human health. There are more than 2000 species of bacterial organisms in the human body, and the gut microbiome is by far the greatest mass of microbiota.<sup>2</sup> These bacterial organisms have developed complicated connection with the human bodies. Many studies have shown that intestinal flora disorders are closely related to the

occurrence of inflammation, metabolic, and systemic diseases (e.g., obesity, diabetes, dyslipidemia, malignancies, psychiatric problems, autoimmune disorders, and cardiovascular diseases).<sup>3</sup>

Therefore, gut microbiota which contributes to the inflammatory metabolism, might possibly be associated with the AMI episodes. Based on this point, and given the need for effective prevention or treatment of AMI, we conduct this comparative study to investigate the composition, and functional differences between the patients with /without AMI by 16S ribosomal RNA (rRNA) microbiome analysis. We attempt to clarify the specific profiles of gut microbiota in AMI patients, and their potentiality for the treatment of AMI.

## **Methods**

### **The Patient and Public Involvement statement**

This study was approved by the ethics committee and institutional review board (IRB) on human research of the Medical Research Department of National Taiwan University Hospital, Taipei, Taiwan. All subjects provided informed consent before participating in the study. The participants provided their written informed consent to participate in this study. All research was performed in accordance with relevant guidelines/regulations, and include in their manuscript a statement confirming that informed consent was obtained from all participants and/or their legal guardians. Research involving human research participants must have been performed in accordance with the Declaration of Helsinki.

### **Study Setting and Participants**

In this single-center and case-control study, 44 patients (AMI: 19 vs control: 25) were enrolled and collected their stool for microbiome analysis between June 2020 and Oct 2020. AMI was defined by the criteria based on a combination of two of three characteristics (e.g., typical symptoms, the electrocardiographic pattern, and cardiac enzyme raise). The control subjects were without coronary artery disease (CAD) based on clinical history, noninvasive stress testing, and coronary imaging studies including coronary computed tomography and/or coronary angiography. Subjects with prior gastrointestinal surgery (e.g., colectomy, ileectomy, and gastrectomy), current administration of antibiotics, inflammatory bowel disease, auto-immune diseases, and malignancy were excluded. The gastrointestinal function of these subjects was normal without vomiting, diarrhea, and/or constipation to the day of stool collection.

### **Fecal Collection and Processing**

The first defecation sample in the morning was collected from each subject. The samples were collected and frozen in liquid nitrogen for future isolation of bacterial genomic DNA. The inner part of the samples was used for sequencing to avoid environmental contamination. Total bacterial DNA was extracted from fecal samples within 2 weeks by the stool DNA Kit (Omega Biotek, Norcross, GA, USA) according to the manufacturer's protocol.

## 16S rRNA Gene Sequencing

To investigate the compositional change of microbiome associated with AMI, PCR amplification was performed on the V3– V4 region of the 16S rRNA gene with TransStart Fastpfu DNA Polymerase (Takara) following by sequencing on the Illumina MiSeq v3 chemistry (Illumina Inc., San Diego, CA, United States) in multiple runs and pooling all 44 samples together according to the manufacturer's instructions.

## Comparison of Gut Microbiome Composition

The raw 16S data were analyzed by Pandaseq, processed through the QIIME (version 1.8.0), clustered into operational taxonomic units (OTUs) with a 97% identity cutoff and taxonomically. The alpha-diversity measures were calculated based on the OTUs counts. Number of observed OTUs indicates microbial richness, which measures the number of taxa in each sample. The linear discriminant analysis effect size (LEfSe) was calculated using the online version of Galaxy3. The linear discriminate analysis (LDA) was performed using a one-against-all strategy, and OTUs showing a score higher than 2 are selected. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways database was used to predict differences in bacterial biochemical pathways between the patients with /without AMI.

## STATISTICS

We applied the metagenomic sequencing for intestinal flora analysis (e.g., flora composition, diversity, functional, and metabolite analyses). Descriptive statistical analyses were used to summarize the clinical features. Other analyses were performed by SPSS 20.0 (Chicago, IL, USA), including unpaired t-test and continuous variables are expressed as the mean  $\pm$  standard deviation (SD).  $p < 0.05$  was defined as statistically significant.

# Results

## Basic Demographics

A total of 44 study participants [AMI: 19 (43.18%) vs control: 25 (56.81%), mean age:  $65.15 \pm 11.53$  vs  $73.20 \pm 9.06$  years] were eligible for final analysis (Table 1). In general, AMI patients present higher body weight and body height values than control ( $p < 0.001$ , 95% CI). Overall, AMI patients showed higher glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), cardiac enzyme (creatinase kinase, CK), and inflammation marker (C-reactive protein, CRP) than control (Table 1).

Table 1

	AMI (n = 19)	Control (n = 25)	<i>p-value</i>
	mean ± SD	mean ± SD	95% CI
Age (yrs)	65.15 ± 11.53	73.20 ± 9.06	< 0.001
BH (cm)	166.08 ± 6.87	158.92 ± 6.62	< 0.001
BW (kg)	72.83 ± 13.04	61.08 ± 11.94	< 0.001
WBC (10 <sup>3</sup> /uL)	7.62 ± 3.28	6.52 ± 1.81	0.14
Hb (g/dL)	13.75 ± 2.57	13.00 ± 2.11	0.23
GLU AC (mg/dL)	114.62 ± 35.73	112.23 ± 36.97	0.81
HbA1c (%)	6.47 ± 1.34	6.23 ± 0.91	0.44
GOT (U/L)	36.46 ± 29.90	23.07 ± 9.13	0.04
GPT (U/L)	26.59 ± 16.43	17.61 ± 10.96	0.02
Bil-T (mg/dL)	0.92 ± 0.46	0.71 ± 0.38	0.22
Bil-D (mg/dL)	0.26 ± 0.26	0.15 ± 0.07	0.37
ALP (U/L)	115.55 ± 152.46	50.33 ± 12.90	0.49
rGT (U/L)	75.82 ± 84.90	17.67 ± 8.50	0.27
BUN (mg/dL)	21.54 ± 12.99	17.96 ± 5.78	0.29
Cr (mg/dL)	1.39 ± 2.08	0.96 ± 0.27	0.32
eGFR (mL/min/1.73 m <sup>2</sup> )	82.79 ± 47.78	79.32 ± 21.79	0.74
UA (mg/dL)	6.29 ± 1.60	5.70 ± 1.42	0.17
Na (mmol/L)	138.23 ± 5.80	138.31 ± 3.79	0.96
K (mmol/L)	4.20 ± 0.44	4.10 ± 0.58	0.46
Ca (mmol/L)	2.29 ± 0.11	2.29 ± 0.17	0.97
Mg (mmol/L)	0.80 ± 0.12	0.90 ± 0.05	0.12
CHO (mg/dL)	151.27 ± 39.44	166.79 ± 44.16	0.16
TG (mg/dL)	126.16 ± 55.33	149.96 ± 111.52	0.27
LDL (mg/dL)	93.06 ± 39.12	94.00 ± 29.19	0.93
HDL (mg/dL)	46.68 ± 12.56	46.70 ± 12.60	0.99

	AMI (n = 19)	Control (n = 25)	<i>p</i> -value
CK (U/L)	895.04 ± 1862.19	105.38 ± 20.33	0.04
CK-MB (U/L)	57.60 ± 118.37	2.69 ± 0.91	0.53
Tn-I (ng/mL)	16.73 ± 21.52	0.01 ± 0.00	0.52
CRP (mg/dL)	3.51 ± 4.59	0.06 ± 0.04	0.03
NT-pro BNP (pg/mL)	1994.68 ± 4037.07	1816.60 ± 3014.64	0.94
LDH (U/L)	221.50 ± 137.27	150.67 ± 52.77	0.41
Lactic acid (mmol/L)	1.56 ± 1.00	0.81 ± 0.16	0.23

CI: confidence interval, AMI, acute myocardial infarction; SD, standard deviation; BH, body height; BW, body weight; WBC, white blood cell; Hb, hemoglobin; GLU AC, fasting blood glucose; HbA1c, glycated hemoglobin; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; Bil-T, total bilirubin; Bil-D, direct bilirubin; ALP, alkaline phosphatase; rGT, r-glutamyl transferase; BUN, blood urea nitrogen; Cr, creatinine; eGFR, estimated glomerular filtration; UA, uric acid; Na, sodium; K, potassium; Ca, calcium; Mg, magnesium; CHO, cholesterol; TG, triglyceride; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; CK, creatine kinase; CK-MB, Creatine Kinase MB; Tn-I, Troponin-I; CRP, C-reactive protein; NT-ProBNP, N-terminal pro-brain natriuretic peptide; LDH, *lactate dehydrogenase*.

## Analysis of Diversity between Groups

The alpha-diversity (richness, uniformity, and Shannon index of diversity) was an indicator for describing species diversity. After analyzing intestinal microbial metagenomics, there was a difference in the relative richness of bacteria between the groups (Fig. 1 and Fig. 2). The abundance of bacteria was more enriched in the AMI group ( $p = 0.01$ , 95% CI). The phylogenetic analogue of taxon analyses also revealed more richness of bacteria in the AMI group (Fig. 3,  $p < 0.001$ , 95% CI). There was no difference of evenness and uniformity between the groups (Fig. 4,  $p = 0.4$ ). The Shannon index was an information statistic index, which assumed all species were represented in a sample and that they were randomly sampled. The Shannon index between the groups was similar (Fig. 5,  $p = 0.23$ , 95% CI).

For further exploring these findings, we performed a LEfSe analysis to identify differences in abundant taxa between the samples of patients with/without AMI. There were over expression of several bacterial genera between the groups (Figs. 6 and 7; Figs. 9 and 10; Figs. 12 and 13). Additionally, *Selenomonadales* was more enriched in the control group at the Family, Genus, and Species levels (Figs. 6 and 7; Figs. 9 and 10; Figs. 12 and 13; all LDA scores  $> 2$ ).

Because the gut flora could produce and consume many metabolites, we assumed certain metabolites were associated with the abundance of the flora. The KEGG metabolic pathways was assisted to estimate the difference in metabolic potential between the groups. There was a difference of the seleno-

compound distribution between the groups. The seleno-compound was more abundant in the AMI group at the Family, Genus, and Species levels (Fig. 8, 11, and 14; all LDA scores > 2).

## Discussion

Trillions of microbial cells harbor at the human intestine as one part of our physiological ecosystem. These communities of bacteria, fungi, archaea, and viruses are collectively referred as “microbiota,” and their genome as the “microbiome.”<sup>4</sup> The microbial colonization of the gastrointestinal tract initiates at birth and the composition of the species-level phylotypes differs individually.<sup>5</sup> Approximately 99% of human gut microbiota are composed by the four phyla of bacterial species, including *Firmicutes*, *Bacteroides*, *Actinobacteria*, and *Proteobacteria*.<sup>6</sup> Among them, *Firmicutes* and *Bacteroidetes* phyla are the two major species accounting for almost 90% of bacterial species in the human intestines.<sup>7</sup>

The gut microbiota plays an important role in the immune support and host metabolism. Abnormal changes of the gut flora might affect host health by inducing immune response.<sup>7</sup> From the previous studies, the gut microbiota could code various enzymes, synthesize vitamins, produce amino acids, and digest various nondigestible dietary components (e.g., large polysaccharides, resistant starch, pectin, cellulose, hemicellulose, alcohols, and sugars, etc.).<sup>5</sup>

The interindividual variabilities of the microbial communities are interfered by host-microbial interactions, host genetics, host diets, host life-style, and environmental conditions (e.g., PH gradient, gastric motility, oxygen content, and nutrition, etc.).<sup>4</sup> The diverse, and various metabolites from gut microbiota also affect the host physiology vice versa.<sup>6</sup>

## The Main Findings of This Study

In this study, the DNAs of the gut microbiota were extracted from the fecal samples of the subjects, and the DNA libraries were constructed by 16S rRNA gene sequencing on the MiSeq (Illumina) platform. The highly similar sequences were grouped into the OTUs. After alpha diversity analysis, the OTUs and faith phylogenetic diversity (PD) were noted to be different between AMI and control groups ( $p$ -value = 0.01 and < 0.001, individually). Therefore, the abundance of gut microbiota was different between the AMI and control groups.

The taxonomic cladogram reported all clades of the gut microbiota, and there was less abundance of *Selenomonadales* in the AMI group relative to the control group at the Family, Genus, and Species levels. The KEGG database resource was comparing for evaluating the possibly metabolic pathways involved with the AMI episodes. After the metagenome functional analysis, the seleno-compound was noted to be more abundant in the AMI group.

From the current studies, there was relatively increasing abundance of *Ruminococcus gnavus* in the patients with CAD.<sup>8</sup> In addition to coronary atherosclerosis, the *Ruminococcus gnavus* was also linked to

specific inflammatory process, production of inflammatory polysaccharide, and inflammatory bowel disease.<sup>8</sup> On the contrary, the abundances of *Lachnospiraceae* and *Ruminococcus gauvreauii* were decreasing in the subjects with CAD.<sup>8</sup> This is the first study to demonstrate “another distinct” gut microbiome- *Selenomonadales* and seleno-compound to be associated with the occurrence of AMI. The possible mechanisms for altering microbiota, and the potential metabolome in association with the AMI episodes are discussed in the following paragraphs.

## The Possibly Metabolic Pathways of Selenomonadales in Correlation with AMI Episodes

### 1. Selenomonadales and short-chain free fatty acids (SCFAs)

Generally, the primary products of anaerobic fermentation of undigested nutrients (e.g., resistant starch, dietary fiber, and various complex polysaccharides) after bacterial hydrolysis are monosaccharides. Those monosaccharides are further fermented to various fatty acids ranging from 1 to 6 carbon chains, commonly referred as short-chain fatty acids (SCFAs) such as acetate, butyrate, and propionate.<sup>4</sup> Acetate is utilized by butyrate producers to produce butyrate while the butyryl- CoA: acetate-CoA-transferase pathway is the main process for the biosynthesis of butyrate.<sup>9</sup> Butyrate acts as histone deacetylases inhibitor, and involves in epigenetic regulation of T-cell development and maintenance.<sup>10</sup>

Those SCFAs (e.g., acetate, butyrate, and propionate) are transported by the monocarboxylate transporters on the mucosal epithelium of the intestines, and into the systemic circulation. Although 5–10% SCFAs, particularly butyrate serves as energy substrates for epithelial cells of the intestines, SCFAs also serve as signaling molecules. SCFAs interact with G-protein receptor (e.g., GPR41 and GPR43), and olfactory receptor 78 (Olf78), which exhibit various physiological functions (e.g., histone deacetylases inhibition, chemotaxis, phagocytosis modulation, reactive oxygen species induction, regulating immune cell proliferation, lipid, and glucose metabolism, etc.).<sup>4</sup>

Additionally, both GPR41 and Olf78 are expressed in smooth muscle cells of small vessels, where they differentially mediate vascular tone. The 3-carbon SCFA propionate may stimulate GPR41 to lower blood pressure, whereas stimulation of Olf78 can increase blood pressure.<sup>4</sup> These signal mechanisms have been proved by the animal models. For instance, Olf78 knock-out mice are hypotensive, whereas GPR41 knock-out mice are hypertensive.<sup>4</sup> Therefore, SCFAs could modulate several physiological effects associated with cardiovascular diseases in addition to energy metabolism (e.g., autonomic systems, blood pressure, immune function, inflammatory responses, and other cellular functions, etc.).<sup>4</sup> Besides, SCFAs have been shown to be beneficial for increasing insulin sensitivity, keeping glucose homeostasis, and body weight control as well.<sup>6</sup>

*Selenomonadales* are the members of Firmicutes, and the class of Negativicutes which are Gram-negative. *Selenomonadales* have been reported to ferment carbohydrates into acetate and lactate.<sup>9</sup> Therefore, those SCFAs metabolites generated by the *Selenomonadales* might lead to the downstream metabolic alterations, and affect coronary atherosclerosis in correlation with AMI episodes.

## 2. Dysbiosis of gut microbiota in AMI subjects

An abnormal imbalance of the gut microbiota is called “dysbiosis”. Foods are important to maintain the integrity of the diversity, and keep balance of microbiota for producing SCFAs efficiently. However, high fat, and modern diets tend to hamper the gut microbiota ecosystem, which may explain the increasing incidence of metabolic diseases recently.<sup>10</sup> Shifts from the animal-based diets to be plant-based diets could alter, and modify the production of SCFAs.<sup>4</sup> An increase in *Firmicutes* has been related to increase enzymes for disintegrating polysaccharides from food, and produce SCFAs as well.<sup>7</sup> Those SCFAs are critical for repairing the cardiac structure after AMI episodes from the animal models.<sup>4</sup> The intestinal microbiota, and their metabolites might stimulate the immune system via intestinal lymphoid tissues.<sup>4</sup> The relative less abundance of *Selenomonadales* in the AMI subjects might be associated with deprivation of SCFAs in the intestine, and resulting in the loss of benefits from SCFAs mentioned above. Therefore, it might be speculated that propagation or sterilization of bacterial species specific for augmenting SCFAs generation, might prevent inflammatory process including coronary atherosclerosis, and could be beneficial for the treatment of AMI subjects.

## Intervention of *Selenomonadales* with probiotic

In addition to changing dietary habits, probiotic is another possible method to modulate gut microbiota profiles. In a rat myocardial infarction model, administration of either *Lactobacillus plantarum* or *Lactobacillus rhamnosus GR-1* is associated with attenuation of cardiac remodeling after AMI.<sup>4</sup> As *Selenomonadales* are considered, *Clostridium butyricum*, which is an oral diet-added probiotic, has been demonstrated to promote the abundance of *Selenomonadales* dramatically. *Clostridium butyricum* is an anaerobic, Gram-positive, butyric acid-producing bacillus, and has a protective role after intestinal injury by modulating gut microbial metabolites, such as SCFAs.<sup>9</sup> After using *Clostridium butyricum* probiotic for two weeks, *Selenomonadales* replace *Clostridiales* to be the major and dominant bacteria in the intestines.<sup>9</sup>

## Seleno-compound Metabolism with the Association of AMI

We are the first to find the relationship between seleno-compound and AMI episodes by metabolomic analysis. Selenium (Se) is a naturally occurring, and essential trace element necessary for activation of specific enzymes (e.g. glutathione peroxidases and thioredoxin reductase) after oxidative stress.<sup>11</sup> Se-containing enzymes, especially glutathione peroxidase, are involved in regulating the redox balance in almost all tissues<sup>12</sup> and very important for the detoxification of reactive oxygen species (e.g., peroxides and hydroperoxides).<sup>11</sup> While the human body is under stress, for instance, oxidative stress caused by

the intense growth activity of the fetus during pregnancy, the first line of defense against the oxidative stress are the endogenous antioxidants, such as the Se containing compounds. During pregnancy, the placenta also plays an important role for activating seleno-compounds such as glutathione-peroxidase and thioredoxinreductase.<sup>12</sup> Therefore, it is reasonable that the increasing abundance of seleno-compound in the AMI subjects might be correlated with the reaction of oxidative stress after AMI episodes.

## The Interaction of *Selenomonadales*, Seleno-compound, and Dietary Se

At present, the *Selenomonadales* have not been reported to be associated with the bioavailable Se-protein compound for supplying selenium to the organisms.<sup>13</sup> The relationship between the *Selenomonadales*, and the seleno-compound needs to be clarified in the future. However, the dietary Se has been proved to be cardioprotective for reducing oxidative stress, lowering connexin-43 dephosphorylation, and decreasing TNF- $\alpha$  expression from the rat models.<sup>14</sup> For the patients with cardiac dysfunction, the Se intake might be helpful for improving cardiac remodeling even when the provided Se is within the normal range of physiological values.<sup>14</sup>

For the dietary Se, the Se-enriched plants (e.g., onion, broccoli, wild leek, and garlic) possess protective effects on the anti-inflammation, ant-cancer, and anti-oxidant activities via Se-methyl selenocysteine or gamma-glutamyl-Se-methyl selenocysteine.<sup>15,16</sup> Additionally, the combination treatment with Vit E, Se, and anthocyanin from purple carrots shows greater antioxidant activities against D-galactose-induced oxidative damage in rats than those of individual treatments, suggesting the synergistic antioxidant effects of these antioxidants.<sup>17</sup> The protection from Vit E against the adverse effects of nitrites/nitrates is attributed to its ability to reduce ONOO- formation, while Se exerts its protective effects via seleno-enzymes/compounds, which reduce ONOO- formed.<sup>18</sup>

## DATA SHARING

Whether data collected for the study, including participant data and a data dictionary defining each field in the set, will be made available to others after publication. These data will be made available after approval of a proposal and a signed data access agreement by e-mail request (e-mail: jeremysnc1000@gmail.com).

## Conclusion

The research of gut microbiota has garnered much interest because of its importance in nutrition, disease, and health. This study demonstrates that there is a decreasing abundance of *Selenomonadales* gut microbiome in the AMI subjects, and the seleno-compound is also increasing after AMI episodes. Our

findings raise the potential role of microbial composition to be a valuable target for preventing, and treatment of AMI. The relationships between the immune microenvironment, intestinal flora, and AMI warrant further investigation. By doing so, gut microbiota-targeted therapy for the AMI could be carried out in the future.

## **Declarations**

### **DECLARATION OF INTERESTS**

All authors disclose no financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work.

### **FUNDING**

None.

### **AUTHORS CONTRIBUTION**

**Chin-Feng Tsai, M.D., PhD.**

Analyzed the data and prepared for the data interpretation.

**Pang-Shuo Huang M.D.**

Analyzed the data and prepared for the data interpretation.

**Ching-Yu Shih MSc.**

Analyzed the data and prepared for the data interpretation.

**Mong-Hsun Tsai Ph.D.**

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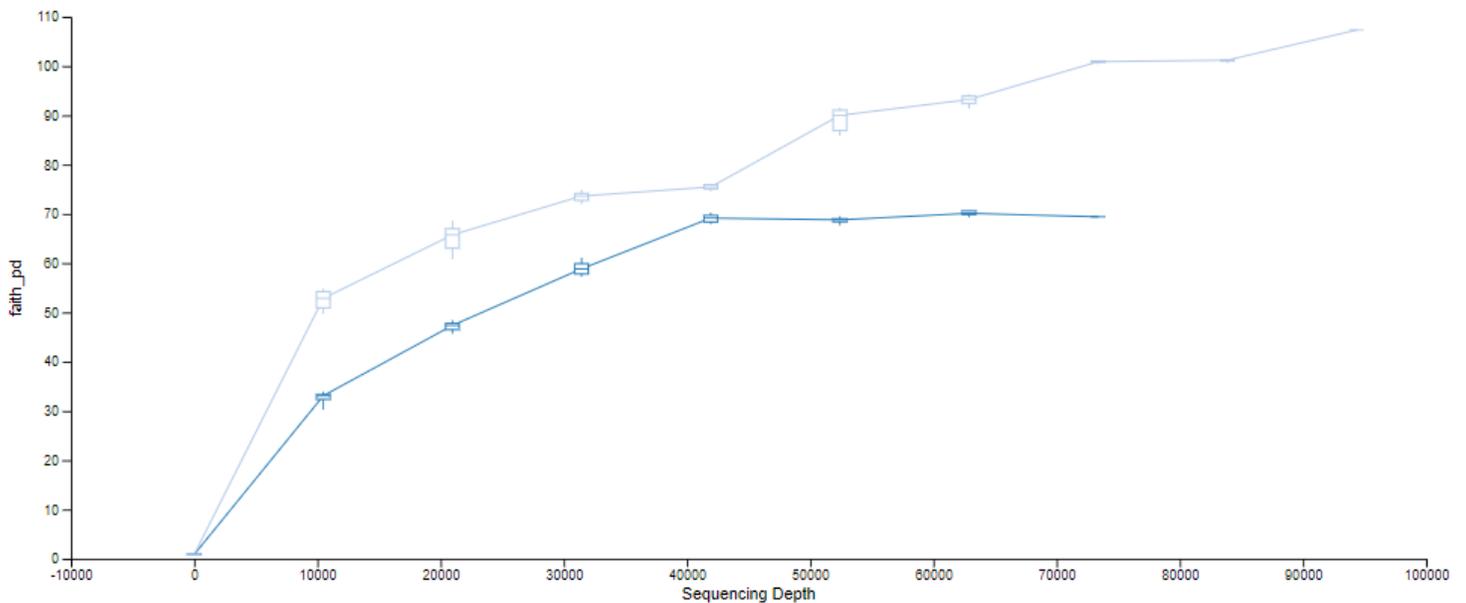
Designed the whole study and was in charge of the whole program.

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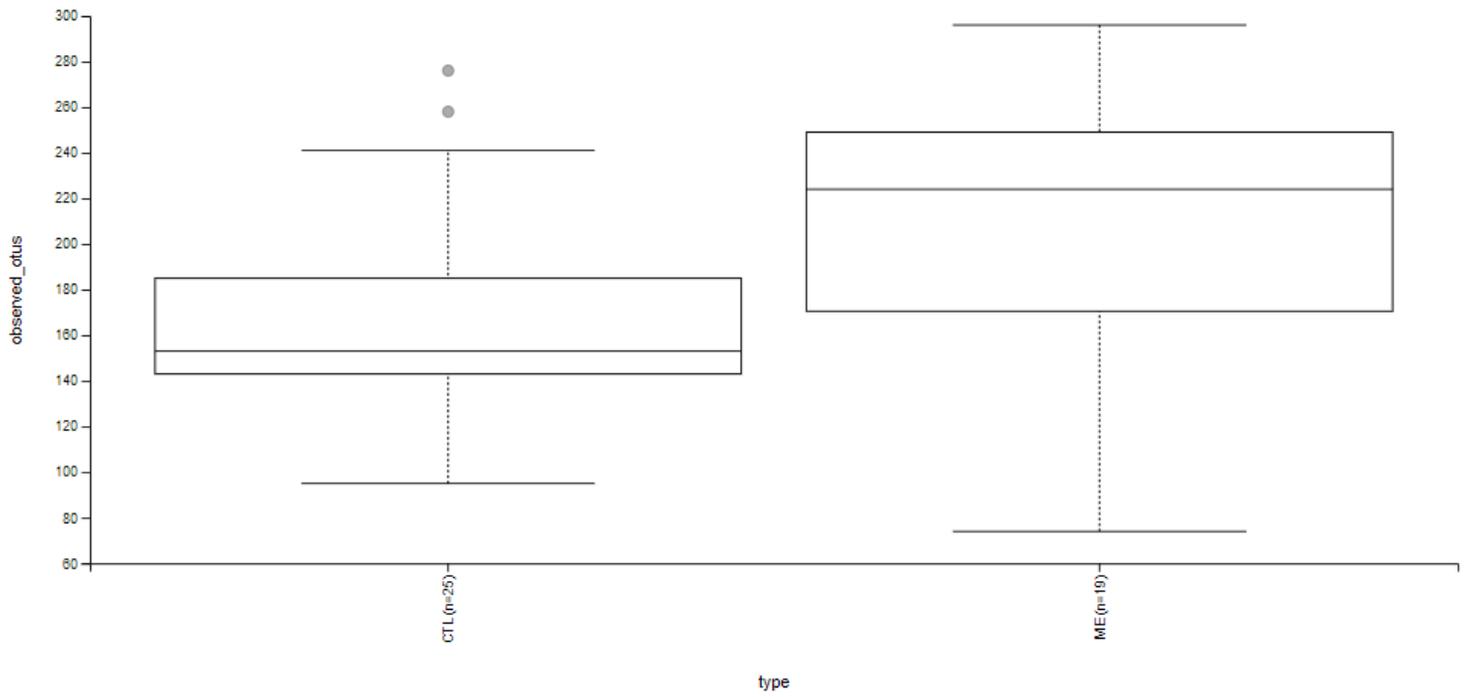
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## Figures



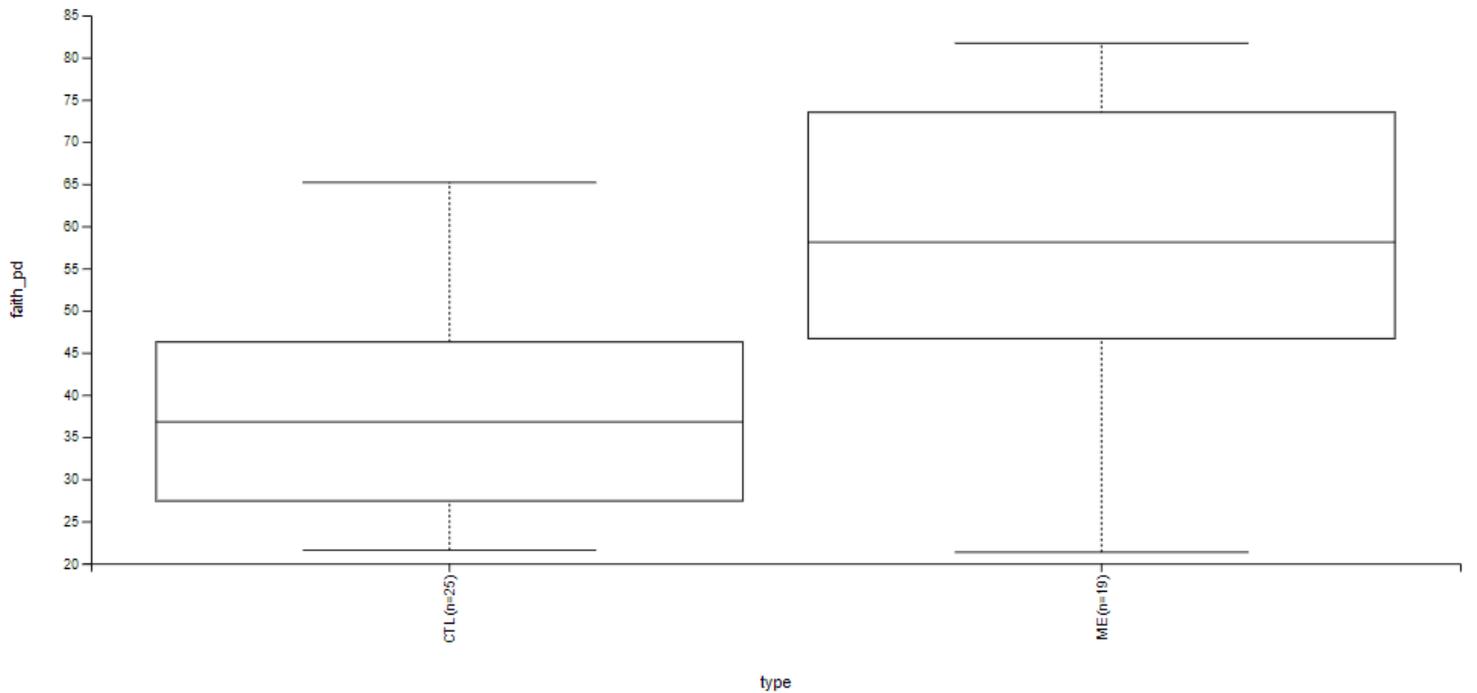
**Figure 1**

Rare fraction (CTL: control; ME: AMI).



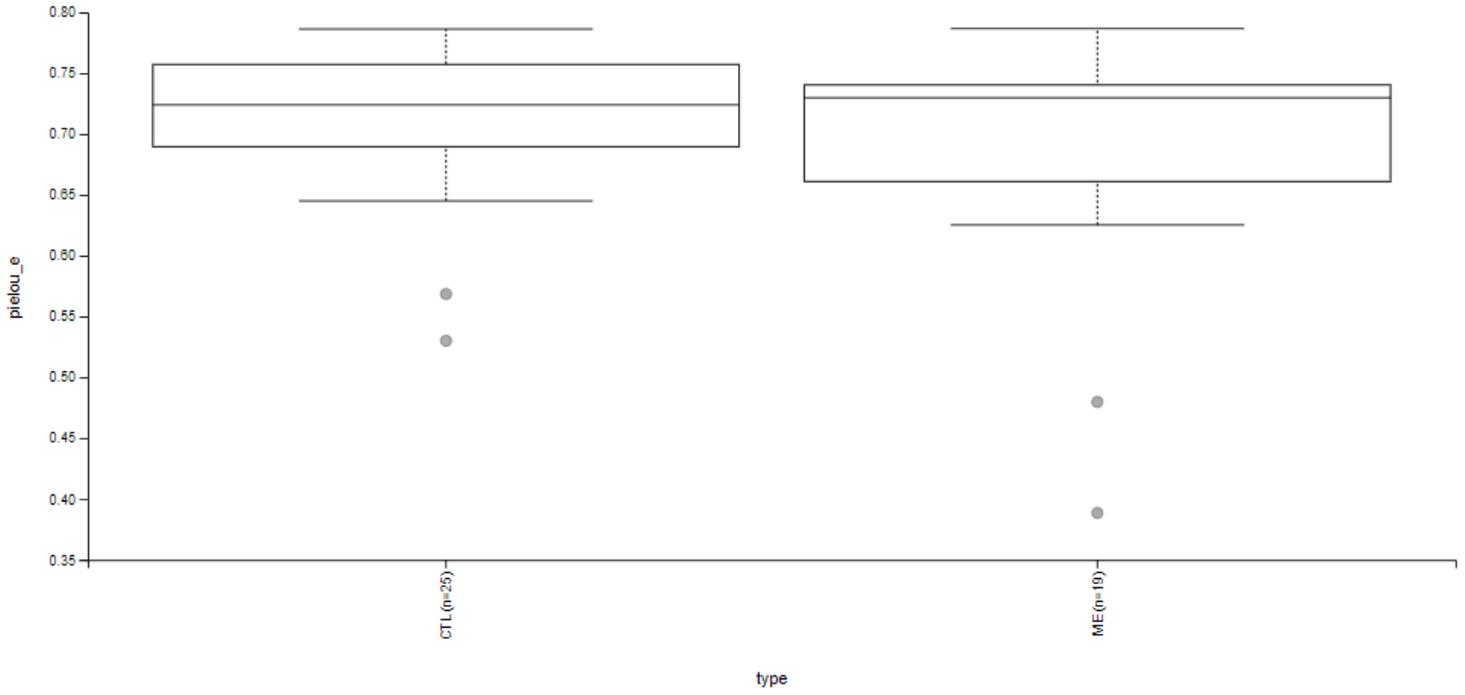
**Figure 2**

Observe operational taxonomic units (OTUs),  $p = 0.01$  (CTL: control; ME: AMI).



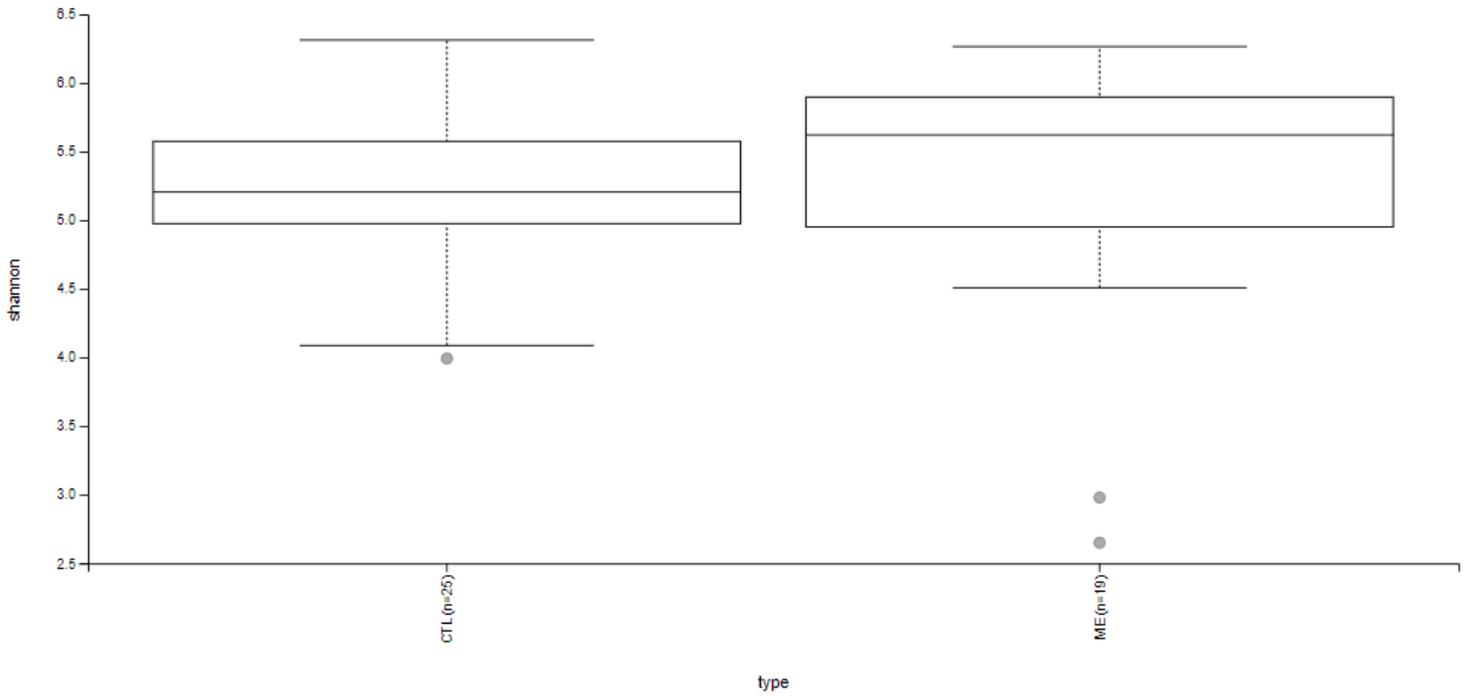
**Figure 3**

Faith's phylogenetic diversity (PD),  $p < 0.001$  (CTL: control; ME: AMI).



**Figure 4**

Pielou's evenness,  $p = 0.4$ , 95% CI (CTL: control; ME: AMI).



**Figure 5**

Shannon,  $p = 0.23$ , 95% CI (CTL: control; ME: AMI).

# Cladogram

CTL  
ME

a: D\_4\_Lachnospiraceae  
b: D\_3\_Selenomonadales  
c: D\_2\_Negativicutes

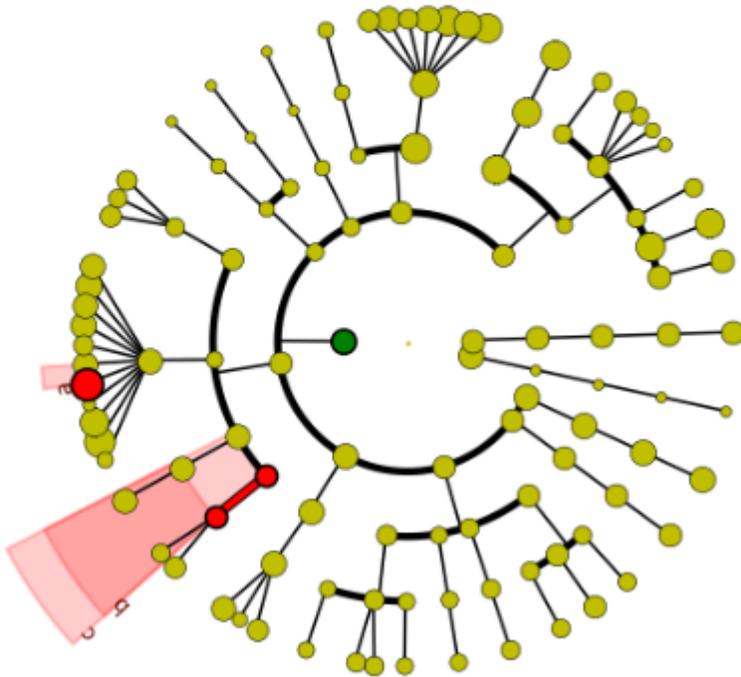


Figure 6

Cladogram showing different abundant taxa at Family level. Alphabets correspond to those in parentheses (CTL: control; ME: AMI).

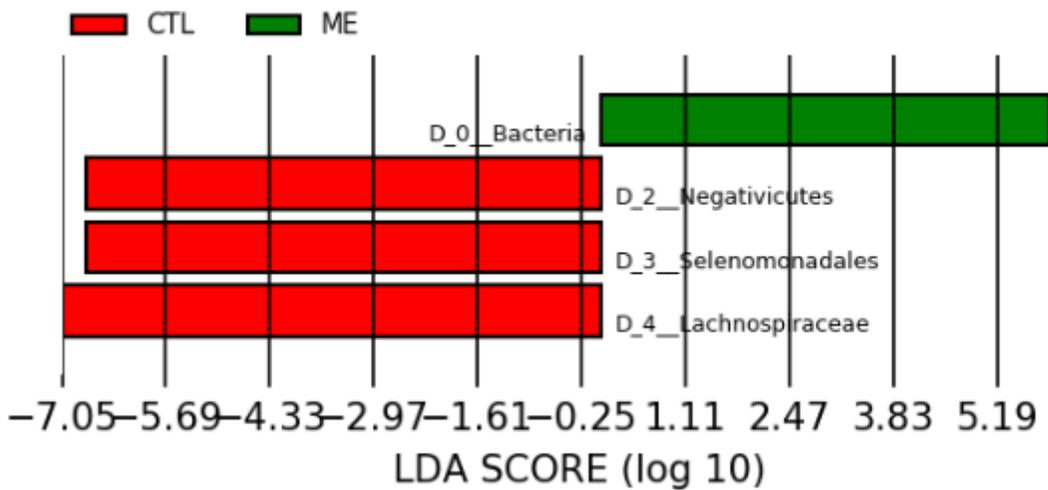
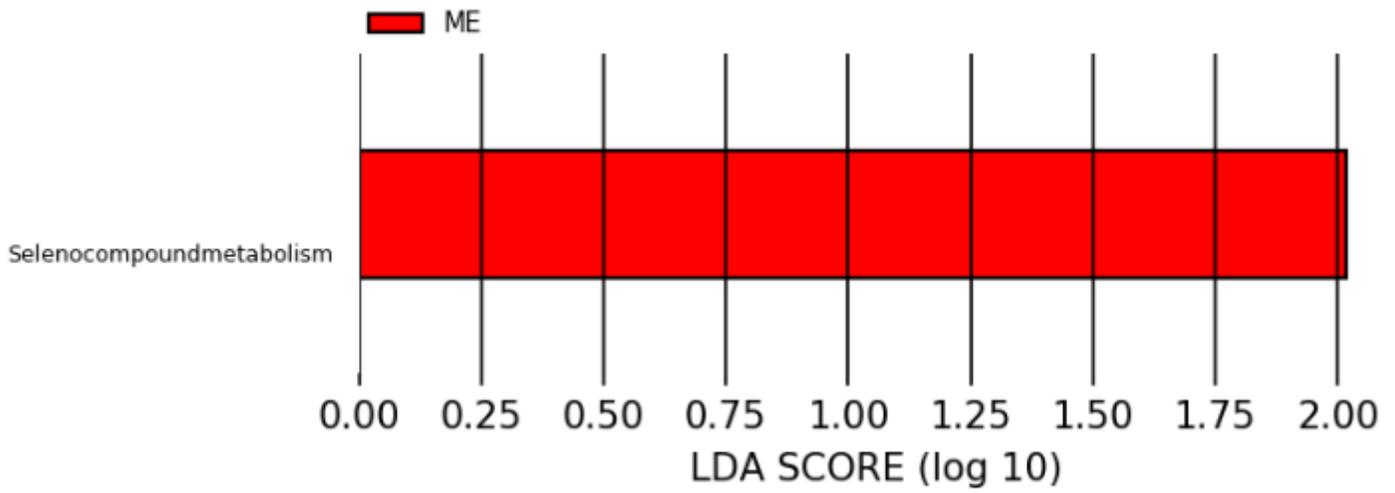


Figure 7

Taxonomic groups showing linear discriminant analysis (LDA) result at Family level (CTL: control; ME: AMI).



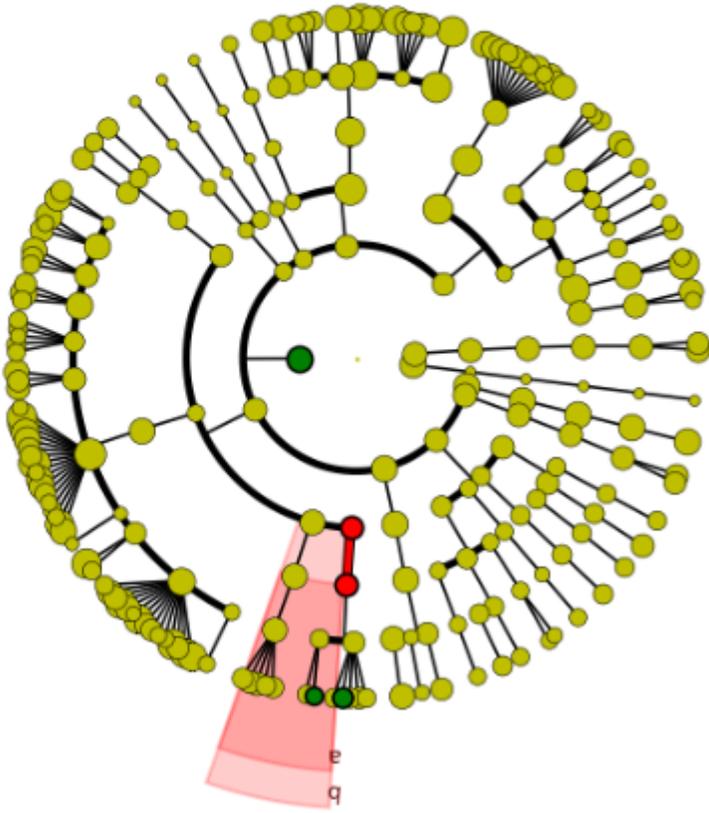
**Figure 8**

Taxonomy differential abundance analysis for the seleno-compound metabolism at Family level (CTL: control; ME: AMI).

# Cladogram

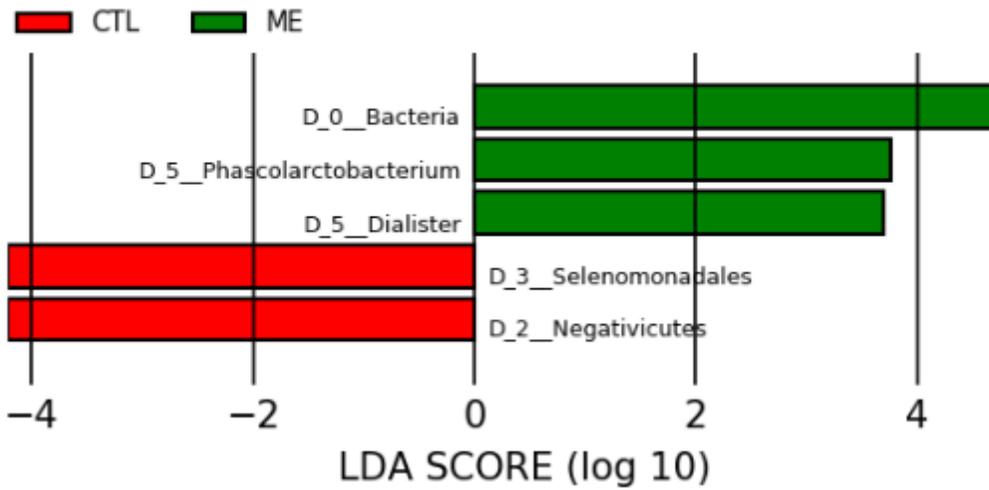
CTL  
ME

a: D\_3\_Selenomonadales  
b: D\_2\_Negativicutes



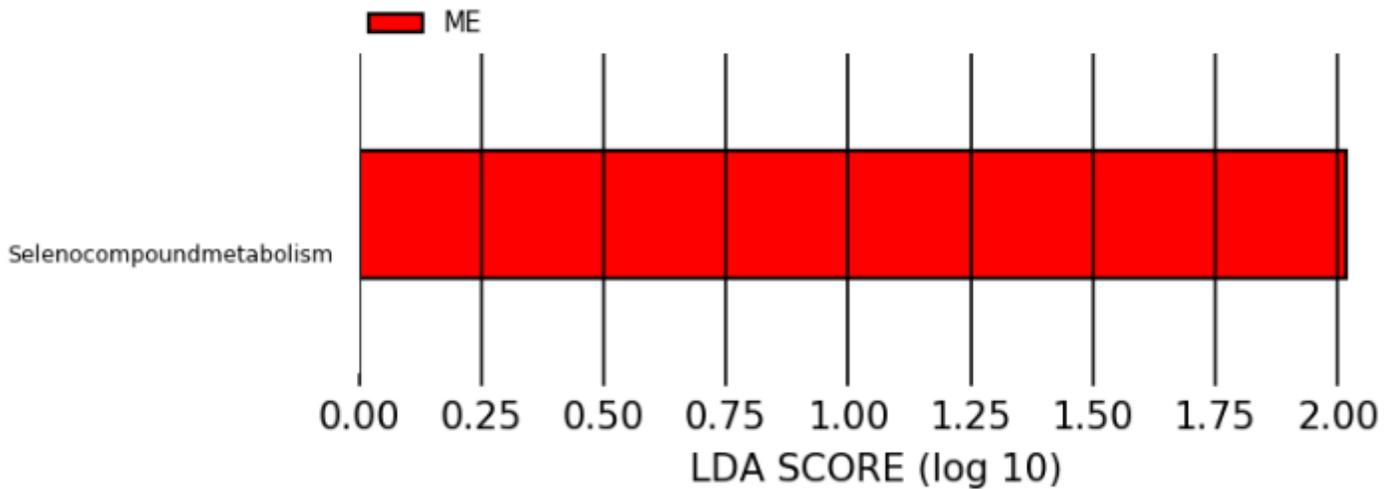
**Figure 9**

Cladogram showing different abundant taxa at Genus level. Alphabets correspond to those in parentheses (CTL: control; ME: AMI).



**Figure 10**

Taxonomic groups showing linear discriminant analysis (LDA) result at Genus level (CTL: control; ME: AMI).



**Figure 11**

Taxonomy differential abundance analysis for the seleno-compound metabolism at Genus level (CTL: control; ME: AMI).

# Cladogram

CTL  
ME

a: D\_3\_\_Selenomonadales  
b: D\_2\_\_Negativicutes

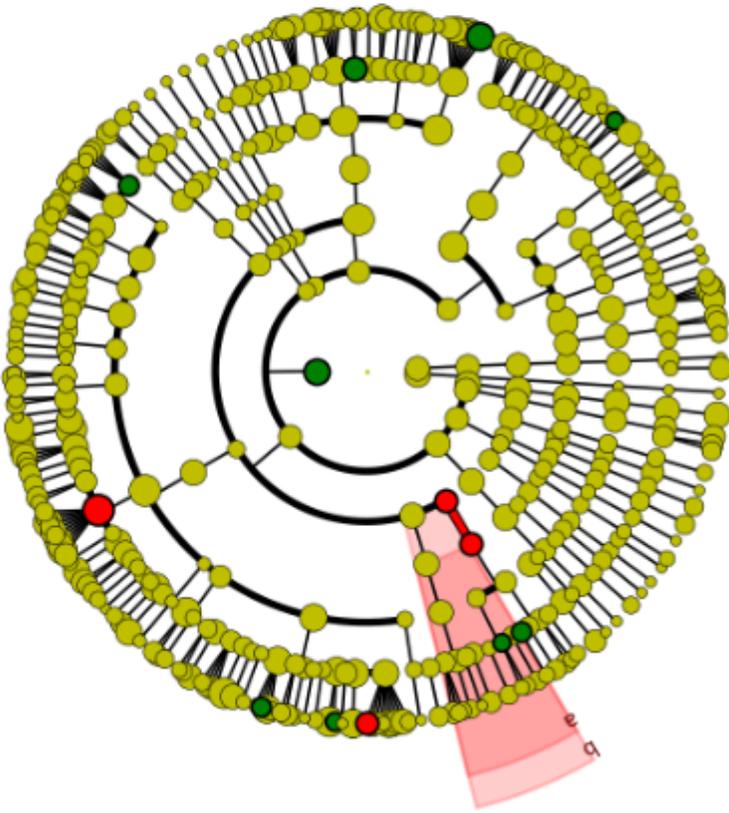


Figure 12

Cladogram showing different abundant taxa at Species level. Alphabets correspond to those in parentheses (CTL: control; ME: AMI).

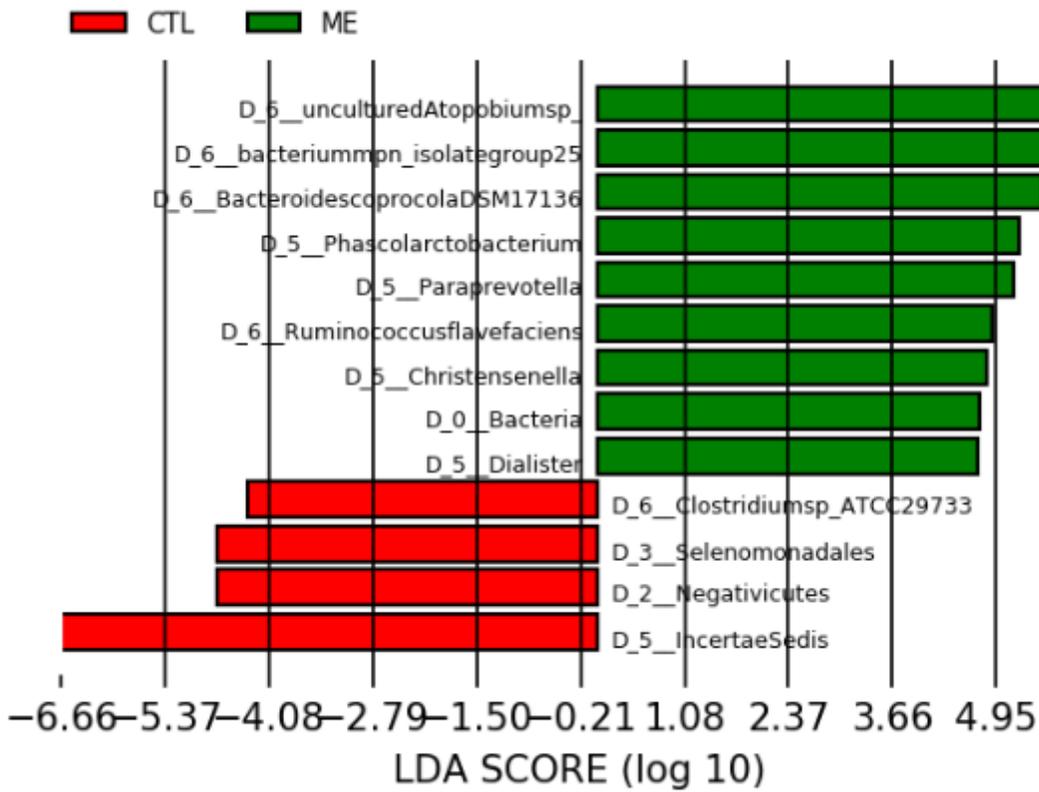


Figure 13

Taxonomic groups showing linear discriminant analysis (LDA) result at Species level (CTL: control; ME: AMI).

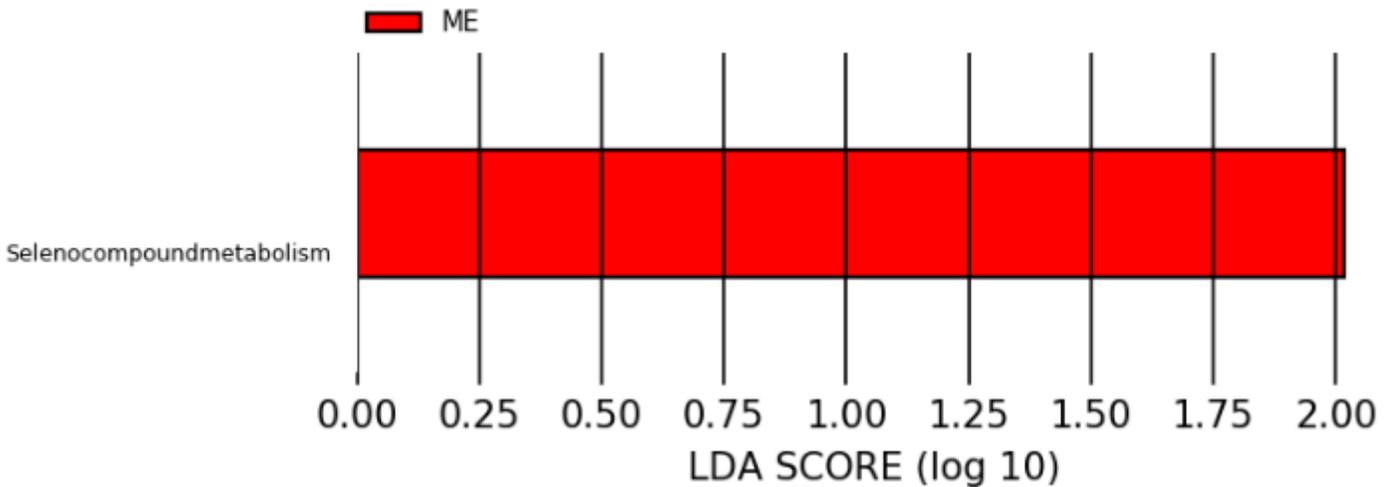


Figure 14

Taxonomy differential abundance analysis for the seleno-compound metabolism at Species level (CTL: control; ME: AMI).