

EXTENDED GENOME REPORT

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High-quality draft genome sequence of a new phytase-producing microorganism *Pantoea sp.* 3.5.1

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Abstract

Strain 3.5.1 was isolated from soils of the Republic of Tatarstan, Russia, on the basis of presence of a high phytate-degrading activity. Strains with such activities attract special interest because of its potential use as feed additives and natural manures. Strain 3.5.1 harbors a 99 % 16S rRNA nucleotide sequence similarity to different *Pantoea* species (*P. vagans*, *P. ananatis*, *P. agglomerans*, *P. anthophila* and *Pantoea sp.*) and exhibits unique biochemical properties that do not allow strain identification up to species. Moreover, the strain 3.5.1 shows a low ANI and MALDI-TOF Mass Spectrometry scores. Thus, it is likely that the strain 3.5.1 represents a new *Pantoea* species. Here, we present the genome sequence of *Pantoea sp.* strain 3.5.1. The 4,964,649 bp draft genome consists of 23 contigs with 4,556 protein-coding and 143 RNA genes. Genome sequencing and annotation revealed two phytase genes and putative regulatory genes controlling its activity.

Keywords: Strain 3.5.1, Pantoea, Genome, 454, Ion Torrent, Phytase

Introduction

Up to 90 % of natural phosphorus in the World is present in the form of phytic acid or phytate and is often accumulated in livestock feces. This form of organic phosphorus cannot be utilized by monogastric farm animals and ends up polluting soils and contributes to the eutrophication of water environments [1, 2]. Moreover, phytate reduces the nutritional value of feeds because it chelates essential minerals such as calcium, iron, zinc, magnesium, manganese, copper and molybdenum [3]. Chemical (acid hydrolysis ion and exchange) or physical (autoclaving) methods to hydrolyze phytate are costly and reduce the nutrient value of feeds. Therefore, the search for of alternative methods of phytate hydrolysis is an important task. In light of this, identification and isolation of bacteria capable of enzymatic phytate hydrolysis is a promising approach that would simultaneously reduce environmental burden caused by current agricultural practices.

Phytases are specific group of phosphatases capable of phytate (myo-inositol 1,2,3,4,5,6-hexakisphosphate) hydrolysis with the formation of less phosphorylated inositol derivatives [4, 5]. There are a few reports on phytase-producing microbes from Russia; they include fungi [6, 7] and bacteria [5, 8, 9]. Here, we characterize a phytase-producing strain 3.5.1, present its classification and describe a set of its features along with the annotated genome sequence that provides important insights into several candidate genes involved in phytate hydrolysis. Strain 3.5.1 was isolated from a forest soil sample on a selective medium containing calcium phytate as the only source of phosphorus.

The genus *Pantoea*, within the *Enterobacteriaceae* family, consists of several species (*P. agglomerans*, *P. ananatis*, *P. dispersa*, *P. vagans* and others) that generally inhabit numerous ecological niches, including plants, water, soil, humans and animals. Classification of these species had a long history before they were separated in the new *Pantoea* genus [10]. *P. agglomerans*

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Organism InformationClassification and features

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(formerly Enterobacter agglomerans) and P. dispersa were proposed as the first *Pantoea* species based on their DNA-DNA hybridization relatedness. Mergaert et al. proposed the name P. ananatis for Erwinia ananas [11]. Brady et al. isolated P. agglomerans-like strains and separated them into four novel species (P. vagans, P. eucalypti, P. deleyi and P. anthophila) based on MultiLocus Sequence Analysis and amplified fragment length polymorphism analysis [12]. Identification of Pantoea species through their nutritional characteristics or biochemical approaches has proven to be difficult. Currently, several strategies based on the use of genomic approaches have been reported to define *Pantoea* species [13-16]. One of challenging approaches to construct the phylogenetic relationships among different bacterial isolates is a whole genome sequencing [17]. To date the NCBI database contains information about nine of 23 validly published Pantoea species genome assemblies.

Strain 3.5.1 was isolated from the forest soil near Agerze village, Aznakaevo district, Republic of Tatarstan, Russia [18, 19]. The isolate was characterized as Gram-negative, motile and rod-shaped bacterium 0.5 μm to 1.5 μm length (Fig. 1 and Table 1). Colonies were round, smooth and shiny after incubation at 37 °C for 24 h. Longer incubation (2–3 days) of the isolate resulted in production of yellow pigment. The strain 3.5.1 displayed phytate-hydrolyzing activity on PSM solid medium (2 % Glucose, 0.4 % Sodium

phytate, 0.2 % CaCl₂, 0.5 % NH₄NO₃, 0.05 % KCl, 0.05 % MgSO₄ × 7H₂O, 0.001 % FeSO₄ × 7H₂O, 0.001 % MnSO₄ × H₂O, 3 % Agar, pH 7.0), *i.e.* it was able to form halo zone around the colonies (clear zone reflecting solubilization of Ca-phytate in the agar medium) (Fig. 1A) [20, 21]. Thus, strain 3.5.1 has the unique characteristics of degradation of phytate and can potentially be used for the industrial production of microbial phytase; the enzyme could possibly be applied as phosphorus-mobilizing agent in soil or as a feed supplement for livestock production.

Strain 3.5.1 was shown to be able to utilize the following carbon substrates: glucose, lactose, maltose and mannitol without gas formation, but unable to oxidize urea (tested on Kligler Iron Agar, Olkenitski's medium and Hiss media) [22, 23]. By API-20E test (bioMerieux, Inc.) it was shown that the strain 3.5.1 cannot utilize ornithine. The strain is resistant to tetracycline, chloramphenicol and erythromycin but susceptible to beta-lactam antibiotics like ampicillin and penicillin. These morphological and biochemical properties are consistent with the notion that this isolate likely belongs to the family *Enterobacteriaceae*.

The taxonomic position of the strain 3.5.1 was first evaluated by the comparison of 16S rRNA gene sequences with related sequences using blastn (nr/nt GenBank Database). The sequence showed 99 % identity to multiple 16S sequences from *Pantoea* species (*Pantoea spp., P. ananatis, P. vagans, P. agglomerans, P. conspicua*

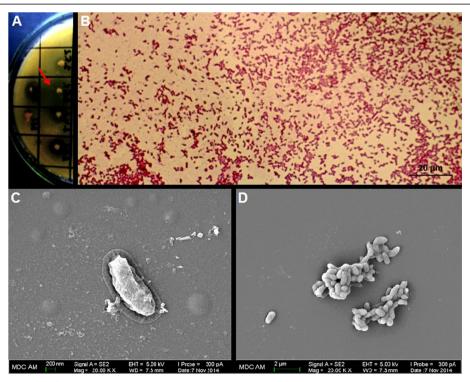


Fig. 1 General characteristics of the strain 3.5.1. **a** Screening for phytate-hydrolyzing activity on PSM solid medium. Strain 3.5.1 is indicated by red arrow. **b** Phase contrast micrograph of the strain 3.5.1. **c** and **d** Scanning electron micrographs of the strain 3.5.1 (Carl Zeiss, Merlin)

Table 1 Classification and general features of *Pantoea sp.* 3.5.1. in accordance with the MIGS recommendations [42] as published by the Genome Standards Consortium [43]

MIGS ID	Property	Term	Evidence code ^a
	Classification	Domain <i>Bacteria</i>	TAS [44]
		Phylum Proteobacteria	TAS [45]
		Class Gammaproteobacteria	TAS [45, 46]
		Order "Enterobacteriales"	TAS [47-49]
		Family Enterobacteriaceae	TAS [49]
		Genus Pantoea	TAS [10, 50]
		Species Pantoea sp.	TAS [51, 52]
		Type strain: 3.5.1	IDA
	Gram stain	Negative	IDA
	Cell shape	Rod-shaped	IDA
	Motility	Motile	IDA
	Sporulation	Non-spore forming	IDA
	Temperature range	Mesophilic	IDA
	Optimum temperature	+37 °C	IDA
	pH range; Optimum	3.5–7; 5	IDA
	Carbon source	D-glucose, lactose, maltose, mannite	IDA
	Energy source	Chemoorganotroph	NAS
MIGS-6	Habitat	Soil	IDA
MIGS-6.3	Salinity	Not tested	
MIGS-22	Oxygen requirement	Facultative aerobic	IDA
MIGS-15	Biotic relationship	Free living	IDA
MIGS-14	Pathogenicity	Opportunistic pathogen	NAS
MIGS-4	Geographic location	Agerze village, Aznakaevo district, Republic of Tatarstan, Russia	IDA
MIGS-5	Sample collection	September 2010	IDA
MIGS-4.1	Latitude	54°83′	IDA
MIGS-4.2	Longitude	53°00′	IDA
MIGS-4.3	Depth	25 cm	IDA
MIGS-4.4	Altitude	233 m	IDA

^aEvidence codes - IDA: Inferred from Direct Assay; TAS: Traceable Author Statement (*i.e.*, a direct report exists in the literature); NAS: Non-traceable Author Statement (*i.e.*, not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). These evidence codes are from the Gene Ontology project [53]

and others). More detailed phylogenetic analysis of the strain 3.5.1 was performed using MEGA 6.0 software [24] with 16S rRNA gene sequences of 21 *Pantoea* species and 2 *Escherichia coli* strains as an outgroup (a complete/scaffold level genome sequences for all these species are available in NCBI database). However, our alignment allowed comparison of only variable regions V3 and V4 of 16S rRNA gene for these set of species, because not all completed sequences of these genes are available. Therefore, we eliminated several species from phylogenetic comparison to generate a tree based on the extended variable regions of 16S rRNA gene [25, 26]. Finally, 14 *Pantoea* species and 2 *Escherichia coli* strains were aligned, the incomplete sites on both 5'- and 3'-ends of the 16S rRNA gene sequences were excluded from the

alignment. The remaining alignment sites (1208 bp), which included V1–V8 regions of 16S rRNA sequences, were selected for the subsequent analysis. Phylogenetic tree was generated using the Maximum likelihood (ML) algorithm with 1,000 bootstrap iterations (Fig. 2). As expected, two strains of *E. coli* (K-12 substr. MG1655 and O157:H16 Santai) could be clearly distinguished phylogenetically from species that belong to *Pantoea* genus. *P. ananatis* and *P. stewartii* belong to two different clades of the tree with high bootstrap support. However, certain clades, such as *P. agglomerans*, *P. vagans* and *Pantoea sp.*, do not form clearly separate groups. Interestingly, despite the fact that the strain 3.5.1 forms a distinct node with *P. agglomerans* Eh318 and *P. vagans* C9-1, *P. vagans* species do not show motility at 37 C° and both with *P. agglomerans* strains are

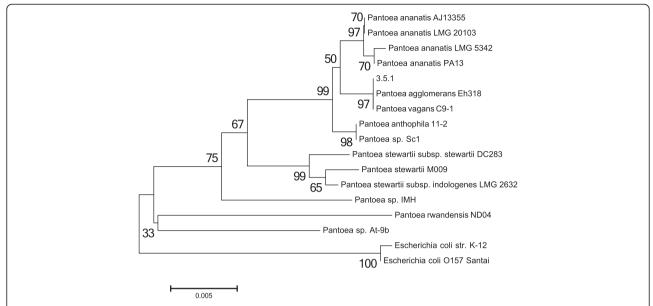


Fig. 2 Maximum likelihood phylogenetic tree highlighting the position of the strain 3.5.1 relative to other species within the genus *Pantoea* based on 16S rRNA gene sequences. Bootstrap consensus tree were inferred from 1,000 replicates. Two *E. coli* strains were used as outgroup. The scale bar, 0,005 substitutions per nucleotide position. The phylogenetic tree was obtained by MEGA 6 software [44]. The corresponding GenBank accession numbers for 16S rRNA sequences are: NR_074740 (*P. ananatis* AJ13355), FJ611814 (*P. ananatis* LMG 20103), FJ611845 (*P. ananatis* LMG 5342), NC_017554 (*P. ananatis* PA13), NZ_JMRT02000019 (the strain 3.5.1), NZ_KK403348 (*P. agglomerans* Eh318), NR_102966 (*P. vagans* C9-1), JXXL01000005 (*P. anthophila* 11–2), FJ611810 (*Pantoea sp.* Sc1), AJ311838 (*P. stewartii* subsp. *stewartii* DC283), KJ830125 (*P. stewartii* M009), NR_119256 (*P. stewartii* subsp. *indologenes* LMG 2632), JX861128 (*Pantoea sp.* IMH), NZ_CP009454 (*P. rwandensis* ND04), NC_014837 (*Pantoea sp.* At-9b), NR_102804 (*E. coli* K-12 substr. MG1655), NZ_CP007592.1 (*E. coli* O157:H16 strain *Santai*)

not able to hydrolyze lactose as a carbon source, unlike the strain 3.5.1 [12]. We also carried out the matrixassisted laser-desorption/ionization time-of-flight MS protein analysis for the strain 3.5.1 using a Microflex spectrometer (Bruker Daltonics, Leipzig, Germany). Measurements were made as previously described [27]. Spectra of the strain 3.5.1 were imported into the MALDI BioTyper software (version 2.0; Bruker) and analyzed by standard pattern matching (with default parameter settings). The commercially available Bruker database contains 26 protein profiles of Pantoea species and 14 protein profiles of *E. coli* species. All these profiles were used as reference data to compare the strain 3.5.1 spectra. Strain 3.5.1 showed log (score) values between 1.6 and 2.0 which allowed defining only its genus identification. The 3.5.1 spectra obtained are shown as a dendrogram in Additional file 1. We then calculated average nucleotide identity values between the genome sequences of the strain 3.5.1 and 21 *Pantoea* species using the JSpecies software [28] based on the BLASTn method (ANIb) and the MUMmer algorithm (ANIm). Analysis of the 3.5.1 genome data yielded low ANI values (ANIb 74.88-85.11 % and ANIm 83.72-86.86 %) indicating that the strain 3.5.1 does not belong to previously characterized species (Additional file 2), since the lowest threshold for ANI clustering is 97 %. Since the strain 3.5.1 differs from known Pantoea species both

biochemically and phylogenetically we designate our isolate as *Pantoea sp.* 3.5.1.

Genome sequencing information

Genome project history

The genome of *Pantoea sp.* strain 3.5.1 was selected for whole genome sequencing because of its ability to produce phytase. Comparison of the strain 3.5.1 genome with other *Pantoea* species may provide insights into the molecular basis of phytase activity and metabolic features of this strain. The high-quality draft genome sequence was completed on March 27, 2015 and was deposited to GenBank as the Whole Genome Shotgun project under the accession number JMRT00000000 (current version JMRT00000000.2) and to the Genome OnLine Database with ID Gp0114842 [29]. A summary of the project information is shown in Table 2.

Growth conditions and genomic DNA preparation

The *Pantoea sp.* strain 3.5.1 is deposited to the Russian National Collection of Industrial Microorganisms (VKPM) under the accession number B-11689.

For genomic DNA isolation bacterial culture was grown overnight in 25 mL LB medium at 37 °C with vigorous shaking. DNA was isolated using a Genomic DNA Purification Kit (Fermentas). DNA purity was tested by gel electrophoresis (1 % agarose gel) and

Table 2 Project information

MIGS ID	Property	Term
MIGS 31	Finishing quality	High quality draft
MIGS-28	Libraries used	Two single-end libraries of 200 bp and 600 bp
MIGS 29	Sequencing platforms	Ion Torrent PGM and 454 GS Junior
MIGS 31.2	Fold coverage	32×
MIGS 30	Assemblers	SPAdes 3.5.0
MIGS 32	Gene calling method	GeneMark, RAST
	Locus Tag	EP46
	Genbank ID	JMRT00000000.2
	GenBank Date of Release	April 16, 2015
	GOLD ID	Gp0114842
	BIOPROJECT	PRJNA246264
MIGS 13	Source Material Identifier	3.5.1
	Project relevance	Phytase producer bacterium, Agricultural

DNA concentration was estimated by the Qubit 2.0 Fluorometer using the Qubit dsDNA (High Sensitivity) Assay Kit (Life Technologies).

Genome sequencing and assembly

The genomic DNA of *Pantoea sp.* 3.5.1 strain was sequenced with 32-fold overall genome coverage by a whole genome shotgun strategy. Two single-end libraries were used: a 200 bp-library for Ion Torrent PGM sequencing (performed in the Research Institute of Physical Chemical Medicine, Moscow, Russia) and 600 bp library for 454 GS Junior sequencing (performed in the Interdisciplinary Center for Proteomics Research, Kazan, Russia). Sequencing of the 200 bp library generated 349,046 reads, while sequencing of the 600 bp library generated 152,266 reads. Both read sets were assembled *de novo* using the SPAdes 3.5.0 assembler [30]. This strategy resulted in 23 contigs (>500 bp) with a calculated genome size of 4,964,649 bp and G + C content of 55,77 mol %. The N50 size of the resulted contigs was 562,444 bp.

Genome annotation

Genes of *Pantoea sp.* 3.5.1 strain were identified using the Prokaryotic Genomes Automatic Annotation Pipeline. The predicted CDSs were translated and analyzed against the NCBI non-redundant database, iPfam, TIGRfam, InterPro, KEGG, COG and IMG databases [31–36]. The genome sequence was also uploaded into the RAST system [37] to check the annotated sequences.

Genome properties

The draft assembly of the genome consists of 23 contigs with the fragment size lager than 500 bp, N50 is 562,444 bp. Of the 4,699 genes predicted, 4,556 were protein-coding genes and 143 were RNA genes.

Putative functions were assigned to the majority of the protein-coding genes (96.96 %), while the remaining ORFs (open reading frames) were annotated as hypothetical proteins. The distribution of genes into COGs functional categories is presented in Tables 3 and 4.

Extended insights

Most phytases of the family *Enterobacteriaceae* family belong to the group of histidine acid phosphatases as judged by their sequence and properties. Three phytase subgroups (AppA-related, Agp-related and PhyK phytases) can be identified within histidine acid phytases based on their substrate specificity and specific activity levels [38]. To gain insight into the phytate-degrading activity of

Table 3 Genome statistics

Attribute	Value	% of Total
Genome size (bp)	4,964,649	100.00
DNA coding (bp)	4,306,583	86.74
DNA G+C (bp)	2,768,589	55.77
DNA scaffolds	23	100.00
Total genes	4,699	100.00
Protein coding genes	4,556	96.96
RNA genes	143	3.04
Pseudo genes	135	2.87
Genes in internal clusters	NA	
Genes with function prediction	3,921	83.44
Genes assigned to COGs	3,507	74.63
Genes with Pfam domains	4,059	86.38
Genes with signal peptides	425	9.04
Genes with transmembrane helices	1,075	22.88
CRISPR repeats	1	-

Table 4 Number of genes associated with general COG functional categories

Code	Value	%age	Description
J	250	6.28	Translation, ribosomal structure and biogenesis
Α	1	0.03	RNA processing and modification
K	366	9.19	Transcription
L	127	3.19	Replication, recombination and repair
В	-	-	Chromatin structure and dynamics
D	45	1.13	Cell cycle control, Cell division, chromosome partitioning
V	85	2.13	Defense mechanisms
Т	186	4.67	Signal transduction mechanisms
М	250	6.28	Cell wall/membrane biogenesis
N	106	2.66	Cell motility
U	48	1.21	Intracellular trafficking and secretion
0	136	3.42	Posttranslational modification, protein turnover, chaperones
C	212	5.32	Energy production and conversion
G	427	10.72	Carbohydrate transport and metabolism
Е	395	9.92	Amino acid transport and metabolism
F	101	2.54	Nucleotide transport and metabolism
Н	209	5.25	Coenzyme transport and metabolism
1	131	3.29	Lipid transport and metabolism
P	252	6.33	Inorganic ion transport and metabolism
Q	80	2.01	Secondary metabolites biosynthesis, transport and catabolism
R	342	8.59	General function prediction only
S	204	5.12	Function unknown
-	1192	25.37	Not in COGs

The total is based on the total number of protein coding genes in the genome

Pantoea sp. 3.5.1 strain, we analyzed its genome for the presence of key genetic factors responsible for phytase activity of *Enterobacteriaceae*.

We detected genes for glucose-1-phosphatase and 3-phytase which are located on the first contig of the assembly (Additional file 3). However, no sequence homology was observed for an *appA*-related gene. Sequence analysis of the 3-phytase gene from *Pantoea sp.* 3.5.1 revealed maximal homology (77 % nucleotide identity) to *phyK* gene of *P. vagans* C9-1. A high degree of homology of glucose-1-phosphatase gene of 3.5.1 strain was found to glucose-1-phosphatases (*agp*) of *P. vagans* C9-1 (84 % nucleotide identity), *Plautia stali* symbiont (82 %), *P. ananatis* strains and *P. rwandensis* ND04 (81 %), *Pantoea sp.* At-9b (80 %), and *E. coli* 042 (72 %). Therefore, we show that *Pantoea* sp. 3.5.1 harbors two phytase-encoding genes (*agp*-related and *phyK* phytases) but lacks *appA*-like phytase genes.

There is still very little information available in regards to the regulation of phytate-degrading gene expression in bacteria. To date, regulation of two periplasmic phytases of *E. coli* (*agp*-encoded acid glucose-1-phosphatase and *appA*-encoded 3-phytase) have been described in great

details [39]. Gene agp is constitutively expressed whereas expression of appA is induced by phosphate starvation and by transition to stationary phase. Gene appA is located within the appCBA operon and its regulation occurs by two inducible promoters. We compared phytase genes, their position and context in the *Pantoea sp.* 3.5.1 genome with agp and appA genes of E. coli. Neither Pantoea sp. 3.5.1 phytase genes (agp and phyK) have similar locations to genome context of E. coli but are comparable with P. vagans C9-1 genome context. However we identified two genes which can possibly participate in the regulation of phytase activity similar to the situation in E. coli: the rpoS gene (RNA polymerase sigma factor RpoS) and araC-like gene (DNA-binding domain-containing protein which belongs to the AraC/XylS family). These regulatory genes are active in anaerobic conditions, phosphate starvation and during entry into stationary phase. Thus, the mechanism of phytase activity regulation in Pantoea sp. 3.5.1 might be similar to E. coli. Figure 3 shows the results of full genome comparison between the Pantoea sp. 3.5.1 strain and P. vagans C9-1 using BLAST Ring Image Generator comparison tool [40]. We also designated the local positions of two detected phytase genes and its possible regulatory genes.

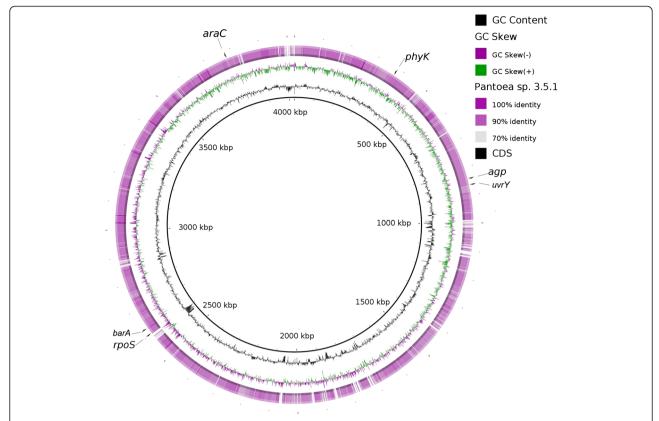


Fig. 3 Circular representation of the genome comparison between the *Pantoea sp.* 3.5.1 strain and *P. vagans* C9-1. The inner ring shows GC content (in black) and GC skew (in purple/green). Color intensity within the circle represents the levels of nucleotide homology (from 70 % to 100 %). The external ring shows CDS of two detected phytase genes and the candidate regulatory genes

Regulation of intracellular phytase activity has also been investigated in rhizospheric strain of *Serratia plymuthica* IC1270 [41]. It was shown that the GrrS/GrrA system (also known as GacS/GacA and BarA/UvrY) and RpoS factor are implicated in phytase production in *S. plymuthica*. Both genes of GrrS/GrrA two-component signal transduction system were also predicted in genome assembly of *Pantoea sp.* 3.5.1.

Conclusions

In the current study, we characterized the genome of the *Pantoea* strain 3.5.1 that was isolated from soils of the Republic of Tatarstan, Russia. The strain exhibits high phytate-degrading activity. Phylogenetically the *Pantoea* strain 3.5.1 is positioned between *P. agglomerans* and *P. vagans*, but the strain 3.5.1 is characterized by phenotypic differences. Thus, it is likely that this strain represents a new *Pantoea* species. In order to improve the understanding of the molecular basis for the ability of *Pantoea sp.* 3.5.1 strain to hydrolyze phytate we performed detailed genome sequencing and annotation. We also

identified three regulatory genes encoding transcriptional factors.

Additional files

Additional file 1: A main spectra profiles (MSP) dendrogram generated by MALDI Biotyper 3.0 software with the 3.5.1 isolate, 26 *Pantoea* reference species and 14 *E. coli* outgroup strains. Each cluster is indicated by different color. Distance level show the phylogenic distance between the selected genus and species. The strain 3.5.1 is highlighted by box. (JPG 182 kb)

Additional file 2: Average nucleotide identity (ANI) values calculated between the 3.5.1 genome assembly, 26 Pantoea species and 2 E. coli strains. Maximum and minimum ANI values are highlighted by yellow color. ANI values of E. coli strains are shown in grey. The corresponding GenBank accession numbers for genome sequences are: NZ_KK403338 (P. agglomerans Eh318), NZ_JPOT000000000 (P. agglomerans 4), NZ_ASJI00000000 (P. agglomerans Tx-10), NZ_JNGC000000000 (P. agglomerans 190), NZ_JPKQ00000000 (P. agglomerans MP2), NC_017554 (P. ananatis PA13), NZ_ASJIH00000000 (P. ananatis BRT175), NC_013956 (P. ananatis LMG-20103), NC_016816 (P. ananatis LMG-5342), NC_017531 (P. ananatis A13355), NZ_XXL00000000 (P. anthophila 11-2), NZ_AVSS00000000 (P. dispersa EGD AAK13), NZ_JTJJ00000000 (P. rodasii ND03), NZ_CP009454 (P. rwandensis ND04), NZ_JFGT0000000 (Pantoea sp. IMH), NZ_CP009880 (Pantoea sp. PSNIH1), NZ_CP009866 (Pantoea sp. PSNIH2), NZ_AJFP00000000 (Pantoea sp. aB),

NZ_JSXF00000000 (*P. stewartii* M073a), NZ_JRW100000000 (*P. stewartii* M009), NZ_JPK000000000 (*Pantoea stewartii* subsp. *indologenes* LMG 2632), NZ_A-HIE00000000 (*Pantoea stewartii* subsp. *stewartii* DC283), NC_014562.1 (*P. vagans* C9-1), NZ_JPKP00000000 (*P. vagans* MP7), NC_000913 (*E. coli* str. K12 substr. MG1655), NC_002695 (*E. coli* O157:H16 Santai). (DOCX 13 kb)

Additional file 3: Links and DNA sequences of phytase genes detected in *Pantoea sp.* 3.5.1 genome. (DOCX 14 kb)

Abbreviations

PSM: Phytase screening medium; nr/nt: non-redundant nucleotide.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

ADS carried out the strain 3.5.1 isolation, performed the laboratory experiments and together with EAB and AAT conducted the phylogenetic studies. AAT drafted the manuscript. SVK assembled and annotated the genome. AMM, NPB and MRS developed the study concept and contributed materials and analyses. All authors read and approved the final manuscript.

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References

- Harland BF, Morris ER. Phytate: A good or a bad food component? Nutr Res. 1995:15:733–54
- Smith VH. Eutrophication of freshwater and coastal marine ecosystems: A global problem. Environ Sci Pollut Res Int. 2003;10:126–39.
- Nolan KB, Duffin PA, Mc. Weeny DJ. Effects of phytate on mineral bioavailability. In vitro studies on Mg, Ca, Fe, Cu and Zn (also CD) solubilities in the presence of phytate. J Sci Food Agric. 1987;40:79–85.
- Oh BC, Choi WC, Park S, Kim YO, Oh TK. Biochemical properties and substrate specificities of alkaline and histidine acid phytases. Appl Microbiol Biotechnol. 2004;63:362–72.
- Mukhametzianova AD, Akhmetova AI, Sharipova MR. Microorganisms as phytase producers. Microbiol (Mikrobiologiya, Moscow). 2012;81:267–75.
- Sinitsyna OA, Fedorova EA, Gusakov AV, Uporov IV, Sokolova LM, Bubnova TM, et al. Isolation and characterization of extracellular phytase a from Penicillium canescens. Russ J Bioorganic Chem. 2006;71:1260–8.

- Rozhkova AM, Sereda AS, Tsurikova NV, Nurtaeva AK, Semenova MV, Rimareva LV, et al. Creation of a heterologous gene expression system on the basis of Aspergillus awamori recombinant strain. Appl Biochem Microbiol (Prikl Biokhim Mikrobiol, Moscow). 2011;47:279–87.
- Shedova EN, Berezina OV, Khmel' IA, Lipasova VA, Borriss R, Velikodvorskaia GA. Phytase activity in some groups of bacteria. Search for and cloning of genes for bacterial phytases. Mol Gen Mikrobiol Virusol (Moscow). 2004;1:18–21.
- Akhmetova Al, Nyamsuren C, Balaban NP, Sharipova MR. Isolation and characterization of a new bacillary phytase. Russ J Bioorganic Chem. 2013;39:384–9
- Gavini F, Mergaert J, Beji A, Mielcarek C, Izard D, Kersters K, et al. Transfer of *Enterobacter agglomerans* (Beijerinck 1888) Ewing and Fife 1972 to *Pantoea* gen. nov. as *Pantoea agglomerans* comb. nov. and description of *Pantoea* dispersa sp. nov. Int J Syst Bacteriol. 1989;39:337–45.
- Mergaert J, Verdonck L, Kersters K. Transfer of Erwinia ananas (synonym, Erwinia uredovora) and Erwinia stewartii to the genus Pantoea emend. as Pantoea ananas (Serrano 1928) comb. nov. and Pantoea stewartii (Smith 1898) comb. nov., respectively, and description of Pantoea stewartii subsp. indologenes subsp. nov. Int J Syst Bacteriol. 1993;43:162–73.
- Brady CL, Venter SN, Cleenwerck I, Engelbeen K, Vancanneyt M, Swings J, et al. Pantoea vagans sp. nov., Pantoea eucalypti sp. nov., Pantoea deleyi sp. nov. and Pantoea anthophila sp. nov. Int J Syst Evol Microbiol. 2009:59:2339–45.
- Brady C, Cleenwerck I, Venter S, Vancanneyt M, Swings J, Coutinho T. Phylogeny and identification of *Pantoea* species associated with plants, humans and the natural environment based on multilocus sequence analysis (MLSA). Syst Appl Microbiol. 2008;31:447–60.
- Brady C, Venter S, Cleenwerck I, Vancanneyt M, Swings J, Coutinho T. A
 FAFLP system for the improved identification of plant-pathogenic and
 plant-associated species of the genus *Pantoea*. Syst Appl Microbiol.
 2007:30:413–7.
- Delétoile A, Decré D, Courant S, Passet V, Audo J, Grimont P, et al. Phylogeny and identification of *Pantoea* species and typing of *Pantoea agglomerans* strains by multilocus gene sequencing. J Clin Microbiol. 2009;47:300–10.
- Tambong JT, Xu R, Kaneza CA, Nshogozabahizi JC. An In-depth analysis of a multilocus phylogeny identifies *leuS* as a reliable phylogenetic marker for the genus *Pantoea*. Evol Bioinform Online. 2014;10:115–25.
- 17. Eisen JA. Assessing evolutionary relationships among microbes from whole-genome analysis. Curr Opin Microbiol. 2000;3:475–80.
- Suleimanova AD, Danilova YV, Sharipova MR, Greiner R. A new intracellular phytase of enterobacteria: isolation and characterization. Russ J Bioorganic Chem. 2013;39:378–83.
- Suleimanova AD, Beinhauer A, Valeeva LR, Chastukhina IB, Balaban NP, Shakirov EV, et al. A novel glucose-1-phosphatase with high phytase activity and unusual metal ion activation is produced by the soil bacterium *Pantoea*. Appl Environ Microbiol. 2015. in press
- Mukesh P, Suma S, Singaracharya MA, Lakshmipathi V. Isolation of phytate-hydrolysing microbial strains from traditional waste water of rice fermentation and liquid cattle feeds. World J Microbiol Biotechnol. 2004;20:531–4.
- Sasirekha B, Bedashree T, Champa KL. Optimization and partial purification of extracellular phytase from *Pseudomonas aeruginosa* p6. Eur J Exp Biol. 2012;2:95–104.
- Garanin BA, Nesmelova ZP, Gurileva NP, Solodova FG. Trial of the use of Olkenitski's medium for the study of bacteria of the *Enterobacteriaceae* group. Lab Delo. 1965;8:498–500.
- Cote RJ, Gherna RL. Nutrition and Media. In: Gerhardt P, Murray RGE, Wood WA, Krieg NR, editors. Methods for general and molecular bacteriology. Washington, D.C: American Society for Microbiology; 1994. p. 157–77.
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol. 2013;30:2725–9.
- 25. Van de Peer Y, Chapelle S, De Wachter R. A quantitative map of nucleotide substitution rates in bacterial rRNA. Nucleic Acids Res. 1996;24:3381–91.
- Kim M, Morrison M, Yu Z. Evaluation of different partial 16S rRNA gene sequence regions for phylogenetic analysis of microbiomes. J Microbiol Methods. 2011;84:81–7.
- Seng P, Drancourt M, Gouriet F, La Scola B, Fournier PE, Rolain JM, et al. Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clin Infect Dis. 2009;49:543–51.

- 28. Richter M, Rosselló-Móra R. Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl Acad Sci. 2009;106:19126–31.
- Liolios K, Chen IM, Mavromatis K, Tavernarakis N, Hugenholtz P, Markowitz VM, et al. The Genomes On Line Database (GOLD) in 2009: status of genomic and metagenomic projects and their associated metadata. Nucleic Acids Res. 2010;38:D346–54.
- Nurk S, Bankevich A, Antipov D, Gurevich AA, Korobeynikov A, Lapidus A, et al. Assembling single-cell genomes and mini-metagenomes from chimeric MDA products. J Comput Biol. 2013;20:714–37.
- Finn RD, Miller BL, Clements J, Bateman A. iPfam: a database of protein family and domain interactions found in the Protein Data Bank. Nucleic Acids Res. 2014;42:D364–73.
- Haft DH, Selengut JD, White O. The TIGRFAMs database of protein families. Nucl Acids Res. 2003;31:371–3.
- Jones P, Binns D, Chang HY, Fraser M, Li W, McAnulla C, et al. InterProScan 5: genome-scale protein function classification. Bioinformatics. 2014;30:1236–40.
- Kanehisa M, Goto S. KEGG: Kyoto Encyclopedia of Genes and Genomes. Nucl Acids Res. 2000;28:27–30.
- 35. Tatusov RL, Koonin EV, Lipman DJ. A genomic perspective on protein families. Science. 1997;278:631–7.
- Markowitz VM, Chen IM, Palaniappan K, Chu K, Szeto E, Pillay M, et al. IMG 4 version of the integrated microbial genomes comparative analysis system. Nucl Acids Res. 2014;42:D560–7.
- Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, et al. The RAST Server: rapid annotations using subsystems technology. BMC Genomics. 2008. doi:10.1186/1471-2164-9-75.
- Böhm K, Herter T, Müller JJ, Borriss R, Heinemann U. Crystal structure of Klebsiella sp. ASR1 phytase suggests substrate binding to a preformed active site that meets the requirements of a plant rhizosphere enzyme. FEBS J. 2010;277:1284–96.
- Greiner R. Phytate-degrading enzymes: regulation of synthesis in microorganisms and plants. In: Turner BL, Richardson AE, Mullaney EJ, editors. Inositol Phosphates: Linking Agriculture and the Environment. UK, Wallingford: CAB International; 2007. p. 78–96.
- Alikhan NF, Petty NK, Ben Zakour NL, Beatson SA. BLAST Ring Image Generator (BRIG): simple prokaryote genome comparisons. BMC Genomics. 2011;12:402.
- 41. Shedova E, Lipasova V, Velikodvorskaya G, Ovadis M, Chernin L, Khmel I. Phytase activity and its regulation in a rhizospheric strain of *Serratia plymuthica*. Folia Microbiol (Praha). 2008;53:110–4.
- Field D, Garrity G, Gray T, Morrison N, Selengut J, Sterk P, et al. The minimum information about a genome sequence (MIGS) specification. Nat Biotechnol. 2008;26:541–7.
- Field D, Amaral-Zettler L, Cochrane G, Cole JR, Dawyndt P, Garrity GM, et al. Clarifying concepts and terms in biodiversity informatics. PLoS Biol. 2011;9:e1001088.
- Woese CR, Kandler O, Wheelis ML. Towards a natural system of organisms: proposal for the domains *Archaea*, *Bacteria*, and *Eucarya*. Proc Natl Acad Sci U S A. 1990;87:4576–9.
- Garrity GM, Bell JA, Lilburn T. Phylum XIV. Proteobacteria phyl nov. In: Brenner DJ, Krieg NR, Stanley JT, Garrity GM, editors. Bergey's manual of systematic bacteriology. (The Proteobacteria part B The Gammaproteobacteria). New York: Springer; 2005. p. 1.
- Garrity GM, Bell JA, Lilburn T. Class III. Gammaproteobacteria class. nov. In: Garrity GM, Brenner DJ, Krieg NR, Staley JT, editors. Bergey's manual of systematic bacteriology. New York: Springer; 2005. p. 1.
- Garrity GM, Holt JG. Taxonomic outline of the Archaea and Bacteria. In: Boone DR, Castenholz RW, editors. Bergey's manual of systematic bacteriology. (The Archaea and the deeply branching and phototrophic Bacteria). New York: Springer; 2001. p. 155–66.
- 48. Rahn O. New principles for the classification of bacteria. Zentralbl Bakteriol Parasitenkd Infektionskr Hyg. 1937;96:273–86.
- Commission J. Conservation of the family name Enterobacteriaceae, of the name of the type genus, and designation of the type species Opinion No. 15. Int Bull Bacteriol Nomencl Taxon. 1958;8:74.
- 50. Brady CL, Cleenwerck I, Venter SN, Engelbeen K, De Vos P, Coutinho TA. Emended description of the genus *Pantoea*, description of four species from human clinical samples, *Pantoea septica* sp. nov., *Pantoea eucrina* sp. nov., *Pantoea brenneri* sp. nov. and *Pantoea conspicua* sp. nov., and transfer of *Pectobacterium cypripedii* (Hori 1911) Brenner et al. 1973 emend. Hauben

- et al. 1998 to the genus as *Pantoea cypripedii* comb. nov. Int J Syst Evol Microbiol. 2010;60:2430–40.
- Tian H, Jing C. Genome sequence of the aerobic arsenate-reducing bacterium *Pantoea sp.* strain IMH. Genome Announc. 2014; doi:10.1128/ genomeA.00267-14.
- Tan WS, Muhamad Yunos NY, Tan PW, Mohamad NI, Adrian TG, Yin WF, Chan KG. Pantoea sp. isolated from tropical fresh water exhibiting N-acyl homoserine lactone production. Sci World J. 2014; doi:10.1155/2014/828971.
- Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, et al. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat Genet. 2000;25:25–9.

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