High-throughput amplicon sequencing datasets of the metacommunity DNA of the gut microbiota of naturally occurring and laboratory aquaculture green sea urchins *Lytechinus variegatus*

Joseph A. Hakim \(^a\), Casey D. Morrow \(^b\), Stephen A. Watts \(^a\), Asim K. Beja \(^a\)

\(^a\) Department of Biology, The University of Alabama at Birmingham, 1300 University Blvd., Birmingham, AL 35294, USA
\(^b\) Department of Cell, Developmental and Integrative Biology, The University of Alabama at Birmingham, 1918 University Blvd., Birmingham, AL 35294, USA

**ABSTRACT**

We present high-throughput amplicon sequence (HTS) datasets of the microbial metacommunity DNA of the gut tissue and the gut digesta of naturally occurring \((n=3)\) and laboratory aquaculture \((n=2)\) green sea urchins, *Lytechinus variegatus*. The HTS datasets were generated on an Illumina MiSeq by targeting the amplicons of the V4 region of the 16S rRNA gene. After the raw sequences were quality checked and filtered, 88% of the sequence reads were subjected to bioinformatics analyses to generate operation taxonomic units (OTUs), which were then verified for saturation by using rarefaction analysis at a 3% sequence variation. Further, the OTUs were randomly subsampled to the minimum sequence count values. Then, the FASTA-formatted representative sequences of the microbiota were assigned taxonomic identities through multiple databases using the SILVA ACT: Alignment, Classification and Tree Service (www.arb-silva.de/aligner). The HTS datasets of this metagenome can be accessed from the BioSample Submission...
The metagenomic datasets presented in this article describe the microbial community compositions in the gut ecosystem of a marine invertebrate echinoderm of ecological, economic, and scientific importance, *Lytechinus variegatus*, fed with formulated diet in laboratory aquaculture conditions and from their natural habitat. Fig. 1 describes the OTUs from the quality-checked and filtered HTS data of the 16S rRNA gene and visualized by rarefaction analysis, which indicated that the total quality
sequences from each sample are approaching saturation when constructed at a 3% sequence variation.

Fig. 2 shows the microbial community profiles of both LAB and ENV gut tissue with a near-exclusive abundance of Epsilonproteobacteria, with the ENV group showing a slightly higher diversity. Table 1 presents the applicability of the HTS datasets for profiling *Lytechinus variegatus* gut microbiota at the highest possible taxonomic levels following the alignment of the representative sequences to five microbial databases.

### 2. Experimental design, materials and methods

#### 2.1. Sample description

The sea urchins were collected from Saint Joseph Bay Aquatic Preserve of the U.S. Gulf of Mexico (29.80° N 85.36° W). For the laboratory aquaculture (LAB) group [1], adult sea urchins (n = 2) were kept in a recirculating saltwater tank system for six months, and fed a formulated feed *ad libitum* once every 24–48 h that consisted of 6% lipid, 28% protein, and 36% carbohydrate relative percentages [2]. The aquaria were maintained at 22 ± 2 °C with a pH of 8.2 ± 0.2 and salinity of 32 ± 1 ppt. For the naturally occurring (ENV) group [3], adult sea urchins (n = 3) were collected from within the same 1 m² area and transported to the laboratory at the University of Alabama at Birmingham (UAB) for sample collection. Water conditions were recorded as 20 ± 2 °C with a pH of 7.8 ± 0.2 and salinity of 28 ± 1 ppt. For both groups, the Illumina MiSeq high throughput-sequencing (HTS) platform was used with the 250 bp paired-end kits targeting the V4 hypervariable region [4,5]. The paired-end raw sequence data were
The raw data were deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under BioProject #PRJNA291441 and #PRJNA326427 for the LAB and ENV group, respectively. The paired-end sequence data for the gut microbial communities can be accessed under the following NCBI BioSample Ids: SAMN03944319.

Fig. 2. Relative abundance distribution of taxa at the highest resolution determined for the merged biological replicates using multiple taxonomic databases. The FASTA-formatted representative sequences determined by the PhyloToAST (v1.4) workflow integrated into QIIME (v1.9.1) were aligned to multiple databases using the SILVA ACT: Alignment, Classification and Tree Service (www.arb-silva.de/aligner). Taxonomic assignments were performed using the SSU (Small Subunit) category and the Least Common Ancestor (LCA) method with the following databases: SILVA, Ribosomal Database Project (RDP), The All-Species Living Tree (LTP) project, Greengenes (GG), and the European Molecular Biology Laboratory (EMBL). Sequences aligned with a similarity threshold below 70% were discarded. The top 25 taxa from each database were merged based on their common taxonomic assignments at the specific level of classification.

Table 1

| Level   | SILVA | RDP   | LTP   | GG    | EMBL |
|---------|-------|-------|-------|-------|------|
| Family  | 234   | 193   | 128   | 219   | 18   |
| Genus   | 167   | 147   | 121   | 132   | 12   |
| Total Unique | 282 | 241   | 128   | 288   | 33   |

Statistical analysis of the representative sequences aligned to multiple databases using the SILVA ACT: Alignment, Classification and Tree Service (www.arb-silva.de/aligner), Taxonomic assignments were performed using the SSU (Small Subunit) category and the Least Common Ancestor (LCA) method with the following databases: SILVA, Ribosomal Database Project (RDP), The All-Species Living Tree (LTP) project, Greengenes (GG), and the European Molecular Biology Laboratory (EMBL). Sequences aligned at a similarity threshold below 70% were discarded. For each database, the total number of uniquely assigned sequences were determined, and the fraction of those assignments to the family and the genus level were listed.
SAMN03944322 (LAB group) and SAMN05277845 - SAMN05277850 (ENV group). Subgroups for the laboratory-fed group are as follows: LAB.Gut.Tissue \((n = 2)\), LAB.Gut.Digesta \((n = 2)\), ENV.Gut.Tissue \((n = 3)\), and ENV.Gut.Digesta \((n = 3)\).

2.2. Quality assessment and filtering

The raw and demultiplexed paired-end sequence datasets were initially assessed by FastQC [7], and only reads showing 80% of bases at a Q score of >33 were retained by using the “fastx_trimmer” command from the FASTX Toolkit [5,8] and merged using USEARCH [9]. Paired-end reads with <50 base overlap and/or >20 mismatching nucleotides were filtered from the analysis, and chimeric sequences were removed using USEARCH [9].

2.3. Taxonomic distribution and alpha diversity

The merged sequence data was analyzed using Quantitative Insights into Microbial Ecology (QIIME; v1.9.1) along with Phylogenetic Tools for Analysis of Species-level Taxa (PhylotoAST; v1.4.0) [10,11]. The initial OTUs were clustered at a 97% similarity through UCLUST in QIIME (v1.9.1) [9], and representative sequences were established by the “most_abundant” option. Then, OTUs with <0.0005% average abundance across all samples were filtered. The redundant OTUs were merged by using the “condense_workflow.py” command through PhylotoAST (v1.4.0) [11]. The OTUs per sample were plotted against the filtered sequence read counts as rarefaction curves, and the data was subsampled to the minimum value using “single_rarefaction.py” in QIIME (v1.9.1). The representative sequences were then assigned taxonomy using the SILVA ACT: Alignment, Classification and Tree Service (www.arb-silva.de/aligner), which utilizes the SILVA Incremental Aligner (SINA; v1.2.11) to align rRNA gene sequences and classify based on Least Common Ancestor (LCA) methods [12]. For this, the SSU (Small Sub-Unit) option selected at a minimum similarity of 0.7 with 20 neighbors per query sequence, and the databases selected were as follows: SILVA database [13], Ribosomal Database Project (RDP) [14], All-Species Living Tree (LTP) project [15], Greengenes (GG) [16,17], and European Molecular Biology Laboratory (EMBL) [18]. Biological replicates were validated and merged according to their subgroup assignment based on significant Analysis of Similarity (ANOSIM) [19] and Adonis [20] measurements \((p = 0.001)\) using the weighted Unifrac distances [21] calculated for each sample. The top 25 taxa at the highest resolution from each database were combined and plotted as relative abundance graphs using Microsoft Excel Software (Seattle, WA, USA). The taxonomic data derived from each of the five databases is summarized in Table 1 showing the total number of OTUs that were assigned a taxonomy, including the proportion that was resolved to the family and the genus level.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
