The de novo *Limacina helicina antarctica* transcriptome utilized in these analysis was assembled using Trinity vs. 2.2.0. The final assembly contained 403,210 contigs. To reduce the number of misaligned reads, the analysis was restricted to focusing on contigs that had more then 1 count per million reads in 9 of the 39 samples. This reduced set of "expressed" candidate sequences were utilized in the subsequent annotation and gene expression analysis. This filtering had a significant impact on the transcriptome by reducing the number of assembled contigs to 83,211 sequences. Average contig length increased to 1,281.52 base-pairs, the GC percentage was 41.27%, and the minimum sequence length was 224 base-pairs (Table 1). Blastx query of this reduced transcriptome resulted in the annotation of 37,517 (45.1%) of these transcripts. Gene ontology annotations were added to these annotations resulting in a total of 77,476 gene ontological terms being assigned to 28,407 of the annotated transcripts. The top 20 gene ontologies for the three major categories are presented in table 3. KEGG enzyme mapping assigned 417 enzymes associated with 130 KEGG pathways. The complete list of KEGG pathways is available in table 4.

**Table 1. Transcriptome assembly statistics**

| **Statistic** | **Total** |
|---|---|
| Contigs | 83,211 |
| Total base pairs | 216,717,523 bp |
| Percent GC | 41.27% |
| Contig N50 | 2,177 bp |
| Median contig length | 1,004 bp |
| Average contig length | 1,425.81 bp |
| Maximum contig length | 19,953 bp |
| Minimum contig length | 224 bp |

**Table 2. Biological Process Gene Ontologies**

| Gene Ontology | Number of Sequences |
|---|---|
| oxidation-reduction process | 624 |
| transport | 498 |
| translation | 418 |
| metabolic process | 401 |
| protein phosphorylation | 359 |
| regulation of transcription, DNA-templated | 353 |
| transmembrane transport | 308 |
| phosphorylation | 293 |
| single-organism cellular process | 283 |
| proteolysis | 260 |
| cellular process | 259 |
| regulation of cellular process | 249 |
| small GTPase mediated signal transduction | 242 |
| protein folding | 210 |
| microtubule-based process | 208 |
| signal transduction | 203 |
| single-organism process | 190 |
| microtubule-based movement | 187 |
| intracellular protein transport | 162 |
| carbohydrate metabolic process | 153 |
| vesicle-mediated transport | 148 |
| intracellular signal transduction | 140 |
| protein ubiquitination | 137 |
| positive regulation of transcription from RNA polymerase II promoter | 125 |
| mRNA splicing, via spliceosome | 124 |
| biological regulation | 121 |
| protein transport | 120 |
| protein dephosphorylation | 120 |
| DNA repair | 116 |
| methylation | 115 |
| primary metabolic process | 107 |
| regulation of transcription from RNA polymerase II promoter | 102 |
| translational initiation | 101 |
| ubiquitin-dependent protein catabolic process | 98 |
| ion transport | 91 |

**Table 3. Molecular Function Gene Ontologies**

| Gene Ontology | Number of Sequences |
|---|---|
| ATP binding | 1682 |
| metal ion binding | 1018 |
| nucleotide binding | 822 |
| RNA binding | 660 |
| GTP binding | 619 |
| binding | 618 |
| zinc ion binding | 565 |
| nucleic acid binding | 513 |
| protein binding | 503 |
| structural constituent of ribosome | 443 |
| DNA binding | 441 |
| catalytic activity | 434 |
| hydrolase activity | 411 |
| calcium ion binding | 369 |
| GTPase activity | 350 |
| transferase activity | 329 |
| oxidoreductase activity | 314 |
| protein serine/threonine kinase activity | 261 |
| kinase activity | 235 |
| ATPase activity | 218 |
| structural constituent of cytoskeleton | 215 |
| organic cyclic compound binding | 204 |
| heterocyclic compound binding | 202 |
| microtubule motor activity | 197 |
| transcription factor activity, sequence-specific DNA binding | 182 |
| actin binding | 162 |
| sequence-specific DNA binding | 157 |
| magnesium ion binding | 152 |
| protein kinase activity | 151 |
| ligase activity | 137 |
| peptidase activity | 137 |
| unfolded protein binding | 136 |
| ubiquitin protein ligase binding | 134 |
| ubiquitin-protein transferase activity | 131 |
| flavin adenine dinucleotide binding | 129 |

**Table 4. Cellular Component Gene Ontologies**

| Gene Ontology | Number of Sequences |
|---|---|
| membrane | 1973 |
| integral component of membrane | 1876 |
| cytoplasm | 1257 |
| nucleus | 1241 |
| cytosol | 605 |
| intracellular | 510 |
| extracellular exosome | 445 |
| mitochondrion | 421 |
| nucleoplasm | 350 |
| intracellular part | 331 |
| plasma membrane | 312 |
| microtubule | 270 |
| nucleolus | 223 |
| ribosome | 217 |
| intracellular membrane-bounded organelle | 176 |
| endoplasmic reticulum | 158 |
| cytoplasmic part | 157 |
| Golgi apparatus | 155 |
| mitochondrial inner membrane | 153 |
| cytosolic large ribosomal subunit | 137 |
| cell part | 132 |
| dynein complex | 126 |
| centrosome | 115 |
| intracellular ribonucleoprotein complex | 110 |
| endoplasmic reticulum membrane | 110 |
| cytoskeleton | 107 |
| integral component of plasma membrane | 107 |
| extracellular space | 101 |
| intracellular organelle | 98 |
| membrane part | 98 |
| extracellular region | 95 |
| catalytic step 2 spliceosome | 91 |
| myosin complex | 86 |
| cell | 86 |
| Golgi membrane | 84 |

**Table 5. KEGG pathways in transcriptome**

| Pathway | KEGG Pathway ID | Number of Enzymes in Pathway |
|---|---|---|
| Biosynthesis of antibiotics | map01130 | 110 |
| Purine metabolism | map00230 | 43 |
| Amino sugar and nucleotide sugar metabolism | map00520 | 25 |
| Cysteine and methionine metabolism | map00270 | 25 |
| Pyrimidine metabolism | map00240 | 23 |
| Glycolysis / Gluconeogenesis | map00010 | 21 |
| Alanine, aspartate and glutamate metabolism | map00250 | 21 |
| Aminoacyl-tRNA biosynthesis | map00970 | 20 |
| Fructose and mannose metabolism | map00051 | 20 |
| Glycine, serine and threonine metabolism | map00260 | 20 |
| Valine, leucine and isoleucine degradation | map00280 | 18 |
| Arginine and proline metabolism | map00330 | 18 |
| Pyruvate metabolism | map00620 | 17 |
| Carbon fixation pathways in prokaryotes | map00720 | 17 |
| Phosphatidylinositol signaling system | map04070 | 16 |
| Citrate cycle (TCA cycle) | map00020 | 16 |
| Inositol phosphate metabolism | map00562 | 15 |
| Pentose phosphate pathway | map00030 | 14 |
| Glycerophospholipid metabolism | map00564 | 14 |
| Butanoate metabolism | map00650 | 13 |
| Carbon fixation in photosynthetic organisms | map00710 | 13 |
| Propanoate metabolism | map00640 | 13 |
| Glyoxylate and dicarboxylate metabolism | map00630 | 12 |
| Tryptophan metabolism | map00380 | 12 |
| Methane metabolism | map00680 | 12 |
| Glutathione metabolism | map00480 | 12 |
| Tyrosine metabolism | map00350 | 12 |
| Starch and sucrose metabolism | map00500 | 12 |
| Fatty acid degradation | map00071 | 11 |
| Drug metabolism - other enzymes | map00983 | 11 |
| Porphyrin and chlorophyll metabolism | map00860 | 11 |
| Galactose metabolism | map00052 | 11 |
| Pentose and glucuronate interconversions | map00040 | 11 |
| N-Glycan biosynthesis | map00510 | 10 |
| Lysine degradation | map00310 | 10 |
| Nicotinate and nicotinamide metabolism | map00760 | 10 |
| Arginine biosynthesis | map00220 | 10 |
| One carbon pool by folate | map00670 | 10 |

| | | |
|---|---|---|
| Terpenoid backbone biosynthesis | map00900 | 10 |
| beta-Alanine metabolism | map00410 | 9 |
| Fatty acid biosynthesis | map00061 | 9 |
| Phenylalanine metabolism | map00360 | 9 |
| Glycerolipid metabolism | map00561 | 8 |
| Oxidative phosphorylation | map00190 | 7 |
| Fatty acid elongation | map00062 | 7 |
| Biosynthesis of unsaturated fatty acids | map01040 | 7 |
| Sphingolipid metabolism | map00600 | 7 |
| Pantothenate and CoA biosynthesis | map00770 | 6 |
| Other glycan degradation | map00511 | 6 |
| alpha-Linolenic acid metabolism | map00592 | 6 |
| Histidine metabolism | map00340 | 6 |
| Various types of N-glycan biosynthesis | map00513 | 6 |
| Sulfur metabolism | map00920 | 6 |
| Metabolism of xenobiotics by cytochrome P450 | map00980 | 5 |
| Glycosaminoglycan degradation | map00531 | 5 |
| Streptomycin biosynthesis | map00521 | 5 |
| Synthesis and degradation of ketone bodies | map00072 | 5 |
| Phenylalanine, tyrosine and tryptophan biosynthesis | map00400 | 5 |
| Drug metabolism - cytochrome P450 | map00982 | 5 |
| Riboflavin metabolism | map00740 | 5 |
| Ascorbate and aldarate metabolism | map00053 | 5 |
| Novobiocin biosynthesis | map00401 | 4 |
| Tropane, piperidine and pyridine alkaloid biosynthesis | map00960 | 4 |
| Biotin metabolism | map00780 | 4 |
| Vitamin B6 metabolism | map00750 | 4 |
| Benzoate degradation | map00362 | 4 |
| Nitrogen metabolism | map00910 | 4 |
| Geraniol degradation | map00281 | 4 |
| Folate biosynthesis | map00790 | 4 |
| Isoquinoline alkaloid biosynthesis | map00950 | 4 |
| Arachidonic acid metabolism | map00590 | 4 |
| Retinol metabolism | map00830 | 4 |
| Styrene degradation | map00643 | 3 |
| mTOR signaling pathway | map04150 | 3 |
| Aminobenzoate degradation | map00627 | 3 |
| Steroid biosynthesis | map00100 | 3 |
| Selenocompound metabolism | map00450 | 3 |
| Caprolactam degradation | map00930 | 3 |
| Steroid hormone biosynthesis | map00140 | 3 |

| | | |
|---|---|---|
| Ether lipid metabolism | map00565 | 3 |
| D-Glutamine and D-glutamate metabolism | map00471 | 3 |
| Thiamine metabolism | map00730 | 3 |
| Valine, leucine and isoleucine biosynthesis | map00290 | 2 |
| Phosphonate and phosphinate metabolism | map00440 | 2 |
| Linoleic acid metabolism | map00591 | 2 |
| D-Arginine and D-ornithine metabolism | map00472 | 2 |
| Lysine biosynthesis | map00300 | 2 |
| Ubiquinone and other terpenoid-quinone biosynthesis | map00130 | 2 |
| Chloroalkane and chloroalkene degradation | map00625 | 2 |
| Mucin type O-glycan biosynthesis | map00512 | 2 |
| Limonene and pinene degradation | map00903 | 2 |
| Mannose type O-glycan biosynthesis | map00515 | 2 |
| Cyanoamino acid metabolism | map00460 | 2 |
| Neomycin, kanamycin and gentamicin biosynthesis | map00524 | 2 |
| Steroid degradation | map00984 | 2 |
| Glycosphingolipid biosynthesis - globo and isoglobo series | map00603 | 2 |
| T cell receptor signaling pathway | map04660 | 2 |
| Indole alkaloid biosynthesis | map00901 | 2 |
| Carbapenem biosynthesis | map00332 | 2 |
| Caffeine metabolism | map00232 | 1 |
| Monobactam biosynthesis | map00261 | 1 |
| Glycosphingolipid biosynthesis - ganglio series | map00604 | 1 |
| Lipoic acid metabolism | map00785 | 1 |
| Toluene degradation | map00623 | 1 |
| Other types of O-glycan biosynthesis | map00514 | 1 |
| Taurine and hypotaurine metabolism | map00430 | 1 |
| Glycosaminoglycan biosynthesis - keratan sulfate | map00533 | 1 |
| Flavone and flavonol biosynthesis | map00944 | 1 |
| Th1 and Th2 cell differentiation | map04658 | 1 |
| Sesquiterpenoid and triterpenoid biosynthesis | map00909 | 1 |
| C5-Branched dibasic acid metabolism | map00660 | 1 |
| Primary bile acid biosynthesis | map00120 | 1 |
| Insect hormone biosynthesis | map00981 | 1 |
| PI3K-Akt signaling pathway | map04151 | 1 |
| Glycosaminoglycan biosynthesis - heparan sulfate / heparin | map00534 | 1 |
| Betalain biosynthesis | map00965 | 1 |
| Glycosylphosphatidylinositol (GPI)-anchor biosynthesis | map00563 | 1 |
| Penicillin and cephalosporin biosynthesis | map00311 | 1 |
| Human papillomavirus infection | map05165 | 1 |
| Ethylbenzene degradation | map00642 | 1 |

| | | |
|---|---|---|
| Aflatoxin biosynthesis | map00254 | 1 |
| Phenylpropanoid biosynthesis | map00940 | 1 |
| Naphthalene degradation | map00626 | 1 |
| Glycosaminoglycan biosynthesis - chondroitin sulfate / dermatan sulfate | map00532 | 1 |
| Diterpenoid biosynthesis | map00904 | 1 |
| Zeatin biosynthesis | map00908 | 1 |
| Glucosinolate biosynthesis | map00966 | 1 |
| Cutin, suberine and wax biosynthesis | map00073 | 1 |
| Biosynthesis of ansamycins | map01051 | 1 |