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Research article

Comparison of transcriptomes of enlarged spheroplasts of *Erythrobacter litoralis* and *Lelliottia amnigena*

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Abstract: Bacterial spheroplasts do not divide but they grow and enlarge with DNA replication in a broth containing an inhibitor of peptidoglycan synthesis and high salt concentration. The enlarged spheroplasts of *Lelliottia amnigena*, belonging to the family Enterobacteriaceae, formed vacuole-like structures, while those of the aerobic photosynthetic marine bacterium *Erythrobacter litoralis* did not form such structures. In addition, the enlarged spheroplasts of *L. amnigena*, which differ in the size of inner and outer membranes, were larger than those of *E. litoralis*. To elucidate the reason for these differences, we analyzed the transcriptome (RNA-seq) of spheroplasts at the beginning of growth and upon enlargement. After sequencing the RNAs expressed in normal and enlarged spheroplasts, we compared the gene expression levels of the 1100 orthologs in *E. litoralis* and *L. amnigena*. Among these, 347 and 213 genes were more than 2-fold upregulated in enlarged spheroplasts of *E. litoralis* and *L. amnigena*, respectively; 193 and 269 genes were less than 0.5-fold downregulated in those of *E. litoralis* and *L. amnigena*, respectively. Thirty six genes were upregulated in *L. amnigena* enlarged spheroplasts but were downregulated in *E. litoralis* enlarged spheroplasts, and may be related to vacuole-like structure generation. Fourteen of the 36 genes encoded a membrane protein. Our findings indicate that spheroplast enlargement varies between different organisms with respect to gene expression.

Keywords: enlarged spheroplast; *Erythrobacter litoralis*; inner membrane; *Lelliottia amnigena*;

outer membrane; transcriptome; vacuole-like structure

1. Introduction

Enlarged spheroplasts are generated by spheroplast cultivation in a broth containing an inhibitor of peptidoglycan synthesis (for example, penicillin) and high salt concentration after lysozyme treatment of bacterial cells [1–5]. In presence of penicillin, spheroplasts do not divide but grow and enlarge with DNA replication in the marine broth, whereas they divide in the absence of penicillin [6,7]. The rate of DNA increase in the absence of penicillin is higher than that observed in the presence of penicillin [6,7].

Lelliottia amnigena (*Enterobacter amnigenus*) belongs to the family Enterobacteriaceae of Gram-negative bacteria [8]. Most enlarged spheroplasts of *L. amnigena* have quite different sizes of the inner and outer membrane vesicles [9]; the inner membrane shows a maximum size of approximately 15 μm in diameter, while the outer membrane has a maximum size of > 30 μm in diameter. Vacuole-like structures were formed in enlarged spheroplasts of *L. amnigena*. Based on the membrane specific protein distribution in *Escherichia coli*, the membrane of these vacuole-like structures is very similar but not identical to the inner membrane, which is completely different from the outer membrane [4].

Erythrobacter litoralis is an aerobic, anoxygenic, and photosynthetic marine bacterium, belonging to Alphaproteobacteria, which generates bacteriochlorophyll *a* and carotenoids [10]. The size of *E. litoralis* spheroplasts is limited to a diameter of 6–7 μm [7]. Continuous exposure to light was shown to inhibit their enlargement [7]. In addition, they lacked vacuole-like structures. We hypothesized that the difference of enlarged spheroplast size and the lack of vacuole-like structure may be related to the difference of general metabolism in bacterial cells. In contrast, the Gram-positive bacterium *Bacillus subtilis* and the Gram-negative bacterium *E. coli* are known to form vacuole-like structures and their cell size is approximately 15 μm in diameter [4,5]. Divergence between inner and outer membranes enlargements is not observed in *E. litoralis* enlarged spheroplasts.

Thus, the giant spheroplasts of *E. litoralis* and *L. amnigena* are structurally different. In order to elucidate this difference, we sequenced the RNAs expressed in the spheroplasts at the beginning of growth and in the enlarged spheroplasts. We then compared the changes in expression of the orthologous genes between the enlarged spheroplasts of *E. litoralis* and *L. amnigena*.

2. Methods

2.1. Cultivation of *E. litoralis* and *L. amnigena* spheroplasts

Cells of *E. litoralis* NBRC 102620 and *L. amnigena* (*Enterobacter amnigenus*) NBRC 105700 were grown on marine broth agar (Difco, Sparks, MD). The harvested cells (approximately 0.003 g) were suspended in a buffer (1 mL) consisting of 0.1 M Tris-HCl (pH 7.6) and 0.3 M sucrose. Lysozyme (200 μg/mL, Wako, Osaka) was added to the cell suspension and allowed to incubate at 25 ℃ (for *E. litoralis*) and 37 ℃ (for *L. amnigena*) for 15 min. After harvesting (centrifugation for 5 min at 3000 rpm) the suspension, the cells were suspended in marine broth (1 mL) containing 600 μg/mL penicillin G (Serva, München). The suspension was then diluted by adding 4 μL of suspension to marine broth (1 mL) containing penicillin G. The diluted suspension was then incubated at 25 ℃ in the dark. We used spheroplasts at two time points of growth: 0 h and 96 h for *E. litoralis*, and 0 h and 43 h for *L. amnigena*.

2.2. DAPI staining

Fluorescence images of enlarged *L. amnigena* cells stained with DAPI were acquired. DAPI (final concentration, 0.25 μg/mL) was added to the cell suspension and incubated at room temperature for 1 h. Fluorescent micrographs were taken using Keyence BZ-X710.

2.3. RNA isolation

Total RNA was isolated using the NucleoSpin RNA kit (Macherey-Nagel, Düren). After removal of rRNA with the RiboMinus Transcriptome Isolation Kit for bacteria (Thermo Fisher Science, Waltham, MA), the RNAs were purified and concentrated with the NucleoSpin RNA Clean-up XS kit (Macherey-Nagel, Düren).

2.4. RNA-seq

Sequencing libraries were prepared from RiboMinus-treated RNAs by NEXTflex qRNA-Seq Kit (Bioo Scientific, Austin, TX). The resultant libraries were analyzed by TapeStation 2200 (Agilent, Waldbronn) equipped with a High Sensitivity D1000 tape (Agilent, Waldbronn). The concentration of each library was quantified with KAPA Library Quantification Kits. The four libraries were sequenced on a Miseq system (Illumina, San Diego, CA) with 2 × 75 base paired end sequencing.

FASTQ files were imported into the CLC Genomic Workbench (CLC bio, Germantown, MD) as paired-end reads, and read data were mapped on each genome by RNA-seq with the following parameters: Count paired reads as two = Yes, Auto-detect paired distances = No, Similarity fraction = 0.8, Length fraction = 0.5, Mismatch cost = 2, Insertion cost = 3, Deletion cost = 3.

2.5. Gene expression comparison

We performed reciprocal best hits using BLASTp (*E*-value < 0.001) to select orthologous genes between *E. litoralis* and *L. amnigena*. For orthologous genes, we calculated the ratio of reads per kilobase of gene per million mapped sequence reads (RPMK) in the enlarged spheroplasts per the RPKM in spheroplasts at 0 h of growth and selected the ratio of > 2 for the upregulated genes and the ratio of < 0.5 for the downregulated genes. The difference in expression was estimated by Fisher's exact test [11]. Considering that the mapped number of *E. litoralis* spheroplasts was 3 times greater than that of spheroplasts at 0 h of growth, when the RPKM = 0 (the mapped number = 0) in *E. litoralis* spheroplasts at 0 h of growth, we did not consider the genes with the mapped number 1, 2, and 3 in *E. litoralis* enlarged spheroplasts but considered the genes with the mapped number > 3 in enlarged spheroplasts as upregulated genes.

3. Results and Discussion

3.1. DNA location in the L. amnigena enlarged spheroplast

In a previous study, it was observed that spheroplasts of *L. amnigena* elongated and divided in marine broth containing 3 µg/mL penicillin [9]. In contrast, in marine broth containing 300 µg/mL penicillin, they did not divide and became enlarged with differing sizes of the inner and outer membrane vesicles [9]. The fluorescence image of *L. amnigena* enlarged spheroplasts showed that the vacuole-like structures and the large periplasmic space were not stained with the DNA staining reagent DAPI (Figure 1). This indicates that the chromosomal DNA does not pass through the inner membrane of the enlarged spheroplasts or the membrane of the vacuole-like structures.

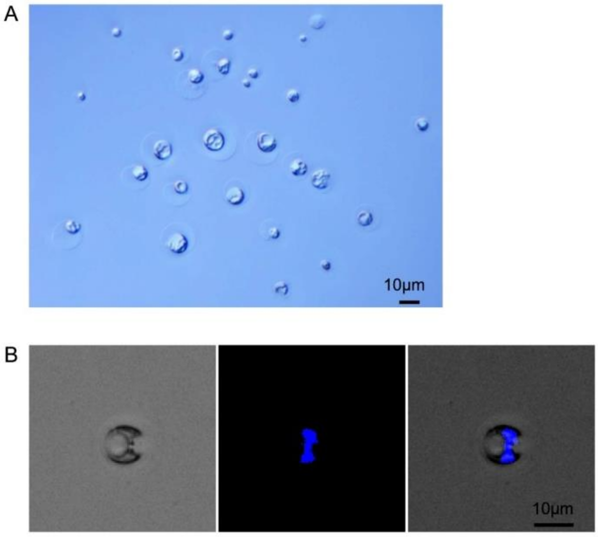

Figure 1. A. Differential interference contrast microscopic images of enlarged spheroplasts of *Lelliottia amnigena* at 48 h of growth. The images are obtained using Olympus IX73. B. Microscopic image of an enlarged spheroplast of *L. amnigena* at 99 h of growth (left). Fluorescence image of the enlarged spheroplast (center). Merged image of the enlarged spheroplast (right). The images are obtained using Keyence BZ-X710.

3.2. Mapped number of RNA sequences to orthologous genes in E. litoralis and L. amnigena

We obtained 78934 and 62870 reads from the spheroplasts of *E. litoralis* at the beginning (0 h) of growth and after enlargement, respectively. Excluding the RNA sequences that map to the ribosomal RNA genes (61767 reads at the beginning; 31412 reads after enlargement) and did not map to the genomic DNA (10565 reads at the beginning; 8902 reads after enlargement), 6602 and 22556 RNA sequences were mapped from the spheroplasts of *E. litoralis* at the beginning (0 h) of growth and after enlargement, respectively. On the other hand, we obtained 6921404 and 957576 reads from the spheroplasts of *L. amnigena* at the beginning (0 h) of growth and after enlargement, respectively. Excluding the RNA sequences that map to the ribosomal RNA genes (6532071 reads at the beginning; 701391 reads after enlargement) and did not map to the genomic DNA (330459 reads at the beginning; 174806 reads after enlargement), 58874 and 81379 RNA sequences were mapped at the beginning (0 h) of growth and after enlargement from the spheroplasts of *L. amnigena*. The RPKMs were then calculated and compared at the gene expression level.

The transcriptome data have been deposited in DDBJ under the accession number DRA004675.

We identified 1100 orthologous gene pairs between *E. litoralis* and *L. amnigena*. Among these 1100 orthologs, 347 and 213 genes were more than 2-fold upregulated in the enlarged spheroplasts of *E. litoralis* and *L. amnigena*, respectively; whereas 193 and 269 genes were less than 0.5-fold downregulated in those of *E. litoralis* and *L. amnigena*, respectively (Figure 2, Supplementary Table 1).

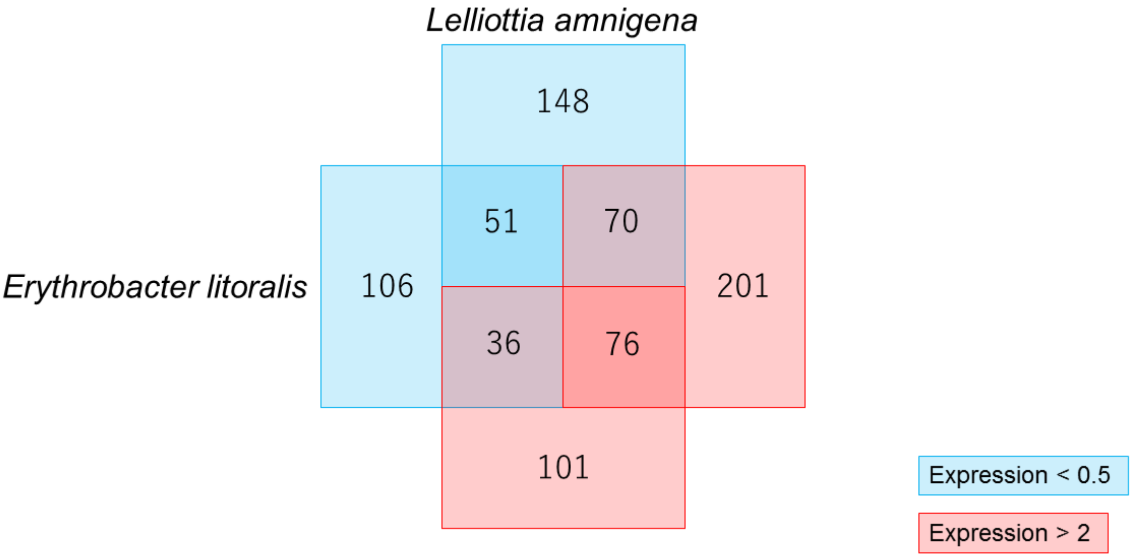

Figure 2. Venn diagram of gene expression changes in the enlarged spheroplasts of *Erythrobacter litoralis* and *Lelliottia amnigena*.

3.3. *Upregulated orthologous genes in E. litoralis and L. amnigena enlarged spheroplasts*

The 76 upregulated orthologous genes in both *E. litoralis* and *L. amnigena* enlarged spheroplasts are shown in Table 1. Of the 76 genes, 25 (32.9%) were homologous to membrane protein coding genes.

Peptidoglycan synthesis-related homologous genes (for example, *mltA* homolog) were significantly ($p < 0.05$) upregulated in both *E. litoralis* and *L. amnigena* enlarged spheroplasts (Table 1). Penicillin causes cell wall stress which might lead to upregulation of peptidoglycan synthesis.

Chaperone homologous genes (for example, *groES* homolog) were also significantly upregulated in enlarged spheroplasts of both bacteria (Table 1), suggesting that a number of proteins may exist and be maintained in the enlarged spheroplasts. The expression of the phage shock protein homologous gene was very high in the enlarged spheroplasts (Table 1), suggesting that these cells were under stress.

Homologs of lipopolysaccharide synthesis and transport-related genes (for example, *lptB* and *lptF* homologs) were upregulated in both *E. litoralis* and *L. amnigena* enlarged spheroplasts (Table 1). It is strongly suggested that these genes may be required to maintain the enlarged inner and outer membranes of these bacteria [12].

Homologs of DNA replication initiator *dnaA* and recombinase *recA* genes were upregulated in both *E. litoralis* and *L. amnigena* enlarged spheroplasts (Table 1). DNA replication was not inhibited in the enlarged spheroplasts [6,7]. Thus, multiple chromosomal DNAs exist in a single cell. The DnaA and RecA homologs might function to maintain the multiple DNAs in the enlarged cells of both bacteria. The fact that enlarged spheroplasts have multiple copies of chromosome per cell supports the notion that chromosomal copy number is determined by the balance between DNA replication and cell division time [13].

3.4. Downregulated orthologous genes in E. litoralis and L. amnigena enlarged spheroplasts

The 51 downregulated genes in both the *E. litoralis* and *L. amnigena* enlarged spheroplasts are shown in Table 2. Of these 51 genes, 7 (13.7%) were homologous to inner and outer membrane protein coding genes, including 3 flagellar protein-encoding genes.

Homologs of amino acid and nucleotide synthesis-related genes and pentose phosphate pathway genes were significantly downregulated in both *E. litoralis* and *L. amnigena* enlarged spheroplasts (Table 2). This indicated that amino acid and nucleotide synthesis were repressed in enlarged spheroplasts. Repression of nucleotide synthesis is consistent with the reduced speed of DNA replication [6,7]. In addition, homologs of flagellar protein genes were significantly downregulated in both *E. litoralis* and *L. amnigena* enlarged spheroplasts (Table 2), which is consistent with absence of motility in the spheroplasts of both bacteria.

The nucleoid protein HU homologous gene was significantly downregulated in both *E. litoralis* and *L. amnigena* enlarged spheroplasts (Table 2). Cross-talk between HU and topoisomerase I has been reported in *E. coli* [14]. Interestingly, topoisomerase I homologous gene was also significantly downregulated in both enlarged spheroplasts (Table 2). The downregulation of both HU and topoisomerase I homologous genes may be one of the characteristics of enlarged spheroplasts. Thus, in enlarged spheroplasts, recombination may be repressed in multiple copies of chromosomal DNAs.

3.5. Orthologous genes with differential expression between E. litoralis and L. amnigena spheroplasts

The genes with differential expression between *E. litoralis* and *L. amnigena* enlarged spheroplasts are shown in Tables 3 and 4.

As mentioned above, the *recA* homolog was upregulated in both enlarged spheroplasts (Table 1). However, the DNA recombinase *radA* homolog was upregulated in *E. litoralis* enlarged spheroplasts, but was downregulated in *L. amnigena* enlarged spheroplasts (Table 3). In the enlarged spheroplasts, the expression level of the *E. litoralis recA* homolog was much higher than that of *L. amnigena*, suggesting that higher requirement for DNA recombinase in *E. litoralis*. Although we should estimate the functional difference between RecA and RadA homologs [15], it is suggested that the RadA homolog might be needed in addition to the RecA homolog in *E. litoralis* enlarged spheroplasts.

The *secA* homolog was upregulated in *L. amnigena* enlarged spheroplasts but was downregulated in *E. litoralis* enlarged spheroplasts (Table 4). In a previous study [4], SecY, a major component of the Sec complex, was strongly detected in the vacuole-like structure membrane of *E.*

coli. In our findings, the expression level of the *secY* homolog was upregulated (1.57-fold) in *L. amnigena* spheroplasts but was downregulated (0.84-fold) in *E. litoralis* enlarged spheroplasts (Supplementary Table 1). Thus, it is strongly suggested that the SecA homolog (and the SecY homolog) is associated with generation of the vacuole-like structure in *L. amnigena*.

The homologs of cell division-related genes *ftsA* and *ftsZ* were upregulated in *L. amnigena* enlarged spheroplasts but were downregulated in *E. litoralis* enlarged spheroplasts (Table 4), suggesting that these two genes may be also associated with the generation of vacuole-like structures.

Penicillin binding protein 1B homologous gene was upregulated in *L. amnigena* enlarged spheroplasts and was downregulated in *E. litoralis* (Table 4). *E. coli* spheroplasts resynthesize their cell wall and shape without penicillin [16], and the penicillin binding protein 1B was required for the shape recovery [16]. Upon transferring the enlarged spheroplasts to a penicillin-free broth, the shape recovery rate of *L. amnigena* may be higher than that of *E. litoralis*.

The Mg^{2+} transport system was contrasting between the *E. litoralis* and *L. amnigena* enlarged spheroplasts. The Mg^{2+} influx-related gene *corA* [17] was upregulated in *E. litoralis* enlarged spheroplasts but was downregulated in *L. amnigena* enlarged spheroplasts (Table 3). On the other hand, the Mg^{2+} efflux-related gene *apaG* (*corD*) [17] was downregulated in *E. litoralis* but was upregulated in *L. amnigena* (Table 4). These results strongly suggest that *E. litoralis* enlarged spheroplasts require Mg^{2+} but the *L. amnigena* enlarged spheroplasts discard it.

4. Conclusion

We showed that spheroplast enlargement varies between *E. litoralis* and *L. amnigena*. The changes in expression of the orthologous genes also vary between enlarged spheroplasts of the two bacteria. Based on the different gene expression pattern, the candidate genes related to the cell structural differences were extracted in this study.

Acknowledgements

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Table 1. Orthologous genes upregulated (ratio > 2) in *E. litoralis* and *L. amnigena* enlarged spheroplasts.

Protein ID of *E. litoralis*	RPKM in *E. litoralis* spheroplasts at the beginning of growth	RPKM in *E. litoralis* enlarged spheroplasts	Ratio of RPKMs of *E. litoralis*	Annotated function	Protein ID of *L. amnigena*	RPKM in *L. amnigena* spheroplasts at the beginning of growth	RPKM in *L. amnigena* enlarged spheroplasts	Ratio of RPKMs of *L. amnigena*	Annotated function
Homologs of chaperone genes									
KEO89606.1*	0.00	623.74		molecular chaperone GroES	KDM56993.1*	347.00	1300.00	3.75	chaperonin
KEO92782.1*	0.00	4575.93		phage shock protein	KDM46724.1*	4670.00	27100.00	5.80	phage shock protein A
KEO93197.1*	0.00	2039.77		ATP-dependent protease	KDM53259.1*	178.00	608.00	3.42	ATP-dependent protease ATPase subunit HslU
KEO93350.1*	588.91	6885.08	11.69	ATPase AAA	KDM51144.1*	449.00	1660.00	3.70	chaperone ClpB
KEO98976.1*	265.47	587.82	2.21	molecular chaperone DnaJ	KDM57339.1*	267.00	751.00	2.81	chaperone dnaJ
Homologs of DNA replication and repair-related genes									

KEO92279.1*	304.84	659.99	2.17	ATPase AAA	KDM53654.1*	0.00	8.08		Mg chelatase-like protein
KEO92303.1*	0.00	255.35		chromosomal replication initiator protein DnaA	KDM52760.1*	280.00	617.00	2.20	chromosomal replication initiator protein dnaA
KEO92620.1*	1142.64	8813.23	7.71	recombinase RecA	KDM50414.1*	401.00	893.00	2.23	protein recA
Homologs of lipopolysaccharide synthesis and transport-related genes									
KEO89583.1*	0.00	224.27		ABC transporter ATP-binding protein	KDM54743.1*	46.80	339.00	7.24	lipopolysaccharide export system ATP-binding protein LptB
KEO90699.1*	309.18	1369.19	4.43	D-arabinose 5-phosphate	KDM54739.1*	34.40	74.70	2.17	arabinose 5-phosphate isomerase
KEO92387.1*	0.00	693.72		permease	KDM54753.1*	201.00	458.00	2.28	lipopolysaccharide export system permease lptF
KEO96424.1*	321.43	901.51	2.80	ABC transporter	KDM55137.1*	29.10	91.30	3.14	lipid A export ATP-binding/permease MsbA
KEO98632.1*	0.00	130.74		MFS transporter	KDM50532.1*	0.00	10.30		lysophospholipid transporter lplT
Homologs of peptidoglycan synthesis-related genes									

KEO92316.1*	0.00	1429.28		MltA	KDM50514.1*	46.40	123.00	2.65	membrane-bound lytic murein transglycosylase A
KEO93302.1*	0.00	801.96		peptidoglycan transglycosylase	KDM54749.1*	0.00	16.90		monofunctional biosynthetic peptidoglycan transglycosylase
KEO93307.1*	0.00	252.66		N-acetylmuramoyl-L-alanine amidase	KDM55076.1*	20.40	118.00	5.78	N-acetylmuramoyl-L-alanine amidase AmiD
KEO93413.1*	0.00	408.49		peptidoglycan glycosyltransferase	KDM57390.1*	115.00	452.00	3.93	peptidoglycan synthase ftsI
KEO93417.1*	0.00	193.99		UDP-N-acetylmuramoylalanine--D-glutamate ligase	KDM57394.1*	116.00	802.00	6.91	UDP-N-acetylmuramoylalanine-D-glutamate ligase
Homologs of other membrane protein genes									
KEO89306.1*	1090.43	2253.52	2.07	membrane protease subunit HflC	KDM56960.1*	135.00	599.00	4.44	protein HflC
KEO89307.1*	535.14	3554.84	6.64	peptidase	KDM56961.1*	431.00	1380.00	3.20	protein HflK

KEO89575.1*	0.00	225.11		flagellar biosynthesis protein FliR	KDM48637.1*	0.00	31.30		flagellar biosynthetic protein fliR
KEO89580.1*	0.00	221.23		mechanosensitive ion channel protein MscS	KDM55515.1*	68.20	188.00	2.76	miniconductance mechanosensitive channel
KEO92762.1*	0.00	194.41		membrane protein	KDM51940.1*	0.00	16.20		hypothetical protein
KEO93138.1*	0.00	505.31		ABC transporter	KDM55480.1*	321.00	823.00	2.56	hypothetical protein
KEO93143.1*	0.00	301.81		proline:sodium symporter PutP	KDM51759.1*	495.00	1180.00	2.38	sodium/proline symporter
KEO96616.1*	0.00	211.59		membrane protein	KDM48736.1*	116.00	568.00	4.90	hypothetical protein
KEO96782.1*	0.00	281.12		MarC family transcriptional regulator	KDM46654.1*	0.00	37.90		UPF0056 membrane protein yhcE
KEO96802.1*	0.00	526.58		hypothetical protein	KDM51307.1*	54.80	674.00	12.30	manganese transporter mntH
KEO98444.1*	159.95	613.91	3.84	protoheme IX farnesyltransferase	KDM55407.1*	612.00	1340.00	2.19	protoheme IX farnesyltransferase
KEO98495.1*	0.00	1075.18		hypothetical protein	KDM55130.1*	24.50	70.90	2.89	UPF0702 transmembrane protein ycaP
KEO98569.	201.21	3148.42	15.65	histidine kinase	KDM51863	0.00	16.80		sensor protein phoQ

1*					.1*				
KEO98609.1*	288.10	612.40	2.13	membrane protein	KDM57482.1*	316.00	742.00	2.35	outer membrane protein assembly factor yaeT
KEO99117.1*	0.00	227.97		membrane protein	KDM54035.1*	14.10	51.10	3.62	hypothetical protein
Others									
KEO89324.1*	0.00	339.26		mannose-1-phosphate guanylyltransferase	KDM46222.1*	118.00	1610.00	13.64	mannose-1-phosphate guanylyltransferase
KEO89964.1*	0.00	1086.92		taurine dioxygenase	KDM57053.1*	19.90	115.00	5.78	alpha-ketoglutarate-dependent taurine dioxygenase
KEO89994.1*	0.00	480.32		aminomethyltransferase	KDM49721.1*	264.00	696.00	2.64	aminomethyltransferase
KEO90662.1*	0.00	165.87		phosphoribosylaminoimidazole carboxylase	KDM55484.1*	0.00	138.00		N5-carboxyaminoimidazole ribonucleotide synthase
KEO90672.1*	0.00	261.97		succinyl-CoA synthetase subunit beta	KDM55689.1*	480.00	1360.00	2.83	succinyl-CoA ligase [ADP-forming] subunit beta

KEO91012.1*	0.00	329.01		glutamyl-tRNA synthetase	KDM57451.1*	0.00	13.80		glutamyl-Q tRNA(Asp) synthetase
KEO92307.1*	0.00	1173.54		2-octaprenylphenol hydroxylase	KDM52399.1*	41.40	112.00	2.71	2-polyprenylphenol 6-hydroxylase
KEO92322.1*	1243.70	5084.10	4.09	alkyl hydroperoxide reductase	KDM55608.1*	108.00	706.00	6.54	alkyl hydroperoxide reductase subunit F
KEO92357.1*	0.00	486.82		hypothetical protein	KDM55447.1*	0.00	44.50		maltose O-acetyltransferase
KEO92389.1*	0.00	1032.40		Clp protease ClpS	KDM55108.1*	212.00	689.00	3.25	ATP-dependent Clp protease adapter protein ClpS
KEO92432.1*	0.00	225.11		acetyl-CoA acetyltransferase	KDM49670.1*	0.00	10.40		hypothetical protein
KEO92626.1*	0.00	371.92		transcription elongation factor GreB	KDM53922.1*	0.00	25.90		transcription elongation factor greB
KEO92766.1*	0.00	499.00		pseudouridine synthase	KDM54709.1*	53.60	142.00	2.65	tRNA pseudouridine synthase B
KEO92773.1*	238.61	1937.27	8.12	translation initiation factor IF-2	KDM54711.1*	645.00	1330.00	2.06	translation initiation factor IF-2
KEO92898.1*	0.00	183.68		oxidoreductase	KDM50444.1*	0.00	20.20		formate hydrogenlyase subunit 2

KEO92901.1*	0.00	211.59	metallophosphoesterase	KDM54593.1*	123.00	267.00	2.17	protein icc
KEO93029.1*	0.00	1061.69	Fur family transcriptional regulator	KDM55662.1*	494.00	1760.00	3.56	ferric uptake regulation protein
KEO93118.1*	0.00	351.20	4-hydroxythreonine-4-phosphate dehydrogenase	KDM57359.1*	86.00	299.00	3.48	4-hydroxythreonine-4-phosphate dehydrogenase
KEO93158.1*	0.00	513.67	ubiquinone biosynthesis protein UbiH	KDM49723.1*	43.20	93.80	2.17	2-octaprenyl-6-methoxyphenol hydroxylase
KEO93247.1*	0.00	269.73	TetR family transcriptional regulator	KDM55557.1*	86.70	564.00	6.51	HTH-type transcriptional regulator BetI
KEO93375.1*	0.00	357.49	4-hydroxy-3-methylbut-2-enyl diphosphate reductase	KDM57347.1*	53.60	142.00	2.65	4-hydroxy-3-methylbut-2-enyl diphosphate reductase
KEO93411.1*	0.00	566.68	16S rRNA methyltransferase	KDM57388.1*	162.00	1000.00	6.17	ribosomal RNA small subunit methyltransferase H

KEO96480.1*	303.62	627.48	2.07	methionine sulfoxide reductase B	KDM51893.1*	41.00	208.00	5.07	peptide methionine sulfoxide reductase msrB
KEO96529.1*	0.00	210.35		phosphoribosylamine--glycine ligase	KDM52454.1*	92.00	380.00	4.13	phosphoribosylamine-glycine ligase
KEO96539.1*	0.00	696.27		hypothetical protein	KDM51989.1*	200.00	435.00	2.18	FeS cluster assembly protein sufD
KEO96580.1*	0.00	486.03		PTS IIA-like nitrogen-regulatory protein PtsN	KDM54746.1*	0.00	150.00		nitrogen regulatory protein
KEO96608.1*	0.00	1394.71		translation factor Sua5	KDM53819.1*	0.00	42.90		tRNA threonylcarbamoyladenosine biosynthesis protein RimN
KEO96752.1*	97.51	359.85	3.69	integrase	KDM51758.1*	94.30	425.00	4.51	putA protein
KEO98470.1*	0.00	124.49		hypothetical protein	KDM51955.1*	11.80	59.60	5.05	UPF0061 protein ydiU
KEO98596.1*	0.00	814.69		LysR family transcriptional regulator	KDM57341.1*	37.70	81.90	2.17	transcriptional activator protein nhaR
KEO98607.1*	0.00	192.91		1-deoxy-D-xylulose 5-phosphate reductoisomerase	KDM57478.1*	42.50	1230.00	28.94	1-deoxy-D-xylulose 5-phosphate reductoisomerase

KEO98608.1*	0.00	1552.72	peptidase	KDM57481.1*	264.00	854.00	3.23	RIP metalloprotease RseP
KEO98641.1*	0.00	1679.78	LysR family transcriptional regulator	KDM53240.1*	130.00	321.00	2.47	hydrogen peroxide-inducible genes activator
KEO98759.1*	0.00	157.99	3-dehydroquinate synthase	KDM53905.1*	46.80	192.00	4.10	3-dehydroquinate synthase
KEO98794.1*	0.00	345.72	branched-chain alpha-keto acid dehydrogenase subunit E2	KDM57421.1*	744.00	6140.00	8.25	dihydrolipoyllysine-residue acetyltransferase
KEO98866.1*	0.00	196.33	farnesyl-diphosphate synthase	KDM55391.1*	75.50	519.00	6.87	farnesyl diphosphate synthase
KEO98882.1*	0.00	473.35	rRNA methyltransferase	KDM51417.1*	107.00	496.00	4.64	tRNA (cytidine/uridine-2'-O-)-methyltransferase TrmJ
KEO98935.1*	0.00	3161.33	transcriptional regulator	KDM55471.1*	0.00	120.00		HTH-type transcriptional regulator CueR
KEO98999.1*	0.00	246.42	nucleoside triphosphate hydrolase	KDM50489.1*	85.80	233.00	2.72	nucleoside triphosphate pyrophosphohydrolase

KEO99007.1*	0.00	538.76	2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase	KDM50467.1*	70.80	154.00	2.18	2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase
KEO99009.1*	0.00	388.83	cyclase	KDM51170.1*	35.60	155.00	4.35	hypothetical protein
KEO99027.1*	0.00	95.59	transketolase	KDM57420.1*	1130.00	10900.00	9.65	pyruvate dehydrogenase E1 component
KEO99055.1*	0.00	1388.60	hypothetical protein	KDM55136.1	0.00	5.43		hypothetical protein

*P-value < 0.05

Table 2. Orthologous genes downregulated (ratio < 0.5) in *E. litoralis* and *L. amnigena* enlarged spheroplasts.

Protein ID of *E. litoralis*	RPKM in *E. litoralis* spheroplasts at the beginning of growth	RPKM in *E. litoralis* enlarged spheroplasts	Ratio of RPKMs of *E. litoralis*	Annotated function	Protein ID of *L. amnigena*	RPKM in *L. amnigena* spheroplasts at the beginning of growth	RPKM in *L. amnigena* enlarged spheroplasts	Ratio of RPKMs of *L. amnigena*	Annotated function
Homologs of amino acid synthesis and metabolism-related genes									
KEO88954.1*	616.47	136.50	0.22	L-asparaginase	KDM51897.1*	33.40	0.00	0.00	L-asparaginase 1
KEO89592.1*	365.66	143.94	0.39	S-adenosylmethionine synthetase	KDM49649.1*	1070.00	255.00	0.24	S-adenosylmethionine synthase
KEO89916.1*	363.48	0.00	0.00	2,3,4,5-tetrahydropyridine-2,6-carboxylate N-succinyltransferase	KDM57471.1*	1050.00	432.00	0.41	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase
KEO90596.1*	298.26	88.06	0.30	threonine aldolase	KDM55097.1*	509.00	172.00	0.34	low specificity L-threonine aldolase

KEO92299.1*	201.21	0.00	0.00	4-hydroxy-tetrahydrodipicolinate reductase	KDM57348.1*	269.00	14.90	0.06	dihydrodipicolinate reductase
KEO92640.1*	250.39	0.00	0.00	argininosuccinate synthase	KDM54714.1*	2220.00	219.00	0.10	argininosuccinate synthase
KEO92798.1*	507.05	149.70	0.30	3-isopropylmalate dehydratase	KDM57378.1*	196.00	60.80	0.31	3-isopropylmalate dehydratase small subunit
KEO93106.1*	831.23	245.41	0.30	serine acetyltransferase	KDM52905.1*	331.00	149.00	0.45	serine acetyltransferase
KEO93391.1*	1078.83	318.51	0.30	glutamine synthetase	KDM53324.1*	385.00	174.00	0.45	glutamine synthetase
KEO93480.1*	1248.41	595.39	0.48	3-phosphoglycerate dehydrogenase	KDM49615.1*	964.00	369.00	0.38	D-3-phosphoglycerate dehydrogenase
KEO98853.1*	596.53	0.00	0.00	ketol-acid reductoisomerase	KDM53647.1*	3330.00	450.00	0.14	ketol-acid reductoisomerase
KEO99046.1*	292.53	0.00	0.00	anthranilate synthase component I	KDM46686.1*	1870.00	7.86	0.00	anthranilate synthase component 1
KEO99050.1*	459.56	45.23	0.10	anthranilate phosphoribosyltransferase	KDM46685.1*	2870.00	53.90	0.02	anthranilate synthase component II
Homologs of flagellar protein genes									

KEO89561.1*	22111.90	337.67	0.02	flagellin	KDM48656.1*	124.00	44.90	0.36	hypothetical protein
KEO90993.1*	740.22	0.00	0.00	flagellar basal body rod protein FlgC	KDM51808.1*	41.90	0.00	0.00	flagellar basal-body rod protein flgC
KEO92484.1*	806.97	79.42	0.10	flagellar motor protein MotB	KDM51720.1*	24600.00	1390.00	0.06	outer membrane protein A
Homologs of nucleotide synthesis and pentose phosphate pathway-related genes									
KEO91063.1*	325.03	95.96	0.30	phosphoribosylpyrophosphate synthetase	KDM47000.1*	985.00	298.00	0.30	ribose-phosphate pyrophosphokinase
KEO93239.1*	414.76	61.23	0.15	glucose-6-phosphate dehydrogenase	KDM48740.1*	345.00	150.00	0.43	glucose-6-phosphate 1-dehydrogenase
KEO93278.1*	293.09	0.00	0.00	aspartate carbamoyltransferase catalytic subunit	KDM56880.1*	1090.00	65.90	0.06	aspartate carbamoyltransferase
KEO96500.1*	688.30	0.00	0.00	ribulose-phosphate 3-epimerase	KDM53902.1*	200.00	54.40	0.27	ribulose-phosphate 3-epimerase
KEO96518.1*	349.69	103.24	0.30	nicotinate-nucleotide pyrophosphorylase	KDM57413.1*	57.00	27.50	0.48	nicotinate-nucleotide pyrophosphorylase [carboxylating]

KEO98799.1*	610.90	45.09	0.07	transketolase	KDM49630.1*	844.00	278.00	0.33	transketolase 1
Homologs of other membrane protein genes									
KEO92879.1*	12676.20	0.00	0.00	membrane protein	KDM46678.1*	80.50	0.00	0.00	outer membrane protein W
KEO93429.1*	12522.20	4363.68	0.35	membrane protein	KDM54978.1*	216.00	86.80	0.40	inner membrane protein YbhL
KEO98493.1*	726.95	268.28	0.37	ABC transporter ATP-binding protein	KDM57319.1*	479.00	192.00	0.40	hypothetical protein
KEO99262.1*	1240.06	162.72	0.13	hypothetical protein	KDM52093.1*	131.00	11.80	0.09	AI-2 transporter tqsA
Others									
KEO89629.1*	824.47	121.71	0.15	3-demethylubiquinone-9 3-methyltransferase	KDM46348.1*	116.00	16.90	0.15	3-demethylubiquinone-9 3-methyltransferase
KEO90016.1*	338.03	49.90	0.15	hypothetical protein	KDM52077.1*	18.60	0.00	0.00	HTH-type transcriptional regulator AbgR
KEO90690.1*	737.52	0.00	0.00	enoyl-ACP reductase	KDM46715.1*	495.00	156.00	0.32	enoyl-[acyl-carrier-protein] reductase [NADH]

KEO92246.1*	756.79	111.72	0.15	glucose-1-phosphate thymidylyltransferase	KDM46209.1*	58.80	28.30	0.48	glucose-1-phosphate thymidylyltransferase 2
KEO92477.1*	572.94	0.00	0.00	molybdopterin biosynthesis protein B	KDM54974.1*	232.00	47.90	0.21	molybdenum cofactor biosynthesis protein B
KEO92492.1*	852.18	125.80	0.15	oxidoreductase	KDM46695.1*	312.00	80.60	0.26	hypothetical protein
KEO92638.1*	479.48	0.00	0.00	cyclopropane-fatty-acyl-phospholipid synthase	KDM52012.1*	148.00	0.00	0.00	cyclopropane-fatty-acyl-phospholipid synthase
KEO92662.1*	1438.43	212.34	0.15	50S ribosomal protein L17	KDM53831.1*	354.00	128.00	0.36	50S ribosomal protein L17
KEO92699.1*	771.18	113.84	0.15	LysR family transcriptional regulator	KDM50509.1*	37.00	0.00	0.00	glycine cleavage system transcriptional activator
KEO93102.1*	223.37	0.00	0.00	bifunctional N-acetylglucosamine-1-phosphate uridyltransferase/glucosamine-1-phosphate acetyltransferase	KDM52744.1*	372.00	108.00	0.29	glmU protein

KEO93190.1*	831.23	409.01	0.49	single-stranded DNA-binding protein	KDM56126.1*	679.00	140.00	0.21	single-stranded DNA-binding protein
KEO93395.1*	438.05	64.66	0.15	cytosol aminopeptidase	KDM51408.1*	158.00	47.70	0.30	peptidase B
KEO96463.1*	111.07	32.79	0.30	glutamate-ammonia-ligase adenylyltransferase	KDM54608.1*	214.00	64.50	0.30	glutamate-ammonia-ligase adenylyltransferase
KEO96716.1*	396.13	116.95	0.30	GntR family transcriptional regulator	KDM52778.1*	68.20	0.00	0.00	hypothetical protein
KEO96769.1*	581.14	171.57	0.30	ribonucleotide-diphosphate reductase subunit beta	KDM46350.1*	481.00	130.00	0.27	ribonucleoside-diphosphate reductase 1 subunit beta
KEO98584.1*	1114.39	0.00	0.00	transcriptional regulator HU subunit alpha	KDM52452.1*	2050.00	630.00	0.31	DNA-binding protein HU-alpha
KEO98612.1*	1352.13	0.00	0.00	50S ribosomal protein L31	KDM53254.1*	159.00	0.00	0.00	50S ribosomal protein L31
KEO98687.1*	228.14	84.19	0.37	phosphoenolpyruvate carboxylase	KDM53245.1*	621.00	236.00	0.38	phosphoenolpyruvate carboxylase
KEO98733.1*	470.03	173.46	0.37	DNA topoisomerase I	KDM46698.1*	569.00	255.00	0.45	DNA topoisomerase 1

KEO98800.1*	603.63	0.00	0.00	glyceraldehyde-3-phosphate dehydrogenase	KDM51892.1*	2900.00	1360.00	0.47	glyceraldehyde-3-phosphate dehydrogenase
KEO98996.1*	96.21	0.00	0.00	methionine--tRNA ligase	KDM46274.1*	576.00	115.00	0.20	methionyl-tRNA synthetase
KEO99011.1*	473.88	139.91	0.30	carbonate dehydratase	KDM52008.1*	26.70	0.00	0.00	hypothetical protein
KEO99037.1*	378.39	0.00	0.00	inorganic polyphosphate kinase	KDM51166.1*	96.60	0.00	0.00	hypothetical protein
KEO99041.1*	4328.46	2008.15	0.46	heat-shock protein	KDM52775.1*	41.30	0.00	0.00	small heat shock protein ibpA
KEO99260.1*	277.08	81.80	0.30	aminotransferase	KDM51415.1*	1540.00	637.00	0.41	cysteine desulfurase

*P-value < 0.05

Table 3. Orthologous genes upregulated in *E. litoralis* enlarged spheroplasts and downregulated in *L. amnigena* enlarged spheroplasts.

Protein ID of *E. litoralis*	RPKM in *E. litoralis* spheroplasts at the beginning of growth	RPKM in *E. litoralis* enlarged spheroplasts	Ratio of RPKMs of *E. litoralis*	Annotated function	Protein ID of *L. amnigena*	RPKM in *L. amnigena* spheroplasts at the beginning of growth	RPKM in *L. amnigena* enlarged spheroplasts	Ratio of RPKMs of *L. amnigena*	Annotated function
Homologs of membrane protein genes									
KEO89564.1*	0.00	106.93		hypothetical protein	KDM48649.1*	40.40	14.60	0.36	flagellar M-ring protein
KEO89607.1*	0.00	129.05		multidrug transporter	KDM52010.1*	150.00	45.10	0.30	multidrug resistance protein mdtK
KEO89895.1*	0.00	634.32		biopolymer transporter ExbB	KDM55699.1*	564.00	177.00	0.31	protein tolQ
KEO89973.1*	0.00	184.81		magnesium transporter CorA	KDM52382.1*	661.00	168.00	0.25	magnesium transporter CorA
KEO92632.1*	0.00	146.76		hypothetical protein	KDM46187.1*	175.00	72.30	0.41	inner membrane transporter YeeF
KEO92658.1*	0.00	1301.72		hypothetical protein	KDM57464.1*	136.00	39.40	0.29	UPF0126 inner membrane protein yadS

KEO92812.1*	0.00	172.56		type VI secretion protein	KDM54463.1*	51.90	12.50	0.24	hypothetical protein
KEO93024.1*	262.72	2171.79	8.27	iron transporter	KDM53929.1*	413.00	171.00	0.41	Fe/S biogenesis protein nfuA
KEO93116.1*	0.00	227.97		organic solvent tolerance protein	KDM57361.1*	483.00	224.00	0.46	LPS-assembly protein lptD
KEO93384.1*	352.12	1143.53	3.25	MttB family protein	KDM52402.1*	220.00	15.90	0.07	sec-independent protein translocase tatC
KEO98525.1*	0.00	128.22		hypothetical protein	KDM52772.1*	118.00	38.00	0.32	D-galactonate transporter
KEO98837.1*	0.00	215.39		phosphate ABC transporter permease	KDM52748.1*	724.00	152.00	0.21	phosphate transport system permease pstA
KEO98838.1*	0.00	194.41		phosphate ABC transporter permease	KDM52747.1*	1310.00	230.00	0.18	phosphate transport system permease pstC
KEO98907.1*	243.77	539.78	2.21	3,4-dihydroxy-2-butanone 4-phosphate synthase	KDM54605.1*	1220.00	432.00	0.35	3,4-dihydroxy-2-butanone 4-phosphate synthase
Others									

KEO88945.1*	1061.88	3056.67	2.88	peptide deformylase	KDM53823.1*	699.00	313.00	0.45	peptide deformylase
KEO88946.1*	0.00	300.90		recombinase RecR	KDM55460.1*	336.00	101.00	0.30	recombination protein recR
KEO88958.1*	0.00	546.11		argininosuccinate lyase	KDM53241.1*	1610.00	116.00	0.07	argininosuccinate lyase
KEO88960.1*	484.06	1822.10	3.76	diaminopimelate decarboxylase	KDM50540.1*	780.00	19.50	0.03	diaminopimelate decarboxylase
KEO89297.1*	0.00	160.53		histidinol-phosphate aminotransferase	KDM46192.1*	240.00	92.60	0.39	histidinol-phosphate aminotransferase
KEO89579.1*	0.00	507.45		tRNA (guanine-N7)-methyltransferase	KDM54472.1*	802.00	154.00	0.19	tRNA (guanine-N(7)-)-methyltransferase
KEO89635.1*	0.00	207.20		formyltetrahydrofolate deformylase	KDM46646.1*	80.60	14.60	0.18	formyltetrahydrofolate deformylase
KEO89998.1*	0.00	437.71		ArsR family transcriptional regulator	KDM55050.1*	52.90	0.00	0.00	hypothetical protein
KEO90041.1*	0.00	118.57		hypothetical protein	KDM48627.1*	48.00	0.00	0.00	DNA-cytosine methyltransferase

KEO90698.1*	0.00	438.68		3-deoxy-manno-octulosonate cytidylyltransferase	KDM55141.1*	91.00	0.00	0.00	3-deoxy-manno-octulosonate cytidylyltransferase
KEO91020.1*	0.00	391.37		molecular chaperone DnaK	KDM57452.1*	5740.00	2210.00	0.39	DnaK suppressor protein
KEO91036.1*	0.00	883.47		molecular chaperone Hsp33	KDM53917.1*	77.30	28.00	0.36	chaperonin
KEO91037.1*	0.00	191.92		ornithine carbamoyltransferase	KDM56877.1*	16.90	0.00	0.00	ornithine carbamoyltransferase, catabolic
KEO92302.1*	0.00	465.99		molybdopterin biosynthesis protein MoeB	KDM52444.1*	112.00	16.30	0.15	sulfur carrier protein ThiS adenylyltransferase
KEO92334.1*	113.82	285.62	2.51	aconitate hydratase	KDM46701.1*	305.00	152.00	0.50	aconitate hydratase 1
KEO92372.1*	0.00	272.18		phosphomethylpyrimidine kinase	KDM46258.1*	42.40	15.30	0.36	hydroxymethylpyrimidine/phosphomethylpyrimidine kinase
KEO92402.1*	0.00	190.30		glutamyl-tRNA synthetase	KDM51312.1*	216.00	86.80	0.40	glutamyl-tRNA synthetase
KEO92434.1*	0.00	266.53		2-hydroxyacid dehydrogenase	KDM52411.1*	69.70	25.20	0.36	glyoxylate/hydroxypyruvate reductase B

KEO92503.1*	0.00	462.99	inosine-5-monophosphate dehydrogenase	KDM51383.1*	417.00	117.00	0.28	inosine-5'-monophosphate dehydrogenase
KEO92549.1*	0.00	490.81	30S ribosomal protein S6	KDM56939.1*	944.00	217.00	0.23	30S ribosomal protein S6
KEO92597.1*	0.00	368.72	tryptophan synthase subunit beta	KDM46683.1*	3260.00	134.00	0.04	tryptophan synthase beta chain
KEO92634.1*	0.00	460.61	oxidoreductase	KDM52065.1*	23.50	0.00	0.00	dihydrofolate reductase folM
KEO92667.1*	0.00	278.51	adenylate kinase	KDM55462.1*	1950.00	914.00	0.47	adenylate kinase
KEO92711.1*	0.00	424.08	3-isopropylmalate dehydrogenase	KDM57380.1*	280.00	113.00	0.40	3-isopropylmalate dehydrogenase
KEO92758.1*	0.00	1060.85	DNA-binding protein	KDM51729.1*	7260.00	966.00	0.13	heat shock protein hspQ
KEO92776.1*	0.00	1339.41	ribosome maturation protein RimP	KDM54713.1*	187.00	81.40	0.44	ribosome maturation factor rimP
KEO92780.1*	0.00	511.79	ATPase AAA	KDM46723.1*	52.10	25.10	0.48	psp operon transcriptional activator
KEO92795.1*	0.00	399.20	thioesterase	KDM55422.1*	298.00	61.60	0.21	long-chain acyl-CoA thioesterase tesC

KEO92811.1*	0.00	81.47		polyphosphate kinase	KDM51368.1*	437.00	107.00	0.24	polyphosphate kinase
KEO93025.1*	499.56	1179.89	2.36	malonic semialdehyde reductase	KDM51751.1*	57.50	20.80	0.36	hypothetical protein
KEO93125.1*	0.00	144.29		succinylarginine dihydrolase	KDM51916.1*	89.70	27.80	0.31	N-succinylarginine dihydrolase
KEO93177.1*	0.00	687.39		aromatic amino acid aminotransferase	KDM55151.1*	385.00	124.00	0.32	aspartate aminotransferase
KEO93181.1*	0.00	286.50		riboflavin synthase subunit alpha	KDM52011.1*	317.00	76.60	0.24	riboflavin synthase alpha chain
KEO93285.1*	1214.49	3764.87	3.10	bacterioferritin	KDM53859.1*	463.00	77.30	0.17	bacterioferritin
KEO93379.1*	0.00	350.17		beta-hexosaminidase	KDM51842.1*	248.00	71.90	0.29	beta-hexosaminidase
KEO93387.1*	0.00	283.79		threonine dehydratase	KDM54671.1*	51.50	0.00	0.00	threonine dehydratase catabolic
KEO93441.1*	0.00	293.53		cystathionine beta-lyase	KDM54516.1*	285.00	92.90	0.33	cystathionine beta-lyase
KEO93476.1*	0.00	3077.91		hypothetical protein	KDM54633.1*	68.60	24.80	0.36	hypothetical protein
KEO96380.1*	0.00	130.17		protein-PII uridylyltransferase	KDM57472.1*	108.00	32.10	0.30	[protein-PII] uridylyltransferase

KEO96408.1*	0.00	407.90		GTP cyclohydrolase	KDM46702.1*	287.00	104.00	0.36	GTP cyclohydrolase-2
KEO96434.1*	131.11	377.39	2.88	glutamate synthase	KDM54762.1*	2310.00	320.00	0.14	glutamate synthase [NADPH] large chain
KEO96435.1*	0.00	156.26		glutamate synthase	KDM54763.1*	1780.00	320.00	0.18	glutamate synthase [NADPH] small chain
KEO96528.1*	0.00	211.70		exodeoxyribonuclease VII large subunit	KDM51384.1*	74.20	0.00	0.00	exodeoxyribonuclease 7 large subunit
KEO96545.1*	0.00	814.69		AsnC family transcriptional regulator	KDM51416.1*	2800.00	150.00	0.05	HTH-type transcriptional regulator iscR
KEO98460.1*	0.00	1164.32		50S ribosomal protein L27	KDM54727.1*	1840.00	667.00	0.36	50S ribosomal protein L27
KEO98556.1*	0.00	200.94		citrate lyase	KDM54480.1*	19.40	0.00	0.00	citrate lyase subunit beta
KEO98575.1*	300.92	1066.10	3.54	farnesyltranstransferase	KDM54729.1*	175.00	25.30	0.14	octaprenyl-diphosphate synthase
KEO98712.1*	0.00	412.01		thioredoxin reductase	KDM55115.1*	351.00	88.80	0.25	thioredoxin reductase
KEO98796.1*	0.00	1374.78		5-formyltetrahydrofolate cyclo-ligase	KDM49727.1*	85.40	41.20	0.48	5-formyltetrahydrofolate cyclo-ligase

KEO98834.1*	0.00	260.35	chemotaxis protein CheY	KDM55825.1*	2140.00	142.00	0.07	phosphate regulon transcriptional regulatory protein phoB
KEO98839.1*	0.00	180.36	histidine kinase	KDM55824.1*	380.00	47.40	0.12	phosphate regulon sensor protein phoR
KEO98906.1*	0.00	424.68	6,7-dimethyl-8-ribityllumazine synthase	KDM55386.1*	325.00	157.00	0.48	6,7-dimethyl-8-ribityllumazine synthase
KEO98922.1*	0.00	653.71	50S ribosomal protein L25	KDM46330.1*	477.00	129.00	0.27	50S ribosomal protein L25
KEO98968.1*	0.00	712.85	nucleoside-triphosphate diphosphatase	KDM54467.1*	57.20	0.00	0.00	nucleoside-triphosphatase rdgB
KEO98978.1*	0.00	229.80	DNA repair protein RadA	KDM57315.1*	282.00	106.00	0.38	DNA repair protein radA
KEO99042.1*	0.00	438.68	CTP synthase	KDM50488.1*	871.00	353.00	0.41	CTP synthase

*P-value < 0.05

Table 4. Orthologous genes downregulated in *E. litoralis* enlarged spheroplasts and upregulated in *L. amnigena* enlarged spheroplasts.

Protein ID of *E. litoralis*	RPKM in *E. litoralis* spheroplasts at the beginning of growth	RPKM in *E. litoralis* enlarged spheroplasts	Ratio of RPKMs of *E. litoralis*	Annotated function	Protein ID of *L. amnigena*	RPKM in *L. amnigena* spheroplasts at the beginning of growth	RPKM in *L. amnigena* enlarged spheroplasts	Ratio of RPKMs of *L. amnigena*	Annotated function
Homologs of membrane protein genes									
KEO89304.1*	629.44	247.78	0.39	penicillin-binding protein	KDM57456.1*	296.00	1010.00	3.41	penicillin-binding protein 1B
KEO89573.1*	405.64	59.88	0.15	flagellar biosynthesis protein flip	KDM48639.1*	0.00	16.70		flagellar biosynthetic protein fliP
KEO90985.1*	453.73	133.96	0.30	hypothetical protein	KDM51816.1*	0.00	22.50		flagellar hook-associated protein 1
KEO90987.1*	2625.04	163.16	0.06	flagellar P-ring protein FlgI	KDM51814.1*	15.50	56.00	3.61	flagellar P-ring protein

KEO90994.1*	859.40	0.00	0.00	flagellar basal body rod protein FlgB	KDM51807.1*	0.00	147.00		flagellar basal-body rod protein flgB
KEO92489.1*	458.87	135.47	0.30	ABC transporter	KDM51852.1*	72.60	175.00	2.41	lipoprotein-releasing system ATP-binding protein LolD
KEO92547.1*	320.92	0.00	0.00	ABC transporter	KDM48630.1*	0.00	13.50		inner membrane protein yedI
KEO92700.1*	2204.56	0.00	0.00	magnesium transporter ApaG	KDM57357.1*	89.90	488.00	5.43	protein ApaG
KEO93367.1*	2069.58	111.09	0.05	secretion system protein	KDM53908.1*	0.00	9.92		hypothetical protein
KEO93416.1*	284.06	0.00	0.00	phospho-N-acetylmuramoyl-pentapeptide-transferase	KDM57393.1*	204.00	817.00	4.00	phospho-N-acetylmuramoyl-pentapeptide-transferase
KEO93419.1*	495.89	109.80	0.22	UDP-diphospho-muramoylpentapeptide beta-N-acetylglucosaminyltransferase	KDM57396.1*	79.70	415.00	5.21	undecaprenyldiphospho-muramoylpentapeptide beta-N-acetylglucosaminyltransferase
KEO93424.1*	682.13	134.26	0.20	cell division protein FtsA	KDM57400.1*	865.00	2250.00	2.60	cell division protein ftsA

KEO98787.1*	1321.30	373.84	0.28	preprotein translocase subunit SecA	KDM57404.1*	69.00	559.00	8.10	protein translocase subunit secA
KEO98980.1*	184.72	54.53	0.30	major facilitator transporter	KDM46178.1*	64.50	159.00	2.47	shikimate transporter
Others									
KEO89313.1*	2157.65	0.00	0.00	peptide methionine sulfoxide reductase	KDM56920.1*	0.00	19.10		peptide methionine sulfoxide reductase msrA
KEO89605.1*	7190.86	2640.13	0.37	molecular chaperone GroEL	KDM56992.1*	1220.00	4660.00	3.82	chaperonin
KEO89995.1*	402.88	33.98	0.08	5-methyltetrahydrofolate--homocysteine methyltransferase	KDM55766.1*	111.00	223.00	2.01	methionine synthase
KEO90687.1*	897.43	132.48	0.15	pyridoxamine 5'-phosphate oxidase	KDM52037.1*	0.00	37.40		pyridoxine/pyridoxamine 5'-phosphate oxidase
KEO91004.1*	607.24	0.00	0.00	hypothetical protein	KDM49677.1*	375.00	1380.00	3.68	protein yciF
KEO91044.1*	382.68	0.00	0.00	glucose-methanol-choline oxidoreductase	KDM55555.1*	51.00	185.00	3.63	choline dehydrogenase

KEO91076.1*	644.55	190.30	0.30	phosphomannomutase	KDM46221.1*	99.10	1120.00	11.30	phosphomannomutase
KEO92378.1*	1931.61	855.42	0.44	RNA polymerase sigma70	KDM51449.1*	2570.00	7420.00	2.89	RNA polymerase sigma-E factor
KEO92529.1*	224.36	66.24	0.30	hypothetical protein	KDM57043.1*	0.00	6.34		hypothetical protein
KEO92652.1*	1365.79	554.44	0.41	succinate--CoA ligase	KDM55690.1*	566.00	1450.00	2.56	succinyl-CoA ligase [ADP-forming] subunit alpha
KEO92654.1*	2162.76	141.89	0.07	dihydrolipoamide succinyltransferase	KDM55688.1*	415.00	1100.00	2.65	hypothetical protein
KEO92656.1*	2572.76	822.87	0.32	dihydrolipoamide dehydrogenase	KDM57422.1*	775.00	3320.00	4.28	dihydrolipoyl dehydrogenase
KEO92681.1*	1049.06	206.48	0.20	50S ribosomal protein L16	KDM53849.1*	537.00	2360.00	4.39	50S ribosomal protein L16
KEO93425.1*	1877.96	607.24	0.32	cell division protein FtsZ	KDM57401.1*	1180.00	5490.00	4.65	cell division protein ftsZ
KEO93475.1*	113.94	0.00	0.00	ATPase AAA	KDM55119.1*	88.50	311.00	3.51	replication-associated recombination protein A

KEO96455.1*	712.48	35.06	0.05	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	KDM54731.1*	162.00	351.00	2.17	UDP-N-acetylglucosamine 1-carboxyvinyltransferase
KEO96456.1*	1034.79	101.84	0.10	UTP--glucose-1-phosphate uridylyltransferase	KDM46650.1*	206.00	678.00	3.29	UTP-glucose-1-phosphate uridylyltransferase
KEO96517.1*	762.48	0.00	0.00	chemotaxis protein CheY	KDM46276.1*	0.00	68.30		hypothetical protein
KEO96548.1*	291.41	86.03	0.30	dihydroorotate dehydrogenase	KDM51709.1*	16.80	72.90	4.34	dihydroorotate dehydrogenase
KEO98611.1*	618.35	0.00	0.00	3-hydroxyacyl-ACP dehydratase	KDM57485.1*	74.50	350.00	4.70	(3R)-hydroxymyristoyl-[acyl-carrier-protein] dehydratase
KEO99053.1*	249.16	73.56	0.30	molybdopterin biosynthesis protein	KDM55029.1*	68.90	149.00	2.16	molybdopterin molybdenumtransferase
KEO99263.1*	1114.39	0.00	0.00	glutamine amidotransferase	KDM54696.1*	327.00	852.00	2.61	protein yhbO

**P*-value < 0.05

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