

12-2012

# Strong Concordance Between Transcriptomic Patterns of Spleen and Peripheral Blood Leukocytes in Response to Avian Pathogenic *Escherichia coli* Infection

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# Strong Concordance Between Transcriptomic Patterns of Spleen and Peripheral Blood Leukocytes in Response to Avian Pathogenic *Escherichia coli* Infection

## Abstract

Avian pathogenic *Escherichia coli* (APEC) causes morbidity in chickens and exhibits zoonotic potential. Understanding host transcriptional responses to infection aids the understanding of protective mechanisms and serves to inform future colibacillosis control strategies. Transcriptomes of spleen and peripheral blood leukocytes (PBLs) of the same individual birds in response to APEC infection were compared to identify common response patterns and connecting pathways. More than 100 genes in three contrasts examining pathology and infection status were significantly differentially expressed in both tissues and similarly regulated. Tissue-specific differences in catalytic activity, however, appear between birds with mild and severe pathology responses. Early expression differences, between birds with severe pathology and uninfected controls, in the mitogen-activated protein kinase pathway in PBLs precede spleen responses in the p53 and cytokine-cytokine receptor pathways. Tissue bialysis is useful in identifying genes and pathways important to the response to APEC, whose role might otherwise be underestimated in importance.

## Keywords

transcriptome, spleen, leukocyte, meta-analysis, APEC, Animal Science, Statistics

## Disciplines

Agriculture | Animal Sciences | Biostatistics | Poultry or Avian Science | Veterinary Microbiology and Immunobiology | Veterinary Preventive Medicine, Epidemiology, and Public Health

## Comments

This article is from *Avian Diseases* 56, no. 4 (2012): 732–736, doi:[10.1637/10261-060512-Reg.1](https://doi.org/10.1637/10261-060512-Reg.1).

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Source: Avian Diseases, 56(4):732-736. 2012.

Published By: American Association of Avian Pathologists

DOI: <http://dx.doi.org/10.1637/10261-060512-Reg.1>

URL: <http://www.bioone.org/doi/full/10.1637/10261-060512-Reg.1>

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## Strong Concordance Between Transcriptomic Patterns of Spleen and Peripheral Blood Leukocytes in Response to Avian Pathogenic *Escherichia coli* Infection

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Received 6 June 2012; Accepted 31 July 2012; Published ahead of print 1 August 2012

**SUMMARY.** Avian pathogenic *Escherichia coli* (APEC) causes morbidity in chickens and exhibits zoonotic potential. Understanding host transcriptional responses to infection aids the understanding of protective mechanisms and serves to inform future colibacillosis control strategies. Transcriptomes of spleen and peripheral blood leukocytes (PBLs) of the same individual birds in response to APEC infection were compared to identify common response patterns and connecting pathways. More than 100 genes in three contrasts examining pathology and infection status were significantly differentially expressed in both tissues and similarly regulated. Tissue-specific differences in catalytic activity, however, appear between birds with mild and severe pathology responses. Early expression differences, between birds with severe pathology and uninfected controls, in the mitogen-activated protein kinase pathway in PBLs precede spleen responses in the p53 and cytokine-cytokine receptor pathways. Tissue bialysis is useful in identifying genes and pathways important to the response to APEC, whose role might otherwise be underestimated in importance.

**RESUMEN.** Marcada concordancia entre los patrones transcriptómicos de bazo y leucocitos de sangre periférica durante la respuesta a la infección por *Escherichia coli* patógena aviar.

La *Escherichia coli* patógena para las aves (APEC) causa morbilidad en pollos y representa un potencial zoonótico. El conocimiento de las respuestas transcripcionales del huésped durante la infección contribuye a la comprensión de los mecanismos de protección y sirve para instrumentar futuras estrategias para el control de la colibacilosis. Se compararon los transcriptomas de bazo y de leucocitos de sangre periférica (PBL) de las mismas aves individuales durante la respuesta a la infección por *E. coli* patógena para las aves para identificar los patrones de respuesta comunes y los mecanismos de conexión. Más de 100 genes en tres contrastes que examinaron la patología y el estado de infección mostraron patrones de expresión significativamente diferentes en ambos tipos tejidos y fueron regulados de la forma similar. Se observaron diferencias específicas de tejido en la actividad catalítica sin embargo, aparecen entre las aves con respuestas de patologías leves y severas. Las diferencias de expresión temprana entre aves con patología severa y los controles no infectados, en la ruta de la proteína quinasa activada por mitógenos en sangre periférica y del bazo preceden a la respuesta esplénica en los mecanismos p53 y de receptores de citoquinas-citoquinas. El análisis dual de tejidos es útil en la identificación de genes y vías importantes para la respuesta a la *E. coli* patógena para las aves, cuyo papel de otro modo podría ser subestimado en importancia.

Key words: transcriptome, spleen, leukocyte, meta-analysis, APEC

Abbreviations: ATP = adenosine triphosphate; APEC = avian pathogenic *Escherichia coli*; DAVID = Database for Annotation, Visualization and Integrated Discovery; DPI = day postinfection; ExPEC = extraintestinal pathogenic *E. coli*; KEGG = Kyoto Encyclopedia of Genes and Genomes; IRG1 = immunoresponsive homolog 1; MAPK = mitogen-activated protein kinase; PBL = peripheral blood leukocyte; USDA = U.S. Department of Agriculture

Avian pathogenic *Escherichia coli* (APEC) is the causative agent of colibacillosis and has zoonotic potential, making it a concern for producers and consumers alike. APEC infection results in extraintestinal infections, causing decreased body weight, condemnations of carcasses, and potential animal death (2). Recent studies show marked structural similarities between APEC and human extraintestinal pathogenic *E. coli* (ExPEC) strains, suggesting that APEC may be a source of virulence genes for human ExPEC (10,11,12). These genomic similarities appear to translate to functional similarities, as certain APEC strains are able to cause disease in a rat model of human neonatal meningitis (26).

Microarray technology has enabled great advances in our understanding of gene function in both microbial pathogens and their hosts. Small tissue-specific or pathway-specific platforms allow for more targeted research, whereas use of large global arrays, such as the chicken 44K Agilent microarray (14), can yield more comprehensive data. Indeed, the larger arrays are more likely to reveal novel genes or pathways because their probes are not restricted to a few elements, often of known function and selected for a particular purpose. Most microarray studies of host response to infection analyze the expression patterns of one specific tissue after pathogen exposure, but few combine information from multiple tissues to understand complex interactions that occur to fight infection. Meta-analyses combine the information gathered from multiple studies addressing similar questions, but often the data

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Table 1. Number of significantly differentially expressed genes in each contrast of interest for each tissue.

Contrast of interest	Spleen	PBL
Severe pathology <i>vs.</i> mild pathology at 5 DPI	799	1,914
Severe pathology <i>vs.</i> control at 1 DPI	1,101	1,097
Severe pathology <i>vs.</i> control at 5 DPI	1,723	506

from such studies originate from different individuals or use mRNA that was collected under different experimental conditions (3,5,7,25). In the current study, transcriptome patterns in spleen and peripheral blood leukocytes (PBLs) from the same individual chickens were compared in a bialysis to better understand the host response to APEC. Understanding of concurrent function and interaction of tissues can provide insight into host response mechanisms and elucidate the best methods to combat disease. Analysis of tissues from the same individuals is expected to yield a more refined assessment of gene action.

## MATERIALS AND METHODS

Previously published microarray experiments examined two separate tissues: spleen (21) and PBL (22); microarray data are available in Gene Expression Omnibus, series 25511 and series 31387, respectively. Tissues were collected from the same pathogen-challenge trials, using the same individual chickens. Briefly, commercial male broiler chickens were vaccinated or nonvaccinated at 2 wk of age, then challenged or nonchallenged at 4 wk of age with APEC O1 delivered via intra-air sac injection. Tissues were then harvested at 1 and 5 days postinfection (DPI). Within the nonvaccinated, challenged group, pathology status was assigned to individual chickens by internal lesion scores. Three contrasts were selected for meta-analysis based on large numbers of significantly differentially expressed genes in both tissues: 1) severe pathology *vs.* mild pathology at 5 DPI, 2) severe pathology *vs.* control at 1 DPI, and 3) severe pathology *vs.* control at 5 DPI (Table 1). Significance for the number of significantly expressed genes in both tissues was established through a chi-square test based on  $2 \times 2$  contingency table. Pathway analysis was conducted using the Database for Annotation, Visualization and Integrated Discovery (DAVID) (8,9).

## RESULTS

**Transcriptome meta-analysis.** Ten genes differentially expressed in the spleen microarray and 15 genes differentially expressed in the PBL microarray were previously validated by quantitative PCR (21,22). In each of the three contrasts examined, a large number of genes were significantly differentially expressed in both tissues: 168 genes in severe pathology *vs.* mild pathology at 5 DPI, 163 genes severe pathology *vs.* control at 1 DPI, and 114 genes severe pathology *vs.* control at 5 DPI (Fig. 1). In each instance, the number of genes observed was significantly higher than the number expected

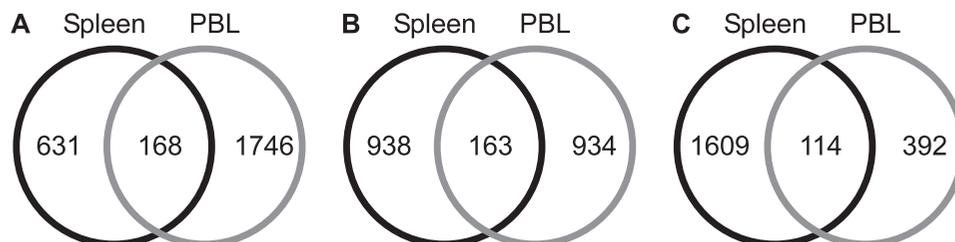


Fig. 1. The number of genes significantly expressed in spleen or PBLs, or both, in the contrasts of (A) severe pathology *vs.* mild pathology at 5 DPI, (B) severe pathology *vs.* control at 1 DPI, and (C) severe pathology *vs.* control at 5 DPI.

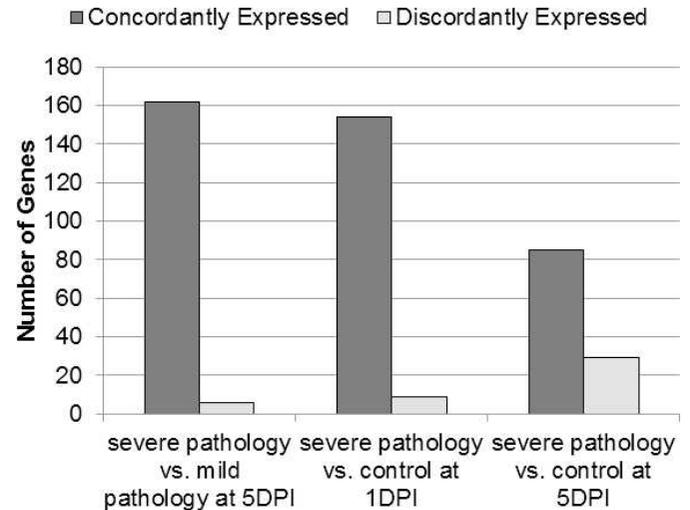


Fig. 2. The number of concordantly and discordantly significantly differentially expressed genes in each contrast across both tissues. Many more significant genes were concordantly expressed in both tissues.

( $P < 0.0001$ ). There was a high level (>90%) of concordance, defined as agreement in direction, of expression patterns for genes significantly differentially expressed in both tissues (Fig. 2). Amongst these gene sets, severe pathology was associated with more induction (>70%) than repression of gene expression (Fig. 3).

A large number of genes were significantly differentially expressed across time point and tissue in the severe pathology *vs.* control contrast (Fig. 4). In this contrast, more differentially expressed genes (174 genes) were found in common between the PBLs at 1 DPI and spleen at 5 DPI, than between the PBLs at 5 DPI and spleen at 1 DPI (66 genes).

**DAVID.** Molecular function and biological processes of genes significant to both tissues were examined using DAVID (Tables 2–4). Analysis of significantly differentially expressed genes in the severe pathology *vs.* mild pathology contrast revealed high amounts of gene function in catalytic, lyase, and transporter activity (Table 2). These activities worked primarily within metabolic and biosynthetic processes (Table 3). Greater than 90% of genes with catalytic activity had increased expression in the birds with severe pathology compared to those with mild pathology. The majority of biological processes associated with severe pathology *vs.* control at 1 DPI were for response to infection (Table 4), but no molecular functions were found for this contrast. Only one biological process term and one molecular function term were significantly enriched among significantly differentially expressed genes between severe pathology and control at 5 DPI: “generation of precursor metabolites and energy” was enriched with three genes and “SH3 domain binding” was enriched with two genes.

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways within each tissue were examined for the contrasts of severe

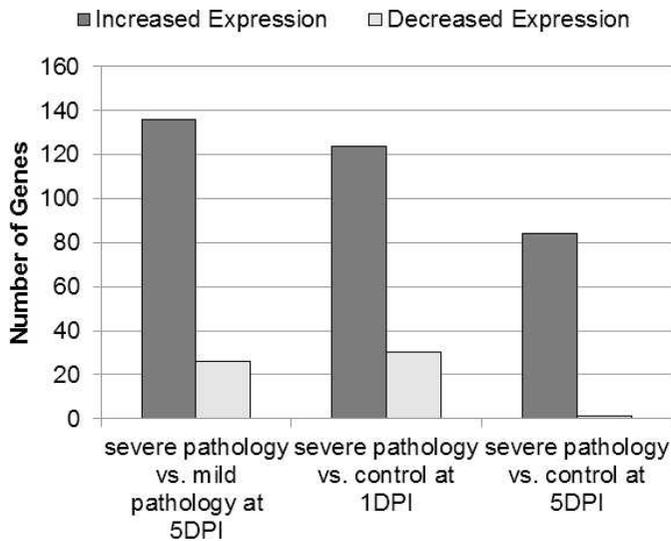


Fig. 3. The direction of expression in concordantly expressed genes. Gene expression was primarily increased among concordantly expressed genes.

pathology compared to control at both 1 and 5 DPI. Results showed pathway networks connecting these two tissues over time. The mitogen-activated protein kinase (MAPK) pathway in PBLs at 1 DPI experienced a 12-element enrichment transitioning to downstream pathways in the spleen at 5 DPI; the p53 and cytokine–cytokine receptor pathways experienced six- and 14-element enrichments, respectively.

## DISCUSSION

Strong concordance between spleen and PBLs in expression patterns of genes differentially expressed in both tissues in response to APEC infection was identified. Although genes significantly differentially expressed in both tissues were in high concordance, only 6%–22% of these genes from each individual tissue analysis were also significantly differentially expressed in the other tissue. This is surprisingly low, because spleen and PBLs often function together, with the spleen serving as site of leukocyte storage and antigen exposure and processing (19). A comparison of basal expression patterns of eight different chicken tissues found that nearly half (49%) of the genes assessed were expressed in all tissues whereas few (14%) were tissue-specific (18). The low percentage of significantly differentially expressed genes across both tissues in the current study is likely due to tissue-specific responses to APEC, rather than reflecting constitutive expression. Tissue-specific expression in

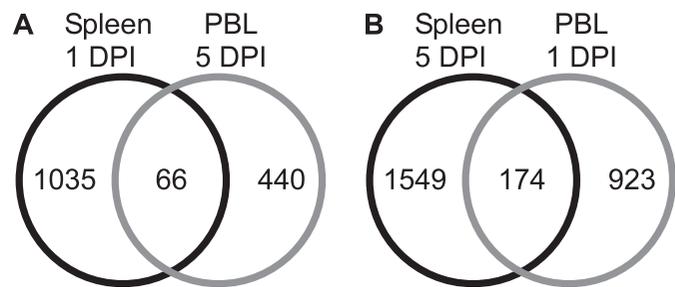


Fig. 4. The number of genes significantly expressed between severe pathology and control at different DPI in spleen or PBLs, or both. (A) Spleen at 1 DPI compared with PBLs at 5 DPI, (B) spleen at 5 DPI compared with PBLs at 1 DPI.

response to stimulus demonstrates the unique role of each tissue as an individual response site contributing to the whole organism's response, and the value of both individual and combined study of multiple tissues in disease studies.

Comparison of tissues across time points revealed that PBLs at 1 DPI shared more significant genes with the spleen at 5 DPI than the PBLs at 5 DPI shared with the spleen at 1 DPI. This difference may demonstrate that tissues respond at different times to infection, or may reflect differences in the flow of cascading signals over time and between tissues. Time-course experiments of other bacterial infections have shown increasing rates of colonization over the first 48 hr after infection (16). Many reports of spleen colonization of APEC are measured 48 hr or more after infection (4,6,17), whereas APEC has been shown to gain access to the bloodstream within hours after intra-air sac inoculation (20,24). At least one study demonstrated early splenic colonization at 6 hr after infection, but data comparing bacterial counts in the spleen to those found in blood were not reported (20).

KEGG pathway networks were discovered across time points and tissues in the current study. Enrichment of the MAPK pathway within the PBLs at 1 DPI is hypothesized to cause the downstream changes in cytokine–cytokine receptor and p53 signaling seen in the spleen at 5 DPI. MAPK and p53 pathways are associated with apoptosis, tumor suppression, and viral infection (15,29). The increase in apoptotic activity in the birds with severe pathology may contribute to the observed pathology.

In both tissues, pathology differences are associated with differences in expression of genes involved in transport and reaction rate. mRNA expressed from catalytic activity genes were elevated in the severe compared to the mild pathology group in both tissues. Increases in catalytic activity have previously been associated with greater pathology. Mouse mutants with an inactivated catalytic gene show reduced incidence and severity of lipopolysaccharide-induced arthritis

Table 2. Enriched molecular function terms between severe pathology and mild pathology at 5 DPI in both tissues ( $P < 0.05$ ).

Term	Count	Genes
Hydro-lyase activity, GO:0016836	3	IRG1, ENO2, ACO1
Inorganic cation transmembrane transporter activity, GO:0022890	4	ATP6V0D2, ATP6V1E1, SLC31A2, TCIRG1
Hydrogen ion transmembrane transporter activity, GO:0015078	3	ATP6V0D2, ATP6V1E1, TCIRG1
Hydrolase activity, acting on glycosyl bonds, GO:0016798	3	BST1, GUSB, CHIA
Iron ion binding, GO:0005506	4	CYP8B, HBG1, ACO1, HEBP1
Catalytic activity, GO:0003824	21	BST1, PNAT3, ABHD12, CAMK2D, DALRD3, GALM, ENO2, DIO2, CYP8B, CHIA, TCIRG1, ACO1, USP18, RNASET2, IRG1, ATP6V1E1, GUSB, NUS1, AKR7A2, GSTA3, GCH1

Table 3. Enriched biological process terms between severe pathology and mild pathology at 5 DPI in both tissues ( $P < 0.05$ ).

Term	Count	Genes
Generation of precursor metabolites and energy, GO:0006091	5	ATP6V0D2, ATP6V1E1, ENO2, TCIRG1, ACO1
Purine ribonucleoside triphosphate metabolic process, GO:0009205	4	ATP6V0D2, ATP6V1E1, GCH1, TCIRG1
ATP <sup>A</sup> synthesis-coupled proton transport, GO:0015986	3	ATP6V0D2, ATP6V1E1, TCIRG1
Purine nucleotide metabolic process, GO:0006163	4	ATP6V0D2, ATP6V1E1, GCH1, TCIRG1
Catabolic process, GO:0009056	6	IRG1, ENO2, GCH1, CHIA, ACO1, USP18
Oxidative phosphorylation, GO:0006119	3	ATP6V0D2, ATP6V1E1, TCIRG1
Alcohol metabolic process, GO:0006066	4	VLDLR, ENO2, GALM, GCH1
Metabolic process, GO:0008152	21	BST1, PNAT3, ATP6V0D2, CAMK2D, DALRD3, GALM, ENO2, DIO2, CHIA, FOXP1, TCIRG1, ACO1, USP18, IRG1, VLDLR, ATP6V1E1, GUSB, AKR7A2, NFIL3, GSTA3, GCH1
Amine metabolic process, GO:0009308	4	DALRD3, DIO2, GCH1, CHIA
ATP biosynthetic process, GO:0006754	3	ATP6V0D2, ATP6V1E1, TCIRG1
Nucleoside phosphate metabolic process, GO:0006753	4	ATP6V0D2, ATP6V1E1, GCH1, TCIRG1
ATP metabolic process, GO:0046034	3	ATP6V0D2, ATP6V1E1, TCIRG1
Nucleobase, nucleoside and nucleotide metabolic process, GO:0055086	4	ATP6V0D2, ATP6V1E1, GCH1, TCIRG1
Purine ribonucleoside triphosphate biosynthetic process, GO:0009206	3	ATP6V0D2, ATP6V1E1, TCIRG1
Heterocycle metabolic process, GO:0046483	4	ATP6V0D2, ATP6V1E1, GCH1, TCIRG1
Nucleoside triphosphate biosynthetic process, GO:0009142	3	ATP6V0D2, ATP6V1E1, TCIRG1

<sup>A</sup>ATP = adenosine triphosphate.

(30). Few of these catalytic genes have been well studied, especially in response to infection, but one gene, immunoresponsive 1 homolog (IRG1), was highly expressed in the severe pathology groups for both tissues in the current study and has recently been associated with response to viral infection. Expression differences between chickens that are resistant and susceptible to Marek's disease were observed in IRG1 after infection with Marek's virus, with greater expression in the susceptible line (23). Genotypes of single nucleotide polymorphisms found within the promoter region of IRG1 were associated with susceptibility and viral counts (23). In the current study, transporter gene levels were elevated in the severe pathology group, including a copper transporter: solute carrier family 31, member 2. Analysis of APEC O1 survival in chicken serum revealed that copper uptake by APEC is necessary for survival and that copper may be of low quantity in serum (13). Thus, individuals showing severe pathology may be in competition with APEC for limited copper reserves in the host.

Both tissues had increases in expression of antimicrobial genes in the severe pathology group compared to the control at 1 DPI. Several avian beta-defensins were significantly increased in both tissues after APEC infection. The beta-defensins are a well-documented antimicrobial gene family with protective responses against bacterial infection and their significance was previously reported in the individual-tissue studies (21,22). Two other antimicrobial genes, cathelicidin-2 and cathelicidin-3, were significantly increased in both tissues after APEC infection. Cathelicidin-2 has bacterial killing ability (27) and is released by

heterophils after exposure to lipopolysaccharides (28). Consistent with the current study's findings on spleen and PBL response to APEC, cathelicidin expression also increases after infection with *Salmonella* in cecal tonsils (1).

A major challenge in large-scale microarray studies is interpreting the meaning of hundreds to thousands of significant gene expression signals. Combining information from multiple studies or tissues allows filtering of the data set in a biologically relevant manner and also reveals gene sets that may have been previously unidentified. The current analysis utilizes samples from not only the same experiment, but from the same individual birds, giving a strong connection between tissues to allow for examination of transcriptional patterns at the whole organism level. The results of this study provide a unique understanding of the roles and relationship of spleen and PBLs in host response to APEC infection.

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Table 4. Enriched biological process terms between severe pathology and control at 1 DPI in both tissues ( $P < 0.05$ ).

Term	Count	Genes
Defense response to bacterium, GO:0042742	8	GAL7, GAL6, GAL5, CATHL3, GAL4, LYG2, CATHL2, GAL2
Defense response, GO:0006952	9	GAL7, GAL6, GAL5, CATHL3, GAL4, LYG2, CATHL2, TLR15, GAL2
Response to other organism, GO:0051707	8	GAL7, GAL6, GAL5, CATHL3, GAL4, LYG2, CATHL2, GAL2
Response to stress, GO:0006950	12	F13A1, LYG2, CATHL2, PTGS2, MMP2, TLR15, GAL7, GAL6, GAL5, GAL4, CATHL3, GAL2
Response to stimulus, GO:0050896	14	F13A1, LYG2, CATHL2, PTGS2, MMP2, TLR15, GAL7, GAL6, GAL5, GAL4, CATHL3, BCL2L1, GAL2, AGRP
Innate immune response, GO:0045087	3	CATHL3, CATHL2, TLR15
Prostaglandin biosynthetic process, GO:0001516	2	HPGDS, PTGS2
Growth, GO:0040007	3	BCL2L1, PTGS2, LEFTY2
Eicosanoid biosynthetic process, GO:0046456	2	HPGDS, PTGS2

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## ACKNOWLEDGMENTS

This work was supported by National Research Initiative Competitive Grant no. 2008-35604-18805 from the U.S. Department of Agriculture (USDA) National Institute of Food and Agriculture Microbial Genome Program. ES partial support provided by USDA National Needs Graduate Fellowship Competitive Grant no. 2007-38420-17767 from the National Institute of Food and Agriculture.