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Growth of *Yersinia pseudotuberculosis* in human plasma: impacts on virulence and metabolic gene expression

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**Abstract**

**Background:** In man, infection by the Gram-negative enteropathogen *Yersinia pseudotuberculosis* is usually limited to the terminal ileum. However, in immunocompromised patients, the microorganism may disseminate from the digestive tract and thus cause a systemic infection with septicemia.

**Results:** To gain insight into the metabolic pathways and virulence factors expressed by the bacterium at the blood stage of pseudotuberculosis, we compared the overall gene transcription patterns (the transcriptome) of bacterial cells cultured in either human plasma or Luria-Bertani medium. The most marked plasma-triggered metabolic consequence in *Y. pseudotuberculosis* was the switch to high glucose consumption, which is reminiscent of the acetogenic pathway (known as “glucose overflow”) in *Escherichia coli*. However, upregulation of the glyoxylate shunt enzymes suggests that (in contrast to *E. coli*) acetate may be further metabolized in *Y. pseudotuberculosis*. Our data also indicate that the bloodstream environment can regulate major virulence genes (positively or negatively); the *yadA* adhesin gene and most of the transcriptional units of the pYV-encoded type III secretion apparatus were found to be upregulated, whereas transcription of the pH6 antigen locus was strongly repressed.

**Conclusion:** Our results suggest that plasma growth of *Y. pseudotuberculosis* is responsible for major transcriptional regulatory events and prompts key metabolic reorientations within the bacterium, which may in turn have an impact on virulence.
Background
The Gram-negative bacterium *Y. pseudotuberculosis* is a human enteropathogen which is able to cross the intestinal mucosa through the M cells in Peyer's patches and thus infect the underlying tissues (causing ileitis and mesenteric lymphadenitis). However, in elderly or debilitated individuals (those suffering from malignancies, immunodeficiencies, chronic liver diseases or diabetes mellitus, for example), the organism frequently gains access to the bloodstream and can cause an often fatal septicaemia [1,2]. Known *Y. pseudotuberculosis* virulence genes are transcriptionally regulated by temperature – most probably in order to adapt to the bacterium's life cycle outside and inside the host. Regulation by the omnipresent thermal stimulus can be modulated (via a wide range of mechanisms) by signals such as pH, other ion concentrations and nutrient availability (reviewed in [3]). This allows bacterial pathogens to (i) adapt their gene transcription profiles in response to environmental cues sensed during the course of infection and (ii) express the most appropriate virulence factors at the expense of useless (or even detrimental) ones.

To date, the transcriptional gene regulation occurring when *Y. pseudotuberculosis* enters the human bloodstream has only been inferred indirectly from *in vivo* results in rodent models of infection [4,5] and *in vitro* gene transcription studies. The *in vitro* regulation of certain *Yersinia* virulence loci has mainly been analyzed with respect to single growth parameter changes mimicking the environmental signals known (or assumed) to be detected by bacteria in blood, such as iron scarcity, oxygen tension and pH [6,7]. In the present work, we have adopted an intermediate approach by comparing the overall gene expression profiles of *Y. pseudotuberculosis* grown in human plasma and in Luria-Bertani broth. We then compared the observed variations with those recently published for *Y. pestis* [8], an almost genetically identical pathogen which, however, causes plague – one of the most severe systemic infections in humans and other mammals.

Results and discussion
The genome of *Y. pseudotuberculosis* strain IP32953 has been recently deciphered: it contains 3,951 coding sequences (CDSs), of which 99 are borne by the virulence-associated plasmid pYV and 43 are carried by a 27-kb cryptic plasmid. Only around 49% of CDSs encode a product with a putative or proven function [9]. To gain insight into the transcriptional regulation of virulence and metabolism genes that takes place when *Y. pseudotuberculosis* enters and multiplies in the bloodstream, we compared the transcriptome of IP32953 grown in human plasma to the one of the same strain grown in Luria-Bertani (LB). To this end, we prepared macroarrays composed of 3,674 PCR fragments of ≈ 400-base pairs (bp), covering 96% of IP32953's CDSs and used them as described elsewhere [8] and in the Methods section. Briefly, in three independent cultures, total RNA was extracted from IP32953 cells grown in LB broth or human plasma, in the exponential or stationary phase and at 28°C or 37°C. Macroarray probing was performed three times with independently retrotranscribed and 33P-labeled RNA samples from each of the eight growth combinations. After macroarray imaging, hybridization intensity data were log-transformed and normalized using a simple median normalization method. Relative data have been deposited in the Genoscript database http://genodb.pasteur.fr/cgi-bin/WebObjects/GenoScript in accordance with standards of the Microarray Gene Expression Data Society (MGED). An analysis of variance (ANOVA) was carried out independently for each gene, with the three biological factors of variation (medium, temperature and growth phase) as fixed effects. This statistical approach allowed us to evaluate the transcriptional variations induced by each factor for the dataset as a whole. Thus, three ratios (corresponding to each parameter) and associated *p*-values were calculated for each gene. Inter-condition transcriptional differences were considered to be statistically significant if the *p*-value was below 0.05. Representative macroarray hybridization results were confirmed by qRT-PCR on stored RNA samples, using the constitutively expressed YPTB0775 gene (spot ID YPO3356 and coding for the outer membrane lipoprotein NplD) as a reference (Additional file 1). Since the physiological status of the bacterium during host infection is unknown, we focused our analysis on genes regulated by the temperature and/or the medium in both the exponential and stationary phases. All *Y. pseudotuberculosis* transcriptional variations discussed herein were compared with those of their respective *Y. pestis* orthologs and are summarized in Table 1. *Y. pseudotuberculosis* IP32953 genes regulated at the transcriptional level by growth temperature and/or medium are listed in Tables 2 and 3.

Free iron limitation is a well-known stimulus encountered by bacteria in plasma [10,11]. As expected, IP32953 genes required for iron storage (such as the ferritin-encoding gene fnA [12] (Fig. 1)) were found to be downregulated in plasma. Transcriptional upregulation of most iron uptake systems (along with accessory protein-encoding genes tonB, exbB and exbD) (Fig. 1) is also consistent with this condition and is in agreement with the recent findings in *Y. pestis* [8]. As iron is used as a cofactor by numerous enzymes (mostly when complexed with sulfur), the metal is essential for a broad range of metabolic processes. Besides activation of iron homeostasis systems, lack of iron is also expected to be associated with a dramatic decrease in the transcription of genes encoding such enzymes, with the underlying goal of lowering iron consumption. This situation is exemplified by the katA gene...
Table 1: Y. pseudotuberculosis transcriptional variations discussed in this article compared with those recently published for Y. pestis [8]

| Locus tags | Gene Name | Putative product/function | Y. pseudotuberculosis | Y. pestis |
|------------|-----------|---------------------------|-----------------------|-----------|
| Iron uptake and storage |
| YPTB1659 YPO1783 | ftnA | ferritin | 0.180 (< 0.001) | 0.341 (0.001) |
| YPTB0336 YPO0279 | hmuY | ABC hemin transporter, ATP-binding subunit HmuV | 1.665 (0.003) | 1.499 (0.021) |
| YPTB0338 YPO0281 | hmuT | ABC transporter, periplasmic hemin-binding protein HmuT | 1.684 (0.016) | 2.149 (0.001) |
| YPTB0339 YPO0282 | hmuS | possible hemin degradation/transport protein HmuS | 1.577 (0.022) | 1.984 (< 0.001) |
| YPTB0340 YPO0283 | hmuR | TonB-dependent outer membrane hemin receptor, HmuR | 7.426 (< 0.001) | 2.028 (< 0.001) |
| YPTB0739 YPO3392 | fhuC | putative ABC type hydroxynaphthate-dependent iron transport ATP binding protein | 1.932 (0.047) | 1.500 (0.258) |
| YPTB0740 YPO3391 | yiuA | putative ABC type periplasmic iron siderophore/cobalamin binding protein | 3.085 (< 0.001) | 1.737 (0.027) |
| YPTB1341 YPO1310 | yiuC | putative siderophore/cobalamin ABC transporter, ATP-binding subunit | 2.468 (< 0.001) | 1.991 (0.012) |
| YPTB1343 YPO1312 | yiuD | putative ABC type hydroxynaphthate-dependent iron uptake ATP binding protein | 2.456 (< 0.001) | 0.921 (0.639) |
| YPTB1512 YPO1496 | yiuE | putative heme-binding protein | 1.877 (0.009) | 1.408 (0.113) |
| YPTB1513 YPO1497 | yiuF | putative ABC transporter ATP-binding protein | 1.521 (0.045) | 1.018 (0.921) |
| YPTB1540 YPO1520 | yiuG | putative ferric iron reductase | 4.019 (< 0.001) | 3.137 (< 0.001) |
| YPTB1541 YPO1521 | yiuH | putative decarboxylase | 2.092 (< 0.001) | 1.246 (0.236) |
| YPTB1543 YPO1523 | yiuI | putative siderophore biosynthetic enzyme | 1.896 (0.021) | 1.213 (0.387) |
| YPTB1544 YPO1524 | yiuJ | putative siderophore biosynthetic enzyme | 2.403 (0.005) | 1.830 (0.006) |
| YPTB1549 YPO1528 | yiuK | putative OMR family iron-siderophore receptor | 2.610 (< 0.001) | 3.273 (0.001) |
| YPTB2117 YPO2193 | yiuL | putative siderophore/cobalamin ABC transporter, ATP-binding subunit | 5.464 (< 0.001) | 2.563 (0.001) |
| YPTB2347 YPO2439 | yiuM | putative siderophore/cobalamin ABC transporter, ATP-binding subunit | 11.88 (< 0.001) | 10.22 (< 0.001) |
| YPTB2348 YPO2440 | yiuN | ABC ferric iron transporter, transport subunit YfeB | 3.141 (< 0.001) | 2.772 (0.001) |
| YPTB2349 YPO2441 | yiuO | ABC ferric iron transporter, transport subunit YfeC | 4.375 (< 0.001) | 1.817 (0.017) |
| YPTB2350 YPO2442 | yiuP | ABC ferric iron transporter, transport subunit YfeD | 2.103 (< 0.001) | 1.369 (0.195) |
| YPTB2351 YPO2445 | yiuQ | putative ABC ferric iron transporter regulator | 1.527 (0.008) | 2.085 (0.001) |
| YPTB2363 YPO0989 | iucA | possible siderophore biosynthetic protein, lucA family | 3.789 (< 0.001) | 2.059 (0.033) |
| YPTB2365 YPO0992 | iucB | possible siderophore biosynthetic protein lucB | 2.600 (< 0.001) | 0.920 (0.711) |
| YPTB2366 YPO0993 | iucC | putative siderophore biosynthetic protein lucC | 2.151 (0.002) | 0.631 (0.036) |
| YPTB2398 YPO1011 | iucD | putative siderophore biosynthetic protein lucD | 2.150 (0.005) | 1.845 (0.018) |
| YPTB3383 YPO0682 | exbB | possible MotA/TolQ/ExbB proton channel family protein | 4.165 (< 0.001) | 3.021 (< 0.001) |
| YPTB3382 YPO0683 | exbD | pExbD/TolR-family transport protein | 9.811 (< 0.001) | 3.576 (< 0.001) |
| YPTB3701 YPO0205 | bfd | putative bacteria-ferritin-associated ferrodoxin | 1.857 (0.002) | 3.195 (< 0.001) |
| YPTB3700 YPO0206 | bfr | bacterioferritin | 1.153 (0.255) | 4.483 (< 0.001) |
| YPTB3767 YPO0133 | feoA | conserved hypothetical protein | 1.510 (0.046) | 1.081 (0.648) |
| YPTB3769 YPO0131 | feoC | conserved hypothetical protein | 1.900 (0.001) | 1.964 (0.002) |
| YPTB3857 YPO4022 | feoD | putative ABC transporter, periplasmic iron siderophore ferrichrome binding protein | 3.127 (< 0.001) | 2.157 (< 0.001) |
| YPTB3858 YPO4023 | feoE | putative ABC iron siderophore transporter, permease subunit | 2.236 (0.001) | 1.214 (0.529) |
| YPTB3860 YPO4025 | feoF | putative ABC iron siderophore transporter, ATP-binding subunit | 2.216 (0.001) | 1.417 (0.041) |
| YPTB1246 YPO1207 | katA | putative catalase | 0.324 (< 0.001) | 0.556 (0.002) |
Table 1: *Y. pseudotuberculosis* transcriptional variations discussed in this article compared with those recently published for *Y. pestis* [8] (Continued)

| Gene       | Log2 Fold Change | P-value          |
|------------|------------------|------------------|
| YPTB0811   | YPO3319          | katY             | 0.603 (0.009) |
| YPTB1181   | YPO1150          | bioA             | 3.377 (< 0.001) |
| YPTB1183   | YPO1152          | bioF             | 3.800 (< 0.001) |
| YPTB1184   | YPO1153          | bioC             | 1.784 (0.004) |
| YPTB1185   | YPO1154          | bioD             | 2.499 (0.002) |
| YPTB3925   | YPO4061          | sodA             | 3.101 (< 0.001) |
| YPTB2299   | YPO2386          | sodB             | 0.090 (< 0.001) |
| YPTB0265   | YPO1757          | manX             | 2.673 (< 0.001) |
| YPTB1633   | YPO1757          | manY             | 1.991 (0.006) |
| YPTB1632   | YPO1756          | manZ             | 3.084 (< 0.001) |
| YPTB2463   | YPO1608          | psbG             | 4.033 (< 0.001) |
| YPTB2971   | YPO2993          | psbH             | 1.697 (0.002) |
| YPTB2971   | YPO2994          | psi             | 1.946 (< 0.001) |
| YPTB0078   | YPO0078          | pfkA             | 1.594 (0.017) |
| YPTB3195   | YPO0920          | fbaA, fba, fda   | 1.325 (0.049) |
| YPTB3196   | YPO0921          | pgk              | 1.365 (0.024) |
| YPTB1133   | YPO1133          | glmA, glm        | 3.179 (< 0.001) |
| YPTB2047   | YPO2064          | pykA             | 0.486 (0.001) |
| YPTB2393   | YPO2393          | pykF             | 2.282 (< 0.001) |
| YPTB3762   | YPO0138          | pck              | 0.395 (0.001) |
| YPTB2103   | YPO2180          | adhE, ana        | 2.116 (0.001) |
| YPTB0460   | YPO3516          | mdh              | 0.665 (0.010) |
| YPTB0796   | YPO3335          | funA, funB       | 0.351 (< 0.001) |
| YPTB0413   | YPO0360          | frdA             | 0.156 (< 0.001) |
| YPTB0412   | YPO0359          | frdB             | 0.127 (< 0.001) |
| YPTB0411   | YPO0358          | frdC             | 0.248 (< 0.001) |
| YPTB0410   | YPO0357          | frdD             | 0.393 (0.001) |
| YPTB1145   | YPO1111          | sdhA             | 0.497 (< 0.001) |
| YPTB1144   | YPO1110          | sdhD             | 0.501 (< 0.001) |
| YPTB1143   | YPO1109          | sdhC             | 0.553 (0.016) |
| YPTB1146   | YPO1112          | sdhD             | 0.592 (0.004) |
| YPTB1149   | YPO1115          | sucC             | 0.419 (< 0.001) |
| YPTB1150   | YPO1116          | sucD             | 0.525 (< 0.001) |
| YPTB1148   | YPO1114          | sucB             | 0.610 (0.014) |

**Biotin operon**

- **YPTB0811**
- **YPTB1181**
- **YPTB1183**
- **YPTB1184**
- **YPTB1185**

**Superoxide dismutases**

- **YPTB3925**
- **YPTB2299**

**Ribonucleotides reductases (RNR)**

- **YPTB0265**
- **YPTB2956**
- **YPTB2957**
- **YPTB2958**
- **YPTB2955**
- **YPTB1254**
- **YPTB1253**
- **YPTB0519**
- **YPTB0518**

**Sugar metabolism**

- **YPTB0074**
- **YPTB3195**
- **YPTB3196**
- **YPTB2047**
- **YPTB2393**
- **YPTB3762**
- **YPTB2103**
- **YPTB0460**
- **YPTB0796**
- **YPTB0413**
- **YPTB0412**
- **YPTB0411**
- **YPTB0410**
- **YPTB1145**
- **YPTB1144**
- **YPTB1143**
- **YPTB1146**
- **YPTB1149**
- **YPTB1150**
- **YPTB1148**
Table 1: *Y. pseudotuberculosis* transcriptional variations discussed in this article compared with those recently published for *Y. pestis* [8] (Continued)

| Gene 1 | Gene 2 | Gene 3 | Description | Fold Change | p-Value |
|--------|--------|--------|-------------|-------------|---------|
| YPTB1147 | YPO1113 | sucA | putative 2-oxoglutarate dehydrogenase E1 component | 0.516 | (0.007) |
| YPTB0716 | YPO3415 | acnB | putative aconitate hydratase 2 | 0.510 | (< 0.001) |
| YPTB3656 | YPO3725 | aceA, acl | isocitrate lyase | 2.068 | (0.003) |
| YPTB3657 | YPO3726 | aceB, mas | malate synthase A | 1.875 | (0.053) |
| YPTB2222 | YPO2300 | fnr, nirR | putative fumarate and nitrate reduction regulatory protein | 0.699 | (0.002) |
| YPTB0601 | YPO458 | arcA | putative probable regulatory protein (OmpR family) | 0.464 | (0.001) |

**Porins**

| Gene 1 | Gene 2 | Description | Fold Change | p-Value |
|--------|--------|-------------|-------------|---------|
| YPTB1964 | YPO1411 | ompC2 | putative outer membrane protein C2, porin | 0.835 | (0.447) |
| YPTB1261 | YPO1222 | ompC | putative outer membrane protein C, porin | 0.285 | (< 0.001) |
| YPTB1453 | YPO1435 | ompA | putative outer membrane porin A protein | 1.085 | (0.372) |

**Chromosomal virulence factors**

| Gene 1 | Gene 2 | Description | Fold Change | p-Value |
|--------|--------|-------------|-------------|---------|
| YPTB1668 | YPO3944 | inv | putative invasin | 0.501 | (< 0.001) |
| YPTB1334 | YPO1303 | psaA | pH 6 antigen precursor | 0.367 | (0.154) |
| YPTB1335 | YPO1304 | psaB | chaperone protein PsaB precursor | 0.216 | (< 0.001) |
| YPTB1332 | YPO1301 | pae | putative regulatory protein | 1.987 | (0.002) |

**pYV-encoded virulence factors – Type Three Secretion System**

| Gene 1 | Gene 2 | Description | Fold Change | p-Value |
|--------|--------|-------------|-------------|---------|
| YPO1368 | YPCD1.36c | yscX | YscX, putative type III secretion protein | 1.629 | (0.009) |
| YPCD1.149 | lcrF | LcrF, VirF; putative thermoregulatory protein | 1.441 | (0.042) |
| YPCD1.32c | lcrG | LcrG, putative Yop regulator | 1.713 | (0.014) |
| YPCD1.30c | lcrH, sycD | LcrH, SycD; low calcium response protein H | 3.522 | (< 0.001) |
| YPCD1.33c | lcrR | LcrR, hypothetical protein | 1.469 | (0.001) |
| YPCD1.31c | lcrV | LcrV, putative V antigen, antihost protein/regulator | 1.904 | (< 0.001) |
| YPCD1.05c | sycE | SycE, yerA; putative yopE chaperone | 5.781 | (< 0.001) |
| YPCD1.95c | sycH | SycH, putative yopH targeting protein | 5.088 | (< 0.001) |
| YPCD1.91 | lcrU | putative resolvase | 0.341 | (< 0.001) |
| YPCD1.45c | virG | VirG; putative Yop targeting lipoprotein | 1.957 | (0.015) |
| YPCD1.29c | yopB | YopB, putative Yop targeting protein | 2.816 | (< 0.001) |
| YPCD1.28c | yopD | YopD, putative Yop negative regulation/targeting component | 2.933 | (< 0.001) |
| YPCD1.26c | yopM | YopM, putative targeted effector protein | 3.467 | (< 0.001) |
| YPCD1.39c | yopN | YopN, LcrE; putative membrane-bound Yop targeting protein | 2.612 | (< 0.001) |
| YPCD1.51 | yscC | YscC, putative type III secretion protein | 2.383 | (0.001) |
| YPCD1.53 | yscD | YscD, putative type III secretion protein | 2.541 | (< 0.001) |
| YPCD1.54 | yscE | YscE, putative type III secretion protein | 2.710 | (0.001) |
| YPCD1.55 | yscF | YscF, putative type III secretion protein | 2.116 | (0.017) |
| YPCD1.56 | yscG | YscG, putative type III secretion protein | 2.462 | (< 0.001) |
| YPCD1.58 | yscI | YscI, LcrO; putative type III secretion protein | 1.864 | (0.003) |
| YPCD1.62 | yscM, lcrQ | YscM, LcrQ, putative type III secretion regulatory | 0.678 | (0.036) |
| YPCD1.40 | yscO | YscO, putative type III secretion protein | 2.855 | (< 0.001) |
| YPCD1.41 | yscP | YscP, putative type III secretion protein | 3.087 | (< 0.001) |
| YPCD1.43 | yscQ | YscQ, putative type III secretion protein | 1.635 | (0.026) |
| YPCD1.44 | yscR | YscR, putative Yop secretion membrane protein | 1.948 | (0.012) |

**pYV-encoded virulence factors – Others**

| Gene 1 | Gene 2 | Description | Fold Change | p-Value |
|--------|--------|-------------|-------------|---------|
| YPCD1.88c | yadA | YadA, Yersinia adhesion | 13.52 | (< 0.001) |
Table 2: *Y. pseudotuberculosis* IP32953 chromosomal genes (sorted by COG class [28]) that are transcriptionally regulated by growth medium and/or temperature.

| COG class | Gene designation | Genoscript spot ID | Gene product/function | Fold ratio in gene transcription (p-value) |
|-----------|------------------|--------------------|-----------------------|-------------------------------------------|
| Human plasma/Luria Bertani Broth | 37°C/28°C |
| C: energy production and conversion |
| YPTB0086 (gplK) | YPO0090 | glycerol kinase | 0.437 (0.002) |
| YPTB0108 (ppc) | YPO3929 | phosphoenolpyruvate carboxylase | 0.678 (0.045) |
| YPTB0118 | YPO3917 | putative pyridine nucleotide-disulphide oxidoreductase | 1.559 (0.016) |
| YPTB0211 (gplC) | YPO3824 | anaerobic glycerol-3-phosphate dehydrogenase subunit C | 0.49 (0.003) |
| YPTB0374 (qor) | YPO0319 | quinone oxidoreductase | 1.393 (0.04) |
| YPTB0410 (frdD) | YPO0357 | fumarate reductase hydrophobic protein | 0.393 (0.001) |
| YPTB0411 (frdC) | YPO0358 | fumarate reductase iron-sulfur protein | 0.248 (< 0.001) |
| YPTB0412 (frdB) | YPO0359 | fumarate reductase flavoprotein subunit | 0.157 (< 0.001) |
| YPTB0460 (mdh) | YPO3516 | malate dehydrogenase | 0.665 (0.01) |
| YPTB0714 (aceF) | YPO3418 | pyruvate dehydrogenase. dihydrolipoytransacetylase component | 0.676 (0.03) |
| YPTB0715 (lpaD) | YPO3417 | dihydrolipoamide dehydrogenase component of pyruvate dehydrogenase complex | 0.657 (0.01) |
| YPTB0716 (acnB) | YPO3415 | aconitate hydratase 2 | 0.51 (< 0.001) |
| YPTB0796 (funA) | YPO3335 | fumarate hydratase. class I | 0.351 (< 0.001) |
| YPTB0887 (nqrA) | YPO3240 | NADH-ubiquinone oxidoreductase subunit A | 0.328 (< 0.001) |
| YPTB0888 (nqrB) | YPO3239 | NADH-ubiquinone oxidoreductase subunit B | 0.58 (0.002) |
| YPTB0889 (nqrC) | YPO3238 | Na+-translocating NADH-quinone oxidoreductase subunit c | 0.551 (0.021) |
| YPTB0892 (nqrF) | YPO3235 | NADH-ubiquinone oxidoreductase subunit F | 0.661 (0.044) |
| YPTB0895 | YPO3232 | putative exported protein | 0.6 (0.008) |
| YPTB0949 (cyoD) | YPO3167 | cytochrome O ubiquinol oxidase subunit CyoD | 0.534 (0.001) |
| YPTB0952 (cyoA) | YPO3164 | cytochrome O ubiquinol oxidase subunit II | 0.537 (< 0.001) |
| YPTB1125 (fdA) | YPO2635 | flavodoxin I | 0.722 (0.033) |
| YPTB1143 (sdhC) | YPO1109 | succinate dehydrogenase cytochrome b-556 subunit | 0.552 (0.015) |
| YPTB1144 (sdhD) | YPO1110 | succinate dehydrogenase hydrophobic membrane anchor protein | 0.5 (< 0.001) |
| YPTB1145 (sdhA) | YPO1111 | succinate dehydrogenase flavoprotein subunit | 0.497 (< 0.001) |
| YPTB1146 (sdhB) | YPO1112 | succinate dehydrogenase iron-sulfur protein | 0.592 (0.003) |
| YPTB1147 (sucA) | YPO1113 | 2-oxoglutarate dehydrogenase E1 component | 0.515 (0.006) |
| YPTB1148 (sucB) | YPO1114 | dihydrolipoamide succinyltransferase component of 2-oxoglutarate dehydro | 0.61 (0.013) |
| YPTB1149 (succ) | YPO1115 | succinyl-CoA synthetase beta chain | 0.419 (< 0.001) |
| YPTB1150 (sucD) | YPO1116 | succinyl-CoA synthetase alpha chain | 0.524 (< 0.001) |
| YPTB1151 (cydA) | YPO1117 | cytochrome D ubiquinol oxidase subunit I | 0.442 (< 0.001) |
| YPTB1152 (cydB) | YPO1118 | cytochrome D ubiquinol oxidase subunit II | 0.565 (0.004) |
| Gene Symbol | Protein Name | Function | Fold Change | p-value |
|------------|--------------|----------|-------------|---------|
| YPTB1408 (pflB) | YPO1383 | formate acetyltransferase I | 0.462 | (< 0.001) |
| YPTB1723 (putA) | YPO1851 | bifunctional PutA protein | 0.643 | (< 0.001) |
| YPTB1945 | YPO1947 | putative thioredoxin | 1.646 | (0.002) |
| YPTB2012 | YPO2028 | putative exported protein | 0.6 | (0.008) |
| YPTB2017 | YPO2035 | hypothetical protein | 1.694 | (0.045) |
| YPTB2089 | YPO2163 | putative nitroreductase | 2.095 | (< 0.001) |
| YPTB2103 (adhE) | YPO2180 | aldehyde-alcohol dehydrogenase | 2.115 | (< 0.001) |
| YPTB2165 | YPO2244 | Fe-S binding NADH dehydrogenase (pseudogene. F/S) | 0.642 | (0.001) |
| YPTB2224 (pntB) | YPO2302 | bifunctional PutA protein | 0.643 | (< 0.001) |
| YPTB2223 (nifH) | YPO2334 | putative pyruvate-flavodoxin oxidoreductase | 1.697 | (0.014) |
| YPTB2247 (icaA) | YPO1641 | isocitrate dehydrogenase [NADP] | 0.632 | (0.013) |
| YPTB2529 | YPO2492 | putative dioxygenase beta subunit | 0.535 | (< 0.001) |
| YPTB2578 (nuoK) | YPO2549 | NADH dehydrogenase I chain H | 0.745 | (0.023) |
| YPTB2581 (nuoD) | YPO2553 | NADH dehydrogenase I chain C/D | 0.573 | (< 0.001) |
| YPTB2587 (nuoA) | YPO2555 | NADH dehydrogenase I chain A | 0.62 | (< 0.001) |
| YPTB2597 (akA) | YPO2566 | acetate kinase | 0.562 | (0.001) |
| YPTB2598 (pta) | YPO2567 | phosphate acetyltransferase | 0.667 | (0.017) |
| YPTB2689 (dmsB) | YPO2966 | putative dimethyl sulfoxide reductase chain B protein | 0.598 | (0.031) |
| YPTB2703 | YPO2980 | putative ion channel protein | 0.565 | (0.01) |
| YPTB2758 (napC) | YPO3036 | cytochrome C-type protein NapC | 0.636 | (0.015) |
| YPTB2861 (fodH) | YPO0589 | Z4-dienoyl-CoA reductase | 0.465 | (0.002) |
| YPTB3656 (aceA) | YPO3725 | isocitrate lyase | 2.069 | (0.002) |
| YPTB3782 (pckA) | YPO0138 | phosphoenolpyruvate carboxykinase [ATP] | 0.395 | (0.001) |
| YPTB3927 (fdoG) | YPO4057 | formate dehydrogenase-O. major subunit | 0.208 | (< 0.001) |
| YPTB3928 (fdoH) | YPO4058 | formate dehydrogenase-O. iron-sulfur subunit | 0.187 | (< 0.001) |
| YPTB3967 (atpD) | YPO4121 | ATP synthase beta subunit protein | 0.679 | (0.025) |
| YPTB3968 (atpG) | YPO4122 | ATP synthase gamma subunit protein | 0.658 | (0.011) |
| YPTB3970 (atpH) | YPO4125 | ATP synthase delta subunit protein | 0.7 | (0.005) |
| YPTB3971 (atpF) | YPO4125 | ATP synthase subunit B protein | 0.616 | (0.002) |
| YPTB3979 (atpB) | YPO3637 | putative carbohydrate kinase | 0.658 | (0.031) |
| YPTB4079 (atpE) | YPO3637 | ATP synthase subunit C protein | 0.656 | (0.037) |

**D: cell division and chromosome partitioning**

| Gene Symbol | Protein Name | Function | Fold Change | p-value |
|------------|--------------|----------|-------------|---------|
| YPTB0222 (flaE) | YPO3813 | cell division ATP-binding protein | 0.595 | (0.019) |
| YPTB1430 (mukB) | YPO1405 | cell division protein | 0.62 | (0.006) |
| YPTB2923 | YPO2686 | putative membrane protein | 0.358 | (< 0.001) |
| YPTB3126 | YPO3195 | possible bacteriophage protein | 1.616 | (0.029) |
| YPTB3976 (gidA) | YPO4130 | glucose inhibited division protein A | 0.607 | (0.007) |

**E: amino acid transport and metabolism**

| Gene Symbol | Protein Name | Function | Fold Change | p-value |
|------------|--------------|----------|-------------|---------|
Table 2: Y. pseudotuberculosis IP32953 chromosomal genes (sorted by COG class [28]) that are transcriptionally regulated by growth medium and/or temperature. (Continued)

| Gene  | Description                                                                 | RMA   | FDR   |
|-------|------------------------------------------------------------------------------|-------|-------|
| YPTB0003 (asnA) | YPO0003 aspartate-ammonia ligase                                              | 1.531 | 0.042 |
| YPTB0024 (glnA) | YPO0024 glutamine synthetase                                                 | 0.49  | < 0.001 |
| YPTB0057 (tdh) | YPO0060 threonine 3-dehydrogenase                                            | 0.663 | 0.041 |
| YPTB0066 (cseE) | YPO0070 serine acetyltransferase                                             | 1.669 | 0.016 |
| YPTB0106 (metL) | YPO0116 bifunctional aspartokinase/homoserine dehydrogenase II              | 1.565 | 0.045 |
| YPTB0111 (argB) | YPO3925 acetylglutamate kinase                                               | 1.495 | 0.016 |
| YPTB0112 (argH) | YPO3924 putative argininosuccinate lyase                                     | 1.779 | 0.007 |
| YPTB0134 (ilvG) | YPO3901 acetolactate synthase isozyme II large subunit                      | 0.627 | 0.039 |
| YPTB0166 (cysE) | YPO0070 serine acetyltransferase                                             | 0.448 | < 0.001 |
| YPTB0184 (pykB) | YPO3825 putative anaerobic glycerol-3-phosphate dehydrogenase subunit B     | 0.554 | 0.01 |
| YPTB0226 (livK) | YPO3808 branched-chain amino acid-binding protein                            | 1.49  | 0.032 |
| YPTB0245       | YPO0170 conserved hypothetical protein                                       | 0.662 | 0.031 |
| YPTB0284 (metE) | YPO3788 5-methyltetrahydropteroyltriglutamate – homocysteine methyltransferase | 2.813 | 0.001 |
| YPTB0315 (cysH) | YPO3370 phosphoadenosine phosphosulfate reductase (pseudogene. F/S)         | 1.593 | 0.002 |
| YPTB0341 (carA) | YPO0481 carbamoyl-phosphate synthase small chain                            | 0.609 | 0.001 |
| YPTB0342 (carB) | YPO0482 carbamoyl-phosphate synthase large chain                            | 0.621 | 0.022 |
| YPTB0376 (ilvH) | YPO0540 acetolactate synthase isozyme III small subunit                     | 1.68  | 0.006 |
| YPTB0402 (aspA) | YPO0348 aspartate ammonia-lyase                                             | 0.224 | 0.001 |
| YPTB0407       | YPO0353 conserved hypothetical protein                                       | 0.687 | 0.03 |
| YPTB0521       | YPO3452 putative ABC transporter transporter. ATP-binding protein            | 1.679 | 0.029 |
| YPTB0524       | YPO3448 (7G) putative extracellular solute-binding protein (pseudogene. F/S) | 1.553 | 0.046 |
| YPTB0557       | YPO0391 possible conserved cysteine desulfurase                             | 0.662 | 0.001 |
| YPTB0604 (arcA) | YPO0459 aerobic respiration control protein                                  | 0.465 | 0.001 |
| YPTB0623 (carA) | YPO0481 carbamoyl-phosphate synthase small chain                            | 1.411 | 0.03 |
| YPTB0624 (carB) | YPO0482 carbamoyl-phosphate synthase large chain                            | 0.621 | 0.005 |
| YPTB0676 (livH) | YPO0540 acetolactate synthase isozyme III small subunit                     | 1.68  | 0.006 |
| YPTB0711 (aroP) | YPO3421 aromatic amino acid transport protein                               | 1.68  | 0.006 |
| YPTB0714 (cysH) | YPO3370 phosphoadenosine phosphosulfate reductase (pseudogene. F/S)         | 1.593 | 0.002 |
| YPTB0789       | YPO3343 probable extracellular solute-binding protein                       | 0.462 | 0.001 |
| YPTB0911 (aroL) | YPO3215 shikimate kinase II                                                 | 0.601 | 0.03 |
| YPTB0920 (brnQ) | YPO3202 branched-chain amino acid transport system II carrier protein       | 1.481 | 0.033 |
| YPTB1108 (glnH) | YPO0365 putative amino acid-binding protein precursorR                       | 0.564 | 0.005 |
| YPTB1186       | YPO1155 putative amino acid transportR                                      | 1.736 | 0.017 |
| YPTB1240       | YPO1200 putative amino acid permease                                         | 0.637 | 0.025 |
| YPTB1241       | YPO1201 putative amino acid decarboxylase                                    | 0.557 | 0.024 |
| YPTB1346       | YPO1315 putative hydroxylase (pseudogene. stop)                             | 1.578 | < 0.001 |
| YPTB1352 (sdcC) | YPO1321 serine transportR                                                    | 0.526 | 0.028 |
| YPTB1362 (potG) | YPO1332 putrescine transport ATP-binding protein                             | 0.728 | 0.035 |
| YPTB1375 (artM) | YPO1349 arginine transport system permease protein                           | 1.648 | 0.014 |
| YPTB1384 (poxB) | YPO1358 pyruvate dehydrogenase [cytochrome]                                 | 1.521 | 0.009 |
| YPTB1411 (ansB) | YPO1386 putative L-asparaginase II precursor                                 | 1.869 | 0.013 |
| YPTB1434 (aspC) | YPO1410 aspartate aminotransferase                                           | 1.447 | 0.004 |
Table 2: Y. pseudotuberculosis IP32953 chromosomal genes (sorted by COG class [28]) that are transcriptionally regulated by growth medium and/or temperature. (Continued)

| Gene   | YP1700 | Function                                      | Log2 Ratio | P-value |
|--------|--------|-----------------------------------------------|------------|---------|
| YPTB1348 pepN | YPO1414 | putative aminopeptidase N                      | 1.432      | 0.041   |
| YPTB1541 yslf | YPO1529 | putative decarboxylase                        | 2.092      | < 0.001 |
| YPTB1621 arnF | YPO1743 | aromatic acid transport protein               | 1.68       | 0.006   |
| YPTB1641 hpaF | YPO1765 | 5-carboxymethyl-2-hydroxymuconate delta-     | 1.812      | 0.015   |
| YPTB1656 oibB | YPO1780 | oligopeptidase B                             | 1.695      | 0.006   |
| YPTB1808 yhiA | YPO2313 | possible diaminopimelate decarboxylase         | 1.621      | 0.001   |
| YPTB2001 prsA | YPO2037 | ribose-phosphate pyrophosphokinase            | 0.55       | < 0.001 |
| YPTB2019 oppA | YPO2037 | conserved hypothetical protein                | 1.56       | 0.021   |
| YPTB2067 oppD | YPO2185 | oligopeptide transport ATP-binding protein    | (< 0.001)  |         |
| YPTB2108 oppD | YPO2185 | oligopeptide transport ATP-binding protein    | (< 0.001)  |         |
| YPTB2120 pepT | YPO1631 | peptidase T                                  | 0.488      | 0.007   |
| YPTB2258 compat | YPO2339 | putative periplasmic murein protein            | 1.812      | 0.015   |
| YPTB2262 tyrR | YPO2344 | transcriptional regulatory protein            | 0.486      | < 0.001 |
| YPTB2295 glyA | YPO2381 | lactoylglutathione lyase                      | 1.666      | 0.001   |
| YPTB2437 pepT | YPO1631 | peptidase T                                  | 0.488      | 0.007   |
| YPTB2458 glnH | YPO2313 | putative glutamine-binding periplasmic protein| 1.797      | 0.014   |
| YPTB2459 glnP | YPO2511 | putative glutamine transport system permease  | 1.704      | 0.024   |
| YPTB2550 glnQ | YPO2512 | putative glutamine transport ATP-binding protein| 1.56      | 0.027   |
| YPTB2632 aroC | YPO2795 | chorismate synthase                          | 0.675      | 0.031   |
| YPTB2698 aroB | YPO2795 | putative aminotransferase                    | 2.151      | 0.001   |
| YPTB2714 cysK | YPO2992 | cysteine synthase A                          | 2.108      | 0.005   |
| YPTB2723 cysA | YPO3002 | putative permease                            | 1.328      | 0.036   |
| YPTB2725 cysK | YPO3004 | putative aminopeptidase (pseudogene. F/S)     | 1.526      | 0.022   |
| YPTB2784 gcvR | YPO3063 | glycine cleavage system transcriptional repressors| 2.302      | < 0.001 |
| YPTB2869 glyA | YPO2907 | serine hydroxymethyltransferase               | 1.783      | 0.001   |
| YPTB2882 pheB | YPO2924 | putative membrane protein                    | 1.737      | 0.006   |
| YPTB2909 oppA | YPO2699 | conserved hypothetical protein               | 1.513      | 0.017   |
| YPTB2942 ureC | YPO2667 | urease alpha subunit                         | 0.333      | < 0.001 |
| YPTB2943 ureB | YPO2666 | urease beta subunit                          | 0.2        | < 0.001 |
| YPTB2944 ureA | YPO2665 | urease gamma subunit                         | 0.324      | < 0.001 |
| YPTB2961 aroX | YPO2645 | glycine betaine-binding periplasmic protein  | 0.637      | 0.009   |
| YPTB2986 oppA | YPO1061 | conserved hypothetical protein               | 0.551      | 0.031   |
| YPTB3006 dapD | YPO1041 | 2,3,4,5-tetrahydroxyproline-2-carboxylate N-  | 0.398      | < 0.001 |
| YPTB3181 gcsH | YPO0906 | glycine cleavage system H protein            | 0.377      | < 0.001 |
| YPTB3182 gcvT | YPO0907 | aminomethyltransferase                       | 0.526      | 0.022   |
| YPTB3189 serA | YPO0914 | D-3-phosphoglycerate dehydrogenase           | 1.582      | 0.04    |
| YPTB3214 pprC | YPO0942 | putative pyrroline-5-carboxylate reductase    | 0.647      | 0.036   |
| YPTB3474 proC | YPO0584 | putative symporter protein                   | 0.61       | 0.007   |
| YPTB3570 proE | YPO3660 | putative class II dehydroquinase             | 0.721      | 0.036   |
| YPTB3658 metA | YPO3727 | homoserine O-succinylation transferase       | 1.776      | 0.018   |
| YPTB3749 metA | YPO0152 | 3-dehydroquinase synthase                    | 0.612      | 0.039   |
| YPTB3813 gdhA | YPO3971 | NADP-specific glutamate dehydrogenase        | 1.671      | 0.005   |
| Gene      | COG Number | Description                                                                 | log2 Fold Change | p-value       |
|-----------|-------------|------------------------------------------------------------------------------|------------------|--------------|
| YPTB3853  | or2495      | pyridoxal-phosphate dependent protein (pseudogene, partial)                  | 1.59             | 0.006        |
| YPTB3957  | YPO4111     | putative periplasmic solute-binding protein                                  | 1.59             | 0.006        |

**F: Nucleotide Transport and Metabolism**

| Gene      | COG Number | Description                                                                 | log2 Fold Change | p-value       |
|-----------|-------------|------------------------------------------------------------------------------|------------------|--------------|
| YPTB0250  | YPO3786     | uridine phosphorylase                                                        | 0.488            | 0.005        |
| YPTB0519  | YPO3454     | anaerobic ribonucleoside-triphosphate reductase                             | 0.614            | 0.024        |
| YPTB0584  | YPO0440     | purine nucleoside phosphorylase                                              | 0.607            | 0.025        |
| YPTB0623  | YPO0481     | carbamoyl-phosphate synthase small chain                                    | 0.609 (< 0.001)  | 0.747 (0.028) |
| YPTB0624  | YPO0482     | carbamoyl-phosphate synthase large chain                                    | 0.621 (< 0.001)  | 0.546 (0.005) |
| YPTB0754  | YPO3377     | CTP synthase                                                                | 0.469 (< 0.001)  | 0.546 (0.005) |
| YPTB0901  | YPO3225     | xanthine-guanine phosphorylsoytransferase                                   | 0.68 (0.035)     | 0.328 (0.001) |
| YPTB0991  | YPO3123     | adenine phosphorylsoytransferase                                            | 1.447 (0.042)    | 0.489 (0.003) |
| YPTB1253  | YPO1213     | ribonucleoside-diphosphate reductase 1 beta chain                            | 0.489 (< 0.001)  | 0.707 (0.014) |
| YPTB1254  | YPO1214     | ribonucleoside-diphosphate reductase 1 alpha chain                          | 1.331 (0.037)    |               |
| YPTB1439  | YPO1415     | dihydroorotate dehydrogenase                                                 | 0.55 (< 0.001)   | 0.598 (0.009) |
| YPTB2001  | YPO2013     | ribose-phosphate pyrophosphokinase                                           | 0.75 (0.034)     | 0.651 (0.002) |
| YPTB2102  | YPO2176     | thymidine kinase                                                             | 1.674 (0.018)    | 0.787 (0.014) |
| YPTB2706  | YPO2983     | nucleoside permease                                                          | 0.598 (0.009)    | 0.546 (0.001) |
| YPTB2781  | YPO3059     | phosphoribosylaminomimidazole-succinocarboxamide synthase (pseudogene, IS)  | 0.515 (0.002)    | 1.842 (0.038) |
| YPTB2794  | YPO2827     | uracil phosphoribosyltransferase                                             | 2.842 (< 0.001)  | 0.564 (< 0.001) |
| YPTB2796  | YPO2829     | putative phosphoribosylglycinamide formyltransferase                          | 2.842 (< 0.001)  | 0.564 (< 0.001) |
| YPTB2803  | YPO2837     | putative exopolysphatase                                                     | 2.842 (< 0.001)  | 0.564 (< 0.001) |
| YPTB2956  | YPO2650     | NrdI protein homologue                                                        | 2.842 (< 0.001)  | 0.564 (< 0.001) |
| YPTB2957  | YPO2649     | ribonucleoside-diphosphate reductase 2 alpha chain                           | 2.842 (< 0.001)  | 0.564 (< 0.001) |
| YPTB2958  | YPO2648     | ribonucleoside-diphosphate reductase 2 beta chain                            | 2.842 (< 0.001)  | 0.564 (< 0.001) |
| YPTB3544  | YPO3689     | putative ribonuclease                                                         | 1.543 (0.022)    |               |
| YPTB3854  | YPO4019     | putative phosphoribosyl transferase protein                                  | 1.543 (0.022)    |               |

**G: Carbohydrate Transport and Metabolism**

| Gene      | COG Number | Description                                                                 | log2 Fold Change | p-value       |
|-----------|-------------|------------------------------------------------------------------------------|------------------|--------------|
| YPTB0074  | YPO0078     | 6-phosphofructokinase                                                        | 1.593 (0.017)    | 0.565 (0.023) |
| YPTB0087  | YPO0091     | glycerol uptake facilitator protein                                          | 1.593 (0.017)    | 0.565 (0.023) |
| YPTB2041  | YPO3793     | sn-glycerol-3-phosphate transport. ATP-binding protein                       | 1.725 (0.001)    | 0.565 (0.023) |
| YPTB0542  | YPO4002     | PTS system. IIB component                                                    | 1.968 (0.021)    |               |
| YPTB0548  | YPO4008     | putative aldolase                                                            | 1.968 (0.021)    |               |
| YPTB0550  | YPO4101     | putative ABC transporter permease protein                                     | 0.488 (0.004)    | 0.565 (0.023) |
| YPTB0569  | YPO4240     | putative pectinesterase                                                       | 0.268 (< 0.001)  | 0.565 (0.023) |
| YPTB0583  | YPO4391     | phosphopentomutase                                                           | 1.651 (0.03)     | 0.565 (0.023) |
| YPTB0729  | YPO3350     | putative dihydroxyacetone kinase                                             | 0.683 (0.028)    | 0.565 (0.023) |
| YPTB0799  | YPO3332     | putative sugar ABC transporter. permease protein                              | 1.521 (0.007)    | 1.521 (0.007) |
| YPTB0803  | YPO3227     | putative deoR-family regulatory protein                                      | 2.842 (< 0.001)  | 1.521 (0.007) |
| YPTB0804  | YPO3326     | L-ribulose-5-phosphate 4-epimerase                                           | 2.842 (< 0.001)  | 1.521 (0.007) |
| YPTB0874 | or0625 | probable sugar aldolase | 1.455 (0.042) | 1.627 (0.011) |
|----------|--------|-------------------------|---------------|---------------|
| YPTB1079 | YPO2586 | conserved hypothetical protein | 0.559 (0.025) |               |
| YPTB1080 | YPO2587 | conserved hypothetical protein | 0.644 (0.026) |               |
| YPTB1119 (nagB) | YPO2627 | putative glucosamine-6-phosphate isomerase | 0.654 (0.027) |               |
| YPTB1140 | YPO1106 | conserved hypothetical protein | 0.705 (0.034) |               |
| YPTB1166 (gpmA) | YPO1133 | phosphoglycerate mutase I | 3.178 (< 0.001) |               |
| YPTB1290 (bgIA) | YPO1254 | 6-phospho-beta-glucosidase | 1.802 (0.008) |               |
| YPTB1327 | YPO1295 | putative ABC transport integral membrane subunit | 1.976 (0.009) |               |
| YPTB1381 | YPO1355 | conserved hypothetical protein | 0.683 (0.031) |               |
| YPTB1522 (mglB) | YPO1507 | galactose-binding protein | 0.56 (0.002) | 1.47 (0.029) |
| YPTB1581 | YPO1572 | putative sugar transporter | 0.587 (0.023) |               |
| YPTB1600 (pybX) | YPO1915 | putative signal transducer | 1.766 (0.003) |               |
| YPTB1632 (manZ) | YPO1756 | PTS system. mannose-specific IID component | 3.084 (< 0.001) |               |
| YPTB1633 (manY) | YPO1757 | PTS system. mannose-specific IIC component | 1.991 (0.005) |               |
| YPTB1634 (manX) | YPO1758 | PTS system. mannose-specific IIA component | 2.674 (< 0.001) |               |
| YPTB1687 | YPO1814 | putative sugar ABC transporter. ATP-binding protein | 1.865 (0.016) |               |
| YPTB1930 | YPO1932 | putative sugar transporter | 1.963 (0.002) |               |
| YPTB1975 | YPO1982 | putative dehydrogenase | 0.63 (0.012) |               |
| YPTB2047 (pykA) | YPO2064 | pyruvate kinase II | 0.486 (< 0.001) |               |
| YPTB2082 | YPO2156 | conserved hypothetical protein | 0.538 (0.002) | 1.487 (0.03) |
| YPTB2083 (gapA) | YPO2157 | glyceraldehyde 3-phosphate dehydrogenase A | 0.712 (0.027) |               |
| YPTB2147 | YPO2225 | conserved hypothetical protein | 1.297 (0.041) |               |
| YPTB2190 (mlc) | YPO2268 | putative ROK family transcriptional regulatory protein | 1.417 (0.037) |               |
| YPTB2205 | or3894 | ABC sugar/ribose transporter. permease subunit | 1.61 (0.036) |               |
| YPTB2206 (pykF) | YPO2293 | pyruvate kinase I | 2.282 (< 0.001) |               |
| YPTB2318 (ptsA) | YPO2409 | phosphoenolpyruvate synthase | 0.463 (0.008) |               |
| YPTB2356 (kdul) | YPO1725 | 4-deoxy-L-threo-5-hexosulose-urionate ketol-isomerase | 1.444 (0.047) |               |
| YPTB2360 | YPO1721 | putative sugar ABC transporter (permease) | 1.901 (0.032) |               |
| YPTB2463 (ptsG) | YPO1608 | PTS system. glucose-specific IIBC component | 4.033 (< 0.001) | 1.607 (0.002) |
| YPTB2515 | YPO2474 | conserved hypothetical protein | 0.593 (0.036) |               |
| YPTB2518 | YPO2477 | putative solute-binding protein | 0.62 (0.029) |               |
| YPTB2535 (rbsC) | YPO2499 | putative sugar transport system. permease protein | 1.7 (0.013) |               |
| YPTB2715 (ptsH) | YPO2993 | PTS system. phosphocarrier protein | 1.697 (0.001) |               |
| YPTB2716 (ptsI) | YPO2994 | PTS system. enzyme I component | 1.945 (< 0.001) |               |
| YPTB2717 (cri) | YPO2995 | PTS system. glucose-specific IIA component | 1.611 (0.002) | 0.731 (0.028) |
| YPTB2962 | YPO2444 | conserved hypothetical protein (pseudogene. IS100) | 2.68 (< 0.001) |               |
| YPTB3078 | YPO0834 | putative PTS transport protein | 1.742 (0.025) |               |
| YPTB3190 (rpiA) | YPO0915 | ribose 5-phosphate isomerase A | 0.601 (0.021) | 0.641 (0.039) |
| YPTB3195 (fbA) | YPO0920 | fructose-bisphosphate aldolase class II | 1.325 (0.048) | 0.699 (0.015) |
| YPTB3196 (pgk) | YPO0921 | phosphoglycerate kinase | 1.366 (0.024) |               |
| YPTB3229 | YPO0957 | putative sugar transport system permease protein | 0.626 (0.018) |               |

Table 2: Y. pseudotuberculosis IP32953 chromosomal genes (sorted by COG class [28]) that are transcriptionally regulated by growth medium and/or temperature. (Continued)
| YPTB0014 (mobA) | YPO0958 | putative sugar transport ATP-binding protein | 0.624 | (0.005) |
|------------------|---------|---------------------------------------------|-------|---------|
| YPTB0056 (kbl)  | YPO0998 | putative membrane protein                    | 2.167 | (< 0.001) |
| YPTB0058 (kbl)  | YPO0995 | Sodium:galactoside symporter family protein | 1.764 | (0.014) |
| YPTB0134 (livC) | YPO0977 | ExuT transport protein                       | 1.866 | (0.001) |
| YPTB0182 (hemX) | YPO0976 | putative trehalose-6-phosphate hydrolase     | 0.736 | (< 0.001) |
| YPTB0209 (thiC) | YPO0975 | thiamine biosynthesis protein                | 0.34  | (< 0.001) |
| YPTB0264 (treB) | YPO0974 | putative trehalose-specific IIIC component   | 0.279 | (< 0.001) |
| YPTB0290 (treC) | YPO0973 | putative carbohydrate transport protein      | 1.459 | (0.02) |
| YPTB0344 (lamB) | YPO0972 | maltoporin                                   | 0.419 | (< 0.001) |
| YPTB0379 (glpP) | YPO0971 | glycogen phosphorylase                      | 1.516 | (0.021) |
| YPTB0387 (glpB) | YPO0970 | 1.4-alpha-glucan branching enzyme            | 0.603 | (0.001) |

**H: coenzyme metabolism**

| YPTB0014 (mobA) | or5120 | molybdopterin-guanine dinucleotide biosynthesis protein A | 0.703 | (0.032) |
|------------------|---------|----------------------------------------------------------|-------|---------|
| YPTB0056 (kbl)  | YPO0959 | 2-amino-3-ketobutyrate coenzyme A ligase                 | 0.639 | (0.02) |
| YPTB0034 (ispB) | YPO0958 | acetolactate synthase isozyme II large subunit           | 0.627 | (0.039) |
| YPTB0064 (ilvG) | YPO0957 | putative uroporphyrin-III C-methyltransferase            | 0.695 | (0.033) |
| YPTB0209 (thiC) | YPO0956 | conserved hypothetical protein                          | 0.695 | (0.01) |
| YPTB0264 (treB) | YPO0955 | thiamine biosynthesis protein                            | 0.636 | (0.015) |
| YPTB0290 (thiC) | YPO0954 | octaprenyl-diphosphate synthase                         | 0.731 | (0.03) |
| YPTB0559 (or393) | or393 | hypothetical protein                                     | 1.723 | (0.007) |
| YPTB0561 (rpsT) | or395  | putative protein involved in molybdopterin biosynthesis  | 1.802 | (< 0.001) |
| YPTB0616 (rpsT) | YPO0947 | 30S ribosomal protein S20                                | 0.59  | (0.002) |
| YPTB0664 (folK) | or477  | hypothetical protein                                     | 0.754 | (0.035) |
| YPTB0739 (fhc)  | YPO3400 | 2-amino-4-hydroxy-6-oxo-4-hydroxymethyl-dihydropteridine| 0.572 | (0.009) |
| YPTB0739 (fhc)  | YPO3392 | ferrichrome transport ATP-binding protein FhuC           | 1.932 | (0.046) |
| YPTB0739 (fhc)  | YPO3337 | putative 6-pyruvoyl tetrahydrobiopterin synthase family protein | 0.556 | (0.002) |
| YPTB0758 (gmcM) | YPO3370 | phosphoenolpyruvate-3-phosphate reductase (pseudogene. F/S) | 1.593 | (0.002) |
| YPTB0835 (ribH) | YPO3182 | 6.7-dimethyl-8-ribityllumazine synthase                | 0.695 | (0.01) |
| YPTB0940 (lspA) | YPO3176 | geranyltansferrase                                       | 0.635 | (0.026) |
| YPTB1003 (wbyH) | YPO3111 | putative exported protein                               | 0.727 | (0.039) |
| YPTB1091 (lipA) | YPO2598 | lipoic acid synthetase                                   | 0.558 | (0.002) |
| YPTB1163 (pnuC) | YPO1128 | integral membrane NMM transport protein PnuC            | 1.675 | (0.004) |
| YPTB1181 (bioA) | YPO1150 | adenosylmethionine-8-amino-7-oxononanooate aminotransferase | 3.377 | (< 0.001) |
| YPTB1183 (bioF) | YPO1152 | 8-amino-7-oxononanooate synthase                        | 3.799 | (< 0.001) |
| YPTB1184 (bioC) | YPO1153 | biotin synthesis protein BioC                           | 1.784 | (0.004) |
| YPTB1185 (bioD) | YPO1154 | dethiobiotin synthetase                                 | 2.499 | (0.02) |
| YPTB1343 (bioA) | YPO1312 | putative siderophore ABC transporter. ATP-binding subunit | 2.467 | (< 0.001) |

Table 2: Y. pseudotuberculosis IP32953 chromosomal genes (sorted by COG class [28]) that are transcriptionally regulated by growth medium and/or temperature. (Continued)
Table 2: *Y. pseudotuberculosis* IP32953 chromosomal genes (sorted by COG class [28]) that are transcriptionally regulated by growth medium and/or temperature. (Continued)

| Gene   | Protein/Function                                                                 | Ratio   | P-value   |
|--------|----------------------------------------------------------------------------------|---------|-----------|
| YPTB1384 (poxB) | YPO1358 pyruvate dehydrogenase [cytochrome]                                       | 1.521 (0.009) |
| YPTB1885  | or1359 possible ThiF family                                                     |         |           |
| YPTB1886  | or1360 conserved hypothetical protein                                             |         |           |
| YPTB1888  | or1362 conserved hypothetical protein                                             | 1.886 (0.001) |
| YPTB2033  | YPO2050 conserved hypothetical protein                                             | 0.542 (0.002) |
| YPTB2136 (btrR) | YPO2214 cob(II)alamin adenosyltransferase                               | 1.975 (0.002) |
| YPTB2191  | YPO2269 putative dethiobiotin synthetase                                          | 0.3 (< 0.001) |
| YPTB2304 (ribE) | YPO2391 riboflavin synthase alpha chain                                        | 1.44 (0.012) |
| YPTB2459  | or3719 hypothetical                                                                | 0.379 (< 0.001) |
| YPTB2561 (menF) | YPO2528 menaquinone-specific isochorismate synthase                              | 0.522 (0.011) |
| YPTB2561 (menF) | YPO2528 menaquinone-specific isochorismate synthase                              | 0.634 (0.01) |
| YPTB3574  | YPO3657 putative sodium/panthothenate symporter                                   | 0.616 (0.047) |
| I: lipid metabolism |
| YPTB0416 (psd) | YPO0364 phosphatidylserine decarboxylase proenzyme                               | 1.846 (0.024) |
| YPTB0434 (aidB) | YPO0383 putative acyl- CoA dehydrogenase                                         | 1.613 (0.049) |
| YPTB0558  | or0392 possible acyl-CoA dehydrogenase                                            | 1.628 (0.042) |
| YPTB0674  | YPO0537 putative AMP-binding enzyme-family protein                               | 0.616 (0.047) |
| YPTB0883 (yafH) | YPO3244 probable acyl-CoA dehydrogenase                                         | 2.292 (< 0.001) |
| YPTB1355  | YPO1324 putative permease                                                        | 0.499 (< 0.001) |
| YPTB1450 (fabA) | YPO0537 putative acyl carrier protein                                             | 1.669 (0.033) |
| YPTB1480  | YPO1430 3-hydroxydecanoyl-[acyl-carrier-protein] dehydratase                      | 0.685 (0.001) |
| YPTB2242 (acpD) | YPO2233 acyl carrier protein phosphodiesterase                                  | 0.703 (0.013) |
| YPTB2470 (acpP) | YPO3751 50S ribosomal protein L11                                                 | 0.703 (0.007) |
| YPTB2473 (fabH) | YPO2757 3-oxoacyl-[acyl-carrier-protein] synthase III                             | 0.605 (0.009) |
| YPTB2993 (poxD) | YPO1054 UDP-3-o-[3-hydroxymyristoyl] glucoseamine N-acyltransferase             | 0.761 (0.007) |
| YPTB3849  | YPO4014 putative membrane protein                                                 | 1.549 (0.012) |
| YPTB3856  | YPO4021 hypothetical protein                                                     | 0.58 (0.012) |
| J: translation, ribosomal structure and biogenesis |
| YPTB0034 (trmH) | YPO0037 tRNA (guanosine-2’-O-)-methyltransferase                                | 1.673 (0.023) |
| YPTB0041 (rplK) | YPO0044 ribonuclease PH                                                         | 0.667 (0.005) |
| YPTB0276 (rpsI) | YPO0197 elongation factor Tu                                                     | 0.624 (0.001) |
| YPTB0279 (rplK) | YPO3751 50S ribosomal protein L11                                                 | 0.703 (0.027) |
| YPTB0280 (rplA) | YPO3750 50S ribosomal protein L1                                                | 0.681 (0.027) |
| YPTB0281 (rplJ) | YPO3749 50S ribosomal protein L10                                                | 0.612 (0.017) |
| YPTB0282 (rplL) | YPO3748 50S ribosomal protein L7/L12                                             | 0.58 (0.014) |
| YPTB0408 (efp) | YPO0354 elongation factor P                                                      | 1.7 (< 0.001) |
| YPTB0438 (rpsE) | YPO3539 30S ribosomal protein S6                                                  | 0.597 (0.008) |
| YPTB0441 (rplJ) | YPO3536 50S ribosomal protein L9                                                  | 0.476 (< 0.001) |
| YPTB0464 (rplU) | YPO3512 50S ribosomal protein L21                                                | 0.539 (0.006) |
| YPTB0465 (rpmA) | YPO3511 50S ribosomal protein L27                                                | 0.66 (0.026) |
| YPTB0480 (inB) | YPO3496 translation initiation factor IF2-2 (pseudogene. inframe deletion)     | 0.611 (< 0.001) |
| YPTB0483 (rpsO) | YPO3493 30S ribosomal protein S15                                                | 0.647 (0.004) |
| YPTB0484 (rplF) | YPO3490 polyribonucleotide nucleotidylytransferase                               | 0.612 (0.006) |
| YPTB0529 (valS) | YPO3443 valyl-tRNA synthetase                                                    | 0.648 (0.004) |
Table 2: Y. pseudotuberculosis IP32953 chromosomal genes (sorted by COG class [28]) that are transcriptionally regulated by growth medium and/or temperature. (Continued)

| Gene ID   | Y. pseudotuberculosis  | Gene ID   | Y. pseudotuberculosis  | p-value  | q-value  |
|-----------|------------------------|-----------|------------------------|----------|----------|
| YPTB0575  | prfC                   | YPO0430   | peptide chain release factor 3 | 0.536  | (0.014)  | 0.611  | (0.046)  |
| YPTB0732  | prfC                   | YPO3399   | poly(A) polymerase      | 0.74     | (0.025)  |        |          |
| YPTB0794  | map                    | YPO3337   | methionine aminopeptidase | 3.582    | (< 0.001)|        |          |
| YPTB0834  | ripS                   | YPO3295   | 30S ribosomal protein S16 | 0.682    | (0.044)  |        |          |
| YPTB0835  | ripM                   | YPO3294   | 16S rRNA processing protein | 0.628    | (0.03)   | 0.68   | (0.011)  |
| YPTB0836  | trimD                  | YPO3293   | tRNA (guanine-N1)-methyltransferase | 0.141    | (< 0.001)| 2.388  | (0.03)   |
| YPTB0844  | ripF                   | YPO3279   | putative sigma 54 modulation protein | 0.718    | (0.04)   |        |          |
| YPTB0846  | ripD                   | YPO3277   | ribosomal large subunit pseudouridine synthase D | 0.626    | (0.021)  |        |          |
| YPTB0858  | or0769                 | YPO1104   | conserved hypothetical protein | 1.634    | (0.005)  |        |          |
| YPTB1138  | YPO1336               | putative RNA methyltransferase | 0.588    | (0.018)  |        |          |
| YPTB1141  | ansB                  | YPO1386   | putative L-asparaginase II precursorR | 1.869    | (0.013)  |        |          |
| YPTB1147  | ripA                  | YPO1392   | 30S ribosomal protein S1 | 0.427    | (< 0.001)|        |          |
| YPTB1153  | or1412                | YPO1412   | asparaginyl-tRNA synthetase | 1.628    | (0.022)  |        |          |
| YPTB1205  | ripF                  | YPO1955   | putative acetyltransferase | 0.594    | (0.017)  |        |          |
| YPTB1213  | ripM                  | YPO2017   | peptide chain release factor 1 | 0.665    | (0.008)  | 0.733  | (0.035)  |
| YPTB1215  | ripN                  | YPO2213   | putative RNA pseudouridylate synthase-family protein | 0.691    | (0.032)  |        |          |
| YPTB1232  | ripL                  | YPO2228   | translation initiation factor SUI1 family protein | 1.574    | (0.008)  |        |          |
| YPTB1233  | ripO                  | YPO2420   | probable formyl transferase | 0.578    | (0.015)  |        |          |
| YPTB1237  | ripS                  | YPO2428   | phenylalanyl-tRNA synthetase beta chain | 1.646    | (0.003)  |        |          |
| YPTB1239  | ripP                  | YPO2429   | phenylalanyl-tRNA synthetase alpha chain | 0.586    | (< 0.001)|        |          |
| YPTB1251  | ripQ                  | YPO2766   | tRNA pseudouridylate synthase A | 0.52     | (0.01)  |        |          |
| YPTB1261  | ripR                  | YPO2898   | putative SpoU-family rRNA methylase | 0.609    | (0.026)  |        |          |
| YPTB1262  | ripS                  | YPO1047   | ribosome recycling factorR | 0.611    | (0.047)  |        |          |
| YPTB1263  | ripT                  | YPO1045   | elongation factor Ts | 0.464    | (< 0.001)|        |          |
| YPTB1264  | ripU                  | YPO1044   | 30S ribosomal protein S2 | 0.761    | (0.039)  |        |          |
| YPTB1265  | ripV                  | YPO1038   | Conserved hypothetical protein | 0.761    | (0.039)  |        |          |
| YPTB1266  | ripW                  | YPO1395   | Possible bacteriophage protein | 1.616    | (0.029)  |        |          |
| YPTB1267  | ripX                  | YPO3562   | 30S ribosomal protein S9 | 0.723    | (0.037)  | 0.582  | (0.001)  |
| YPTB1268  | ripY                  | YPO0233   | 30S ribosomal protein S4 | 0.576    | (0.002)  |        |          |
| YPTB1269  | ripZ                  | YPO0232   | 30S ribosomal protein S11 | 0.594    | (0.017)  |        |          |
| YPTB1270  | ripA                  | YPO0231   | 30S ribosomal protein S13 | 0.518    | (0.005)  |        |          |
| YPTB1271  | ripB                  | YPO0228   | 50S ribosomal protein L15 | 0.593    | (0.008)  |        |          |
| YPTB1272  | ripC                  | YPO0225   | 50S ribosomal protein L18 | 0.563    | (0.005)  |        |          |
| YPTB1273  | ripD                  | YPO0279   | 30S ribosomal protein S8 | 0.638    | (0.042)  | 0.619  | (0.032)  |
| YPTB1274  | ripE                  | YPO0221   | 50S ribosomal protein L24 | 0.574    | (0.005)  |        |          |
| YPTB1275  | ripF                  | YPO0220   | 50S ribosomal protein L14 | 0.634    | (0.015)  |        |          |
| YPTB1276  | ripG                  | YPO0219   | 30S ribosomal protein S17 | 0.58     | (0.016)  |        |          |
| YPTB1277  | ripH                  | YPO0217   | 50S ribosomal protein L16 | 0.511    | (< 0.001)|        |          |
| YPTB1278  | ripI                  | YPO0216   | 30S ribosomal protein S3 | 0.642    | (0.028)  |        |          |
| YPTB1279  | ripJ                  | YPO0215   | 50S ribosomal protein L22 | 0.466    | (0.003)  |        |          |
| YPTB1280  | ripK                  | YPO0214   | 30S ribosomal protein S19 | 0.612    | (0.013)  |        |          |
| YPTB1281  | ripL                  | YPO0213   | 50S ribosomal protein I2 | 0.555    | (< 0.001)| 0.596  | (< 0.001)|
Table 2: *Y. pseudotuberculosis* IP32953 chromosomal genes (sorted by COG class [28]) that are transcriptionally regulated by growth medium and/or temperature. (Continued)

| Gene | Description | K: transcription | Values |
|------|-------------|------------------|-------|
| YPTB0035 (spoT) | YPO0038 guanosine-3',5'-bis(bisphosphate) 3'-pyrophosphohydrolase | 0.684 (0.011) | |
| YPTB0100 (cytR) | YPO0108 transcriptional repressor | 0.572 (0.014) | |
| YPTB0167 (rho) | YPO3867 transcription termination factor | 0.467 (< 0.001) | |
| YPTB0263 (rfaH) | YPO3770 putative regulatory protein | 0.552 (0.001) | |
| YPTB0278 (rusG) | YPO3752 transcription antitermination protein | 0.62 (0.002) | |
| YPTB0284 (rpoC) | YPO3746 DNA-directed RNA polymerase beta' chain | 0.536 (0.002) | |
| YPTB0291 (spoT) | YPO0038 guanosine-3',5'-bis(bisphosphate) 3'-pyrophosphohydrolase | 0.684 (0.011) | |
| YPTB0333 (spoT) | YPO0038 guanosine-3',5'-bis(bisphosphate) 3'-pyrophosphohydrolase | 0.684 (0.011) | |
| YPTB0479 (nusA) | YPO3497 N utilization substance protein A | 0.653 (0.006) | |
| YPTB0599 (rhaR) | YPO0333 L-rhamnose operon transcriptional activator | 0.546 (0.025) | |
| YPTB0601 (arvA) | YPO0458 aerobic respiration control protein | 0.465 (0.001) | |
| YPTB0658 (rapA) | YPO0517 RNA polymerase associated helicase | 0.547 (0.004) | |
| YPTB0712 (pbtR) | YPO3420 pyruvate dehydrogenase complex regulatory protein | 0.808 (0.004) | |
| YPTB0776 (pnp) | YPO3635 DNA polymerase sigma factor Pnp | 0.695 (< 0.001) | |
| YPTB0803 (nuFA) | YPO3266 RNA polymerase sigma factor NusA | 0.595 (0.001) | |
| YPTB0872 (emrR) | YPO3368 RNA polymerase sigma factor EmrR | 0.579 (0.005) | |
| YPTB1258 (rcsB) | YPO1218 putative cold shock protein | 0.693 (0.016) | |
| YPTB1332 (psaE) | YPO1301 putative regulatory protein | 1.986 (0.001) | |
| YPTB1392 (psaD) | YPO1366 cold shock-like protein | 0.46 (0.008) | |
| YPTB1423 (psaE) | YPO1398 putative cold shock protein | 0.579 (0.005) | |
| YPTB1610 (emrR) | YPO1374 putative cold shock protein | 1.430 (0.025) | |
| YPTB1721 (hutC) | YPO1973 putative GntR-family transcriptional regulatory protein | 1.366 (0.025) | |
| YPTB1967 (hutC) | YPO1973 putative GntR-family transcriptional regulatory protein | 0.739 (0.018) | |
| YPTB2048 (hexR) | YPO2065 hex regulon repressor | 1.51 (0.013) | |
| YPTB2072 (fadR) | YPO2144 fatty acid metabolism regulatory protein | 1.494 (0.01) | |
| YPTB2177 (aroA) | YPO2258 arabinose operon regulatory protein | 1.591 (0.002) | |
| YPTB2190 (mic) | YPO2268 putative ROK family transcriptional regulatory protein | 1.417 (0.037) | |
| YPTB2230 (rstA) | YPO2308 two-component regulatory system. response regulator protein | 0.658 (0.024) | |
| YPTB2262 (rprR) | YPO2344 putative ROK family transcriptional regulatory protein | 0.486 (< 0.001) | |
| YPTB2288 (roB) | YPO2374 MarR-family transcriptional regulatory protein | 0.685 (0.014) | |
| YPTB2367 (kdR) | YPO1714 IclR-family transcriptional regulatory protein | 0.685 (0.014) | |
| YPTB2414 (psaC) | YPO1651 AsnC-family transcriptional regulatory protein | 0.435 (< 0.001) | |
| YPTB2534 | YPO2498 | putative LacI-family transcriptional regulatory protein | 1.601 (0.036) |
| YPTB2737 | YPO3017 | putative rpiR-family transcriptional regulatory protein | 0.715 (0.043) |
| YPTB2763 (narP) | YPO3041 | nitrate/nitrite response regulator protein NarP | 1.578 (0.011) |
| YPTB2860 | YPO2897 | conserved hypothetical protein | 0.652 (0.035) |
| YPTB2865 | YPO3017 | putative RNA-binding protein | 0.673 (0.013) |
| YPTB2897 (rpoE) | YPO2711 | RNA polymerase sigma E factor | 1.827 (< 0.001) |
| YPTB2939 (ureG) | YPO2670 | urease accessory protein | 0.632 (0.010) |
| YPTB3017 (gcvA) | YPO1029 | Glycine cleavage system transcriptional activator | 1.503 (0.036) |
| YPTB3490 | YPO3545 | LysR-family transcriptional regulatory protein | 1.827 (< 0.001) |
| YPTB3514 | YPO3570 | BolA-like protein | 0.611 (0.034) |
| YPTB3538 (rnk) | YPO3695 | regulator of nucleoside diphosphate kinase | 0.580 (0.002) |
| YPTB3577 (fiS) | YPO3651 | DNA-binding protein Fis | 0.675 (0.017) |
| YPTB3764 (greB) | YPO0136 | transcription elongation factor | 0.562 (< 0.001) |
| YPTB3779 (glpR) | YPO0120 | glycerol-3-phosphate repressor protein | 0.691 (0.003) |
| YPTB3847 (uhpA) | YPO4012 | two-component system response regulator | 0.567 (0.019) |
| YPTB3887 | YPO4034 | putative AraC-family transcriptional regulatory protein | 0.554 (0.013) |

**L: DNA replication, recombination and repair**

| YPTB0046 (radC) | YPO0049 | putative DNA repair protein | 1.544 (0.026) |
| YPTB0261 | YPO0049 | cytoplasmic Dnase | 0.569 (< 0.001) |
| YPTB0292 | YPO3736 | conserved hypothetical protein | 1.453 (0.017) |
| YPTB0297 (hupA) | YPO3731 | DNA-binding protein HU-alpha | 0.455 (< 0.001) |
| YPTB0302 (or0218) | YPO3955 | DNA-binding protein HU-beta | 0.557 (0.045) |
| YPTB0439 (priB) | YPO3849 | primosomal replication protein n | 1.478 (0.046) |
| YPTB0498 | YPO3475 | conserved hypothetical protein | 0.554 (0.013) |
| YPTB0579 | YPO3041 | putative methyltransferase | 0.64 (0.023) |
| YPTB0658 (rapA) | YPO0057 | RNA polymerase associated helicase | 0.547 (0.004) |
| YPTB0913 (rdgC) | YPO3212 | possible recombination associated protein RdgC | 1.82 (0.004) |
| YPTB0941 (xseB) | YPO3157 | exodeoxyribonuclease VII small subunit | 0.647 (0.004) |
| YPTB0962 (hupB) | YPO3154 | DNA-binding protein HU-beta | 1.674 (< 0.001) |
| YPTB0964 (gabV) | YPO3152 | putative exported protein | 0.625 (0.02) |
| YPTB0418 (infB) | YPO1393 | integrasion host factor beta-subunit | 0.46 (0.003) |
| YPTB1799 | YPO1306 | putative modification methylase | 1.94 (0.026) |
| YPTB2040 (ruvA) | YPO2057 | Holliday junction DNA helicase | 1.225 (0.004) |
| YPTB2140 (topA) | YPO2218 | DNA topoisomerase I | 1.654 (0.012) |
| YPTB2221 (ogt) | YPO2299 | putative methylated-DNA – protein-cysteine methyltransferase | 0.611 (0.017) |
| YPTB2335 (infA) | YPO2427 | integration host factor alpha-subunit | 1.626 (0.001) |
| YPTB2458 | YPO3720 | hypothetical | 0.501 (< 0.001) |
Table 2: Y. pseudotuberculosis IP32953 chromosomal genes (sorted by COG class [28]) that are transcriptionally regulated by growth medium and/or temperature. (Continued)

| Gene          | COG ID | Description                                      | Log2 Ratio (p-value) |
|---------------|--------|--------------------------------------------------|---------------------|
| YPTB2792      | YPO3071| conserved hypothetical protein                    | 1.337 (0.025)       |
| YPTB2834      | YPO2872| exodeoxyribonuclease VII large subunit           | 1.299 (0.044)       |
| YPTB3389      | YPO0674| putative MutT-family protein                      | 0.611 (0.034)       |
| YPTB3577      | or2359 | DNA-binding protein Fis                           | 1.58 (0.013)        |
| YPTB3757      | YPO0144| putative hydrolase                                | 0.58 (0.02)         |
|               |        | **M: cell envelope biogenesis, outer membrane**  |                     |
| YPTB0051      | YPO0054| lipopolysaccharide core biosynthesis glycosyl    | 0.592 (0.001)       |
|               |        | transferase                                       |                     |
| YPTB0173      | YPO3861| glucose-1-phosphate thymidyltransferase          | 1.604 (0.028)       |
| YPTB0415      | YPO0363| putative membrane transport protein               | 1.853 (0.003)       |
| YPTB0491      | YPO3483| multidrug efflux protein                          | 1.833 (0.032)       |
| YPTB0493      | YPO3481| probable outer membrane efflux lipoprotein       | 1.863 (0.015)       |
| YPTB0694      | YPO3483| DNA-binding protein Fis                            | 0.787 (0.04)        |
|               |        | **M: cell envelope biogenesis, outer membrane**  |                     |
| YPTB0801      | YPO2155| putative exported protein                          | 0.796 (0.009)       |
| YPTB1173      | YPO1860| attachment invasion locus protein                 | 1.511 (0.012)       |
| YPTB1819      | or1328 | hypothetical phage protein                        | 1.902 (0.025)       |
| YPTB1964      | or1419 | putative outer membrane porin C protein          | 1.634 (0.001)       |
|               |        | **M: cell envelope biogenesis, outer membrane**  |                     |
| YPTB0697      | YPO0144| putative hydrolase                                | 0.58 (0.02)         |
|               |        | **M: cell envelope biogenesis, outer membrane**  |                     |
| YPTB0775      | YPO3356| lipoprotein                                       | 0.732 (0.015)       |
| YPTB0906      | YPO3220| conserved hypothetical protein                    | 0.651 (0.038)       |
| YPTB0955      | YPO3161| lipoprotein                                       | 1.48 (0.021)        |
| YPTB0987      | YPO3129| putative potassium efflux system                  | 0.626 (0.001)       |
| YPTB1002      | YPO3112| paratose synthase                                 | 0.706 (0.013)       |
| YPTB1008      | YPO3104| putative mannosyltransferase                      | 0.669 (0.03)        |
| YPTB1014      | YPO3096| O-antigen chain length determinant                | 0.577 (0.019)       |
| YPTB1109      | YPO2616| putative apolipoprotein N-acetyltransferase       | 0.669 (0.029)       |
| YPTB1160      | YPO1125| peptidoglycan-associated lipoprotein Pal          | 1.649 (< 0.001)     |
| YPTB1217      | YPO1176| penicillin-binding protein 7 precursor            | 0.565 (0.009)       |
| YPTB1261      | YPO1222| outer membrane protein C. porin                  | 1.371 (0.034)       |
| YPTB1266      | YPO1231| putative outer membrane-associated protease       | 0.569 (0.015)       |
| YPTB1309      | YPO1275| putative lipoprotein                              | 0.499 (< 0.001)     |
| YPTB1381      | YPO1355| conserved hypothetical protein                    | 0.683 (0.031)       |
| YPTB1385      | YPO1411| putative outer membrane porin C protein          | (< 0.001)           |
| YPTB1453      | YPO1435| putative outer membrane porin A protein          | 0.355 (< 0.001)     |
| YPTB1481      | YPO1498| putative exported protein                         | 0.301 (< 0.001)     |
| YPTB1528      | YPO1513| putative membrane protein                         | 1.513 (0.043)       |
| YPTB1731      | YPO1860| attachment invasion locus protein                 | 1.634 (0.001)       |
| YPTB1819      | or1328 | hypothetical phage protein                        | 1.902 (0.025)       |
| YPTB1964      | or1419 | putative outer membrane porin C protein          | 1.634 (0.001)       |
| YPTB1975      | YPO1982| putative dehydrogenase                            | 0.63 (0.012)        |
| YPTB2081      | YPO2155| putative exported protein                         | 0.796 (0.009)       |
| YPTB2113      | YPO2190| attachment invasion locus protein precursor       | 1.511 (0.012)       |
| YPTB2117      | YPO2193| TonB                                             | 5.465 (< 0.001)     |
| YPTB2212      | YPO2201| putative exported protein                         | 0.165 (< 0.001)     |
| YPTB2233      | YPO2312| insecticidal toxin                               | 1.424 (0.045)       |
|               |        | (pseudogene. inframe insertion)                  |                     |
| YPTB2294      | YPO2380| insecticidal toxin                               | 1.424 (0.045)       |
|               |        | (pseudogene. inframe insertion)                  |                     |
| YPTB2323      | YPO2415| putative lipoprotein                              | 1.388 (0.025)       |
| YPTB2979      | YPO1067| putative copper homeostasis lipoprotein          | 1.794 (0.001)       |
Table 2: Y. pseudotuberculosis IP32953 chromosomal genes (sorted by COG class [28]) that are transcriptionally regulated by growth medium and/or temperature.  (Continued)

| Gene ID | Gene Product Description | Fold Change | P-value |
|---------|--------------------------|-------------|---------|
| YPTB2994 (ompH) | cationic 19 kDa outer membrane protein precursor | 1.69 | (< 0.001) |
| YPTB2995 | putative surface antigen | 0.745 | (0.016) |
| YPTB2996 | putative membrane protein | 1.531 | (0.03) |
| YPTB3194 | putative membrane protein | 1.569 | (0.024) |
| YPTB3277 | or3091 | 1.57 | (0.026) |
| YPTB3282 | or3086 | 1.593 | (0.028) |
| YPTB3313 (slyB) | putative lipoprotein | 1.452 | (0.003) |
| YPTB3407 (rfaE) | ADP-heptose synthase | 0.709 | (0.042) |
| YPTB3438 | putative membrane protein | 1.993 | (0.004) |
| YPTB3497 (mtgA) | monofunctional biosynthetic peptidoglycan transglycosylase | 1.736 | (0.008) |
| YPTB3513 (murA) | UDP-N-acetylglicosamine 1-carboxyvinyltransferase | 0.775 | (0.034) |
| YPTB3517 | putative glycol transferase | 0.657 | (0.013) |
| YPTB3958 | putative membrane protein | 1.69 | (0.004) |
| YPTB3965 (glmU) | UDP-N-acetylglicosamine pyrophosphorylase | 1.572 | (0.042) |

N: cell motility and secretion

| Gene ID | Gene Product Description | Fold Change | P-value |
|---------|--------------------------|-------------|---------|
| YPTB0071 (cpxP) | putative exported protein | 0.337 | (< 0.001) |
| YPTB0156 | putative chaperone protein | 0.657 | (0.01) |
| YPTB0158 | putative outer membrane usher protein | 1.626 | (0.016) |
| YPTB0339 | putative outer membrane fimbrial usher protein | 1.398 | (0.049) |
| YPTB0706 (hofB) | putative type II secretion system protein | 0.711 | (0.043) |
| YPTB1335 (psaB) | chaperone protein PsdB precursor | 0.216 | (< 0.001) |
| YPTB1680 (flgH) | flagellar protein FlgH | 1.906 | (0.01) |
| YPTB1688 (flgL) | flagellar hook-associated protein 1 | 0.616 | (0.01) |
| YPTB1693 | flagellar hook-associated protein 3 | 1.514 | (0.041) |
| YPTB1696 (flmA) | flagellar switch protein FlmA | 2.053 | (0.011) |
| YPTB1698 (flk) | flagellar hook-length control protein Flk | 1.79 | (0.021) |
| YPTB1919 | probable fimbrial usher protein | 1.941 | (0.006) |
| YPTB2396 (cheZ) | chemotaxis protein CheZ | 1.477 | (0.027) |
| YPTB2405 (cheA) | chemotaxis protein CheA | 1.725 | (0.015) |
| YPTB2843 | putative fimbrial biogenesis protein | 0.686 | (0.022) |
| YPTB3347 (flIC) | putative flagellar motor switch protein | 1.68 | (0.03) |
| YPTB3357 | flagellar assembly protein | 0.611 | (0.049) |
| YPTB3896 | fimbrial protein | 1.644 | (0.021) |

No COG

| Gene ID | Gene Product Description | Fold Change | P-value |
|---------|--------------------------|-------------|---------|
| YPTB0092 | hypothetical protein | 1.623 | (0.025) |
| YPTB0094 | putative exported protein | 1.434 | (< 0.001) |
| YPTB0123 (yijD) | putative membrane protein | 0.503 | (< 0.001) |
| YPTB0139 | putative membrane protein | 0.669 | (0.033) |
| YPTB0141 | putative membrane protein | 0.717 | (0.036) |
| YPTB0148 | colicin | 1.605 | (0.045) |
| YPTB0149 | putative colicin immunity protein | 1.605 | (0.045) |
Table 2: *Y. pseudotuberculosis* IP32953 chromosomal genes (sorted by COG class [28]) that are transcriptionally regulated by growth medium and/or temperature. (Continued)

| Gene ID  | Description                                      | Ratio  | P-value  |
|---------|--------------------------------------------------|--------|----------|
| YPTB0151 | or0099 pyocin S2 immunity protein                | 1.549  | 0.013    |
| YPTB0212 | dcrB putative lipoprotein                        | 0.758  | 0.028    |
| YPTB0237 | or5000 putative exported protein                  |        |          |
| YPTB0244 | or169 hypothetical                               | 0.517  | <0.001   |
| YPTB0362 | YPO0306 conserved hypothetical protein (pseudogene. F/S) | 0.66   | 0.036    |
| YPTB0391 | YPO0337 putative exported protein                 |        |          |
| YPTB0406 | YPO0352 putative lipoprotein                     | 1.294  | 0.042    |
| YPTB0449 | YPO3527 conserved hypothetical protein            |        |          |
| YPTB0499 | YPO3474 hypothetical                              | 1.509  | 0.036    |
| YPTB0505 | YPO3468 hypothetical                              | 1.632  | 0.026    |
| YPTB0546 | YPO0306 conserved hypothetical protein            |        |          |
| YPTB0560 | or0394 hypothetical                               | 2      | <0.001   |
| YPTB0593 | YPO0345 putative membrane protein                 | 0.736  | 0.025    |
| YPTB0651 | YPO0511 hypothetical                              | 0.576  | 0.02     |
| YPTB0657 | YPO0516 hypothetical                              | 0.457  | 0.001    |
| YPTB0666 | or4788 putative IS1400 transposase B              | 2.101  | 0.001    |
| YPTB0678 | YPO0344 putative membrane protein                 | 0.631  | 0.009    |
| YPTB0768 | (ygbE) putative membrane protein                  | 1.735  | 0.007    |
| YPTB0793 | YPO3339 hypothetical                              | 1.679  | 0.023    |
| YPTB0795 | YPO3336 conserved hypothetical protein            | 3      | <0.001   |
| YPTB0903 | (crl) YPO3223 curlin genes regulatory protein     | 2.098  | <0.001   |
| YPTB0957 | YPO3159 hypothetical                              | 1.521  | 0.034    |
| YPTB0978 | (ymsA) YPO3138 modulating protein YmoA (histone-like protein) | 0.509 | <0.001 |
| YPTB0979 | YPO3137 conserved hypothetical protein            | 0.469  | <0.001   |
| YPTB0980 | YPO3136 hypothetical                               | 0.587  | (0.01)   |
| YPTB1004 | (wzx) or0734 putative O-unit flipase              |        |          |
| YPTB1018 | (ushB) or0747 S’-nucleotidase/UDP-sugar diphosphatase | 1.34  | (0.031) |
| YPTB1041 | YPO2820 hypothetical                              | 1.394  | 0.029    |
| YPTB1042 | (int) or4598 phage integrase (pseudogene. Partial) | 1.483 | (0.046) |
| YPTB1043 | or0754 hypothetical                               | 0.569  | 0.002    |
| YPTB1130 | (trp 1400A) or4531 IS1400 transposase A           | 2.136  | <0.001   |
| YPTB1167 | (psf) YPO1134 putative starvation-inducible protein | 2.04 | (0.014) |
| YPTB1202 | (kapB) YPO1172 xanthosine permease (pseudogene. IS1541) | 1.381 | (0.03) |
| YPTB1220 | YPO1179 conserved hypothetical protein            | 0.702  | 0.038    |
| YPTB1287 | or0929 putative bacteriophage tail fiber protein  | 1.853  | (0.01)   |
| YPTB1291 | YPO1255 hypothetical                              | 0.561  | (0.008)  |
| YPTB1303 | YPO1269 conserved hypothetical protein            | 1.754  | (0.008)  |
| YPTB1334 | (psaA) YPO1303 pH 6 antigen precursor (antigen 4) (adhesin) |        |          |
| YPTB1359 | YPO1328 putative membrane protein                 | 1.723  | (0.003)  |
| YPTB1515 | YPO1499 putative membrane protein                 | 1.521  | (0.045)  |
| YPTB1543 | (ysuH) YPO1531 putative siderophore biosynthetic enzyme | 1.896 | (0.02) |
| YPTB1583 | YPO1574 putative exported protein                 | 0.605  | <0.001   |
| YPTB1602 | (int) or4274 integrase                           | 0.592  | (0.026)  |
| YPTB1616 | or1193 conserved hypothetical protein             | 0.677  | (0.016)  |
| YPTB1619 | YPO1741 hypothetical                              | 1.496  | (0.025)  |
Table 2: *Y. pseudotuberculosis* IP32953 chromosomal genes (sorted by COG class [28]) that are transcriptionally regulated by growth medium and/or temperature. (Continued)

| Gene       | Description                | Ratio  | (Significance) |
|------------|----------------------------|--------|----------------|
| YPTB1622   | YPO1744 putative exported   | 1.671  | (0.019)        |
| YPTB1663   | YPO1788 putative acyl carrier protein | 1.57  | (0.049)        |
| YPTB1664   | YPO1789 putative membrane protein | 1.638 | (0.031)        |
| YPTB1668   | YPO1788 putative exported protein | 0.5   | (< 0.001)     |
| YPTB1705   | YPTB1705 putative phage minor tail protein | 1.794 | (0.002)        |
| YPTB1722   | YPO1850 hypothetical protein | 1.593  | (0.045)        |
| YPTB1734   | YPO1864 conserved hypothetical protein | 0.679  | (0.003)        |
| YPTB1752   | YPO1788 O protein [Enterobacteria phage 186] | 0.49  | (0.01)         |
| YPTB1785   | YPO1788 hypothetical protein | 0.453  | (< 0.001)     |
| YPTB1786   | YPO1788 hypothetical protein | 1.605  | (0.042)        |
| YPTB1798   | YPO1788 conserved hypothetical protein | 1.506  | (0.043)        |
| YPTB1801   | YPO1788 hypothetical protein | 0.279  | (< 0.001)     |
| YPTB1802   | YPO1788 hypothetical protein | 0.449  | (0.01)         |
| YPTB1815   | YPO1788 putative phage protein | 1.57  | (0.037)        |
| YPTB1821   | YPTB1821 putative acyl carrier protein | 1.57  | (0.049)        |
| YPTB1822   | YPO1788 putative membrane protein | 1.638  | (0.031)        |
| YPTB1826   | YPO1788 bacterioiophage hypothetical protein | 1.574  | (0.004)        |
| YPTB1850   | YPO1788 gpR [Enterobacteria phage P2] | 1.779  | (0.024)        |
| YPTB1858   | YPO1788 similar to V protein phage 186 | 1.743  | (0.024)        |
| YPTB1862   | YPO1788 putative phage replication protein | 0.536  | (0.011)        |
| YPTB1884   | YPO1788 possible MFS Superfamily multidrug-efflux transporter | 1.854  | (0.007)        |
| YPTB1887   | YPO1788 hypothetical protein | 1.593  | (0.034)        |
| YPTB1893   | YPO1788 conserved hypothetical protein | 1.574  | (0.049)        |
| YPTB1980   | YPO1788 hypothetical protein (pseudoogene, IS285) | 1.53  | (0.049)        |
| YPTB1986   | YPO1788 hypothetical protein | 0.649  | (0.039)        |
| YPTB1987   | YPO1788 hypothetical protein | 0.57   | (0.047)        |
| YPTB1996   | YPO1788 putative membrane protein | 2.041  | (0.002)        |
| YPTB2000   | YPO1788 putative membrane protein | 0.256  | (< 0.001)     |
| YPTB2092   | YPO1788 putative exported protein | 0.671  | (0.027)        |
| YPTB2114   | YPO1788 hypothetical protein | 0.28   | (< 0.001)     |
| YPTB2148   | YPO1788 hypothetical protein | 1.855  | (0.007)        |
| YPTB2151   | YPO1788 osmotically inducible lipoprotein B precursor | 2.229  | (< 0.001)     |
| YPTB2219   | YPO1788 hypothetical protein | 1.413  | (0.032)        |
| YPTB2227   | YPO1788 putative exported protein | 1.413  | (0.031)        |
| YPTB2229   | YPO1788 conserved hypothetical protein | 0.708  | (0.026)        |
| YPTB2237   | YPO1788 putative acid shock protein | 0.654  | (0.022)        |
| YPTB2269   | YPO1788 phage shock protein B | 1.53  | (0.018)        |
| YPTB2334   | YPO1788 putative exported protein | 1.526  | (0.008)        |
| YPTB2358   | YPO1788 periplasmic pectate lyase precursor | 2.081  | (0.001)        |
| YPTB2363   | YPO1788 putative exported protein | 1.804  | (0.038)        |
| YPTB2387   | YPO1788 conserved hypothetical protein | 1.794  | (0.008)        |
| YPTB2393   | YPO1788 putative exported protein | 1.442  | (0.019)        |
| YPTB2417   | YPO1788 putative membrane protein | 1.559  | (0.003)        |
Table 2: *Y. pseudotuberculosis* IP32953 chromosomal genes (sorted by COG class [28]) that are transcriptionally regulated by growth medium and/or temperature. (Continued)

| Gene ID | YPO Number | Description                                      | IP32953 | SDIP216 | Regulation 1 (G/M) | Regulation 2 (G/T) |
|---------|------------|--------------------------------------------------|---------|---------|---------------------|---------------------|
| YPTB2419 | YPO1650    | hypothetical protein                             | 0.6     | (0.026) |                     |                     |
| YPTB2420 | YPO1649    | conserved hypothetical protein                   | 1.758   | (0.004) | 2.658 (< 0.001)    |                     |
| YPTB2421 | YPO1648    | probable histidine acid phosphatase              | 1.662   | (0.01)  |                     | 1.474 (0.045)      |
| YPTB2425 | YPO1643    | hypothetical protein                             | 1.504   | (0.019) |                     |                     |
| YPTB2446 | YPO1619    | hypothetical protein                             | 1.646   | (0.006) |                     |                     |
| YPTB2483 | YPO1586    | DNA-damage-inducible protein I                   | 2.658   | (< 0.001) |                     |                     |
| YPTB2495 | or1732     | glucans biosynthesis protein                     | 0.433   | (0.001) |                     |                     |
| YPTB2496 | or1737     | hypothetical                                      | 0.433   | (0.001) |                     |                     |
| YPTB2540 | or3654     | conserved hypothetical protein                   | 1.718   | (0.031) |                     |                     |
| YPTB2552 | YPO2515    | hypothetical                                      | 1.391   | (0.02)  |                     |                     |
| YPTB2554 | YPO2521    | putative exported protein                        | 1.504   | (0.019) |                     | 1.718 (0.031)      |
| YPTB2562 | YPO2530    | conserved hypothetical protein                   | 1.46    | (0.023) |                     | 1.396 (0.042)      |
| YPTB2623 | YPO2760    | putative flagellar assembly regulatory protein. flk | 0.542   | (0.024) |                     | 1.503 (0.01)       |
| YPTB2699 | YPO2976    | conserved hypothetical protein                   | 4.448   | (< 0.001) |                     |                     |
| YPTB2704 | YPO2981    | putative exported protein                        | 1.628   | (0.015) |                     |                     |
| YPTB2744 | YPO3026    | putative lipoprotein                             | 1.646   | (0.006) |                     |                     |
| YPTB2753 | YPO3031    | putative acetyltransferase                       | 1.391   | (0.02)  |                     |                     |
| YPTB2750 | or3477     | hypothetical                                      | 0.688   | (0.006) |                     |                     |
| YPTB2787 | YPO3066    | hypothetical protein                             | 1.504   | (0.019) |                     |                     |
| YPTB2822 | YPO2857    | putative exported protein                        | 1.718   | (0.031) |                     |                     |
| YPTB2877 | YPO2918    | putative exported protein                        | 1.46    | (0.023) |                     | 1.396 (0.042)      |
| YPTB2882 | YPO2976    | conserved hypothetical protein                   | 1.504   | (0.019) |                     | 1.718 (0.031)      |
| YPTB2922 | or3339     | hypothetical                                      | 0.542   | (0.024) |                     |                     |
| YPTB2953 | YPO2657    | putative mobilization protein                    | 0.566   | (< 0.001) |                     |                     |
| YPTB2954 | or3477     | hypothetical                                      | 0.639   | (0.027) |                     |                     |
| YPTB3007 | YPO1040    | conserved hypothetical protein                   | 1.815   | (0.033) |                     |                     |
| YPTB3039 | YPO0791    | hypothetical protein                             | 0.728   | (0.024) |                     |                     |
| YPTB3041 | YPO0792    | putative membrane protein                        | 0.728   | (0.024) |                     |                     |
| YPTB3071 | YPO0822    | putative exported protein                        | 0.617   | (0.015) |                     |                     |
| YPTB3111 | YPO0867    | putative membrane protein                        | 0.51    | (0.002) |                     |                     |
| YPTB3177 | YPO0901    | putative exported protein                        | 0.675   | (0.027) |                     |                     |
| YPTB3179 | YPO0904    | hypothetical protein                             | 0.433   | (0.001) |                     |                     |
| YPTB3200 | YPO0948    | conserved hypothetical protein                   | 1.56    | (0.021) |                     |                     |
| YPTB3256 | or3105     | insertion element protein                        | 1.939   | (< 0.001) |                     |                     |
| YPTB3257 | YPO0983    | putative lipoprotein                             | 2.06    | (0.001) |                     |                     |
| YPTB3280 | or3088     | hypothetical protein                             | 0.606   | (0.009) |                     |                     |
| YPTB3305 | YPO1002    | hypothetical protein                             | 1.679   | (0.023) |                     |                     |
| YPTB3342 | YPO0720    | putative flagellar regulatory protein            | 1.492   | (0.007) |                     |                     |
| YPTB3343 | YPO0719    | hypothetical protein                             | 1.563   | (0.038) |                     |                     |
| YPTB3371 | YPO0694    | Putative membrane protein                        | 0.653   | (0.049) |                     |                     |
| YPTB3421 | YPO0640    | hypothetical protein                             | 0.716   | (0.028) |                     |                     |
| YPTB3454 | or2954     | Fragment of hemagglutinin/hemolysin-related protein | 0.653   | (0.025) |                     |                     |
| Accession | Description                                                                 | Log2 (Fold Change) | p-value  |
|-----------|------------------------------------------------------------------------------|--------------------|----------|
| YPTB3458  | or2950 hypothetical                                                           | 1.92               | (0.005)  |
| YPTB3504  | YPO3559 putative exported protein                                            | 0.483              | (< 0.001)|
| YPTB3534  | YPO3699 putative exported protein                                            | 0.676              | (0.038)  |
| YPTB3551  | YPO3681 Insecticidal toxin TcaA                                              | 0.619              | (0.047)  |
| YPTB3556  | YPO3675 putative exported protein                                            | 0.637              | (0.043)  |
| YPTB3627  | YPO3601 conserved hypothetical protein                                        | 0.595              | (0.006)  |
| YPTB3641 (malM) | YPO3710 maltose operon periplasm protein                                       | 0.379              | (< 0.001)|
| YPTB3769 (feoC) | YPO0131 ferrous iron transport protein C                                 | 1.899              | (< 0.001)|
| YPTB3770  | YPO0130 putative exported protein                                            | 0.43               | (< 0.001)|
| YPTB3781  | YPO3935 putative membrane protein                                            | 0.717              | (0.036)  |
| YPTB3789  | or2712 putative invasin                                                      | 0.614              | (0.01)   |
| YPTB3858  | YPO4020 putative membrane protein                                            | 0.487              | (< 0.001)|
| YPTB3893  | YPO4081 putative membrane protein                                            | 1.408              | (0.01)   |
| YPTB3917 (pioF) | YPO4070 putative exported protein                                 | 1.554              | (0.016)  |
| YPTB3922  | YPO4064 putative multi-membrane protein                                      | 1.409              | (0.046)  |
| YPTB3923  | YPO4063 putative membrane protein                                            | 1.53               | (0.041)  |
| YPTB3944  | or2545 hypothetical protein                                                  | 0.593              | (0.016)  |

**O: posttranslational modification, protein turnover, chaperones**

| Accession | Description                                                                 | Log2 (Fold Change) | p-value  |
|-----------|------------------------------------------------------------------------------|--------------------|----------|
| YPTB0404 (groES) | YPO0350 10 kDa chaperonin                                                     | 0.683              | (0.029)  |
| YPTB0427 (hflK) | YPO0375 putative membrane protein (pseudogene. inframe deletion)             | 0.619              | (0.033)  |
| YPTB0494  | YPO3480 conserved hypothetical protein                                        | 0.444              | (< 0.001)|
| YPTB0495  | YPO3479 putative protease                                                     | 0.328              | (< 0.001)|
| YPTB0518 (ndG) | YPO3455 anaerobic ribonucleoside-triphosphate reductase                      | 0.439              | (0.001)  |
| YPTB0612 (dnaK) | YPO0469 chaperone protein DnaK                                               | 0.73               | (0.049)  |
| YPTB0647 (cylB) | YPO0506 putative Cip ATPase                                                   | 1.481              | (0.047)  |
| YPTB0774 (pcm) | YPO3357 protein-L-isoaaspartate O-methyltransferase                          | 0.62               | (0.014)  |
| YPTB0925 (alpC) | YPO3194 putative alkyl hydroperoxide reductase subunit c                     | 0.545              | (< 0.001)|
| YPTB0948 (cyoE) | YPO3168 protoheme IX farnesyltransferase                                     | 1.422              | (0.039)  |
| YPTB0958 (tig)  | YPO3158 Trigger factor                                                      | 0.607              | (0.008)  |
| YPTB0995 (htpG) | YPO3119 heat shock protein HtpG                                              | 0.569              | (0.004)  |
| YPTB1025  | YPO3083 conserved hypothetical protein                                       | 0.66               | (0.014)  |
| YPTB1026 (ybbN) | YPO3082 putative thioredoxin                                                | 0.715              | (0.019)  |
| YPTB1034 (pplB) | YPO3074 peptidyl-prolyl cis-trans isomerase B                                | 1.419              | (0.013)  |
| YPTB1141  | YPO1107 heat shock protein GrpE                                              | 1.442              | (0.048)  |
| YPTB1406 (pf4) | YPO1381 pyruvate formate-lyase 1 activating enzyme                          | 0.578              | (0.004)  |
| YPTB1871  | or1348 similar to hypothetical bacteriophage P27 protein                     | 0.58               | (0.001)  |
| YPTB1945  | YPO1947 putative thioredoxin                                                | 1.646              | (0.002)  |
| YPTB2070 (dsbB) | YPO2141 disulfide bond formation protein B                                  | 1.626              | (< 0.001)|
| YPTB2084  | YPO2158 conserved hypothetical protein                                       | 0.683              | (0.003)  |
| YPTB2261 (tpx) | YPO2342 thiol peroxidase                                                    | 2.414              | (< 0.001)|
| YPTB2297  | YPO2383 conserved hypothetical protein                                       | 0.741              | (0.025)  |

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*Y. pseudotuberculosis* IP32953 chromosomal genes (sorted by COG class [28]) that are transcriptionally regulated by growth medium and/or temperature. (Continued)
| Gene       | Description                                           | Log2 Fold Change | p Value   |
|------------|-------------------------------------------------------|------------------|-----------|
| YPTB2311   | YPO2401 conserved hypothetical protein                | 1.822            | 0.001     |
| YPTB2312   | YPO2402 putative ATP-dependent transporter            | 2.036            | 0.006     |
| YPTB2313   | YPO2403 conserved hypothetical protein                | 2.213            | 0.002     |
| YPTB2734 (cysT) | YPO3014 sulfate transport system permease protein CysT | 0.714            | 0.029     |
| YPTB2785 (bcp) | YPO3064 bacterioferritin comigratory protein         | 1.417            | 0.033     |
| YPTB2806   | YPO2840 putative heat shock protein                   | 0.674            | 0.027     |
| YPTB2905 (pcp) | YPO2669 putative pyrolylone-carboxylate peptidase     | 1.608            | 0.016     |
| YPTB2938 (ureD) | YPO2671 urease accessory protein                      | 0.377            | < 0.001   |
| YPTB2939 (ureG) | YPO2670 urease accessory protein                      | 0.293            | < 0.001   |
| YPTB2940 (ureF) | YPO2668 urease accessory protein                      | 0.268            | < 0.001   |
| YPTB2941 (ureE) | YPO2840 putative heat shock protein                   | 0.347            | < 0.001   |
| YPTB3408 (glnE) | YPO0653 glutamate-ammonia-ligase adenylyltransferase | 1.792            | 0.046     |
| YPTB3415 (gcp) | YPO0646 putative glycoprotease                        | 0.568            | 0.009     |
| YPTB3710 (fkhA) | YPO0195 peptidyl-prolyl cis-trans isomerase           | 0.571            | 0.007     |
| YPTB3728   | YPO0176 conserved hypothetical protein                | 1.988            | 0.002     |
| YPTB3734 (ppiA) | YPO0281 hemin-binding periplasmic protein              | 1.684            | 0.015     |
| YPTB3930 (fhaE) | YPO0285 putative formate dehydrogenase formation     | 0.556            | < 0.001   |
| YPTB0071   | YPO0075 putative exported protein                     | 0.337            | < 0.001   |
| YPTB0270 (trkK) | YPO3762 Trk system potassium uptake protein TrkH     | 0.634            | 0.018     |
| YPTB0336 (hnuV) | YPO0279 hemin transport system ATP-binding protein   | 1.666            | 0.003     |
| YPTB0338 (hnuT) | YPO0281 hemin-binding periplasmic protein             | 1.684            | 0.015     |
| YPTB0339 (hnuS) | YPO0282 hemin transport protein                       | 1.577            | 0.021     |
| YPTB0340 (hnuR) | YPO0283 hemin receptor precursor                     | 7.426            | < 0.001   |
| YPTB0343   | YPO0285 conserved hypothetical protein                | 1.774            | 0.009     |
| YPTB0354 (terB) | YPO0296 tellurite resistance protein                 | 0.504            | 0.002     |
| YPTB0371   | YPO0150 putative regulatory protein                   | 0.409            | < 0.001   |
| YPTB0516 (phnG) | YPO3457 PhnG protein                                 | 1.713            | 0.016     |
| YPTB0521   | YPO3452 putative ABC transporter transporter. ATP-binding protein | 1.679          | 0.029     |
| YPTB0594   | YPO0451 putative cation-transporting P-type ATPase   | 0.653            | 0.046     |
| YPTB0662 (fhuP) | YPO0521 thiamine transport system permease protein  | 1.973            | 0.003     |
| YPTB0739 (fhuC) | YPO3392 ferrichrome transport ATP-binding protein FhuC | 1.932          | 0.046     |
| YPTB0740 (fhuD) | YPO3391 ferrichrome-binding periplasmic protein        | 1.835            | 0.021     |
| YPTB0790 (yihA) | YPO3342 putative cytochrome C peroxidase             | 0.479            | < 0.001   |
| YPTB0811 (ktatY) | YPO3319 catalase-peroxidase                         | 0.602            | 0.008     |
| YPTB0986   | YPO3130 conserved hypothetical protein                | 0.652            | 0.01      |
| YPTB1246 (katA) | YPO1207 catalase                                     | 0.324            | < 0.001   |
| YPTB1341   | YPO1310 putative periplasmic substrate-binding protein | 3.085            | < 0.001   |
| YPTB1343 (jiuC) | YPO1312 putative siderophore ABC transporter. ATP-binding subunit | 2.467          | < 0.001   |
| YPTB1409 (focA) | YPO1384 putative formate transporter                 | 0.563            | 0.005     |
| YPTB1549 (ysuR) | YPO1537 putative iron-siderophore receptor           | 2.609            | < 0.001   |
| YPTB1659 (frnA) | YPO1783 ferritin                                     | 0.18             | < 0.001   |

**P: inorganic ion transport and metabolism**

| Gene       | Description                                           | Log2 Fold Change | p Value   |
|------------|-------------------------------------------------------|------------------|-----------|
| YPTB0071   | YPO0075 putative exported protein                     | 0.337            | < 0.001   |
| YPTB0270 (trkK) | YPO3762 Trk system potassium uptake protein TrkH     | 0.634            | 0.018     |
| YPTB0336 (hnuV) | YPO0279 hemin transport system ATP-binding protein   | 1.666            | 0.003     |
| YPTB0338 (hnuT) | YPO0281 hemin-binding periplasmic protein             | 1.684            | 0.015     |
| YPTB0339 (hnuS) | YPO0282 hemin transport protein                       | 1.577            | 0.021     |
| YPTB0340 (hnuR) | YPO0283 hemin receptor precursor                     | 7.426            | < 0.001   |
| YPTB0343   | YPO0285 conserved hypothetical protein                | 1.774            | 0.009     |
| YPTB0354 (terB) | YPO0296 tellurite resistance protein                 | 0.504            | 0.002     |
| YPTB0371   | YPO0150 putative regulatory protein                   | 0.409            | < 0.001   |
| YPTB0516 (phnG) | YPO3457 PhnG protein                                 | 1.713            | 0.016     |
| YPTB0521   | YPO3452 putative ABC transporter transporter. ATP-binding protein | 1.679          | 0.029     |
| YPTB0594   | YPO0451 putative cation-transporting P-type ATPase   | 0.653            | 0.046     |
| YPTB0662 (fhuP) | YPO0521 thiamine transport system permease protein  | 1.973            | 0.003     |
| YPTB0739 (fhuC) | YPO3392 ferrichrome transport ATP-binding protein FhuC | 1.932          | 0.046     |
| YPTB0740 (fhuD) | YPO3391 ferrichrome-binding periplasmic protein        | 1.835            | 0.021     |
| YPTB0790 (yihA) | YPO3342 putative cytochrome C peroxidase             | 0.479            | < 0.001   |
| YPTB0811 (ktatY) | YPO3319 catalase-peroxidase                         | 0.602            | 0.008     |
| YPTB0986   | YPO3130 conserved hypothetical protein                | 0.652            | 0.01      |
| YPTB1246 (katA) | YPO1207 catalase                                     | 0.324            | < 0.001   |
| YPTB1341   | YPO1310 putative periplasmic substrate-binding protein | 3.085            | < 0.001   |
| YPTB1343 (jiuC) | YPO1312 putative siderophore ABC transporter. ATP-binding subunit | 2.467          | < 0.001   |
| YPTB1409 (focA) | YPO1384 putative formate transporter                 | 0.563            | 0.005     |
| YPTB1549 (ysuR) | YPO1537 putative iron-siderophore receptor           | 2.609            | < 0.001   |
| YPTB1659 (frnA) | YPO1783 ferritin                                     | 0.18             | < 0.001   |
Table 2: *Y. pseudotuberculosis* IP32953 chromosomal genes (sorted by COG class [28]) that are transcriptionally regulated by growth medium and/or temperature. (Continued)

| Gene ID | Description                      | Log2 Fold Change | p-value  |
|---------|----------------------------------|------------------|----------|
| YPTB1725 | YPO1854 putative membrane protein | 2.084            | (0.013)  |
| YPTB1938 | YPO1941 putative membrane protein | 1.776            | (0.003)  |
| YPTB1942 | YPO1942 putative exported protein | 1.722            | (0.025)  |
| YPTB1975 | YPO1949 putative tellurite resistance protein | 0.549            | (< 0.001) |
| YPTB2061 | YPO2061 exported high-affinity zinc uptake system protein | 1.693            | (0.009)  |
| YPTB2069 | YPO2069 putative integral membrane protein | 1.506            | (0.041)  |
| YPTB2185 | YPO2185 oligopeptide transport ATP-binding protein | 1.188            | (< 0.001) |
| YPTB2236 | YPO2236 superoxide dismutase [Fe] | 1.141            | (< 0.001) |
| YPTB2241 | YPO2241 chelated iron transport system membrane protein | 1.722            | (0.009)  |
| YPTB2250 | YPO2250 putative DNA-binding protein | 1.566            | (< 0.001) |
| YPTB2258 | YPO2258 iron(III)-binding periplasmic protein | 2.387            | (< 0.001) |
| YPTB2267 | YPO2267 conserved hypothetical protein | 1.598            | (0.049)  |
| YPTB2271 | YPO2271 putative binding protein-dependent transport system. inner-membrane comp protein | 0.592            | (0.026)  |
| YPTB2283 | YPO2283 putative exopolysphosphatase | 0.564            | (< 0.001) |
| YPTB2290 | YPO2290 putative potassium channel protein | 0.236            | (< 0.001) |
| YPTB2292 | YPO2292 ABC transporter permease protein | 1.328            | (0.027)  |
| YPTB2297 | YPO2297 cutF putative copper homeostasis lipoprotein | 1.794            | (0.001)  |
| YPTB2306 | YPO2306 putative carbonic anhydrase | 0.344            | (< 0.001) |
| YPTB2309 | YPO2309 putative sulfatase | 0.592            | (0.026)  |
| YPTB2312 | YPO2312 putative periplasmic substrate-binding transport protein | 1.622            | (0.023)  |
| YPTB2327 | YPO2327 putative TonB-dependent outer membrane receptor | 2.15             | (0.004)  |
| YPTB2365 | YPO2365 putative periplasmic binding protein | 1.534            | (0.048)  |
| YPTB2370 | YPO2370 bfr putative bacterioferritin | 0.66             | (0.003)  |
| YPTB2371 | YPO2371 bfrb putative bacterioferritin-associated ferredoxin | 1.856            | (0.002)  |
| YPTB2372 | YPO2372 conbained hypothetical protein | 1.467            | (0.009)  |
| YPTB2373 | YPO2373 putative membrane receptor protein (pseudogene. insert) | 0.568            | (0.001)  |
| YPTB2374 | YPO2374 hypothesized ferrous iron transport protein A | 1.509            | (< 0.045) |
| YPTB2385 | YPO2385 putative iron transport protein | 3.127            | (< 0.001) |
| YPTB2386 | YPO2386 putative iron transport permease | 2.236            | (< 0.001) |
| YPTB2395 | YPO2395 putative iron ABC transporter. ATP-binding protein | 2.216            | (< 0.001) |
| YPTB2396 | YPO2396 superoxide dismutase [Mn] | 3.101            | (< 0.001) |
| YPTB2397 | YPO2397 putative phosphate-binding periplasmic protein | 1.741            | (< 0.001) |

**Q: secondary metabolite biosynthesis, transport and catabolism**

| Gene ID | Description                      | Log2 Fold Change | p-value  |
|---------|----------------------------------|------------------|----------|
| YPTB1030 | YPO1030 putative permease | 1.661            | (0.022)  |
| YPTB1480 | YPO1480 putative acyl carrier protein | 1.669            | (0.048)  |
| Gene ID  | Description                        | Log2 Fold Change | P-value  |
|---------|------------------------------------|-----------------|----------|
| YPTB1544 | putative siderophore biosynthetic enzyme | 2.403           | 0.005    |
| YPTB1550 | putative siderophore biosynthetic enzyme | 8.255           | <0.001   |
| YPTB1596  | yersiniabactin biosynthetic protein  | 1.526           | 0.029    |
| YPTB1966  | imidazolonepropionase                | 0.632           | 0.004    |
| YPTB2064  | putative fumarylacetoacetate hydrolase family protein | 0.73            | 0.018    |
| YPTB2470  | 3-oxoacyl-[acyl-carrier protein] reductase | 0.661           | 0.002    |
| YPTB2561  | menaquinone-specific isochorismate synthase | 0.522           | 0.011    |
| YPTB2626  | 3-oxoacyl-[acyl-carrier-protein] synthase I | 0.605           | 0.009    |
| YPTB3258  | N-aclyhomoserine lactone synthase YspI | 0.494           | 0.032    |
| YPTB3263  | aerobactin synthetase (subunit alpha) | 3.789           | <0.001   |
| YPTB3265  | aerobactin synthetase (subunit beta) | 2.601           | <0.001   |
| YPTB3266  | putative siderophore biosynthesis protein IucD | 2.151           | 0.002    |
| YPTB3297  | putative peptide/polyketide synthase subunit | 2.142           | <0.001   |
| YPTB0026  | conserved hypothetical protein       | 0.616           | 0.018    |
| YPTB0057  | threonine 3-dehydrogenase            | 0.663           | 0.041    |
| YPTB0063  | protein-export protein               | 0.683           | 0.012    |
| YPTB0071  | putative exported protein            | 0.337           | <0.001   |
| YPTB0156  | putative chaperone protein           | 0.657           | 0.01     |
| YPTB0158  | putative outer membrane usher protein | 1.626           | 0.016    |
| YPTB0221  | cell division protein               | 0.71            | 0.035    |
| YPTB0257  | ubiquinone biosynthesis protein      | 0.68            | 0.048    |
| YPTB0258  | Sec-independent protein translocate protein tatA | 0.685           | 0.003    |
| YPTB0327  | putative type III secretion apparatus protein | 0.658           | 0.014    |
| YPTB0331  | putative integral membrane protein   | 2.083           | <0.001   |
| YPTB0353  | putative tellurite resistance protein | 0.565           | <0.001   |
| YPTB0359  | putative outer membrane fimbrial usher protein | 1.398           | 0.049    |
| YPTB0374  | quinone oxidoreductase               | 1.393           | 0.04     |
| YPTB0448  | putative exported protein            | 0.451           | <0.001   |
| YPTB0466  | putative membrane protein            | 1.768           | 0.019    |
| YPTB0493  | probable outer membrane efflux lipoprotein | 1.863           | 0.015    |
| YPTB0576  | osmotically inducible protein Y      | 3.79            | <0.001   |
| YPTB0706  | putative type II secretion system protein | 0.711           | 0.043    |
| YPTB0808  | conserved hypothetical protein       | 0.555           | 0.031    |
| YPTB0832  | putative membrane protein            | 1.401           | 0.011    |
| YPTB0839  | anaerobic C4-dicarboxylate transporter | 0.442           | 0.001    |
| YPTB0878  | S-methylthioribose kinase            | 1.955           | 0.029    |
| YPTB0929  | putative membrane protein            | 1.526           | 0.047    |
| YPTB0965  | conserved hypothetical protein       | 1.553           | 0.047    |
| YPTB1061  | putative autotransporter protein     | 1.82            | 0.003    |
| YPTB1111  | conserved hypothetical protein       | 0.577           | 0.001    |
| YPTB1155  | conserved hypothetical protein       | 1.404           | 0.003    |
| YPTB1159  | TolB colicin import protein         | 1.46            | 0.025    |
Table 2: *Y. pseudotuberculosis* IP32953 chromosomal genes (sorted by COG class [28]) that are transcriptionally regulated by growth medium and/or temperature. (Continued)

| Gene  | Description                                                                 | Log2 Ratio | p-value |
|-------|------------------------------------------------------------------------------|------------|---------|
| YPTB1194 | YPO1163 putative membrane protein                                             | 0.678      | < 0.001 |
| YPTB1210 | or4494 possible ABC transporter multidrug efflux pump. per sease subunit     | 0.669      | 0.03    |
| YPTB1312 | YPO1289 conserved hypothetical protein                                         | 1.662      | 0.026   |
| YPTB1325 | (psaB) chaperone protein PsB precursroR                                      | 0.216      | < 0.001 |
| YPTB1512 | YPO1496 putative heme-binding protein                                         | 2.456      | < 0.001 |
| YPTB1513 | YPO1497 ABC transporter ATP-binding protein                                   | 1.878      | 0.009   |
| YPTB1540 | (psaB) YPO1528 putative ferric iron reductase                                 | 4.019      | < 0.001 |
| YPTB1646 | (hpaC) YPO1770 4-hydroxophenylacetate 3-monoxygenase coupling protein          | 1.613      | 0.023   |
| YPTB1660 | YPO1784 putative copper resistance protein                                     | 3.934      | < 0.001 |
| YPTB1680 | (flg) YPO1807 flagellar protein Flg                                          | 1.906      | 0.01    |
| YPTB1693 | YPO1820A flagellar motor switch protein FlIN                                  | 2.053      | 0.01    |
| YPTB1695 | YPO1822 trp repressor binding protein                                         | 1.401      | 0.006   |
| YPTB1733 | YPO1863 putative membrane protein                                             | 0.626      | -       |
| YPTB1919 | YPO1920 probable fimbral usher protein                                        | 1.461      | 0.019   |
| YPTB1944 | YPO1946 ABC transporter. ATP-binding protein                                  | 1.957      | 0.003   |
| YPTB1985 | YPO1993 putative dehydrogenase                                                | 1.586      | 0.007   |
| YPTB2019 | YPO2037 conserved hypothetical protein                                        | 1.56       | 0.021   |
| YPTB2101 | (hns) YPO2175 Hns DNA binding protein                                         | 1.549      | 0.027   |
| YPTB2169 | or1554 putative toxin transport protein (pseudogene. F/S)                     | 1.508      | 0.006   |
| YPTB2289 | YPO2375 putative aldo/keto reductase                                          | 1.507      | 0.018   |
| YPTB2291 | YPO2377 putative membrane protein                                             | 1.529      | 0.038   |
| YPTB2345 | (marC) YPO2437 multiple antibiotic resistance protein                         | 1.771      | 0.022   |
| YPTB2368 | (ogl) YPO1713 oligogalacturonate lyase                                        | 0.699      | 0.045   |
| YPTB2390 | YPO1689 putative lipoprotein                                                  | 1.312      | 0.032   |
| YPTB2452 | (ycfL) YPO1612 putative lipoprotein                                           | 1.45       | 0.046   |
| YPTB2459 | or3719 hypothetical                                                           | 0.379      | < 0.001 |
| YPTB2471 | (fagC) YPO1599 3-oxoacyl-[acyl-carrier protein] reductase                      | 0.573      | 0.002   |
| YPTB2488 | YPO2451 conserved hypothetical protein                                         | 0.671      | 0.026   |
| YPTB2492 | or3693 conserved hypothetical protein                                         | 0.602      | 0.009   |
| YPTB2553 | or3647 conserved hypothetical protein                                         | 0.573      | 0.006   |
| YPTB2604 | or3598 conserved hypothetical (pseudogene. F/S)                               | 1.86       | 0.004   |
| YPTB2646 | (ccmD) YPO3557 putative heme exporter protein D                                | 0.36       | < 0.001 |
| YPTB2722 | YPO3001 putative pyridine nucleotide-disulphide oxidoreductase                 | 1.897      | 0.04    |
| YPTB2723 | YPO3002 putative permease                                                      | 1.328      | 0.036   |
| YPTB2727 | YPO3007 putative membrane protein                                             | 1.932      | 0.008   |
| YPTB2733 | YPO3031 putative acetyltransferase                                            | 1.391      | 0.02    |
| YPTB2837 | (engA) YPO2875 putative GTP-binding protein                                    | 0.573      | 0.015   |
| YPTB2843 | YPO2881 putative fimbral biogenesis protein                                   | 0.868      | 0.022   |
| YPTB2891 | (lepB) YPO2717 signal peptidase I                                             | 1.527      | 0.032   |
| YPTB2902 | YPO2706 conserved hypothetical protein                                        | 0.549      | 0.026   |
| YPTB3116 | or3203 hypothetical protein                                                   | 0.556      | 0.022   |
| YPTB3176 | YPO2451 putative hemolysin III                                                | 1.415      | 0.038   |
Table 2: *Y. pseudotuberculosis* IP32953 chromosomal genes (sorted by COG class [28]) that are transcriptionally regulated by growth medium and/or temperature. (Continued)

| GenBank Accession | YPO Accession | Description | Log2 Fold Change | P-Value |
|-------------------|---------------|-------------|------------------|---------|
| YPTB3223          | YPO0951       | Putative methyltransferase | 0.612       | (0.023) |
| YPTB3238          | YPO0966       | Putative kinase | 0.505       | (0.032) |
| YPTB3285          | or0382        | Putative autotransporter secreted protein | 1.593       | (0.028) |
| YPTB3291          | YPO0771       | ABC-transporter transmembrane protein | 1.705       | (0.014) |
| YPTB3357          | YPO0704       | Flagellar assembly protein | 0.611       | (0.049) |
| YPTB3381          | YPO0684       | Putative membrane protein | 1.67        | (0.014) |
| YPTB3382 (exbD)   | YPO0683       | ExbD/TolR-family transport protein | 9.812       | (< 0.001) |
| YPTB3383 (exbB)   | YPO0682       | MotA/TolQ/ExbB proton channel family protein | 4.164       | (< 0.001) |
| YPTB3388          | YPO0676       | Putative aldo/keto reductase family protein | 1.632       | (0.042) |
| YPTB3438          | YPO0617       | Conserved hypothetical protein | 1.489       | (0.008) |
| YPTB3464          | YPO0595       | Conserved hypothetical protein | 0.553       | (< 0.001) |
| YPTB3493          | YPO3548       | Putative exported protein | 0.602       | (0.005) |
| YPTB3496          | YPO3551       | Putative exported protein | 1.498       | (0.006) |
| YPTB3538 (tidD)   | YPO3672       | Putative modulator of DNA gyrase | 0.702       | (0.025) |
| YPTB3568          | YPO3662       | Conserved hypothetical protein | 0.528       | (0.005) |
| YPTB3659          | YPO0247       | Putative transferase | 1.289       | (0.029) |
| YPTB3745 (gph)    | YPO0156       | Phosphoglycolate phosphatase | 0.596       | (0.022) |
| YPTB3757          | YPO0144       | Putative hydrolase | 1.58        | (0.013) |
| YPTB3879 (or2640) | YPO3551       | Possible type I restriction enzyme (restriction subunit) | 1.554       | (0.044) |
| YPTB3896          | YPO3662       | Conserved hypothetical protein | 0.666       | (0.024) |
| YPTB3939          | YPO0020       | Conserved hypothetical protein | 0.493       | (< 0.001) |
| YPTB3948 (yidC)   | YPO4120       | Probable membrane protein | 0.673       | (0.046) |
| YPTB3953 (yieG)   | YPO4107       | Xanthine/uracil permeases family protein | 1.583       | (0.042) |

S: function unknown

| GenBank Accession | YPO Accession | Description | Log2 Fold Change | P-Value |
|-------------------|---------------|-------------|------------------|---------|
| YPTB0015 (mobA)   | YPO0013A      | Molybdopterin-guanine dinucleotide biosynthesis protein A | 0.703       | (0.032) |
| YPTB0020          | YPO0020       | Conserved hypothetical protein | 0.493       | (< 0.001) |
| YPTB0040          | YPO0043       | Conserved hypothetical protein | 0.705       | (0.029) |
| YPTB0089          | YPO0093       | Conserved hypothetical protein | 1.682       | (< 0.001) |
| YPTB0196 (or1333) | YPO3816A      | Conserved hypothetical protein | 3.061       | (< 0.001) |
| YPTB0219          | YPO3732       | Conserved hypothetical protein | 1.383       | (0.046) |
| YPTB0296          | YPO3732       | Conserved hypothetical protein | 0.558       | (< 0.001) |
| YPTB0378          | YPO0323       | Conserved hypothetical protein | 1.483       | (0.016) |
| YPTB0454          | YPO3522       | Conserved hypothetical protein | 1.472       | (0.03) |
| YPTB0478          | YPO3498       | Conserved hypothetical protein | 0.734       | (0.038) |
| YPTB0506 (or367)  | YPO3498       | Conserved hypothetical protein | 0.541       | (0.013) |
| YPTB0547          | YPO0407       | Conserved hypothetical protein | 1.999       | (< 0.001) |
| YPTB0589          | YPO0445       | Conserved hypothetical protein | 1.579       | (0.007) |
| YPTB0600 (creA)   | YPO0457       | Putative exported protein | 1.924       | (< 0.001) |
| YPTB0627          | YPO0485       | Putative membrane protein | 1.523       | (0.018) |
| YPTB0639          | YPO0498       | Hypothetical protein | 0.302       | (< 0.001) |
| YPTB0640          | YPO0499       | Hypothetical protein | 0.302       | (< 0.001) |
| YPTB0641          | YPO0500       | Conserved hypothetical protein | 0.346       | (< 0.001) |
| YPTB0642          | YPO0501       | Conserved hypothetical protein | 0.346       | (< 0.001) |
| YPTB0643          | YPO0502       | Conserved hypothetical protein | 0.702       | (0.012) |
| YPTB0644          | YPO0503       | Conserved hypothetical protein | 0.702       | (0.012) |
| Gene    | Description                                                                 | Fold Change | p-value   |
|---------|-----------------------------------------------------------------------------|-------------|-----------|
| YPTB0646 | YPO0050 conserved hypothetical protein                                        | 0.355       | (<0.001)  |
| YPTB0648 | YPO0057 conserved hypothetical protein                                        | 0.679       | (0.044)   |
| YPTB0649 | YPO0058 hypothetical protein                                                 | 0.56        | (0.031)   |
| YPTB0650 | YPO00510 hypothetical protein                                                | 1.447       | (0.043)   |
| YPTB0653 | YPO00512 putative lipoprotein                                                | 1.45        | (0.035)   |
| YPTB0654 | YPO00513 conserved hypothetical protein                                       | 0.364       | (0.002)   |
| YPTB0655 | YPO00514 putative OmpA-family membrane protein                               | 0.383       | (<0.001)  |
| YPTB0679 | YPO0546 conserved hypothetical protein                                        | 1.35        | (0.036)   |
| YPTB0701 | YPO0512 putative lipoprotein                                                 | 0.663       | (<0.001)  |
| YPTB0744 | YPO0513 conserved hypothetical protein                                       | 2.792       | (<0.001)  |
| YPTB0876 | or0627 methionine salvage pathway enzyme E-2/E-2'                            | 1.533       | (0.007)   |
| YPTB0976 | YPO1400 putative lipoprotein                                                 | 1.504       | (0.043)   |
| YPTB1021 | YPO1407 conserved hypothetical protein                                       | 1.394       | (0.029)   |
| YPTB1057 | YPO2801 putative membrane protein                                            | 1.682       | (0.044)   |
| YPTB1078 | YPO2584 putative carbohydrate kinase                                         | 1.704       | (0.035)   |
| YPTB1085 | YPO2592 putative membrane protein                                            | 0.609       | (0.015)   |
| YPTB1161 | YPO1126 putative exported protein                                            | 0.596       | (0.004)   |
| YPTB1215 | YPO1174 hypothetical protein                                                 | 1.527       | (0.047)   |
| YPTB1222 | YPO1181 putative membrane protein                                            | 0.697       | (0.032)   |
| YPTB1227 | YPO1186 conserved hypothetical protein                                       | 0.634       | (0.005)   |
| YPTB1297 | YPO1261 conserved hypothetical protein                                       | 0.659       | (0.014)   |
| YPTB1387 | YPO1361 putative membrane protein                                            | 1.593       | (0.003)   |
| YPTB1389 | YPO1363 putative virulence factor                                            | 0.632       | (0.043)   |
| YPTB1422 | YPO1397 conserved hypothetical protein                                       | 1.803       | (0.012)   |
| YPTB1432 | YPO1408 putative exported protein                                            | 0.671       | (0.044)   |
| YPTB1499 | YPO1483 hypothetical protein                                                 | 1.491       | (0.029)   |
| YPTB1504 | YPO1487 conserved hypothetical protein                                       | 2.062       | (0.005)   |
| YPTB1571 | YPO1560 conserved hypothetical protein                                       | 1.34        | (0.036)   |
| YPTB1640 | YPO1764 3.4-dihydroxyphenylacetate 2,3-dioxygenase                           | 0.625       | (0.046)   |
| YPTB1729 | YPO1858 putative exported protein                                            | 0.704       | (0.037)   |
| YPTB1901 | or1366 conserved hypothetical protein                                        | 1.511       | (0.01)    |
| YPTB1902 | YPO1882 conserved hypothetical protein                                       | 1.552       | (<0.001)  |
| YPTB1941 | YPO1943 putative membrane protein                                            | 2.118       | (0.004)   |
| YPTB2085 | YPO2159 conserved hypothetical protein                                       | 0.606       | (0.032)   |
| YPTB2146 | YPO2224 conserved hypothetical protein                                       | 1.568       | (0.018)   |
| YPTB2214 | YPO2291 putative virulence factor                                            | 0.638       | (0.023)   |
| YPTB2234 | YPO2312 putative exported protein                                            | 1.503       | (0.008)   |
| YPTB2265 | YPO2347 putative membrane protein                                            | 1.284       | (0.04)    |
| YPTB2314 | YPO2404 conserved hypothetical protein                                       | 2.602       | (<0.001)  |
| YPTB2352 | YPO2444 conserved hypothetical protein                                       | 0.704       | (0.017)   |
| YPTB2388 | YPO1693 conserved hypothetical protein                                       | 1.569       | (0.038)   |
| YPTB2444 | YPO1624 putative exported protein                                            | 2.296       | (0.001)   |
| YPTB2481 | YPO1588 conserved hypothetical protein                                       | 0.759       | (0.035)   |
| YPTB2526 | YPO2489 conserved hypothetical protein                                       | 1.361       | (0.02)    |
| YPTB2547 | YPO2510 putative exported protein                                            | 0.31        | (<0.001)  |
| YPTB2594 | YPO2563 conserved hypothetical protein                                       | 0.563       | (<0.001)  |
Table 2: Y. pseudotuberculosis IP32953 chromosomal genes (sorted by COG class [28]) that are transcriptionally regulated by growth medium and/or temperature. (Continued)

| Gene   | COG ID   | Description                               | Expression Ratio | P-value  |
|--------|----------|-------------------------------------------|------------------|----------|
| YPTB2638 | YPO2745 | conserved hypothetical protein            | 0.483            | (0.001)  |
| YPTB2651 | YPO2732 | putative exported protein                 | 0.381            | (< 0.001)|
| YPTB2660 | YPO2724 | putative membrane protein                 | 1.486            | (0.038)  |
| YPTB2661 | YPO2723 | possible OmpA family (pseudogene. IS100 insertion) | 1.796            | (0.048)  |
| YPTB2674 | YPO2949 | hypothetical protein                      | 0.721            | (0.033)  |
| YPTB2693 | YPO2970 | putative lipoprotein                      | 0.658            | (0.038)  |
| YPTB2694 | YPO2971 | putative lipoprotein                      | 0.648            | (0.002)  |
| YPTB2745 | YPO3027 | putative exported protein                 | 1.573            | (0.023)  |
| YPTB2907 | YPO2701 | putative membrane protein                 | 0.612            | (0.006)  |
| YPTB2981 | YPO1065 | conserved hypothetical protein            | 0.69             | (0.039)  |
| YPTB3117 | YPO0874 | putative antigen leucine-rich repeat protein | 0.753            | (0.006)  |
| YPTB3169 | YPO0659 | conserved hypothetical protein            | 0.721            | (0.038)  |
| YPTB3201 | YPO0590 | putative membrane protein                 | 0.64             | (0.033)  |
| YPTB3203 | YPO0572 | putative exported protein                 | 0.68             | (0.034)  |
| YPTB3204 | YPO0569A| putative membrane protein                 | 0.62             | (0.026)  |
| YPTB3206 | YPO0569 | conserved hypothetical protein            | 0.69             | (0.039)  |
| YPTB3222 | YPO0595 | conserved hypothetical protein            | 0.753            | (0.019)  |
| YPTB3231 | YPO0669 | conserved hypothetical protein            | 0.753            | (0.019)  |
| YPTB3402 | YPO0590 | putative membrane protein                 | 0.209            | (< 0.001) |
| YPTB3403 | YPO0570 | putative membrane protein                 | 0.674            | (0.02)   |
| YPTB3404 | YPO0571 | putative membrane protein                 | 0.576            | (0.002)  |
| YPTB3405 | YPO0572 | putative membrane protein                 | 0.721            | (0.033)  |
| YPTB3406 | YPO0573 | putative membrane protein                 | 0.732            | (0.028)  |
| YPTB3407 | YPO0574 | putative membrane protein                 | 0.609            | (0.015)  |
| YPTB3617 | YPO0575 | putative gamma carboxymuconolactone decaboxylase | 0.573            | (0.01)   |
| YPTB3622 | YPO0576 | putative Rhs accessory genetic element    | 0.589            | (0.014)  |
| YPTB3748 | YPO0577 | putative hypothetical protein             | 0.614            | (0.033)  |
| YPTB3773 | YPO0578 | putative hypothetical protein             | 2.928            | (< 0.001) |
| YPTB3897 | YPO0579 | putative membrane protein                 | 0.753            | (0.006)  |
| T: signal transduction mechanisms |
| YPTB0022 | YPO0022 | nitrogen regulation protein               | 0.723            | (0.037)  |
| YPTB0035 | YPO0038 | guanosine-3',5'-bis(bisphosphate) 3'-pyrophosphodrolase | 0.684            | (0.011)  |
| YPTB0071 | YPO0039 | putative exported protein                 | 0.337            | (< 0.001) |
| YPTB0356 | YPO0040 | tellurium resistance protein              | 1.485            | (0.034)  |
| YPTB0357 | YPO0041 | tellurium resistance protein              | 1.501            | (0.005)  |
| YPTB0468 | YPO0042 | two-component system sensor protein       | 2.184            | (0.001)  |
| YPTB0541 | YPO0043 | putative transcriptional regulator       | 0.652            | (0.035)  |
| YPTB0570 | YPO0044 | HmsT protein                              | 0.574            | (0.014)  |
| YPTB0592 | YPO0045 | aerobic respiration control protein       | 0.465            | (0.001)  |
| YPTB0734 | YPO0046 | DnaK suppressor protein homologue         | 0.508            | (< 0.001) |
| YPTB0789 | YPO0047 | probable extracellular solute-binding protein | 0.462            | (0.001)  |
Table 2: Y. pseudotuberculosis IP32953 chromosomal genes (sorted by COG class [28]) that are transcriptionally regulated by growth medium and/or temperature. (Continued)

| Gene  | Description                                                | Ratio     | P-value     |
|-------|------------------------------------------------------------|-----------|-------------|
| YPTB1108 (glnH) | YPO2615 putative amino acid-binding protein precursor      | 0.564     | (0.005)     |
| YPTB1258 (rcsB) | YPO1218 putative two component response regulator component B | 0.693     | (0.016)     |
| YPTB1259 | YPO1219 putative two component sensor kinase               | 0.696     | (0.041)     |
| YPTB1922 | YPO1923 putative sensor protein                            | 1.451     | (0.044)     |
| YPTB1957 (narX) | YPO1959 nitrate/nitrite sensor protein                     | 1.533     | (0.043)     |
| YPTB2099 | YPO2173 probable response regulator                         | 0.624     | (0.045)     |
| YPTB2156 (cstA) | YPO2234 putative carbon starvation protein A               | 0.544     | (0.012)     |
| YPTB2222 (fnr) | YPO2300 fumarate and nitrate reduction regulatory protein  | 0.699     | (0.001)     |
| YPTB2230 (rstA) | YPO2308 two-component regulatory system. response regulator protein | 0.658     | (0.024)     |
| YPTB2378 | YPO1703 conserved hypothetical protein                      | 0.711     | (0.022)     |
| YPTB2396 (cheZ) | YPO1681 chemotaxis protein CheZ                           | 1.477     | (0.027)     |
| YPTB2405 (cheA) | YPO1666 chemotaxis protein CheA                            | 1.725     | (0.015)     |
| YPTB2435 (phoQ) | YPO1633 sensor protein kinase                              | 0.527     | (< 0.001)   |
| YPTB2548 (glnH) | YPO2511 putative glutamine-binding periplasmic protein      | 1.797     | (0.014)     |
| YPTB2635 (sdsA) | YPO2748 putative phosphohistidine phosphatase              | 1.508     | (0.002)     |
| YPTB2763 (narP) | YPO3041 nitrate/nitrite response regulator protein NarP   | 0.715     | (0.043)     |
| YPTB2899 (rsC) | YPO2714 sigma E factor regulatory protein                  | 1.431     | (0.034)     |
| YPTB2895 (rsB) | YPO2713 sigma E factor regulatory protein                  | 1.466     | (0.04)      |
| YPTB2896 (rsA) | YPO2712 sigma E factor negative regulatory protein         | 1.652     | (< 0.001)   |
| YPTB3350 (flrE) | YPO0712 sigma-54 transcriptional regulatory protein        | 2.134     | (0.004)     |
| YPTB3408 (glnE) | YPO0653 glutamate-ammonia-ligase adenyltransferase         | 1.792     | (0.046)     |
| YPTB3410 | YPO0651 putative exported protein                          | 1.988     | (< 0.001)   |
| YPTB3463 (terX) | YPO0596 putative tellurium resistance protein              | 0.522     | (< 0.001)   |
| YPTB3500 (arcB) | YPO3555 aerobic respiration control sensor/response regulatory protein | 0.674     | (0.012)     |
| YPTB3566 (yhdA) | YPO3664 putative exported protein                          | 1.836     | (< 0.001)   |
| YPTB3729 (cpr) | YPO0175 cAMP-regulatory protein                            | 0.641     | (< 0.001)   |
| YPTB3812 (uspA) | YPO3970 universal stress protein A                         | 0.291     | (< 0.001)   |
| YPTB3847 (uhpA) | YPO4012 two-component system response regulator            | 1.59      | (0.006)     |
| YPTB3957 | YPO4111 putative periplasmic solute-binding protein        | 0.539     | (< 0.001)   |
| YPTB2341 (infC) | YPO2432 translation initiation factor IF-3                 | 1.554     | (0.038)     |

Continued...
that encodes catalase (a ferric enzyme involved in oxidative stress defense), whose transcription is decreased in both Y. pestis and Y. pseudotuberculosis during growth in plasma (Fig. 1). However, the increase in transcription of the bio locus (required for biotin synthesis [13]) and observed in both species) suggests that differential genetic control of a subset of iron-dependent enzymes may favor supply of this metal to the pathways that are most important for bacterial survival (and thus presumably at the expense of other, less critical ones). Furthermore, the impact of transcriptional downregulation on reorientation of metabolic fluxes may be minimized by the concomitant activation of genes coding for isoenzymes that are better suited to this situation.

One example is that of the manganese- and iron-dependent superoxide dismutase genes (i.e. sodA and sodB), which are Fur-activated and -repressed, respectively (Fig. 1) in both Y. pestis and Y. pseudotuberculosis. Similarly, the class Ib ribonucleotide reductase (RNR)-encoding genes (nrdHIEF) are probably important for bacterial life in plasma, since they were found to be upregulated at the expense of those in classes III (nrdDG) and Ia (nrdAB) (Table 2) – even though all three classes are equally...
involved in generating the synthetic precursors for DNA. The fact that only the first class is Fur-activated [14] is consistent with this observation. Similar variations have also been recorded in Y. pestis [8]. However, whereas purine/pyrimidine metabolism has been shown to be essential for Y. pestis virulence [15], the role of this metabolic pathway in the physiopathology of Y. pseudotuberculosis has not yet been investigated. Along with class 1b RNRs, more than half of the enzymes in the tricarboxylic acid cycle (TCA) are known to be catalytically iron-dependent and/or believed to be transcriptionally activated by Fur [16]. Accordingly, and in line with transcriptome data from Y. pestis, we observed that transcription of these genes fell significantly when Y. pseudotuberculosis was grown in plasma.

In contrast to the low availability of iron in blood, glucose is readily available in this biological fluid and at a higher concentration (approx. 7 mM) than in LB broth. When Y. pseudotuberculosis was cultured in plasma, genes involved in glycolysis and the upstream, sugar-supplying, phosphoenolpyruvate-dependent systems were found to be upregulated, as depicted in Fig. 2. This finding is reminiscent of an aerobic phenomenon referred to as "glucose
Medium-dependent differential expression of genes coding for enzymes putatively involved in Y. pseudotuberculosis glycolysis and the tricarboxylic acid cycle (TCA cycle). Significant ($p < 0.05$) upshifts (yellow to red scale) or downshifts (blue scale) in individual gene transcription levels in human plasma versus LB is indicated by the color scale bar. Open boxes indicate genes whose expression levels did not vary significantly ($p > 0.05$). Although considered as not significant by statistical analysis of macroarray data ($p = 0.053$), transcriptional upregulation of aceB in human plasma was confirmed by qRT-PCR. Abbreviations: Ac-CoA: acetyl coenzyme A; PEP: phosphoenolpyruvate. Mean fold changes in transcription and $p$-values are indicated in Table 2.
overflow metabolism"; this consists in channeling the carbon flow towards acetate formation instead of citrate formation, in order to prevent the excessive accumulation of NADH that would otherwise result from very high glucose consumption rates [17]. However, one main feature of glucose overflow in *E. coli* is acetate accumulation due to a strong transcriptional repression of the glyoxylate shunt aceBAK operon [18]. Interestingly, at least the first two of these genes are not down- but are up-regulated in *Y. pseudotuberculosis* (Fig. 2, Additional file 1), suggesting a need for this species to limit acetate overloads. The continuous de-repression of these genes (due to inactivation of the IclR repressor) suggests that this might also be the case in *Y. pestis*. These pathways are controlled by complex and finely balanced networks involving numerous pleiotropic regulators, including Fur, Crp, Fnr and ArcA [16,19]. This unexpected upregulation may well result from the combination of both high glucose and low iron levels in plasma. Whether this occurs through the strong transcriptional repression observed with both *fnr* and *arcA* remains to be addressed in future experiments.

Temperature upshift is typically considered to be the main signal indicating to bacteria that they have entered the host; this hypothesis is supported by the thermal dependency of almost all *Y. pseudotuberculosis* virulence genes and also many of the latter's regulators [3]. Several of these genes were also found to be influenced by growth in plasma and the changes were sometimes in the opposite direction to those seen with temperature upshifts: whereas expression of the invasin-encoding gene *inv* was significantly repressed during bacterial growth under both conditions, transcription of *psaA* (coding for the pH6 antigen) was promoted by temperature upshifts [6,20], but was one of the most strongly repressed in plasma. Interestingly, the impact of this medium on *psaA* transcription was not considered to be significant in *Y. pestis* and suggests that the pH6 antigen does not have the same importance in blood dissemination in the two species. In contrast to the latter two adhesins, transcriptional activation of *yadA* (harbored by the pYV plasmid and involved in adhesion) was found to be the highest of all the *Y. pseudotuberculosis* genes induced under plasma growth conditions. This observation is consistent with YadA's involvement in microbial resistance to complement [21,22]. Similarly, *ompC* whose product is believed to be targeted by lactoferrin [23], a bactericidal peptide derived from lactoferrin by enzymatic cleavage [24], is strongly repressed, whilst no significant modification was observed for the outer membrane-encoding genes *ompA* and *ompC2*.

Lastly, an essential determinant of bacterial virulence is the plasmid-encoded type III secretion system (TTSS) which performs intracellular delivery of a set of *Yersinia* outer proteins (Yops) that subvert the host’s defenses [25]. Interestingly, *Y. pseudotuberculosis* growth in plasma induced the upregulation of 25 genes required for secretion, translocation and chaperoning of the Yop effector proteins in a similar fashion to that observed upon temperature upshift (Fig. 3). Furthermore, the apparently coordinated regulation of *yadA* and the TTSS-encoding genes by temperature and growth in plasma suggests the involvement of a common means of genetic control. YmoA (a chromatin-associated (histone-like) protein which is very similar in structure and function to the haemolysin expression modulating protein Hha from *Escherichia coli*) was shown to negatively influence YadA and Yop expression by favoring supercoiling of the pYV plasmid [26]. A two-fold reduction in ymoA transcription in plasma may be enough to contribute to the TTSS upregulation recorded in *Y. pseudotuberculosis*. Strikingly, this plasma-induced TTSS activation was not observed in *Y. pestis*, since only 3 out of the 25 genes mentioned above were found to be upregulated (in line with the statistically non-significant downregulation of *ymoA*); this raises the possibility that these two pathogenic *Yersinia* species may differ in their transcriptional regulation of pYV-harbored virulence genes.

**Conclusion**

Overall transcription profiling of *Y. pseudotuberculosis* grown in an environment mimicking the blood stage of the infectious process revealed gene regulations that could not be anticipated from the results of previously reported single-stimulus studies. Our findings thus provide insight into how a number of simultaneously sensed environmental cues may be taken into account by the bacterium in a hierarchical manner. Furthermore, comparison of our analyses with those previously performed in *Y. pestis* suggests that transcription of common critical virulence factors may be differently influenced (at least in part) by the plasma environment in these two species.

**Methods**

**DNA macroarray construction**

Pairs of specific oligonucleotide primers were designed with the Primer 3 software for each of the 3,951 *Y. pseudotuberculosis* IP32953 CDSs. In order to avoid cross-hybridization, the specificity of the PCR products relative to the complete genome sequence was tested with CAAT-box software [27]. Primers purchased from Eurogentec were chosen in order to specifically amplify a ≈ 400 to 500 base pair fragment of each open reading frame (ORF), with a melting temperature of 51 to 60°C. Amplification reactions were performed in 96-well plates (Perkin-Elmer) in a 100 μl reaction volume containing 100 ng of *Y. pseudotuberculosis* IP32953 DNA, DNA polymerase (Dynazyme, New England Biolabs), 10 μM of each primer and 2 mM dNTPs (Perkin-Elmer). Reactions were cycled...
Medium- and temperature-dependent differential expression of genes harbored by the Y. pseudotuberculosis virulence plasmid pYV. Significant ($p < 0.05$) upshifts (yellow to red scale) or downshifts (blue scale) in individual gene transcription levels when bacteria were grown in human plasma versus LB (triangles) and/or at 37°C versus 28°C (squares) are indicated by the color scale bar. Only genes spotted on the macroarray (56 out of 99 pYV-borne genes) are shown and those encoding the secretion apparatus and Yop effectors are represented by grey and black boxes, respectively. Mean fold changes in transcription and $p$-values are indicated in Table 3.
tome analysis.
DNA was hybridized to the macroarray before transcrip-
fully deposited on the membranes, 33P-labeled genomic
20°C until use. To ensure that DNA samples were success-
ately following spot deposition, membranes were
branes (Genetix) using a Qpix robot (Genetix). Immedi-
strain IP32953 were spotted onto 22 × 7-cm nylon mem-
ferase DNA (10 to 100 ng) and total genomic DNA from
experimental conditions. ORF-specific PCR products, luci-
IP32953 genome were successfully amplified under our
56°C for 30 min to ensure complement inactivation).

Bacterial culture
The Y. pseudotuberculosis transcriptome was studied in
three independent cultures of strain IP32953 in media
 aliquoted from a single batch. After storage in Luria-Ber-
tani (LB) broth with 40% glycerol at -80°C, the strain was
thawed and then grown on LB agar supplemented with 20
μg ml⁻¹ hemin for 48 h at 28°C. From this culture, 8 × 10⁶
cells were inoculated into 40 ml of either LB broth or
pooled human plasma from healthy donors (heated at
56°C for 30 min to ensure complement inactivation).
Media were then incubated at 28°C or 37°C with shaking
5600 of 0.2–0.4 and 0.1–0.2 for LB and human plasma,
respectively) by centrifugation at 4°C and the pelleted
bacteria were disrupted with RNAwiz reagent (Ambion).
After mixing the lysate with chloroform (0.2 v), total RNA
was precipitated from the aqueous phase with glycoplen
(1/50 v) and isopropanol (1 v). The RNA pellet was
washed with 70% ethanol and then dissolved in sterile,
DNase- and RNase-free water. Contaminating DNA was
removed using the DNA-free kit from Ambion. Nucleic
acid purity and integrity was checked with a BioAnalyzer
System (Agilent) according to the supplier’s instructions. After
quantification by spectrophotometry at 260 and 280
nm, the RNA solution was stored at -80°C until use.

cDNA was purified to remove unincorporated nucleotides
using DyeEx 2.0 spin column (Qiagen).

DNA macroarray hybridization
Macroarrays were prewetted in 2 × SSPE (0.18 M NaCl, 10
mM NaH₂PO₄, 1 mM EDTA, pH 7.7) and prehybridized
for 1 h in 13 ml of hybridization solution (5 × SSPE, 2% 
SDS, 1 × Denhardt’s reagent, 0.1 mg of sheared salmon
sperm DNA ml⁻¹) at 65°C in roller bottles. Hybridization
was carried out for 20 h at 65°C with 15 ml of hybridiza-
sion solution containing the purified cDNA probe. After
hybridization, membranes were washed three times at
room temperature and three times at 65°C for 20 min in
0.5 × SSPE and 0.2% SDS. Probed macroarrays were
exposed to a phosphor screen (Molecular Dynamics) for
24–72 h and imaged using a STORM 860 phosphorim-
ager (Amersham Biosciences). The intensity of all of the
pixels associated with each spot was further quantified using ArrayVision software (Imaging Research, Grinnel,
IA, USA). The experiment design included three biological
replicates for each combination of conditions. Data were
analyzed using the SAS software (SAS Institute Inc, Cary,
NC, USA). They were first log-transformed and normal-
ized with a median normalization. A linear model was
then applied on each gene with the temperature, phase
and growth medium as fixed effects. The significance level
alpha was set to 0.05.

Real-Time Quantitative PCR
Messenger RNAs (mRNAs) were reverse transcribed from
1 μg of nucleic acid by using the High-Capacity cDNA
Archive Kit (Applied Biosystems, Foster City, CA) accord-
to the manufacturer’s instructions. The resulting
cDNA was amplified by the SYBR Green Real-Time PCR
Kit and detected on a Prism 7000 detection system
(Applied Biosystems). The forward and reverse primers
used were as follows: 5'CGCCCATCAAATGCCGCTAAT3'
and 5'TGACCGGGATCGTGTCAA3' for yfeA, 5'TCAA
CGAGGAAACATTCATC3' and 5'GGGTGTCCACCCCGCC
AAAATC3' for psaA, 5'GGTTAGCCGCCGAAACAGAT3'
and 5'CCGCTGGCGACCAAAAGGTT3' for aceB, 5'TGCA
TGTCGCCGCTAAGG3' and 5'GGGTGTCCACCGCTG
CAG3' for ydA, 5'GATCCGTCGTCATCATAAAATAT3'
CAG3' and 5'ATTTGTCGCCGTGACGCA3' for yplB,
5'GAAATCCCGAGCCGCTGTTAA3' and 5'TACCTGCA
TGCCGCTAGT3' for yplN, 5'GACACCACTGGGACG-
CCTA3' and 5'GGGTTCACAGAAAGATAACGCTT3'
for sycH, 5'GGTTAGCCGCCGGTAT3' and 5'CAGGC
CTCTCTGTTTTG3' for ntnK, 5'TTCCTGTGGGACAC
CATCC3' and 5'TCCGTTCGGCAGCACACAA3' for
nlpD. On completion of the PCR amplification, a DNA
melting curve analysis was performed to confirm the pres-
ence of a single amplicon. Relative mRNA levels (2^ΔCt)
were determined by comparing the PCR cycle thresholds
(Ct) for the gene of interest and the constitutively

RNA and cDNA probe preparation
Cells were harvested from exponential-phase cultures
(A₆₀₀ of 0.2–0.4 and 0.1–0.2 for LB and human plasma,
respectively) by centrifugation at 4°C and the pelleted
bacteria were disrupted with RNAwiz reagent (Ambion).
After mixing the lysate with chloroform (0.2 v), total RNA
was precipitated from the aqueous phase with glycoplen
(1/50 v) and isopropanol (1 v). The RNA pellet was
washed with 70% ethanol and then dissolved in sterile,
DNase- and RNase-free water. Contaminating DNA was
removed using the DNA-free kit from Ambion. Nucleic
acid purity and integrity was checked with a BioAnalyzer
2100 (Agilent) according to the supplier’s instructions. After
quantification by spectrophotometry at 260 and 280
nm, the RNA solution was stored at -80°C until use.

50 U AMV reverse transcriptase (Roche), 0.35 pmol.
of each amplified CDS-specific 3' oligonucleotide primer,
222 μM dGTP, dCTP & dTTP, 2.2 μM dCTP and 50 μCi
33P-labelled dCTP (Amersham Biosciences). Labeled

45 times (94°C for 30 s; 60°C for 30 s; 72°C for 60 s) with
a final cycle of 72°C for 7 min in a thermocycler. Each
PCR product was checked by agarose gel electrophoresis
and when DNA amplification was unsuccessful, PCR was
repeated with another primer set. Overall, 3,951 of the
3,994 CDSs (98%) identified in the Y. pseudotuberculosis
IP32953 genome were successfully amplified under our
experimental conditions. ORF-specific PCR products, luci-
ferase DNA (10 to 100 ng) and total genomic DNA from
strain IP32953 were spotted onto 22 × 7-cm nylon mem-

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expressed YPTB0775 gene (spot ID YPO3356) coding for the outer membrane lipoprotein NlpD.

**Abbreviations**

qRT-PCR: quantitative Real Time Reverse Transcription PCR.

**Authors’ contributions**

MLR performed the macroarray hybridizations and participated to the critical proofreading of the manuscript. SC contributed to the experiment set-up and was responsible for bacterial cultures and RNA extractions; she participated in statistical analyses and critical proofreading of the manuscript. RD performed the qPCR experiments. CL, LF, CL, AS, J-YC and CM were involved in the macroarray design and construction. MAD contributed to the experimental set-up and was responsible for the experiment set-up and was responsible for the bacterial cultures. EC participated in experimental design and performed the statistical analyses. JF contributed to the experimental design and construction. MAD contributed to the bacterial cultures. EC participated in experimental design and, as the main project coordinator, in critical proofreading of the manuscript. MM contributed to the bacterial cultures. EC participated in experimental design and performed the statistical analyses. JF contributed to the bacterial cultures. EC participated in critical proofreading of the manuscript. MM contributed to the experimental set-up, performed the spot intensity quantification and the biological interpretation of the results; he wrote this manuscript with assistance of MS, who was also involved in coordination of the project. All the authors have read and approved the content of this article.

**Additional material**

**Additional file 1**

Validation of macroarray hybridization data. Transcriptional changes for three chromosomal (yfeC, psaA and aceB) and five plasmid-borne (yada, yopl, yopN, sycH and tnpB) genes (assessed using macroarray hybridization and qRT-PCR assays) are shown.

Click here for file [http://www.biomedcentral.com/content-supplementary/1471-2180-8-211-S1.pdf](http://www.biomedcentral.com/content-supplementary/1471-2180-8-211-S1.pdf)

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