

A prokaryote like protein (TonB-dependent receptor/protein) is associated with nickel resistance in white birch (*Betula papyrifera*)

Gabriel Theriault¹ and Kabwe Nkongolo^{1,2}

¹Biomolecular Sciences Program and ²Department of Biology, Laurentian University, Sudbury, Ontario, P3E 2C6

Background

- Greater Sudbury Region (GSR) in Northern Ontario, Canada is home to one of the largest nickel/copper deposits and extractive operations in the world. A decade of mining and smelting operations has left the surrounding land acidified and contaminated with high levels of metals (Freedman and Hutchinson, 1980). Years of exposure to toxic levels has led to development of metal resistant plant populations.
- Mechanisms of metal resistance have been reported in many plant species but knowledge of how woody plants deal with metals is scarce (Visioli *et al.*, 2014). Resistance mechanisms differ from species to species and sometimes within the same species. Root exudation is one of the most commonly studied mechanisms of metal resistance. Plants using this strategy will lower the bioavailability of metals around the rhizosphere by secreting organic acids such as citrate and malate from their roots (Maron *et al.*, 2013). Other plants species increase production of chelating molecules such as phytochelatins (PCs), metallothioneins (MTs) and small molecules from metabolic processes to cope with metal contamination (Callahan *et al.*, 2008).
- The TonB-dependent receptors family (TBDTs) is a large group of proteins that facilitate the transport of molecules across the membrane of gram-negative bacteria. Some evidence exists that TBDTs are involved in metal stress. The existence of a TonB-like mechanism in non - prokaryotes has not been established. The recent development of the *Betula papyrifera* (white birch) transcriptome has allowed the discovery of genes involved in plant adaptation to stress.

Objective

To identify novel genes associated with nickel resistance in *Betula papyrifera*

Methods

Nickel treatments

- White birch (*Betula papyrifera*) seeds were collected from a Laurentian University research field site located in the Greater Sudbury Region in Northern Ontario (Canada). The site has been contaminated with metals for > 100 years. Six month seedlings were treated with nickel nitrate as described in Theriault *et al.*, (2016). Gene expression in genotypes resistant and susceptible to a soil nickel concentration of 1,600 mg/kg was analyzed in details.

Transcriptome analysis

- Methods for extraction, RNA- seq libraries, new generation sequencing, and De Novo Transcripts Assembly are described in Theriault *et al.*, (2016). The raw reads were mapped to Trinity assembled transcripts using bowtie and RSEM was used to quantify transcript and gene expression levels. Gene expression was calculated and expressed as Reads Per Kilobase per Million reads mapped (RPKM). A differentially expressed gene analysis was performed between resistant and susceptible genotypes. The top 25 most upregulated and downregulated genes based on Log2FC from the pairwise comparison were ranked (Tables 1 and 2).

Results

Table 1. Top 10 most upregulated genes in nickel-resistant white birch (*Betula papyrifera*) compared to susceptible genotypes.

Transcript ID	FPKM				Log2FC	adj.P.Val	Description
	Res 1	Res 2	Sus 1	Sus 2			
TR99219 c0_g1	88.837	261.961	0	0.54	11.54	1.97E-07	TonB-dependent receptor
TR91600 c2_g5	209.847	415.208	0	0	10.50	1.92E-18	TonB family protein
TR55738 c2_g6	49.072	24.947	0	0.048	10.14	3.40E-09	F-box/LRR-repeat protein
TR117076 c0_g1	75.234	281.904	0	0	10.02	3.56E-16	Calnexin
TR96264 c0_g1	2.728	3.487	0	0	9.46	4.57E-14	4-hydroxyphenylacetate 3-monooxygenase oxygenase component
TR78530 c1_g1	44.645	71.609	0.115	0.072	9.44	3.09E-05	PREDICTED: protein PHLOEM PROTEIN 2-LIKE A1-like
TR86018 c0_g2	42.155	64.06	0	0	9.34	1.53E-13	Proline-rich protein PRP1
TR67414 c0_g5	48.434	40.922	0	0	8.90	5.98E-12	Protein induced upon tuberization
TR109068 c1_g1	10.829	25.767	0	0	8.88	1.27E-11	Putative transmembrane protein
TR100502 c2_g1	5.78	18.454	0	0	8.83	2.34E-11	NA

FPKM: Fragments Per Kilobase of transcript per Million mapped reads
Log 2FC: Fold change from log2
adj.P.Val: Adjusted p values

Table 2. Top 10 most downregulated genes in nickel-resistant white birch (*Betula papyrifera*) compared to susceptible genotypes.

Transcript ID	FPKM				Log2FC	adj.P.Val	Description
	Res 1	Res 2	Sus 1	Sus 2			
TR16661 c0_g1	0	0	14.611	14.912	-11.94	2.78E-20	A protein
TR94889 c2_g1	0	0.028	232.706	100.675	-11.65	1.14E-14	mRNA cap guanine-N7 methyltransferase 2 isoform X2
TR53148 c0_g1	0	0	14.082	13.831	-10.85	3.07E-16	Replication-associated protein A
TR55969 c0_g1	0	0	26.541	55.026	-9.87	7.29E-13	Ankyrin repeat family protein
TR94980 c2_g4	0	0	65.692	57.535	-9.50	9.80E-12	Serine/threonine-protein kinase
TR61028 c0_g1	0	0	54.682	175.439	-9.19	1.07E-10	NA
TR56209 c7_g8	0	0	114.437	89.869	-9.14	1.56E-10	Putative disease resistance protein RGA3
TR35375 c2_g19	0	0	110.296	66.672	-8.93	6.51E-10	Chaperone protein dnaJ 8, chloroplastic isoform X2
TR79073 c0_g1	0	0	132.672	1.609	-8.90	8.14E-10	Putative ribonuclease H protein At1g65750-like
TR35437 c1_g1	0	0	44.098	88.2	-8.90	7.95E-10	NA

FPKM: Fragments Per Kilobase of transcript per Million mapped reads
Log 2FC: Fold change from log2
adj.P.Val: Adjusted p values

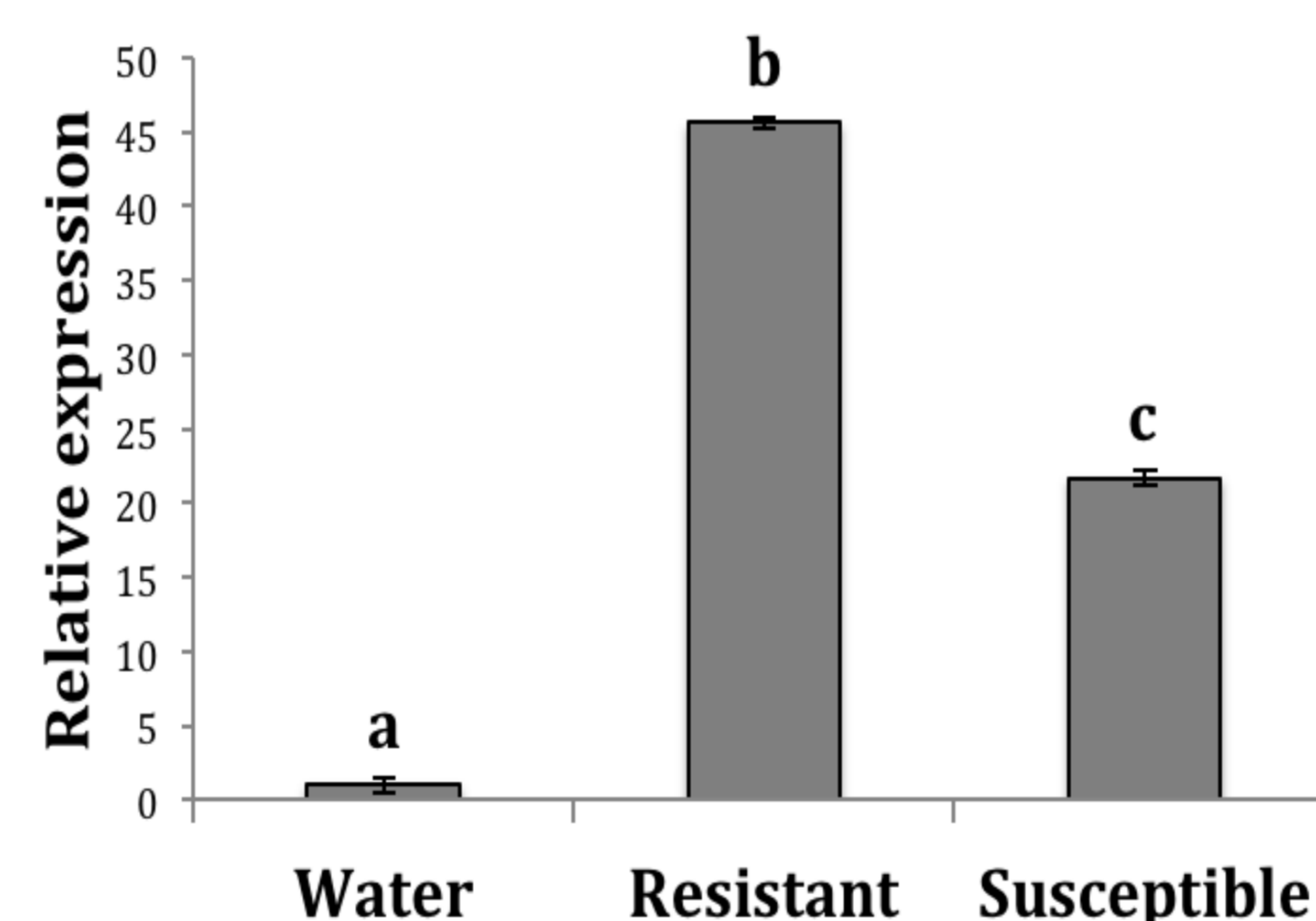


Figure 1. Quantitative qPCR measurement of the expression of the TonB-dependent family receptor (TR99219|c0_g1) in nickel resistant and susceptible white birch (*Betula papyrifera*) treated with 1,600 mg/kg of nickel. Expression was standardized using the housekeeping gene efla. Normalization was then performed against water controls (all values divided by water). * Significant differences were found using ANOVA (Tukey's HSD multiple comparison analysis, p<0.05).

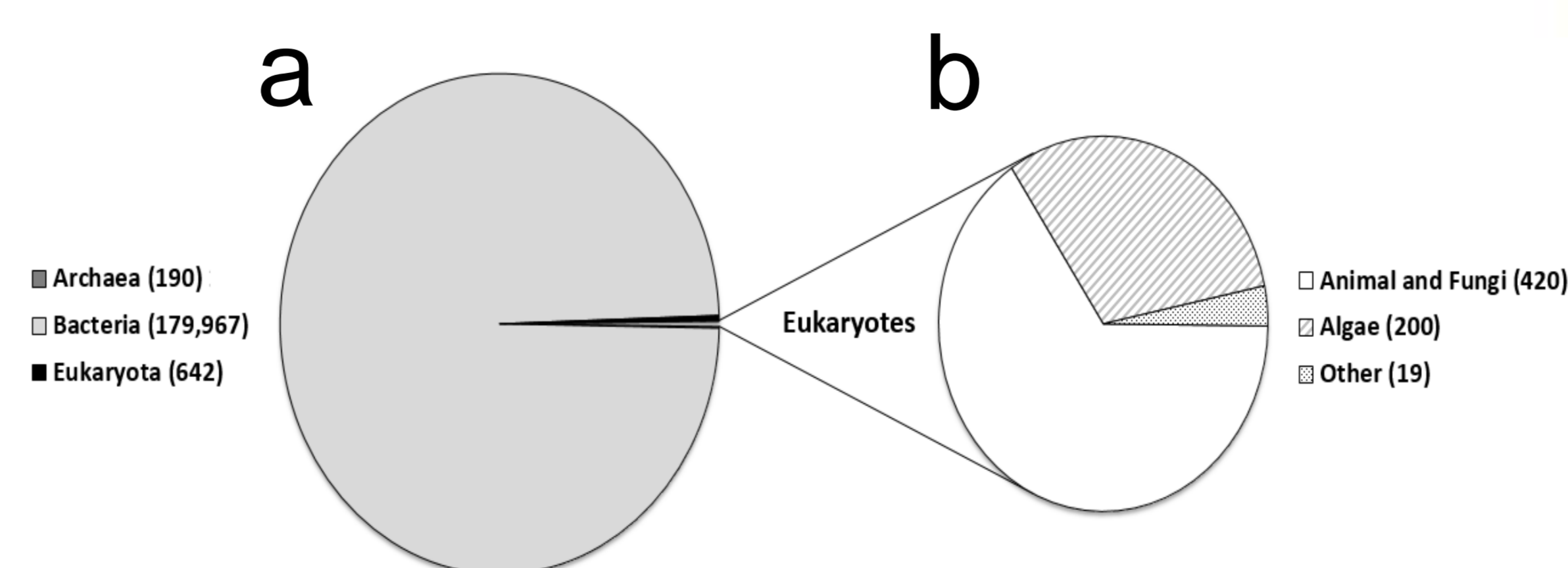


Figure 2: a) Distribution of TonB hits in Uniprot database classified by group of organisms (note that 99.5% hits are from bacteria); b) Distribution of TonB hits in Uniprot database for Eukaryote.

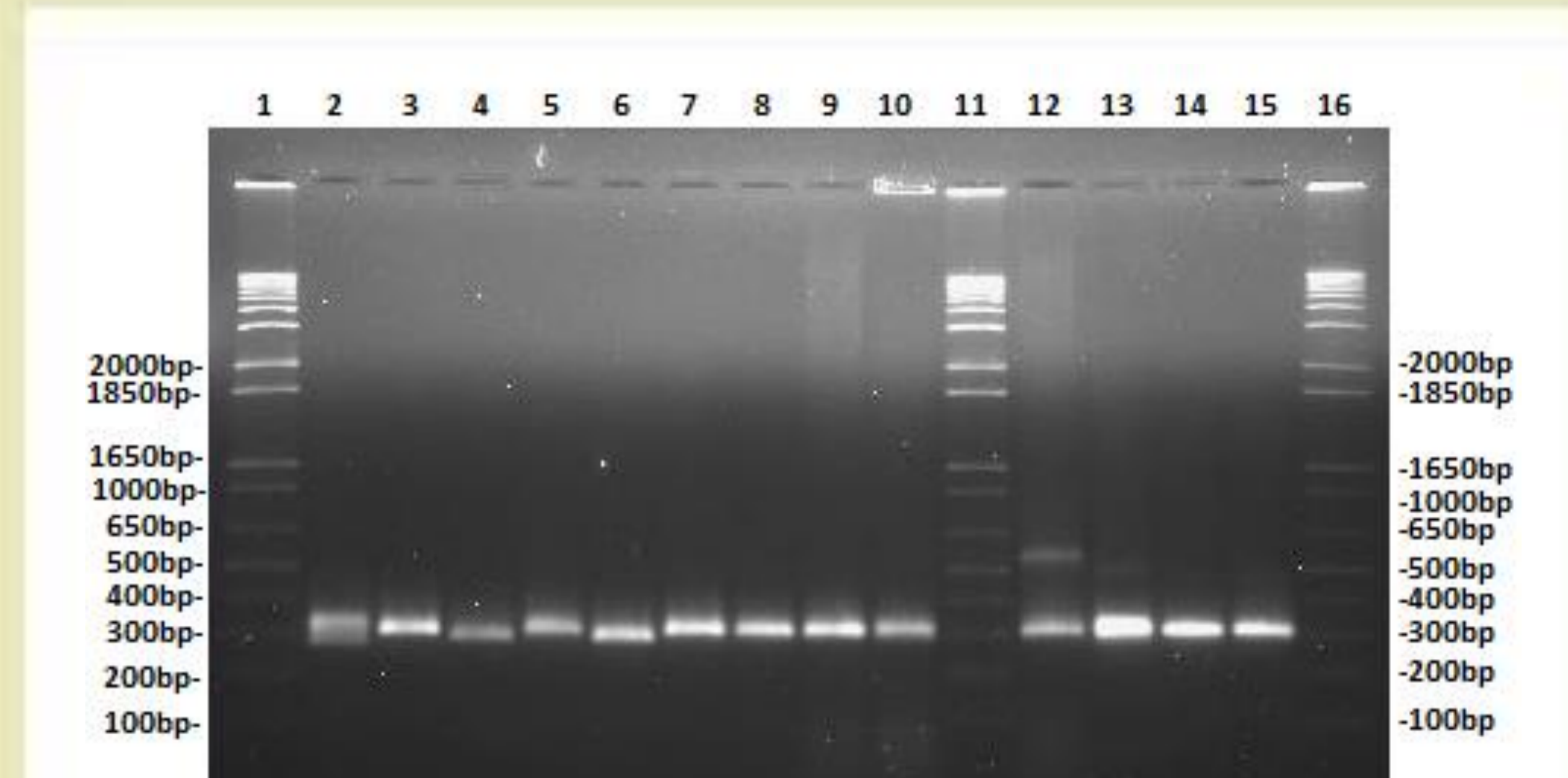


Figure 3a. PCR amplification products on 1% agarose gel. A 300 bp band corresponds to the presence of the TonB-dependent receptor. Lanes 1, 11 and 16 are loaded with 1kb+ ladder; 2 – 10 with white birch (*Betula papyrifera*) from different populations (leaf DNA); 12 *Betula alleghaniensis* (seedling DNA); 13 *Betula minor* (seedling DNA); 14 and 15 white birch (root cDNA and leaf cDNA).

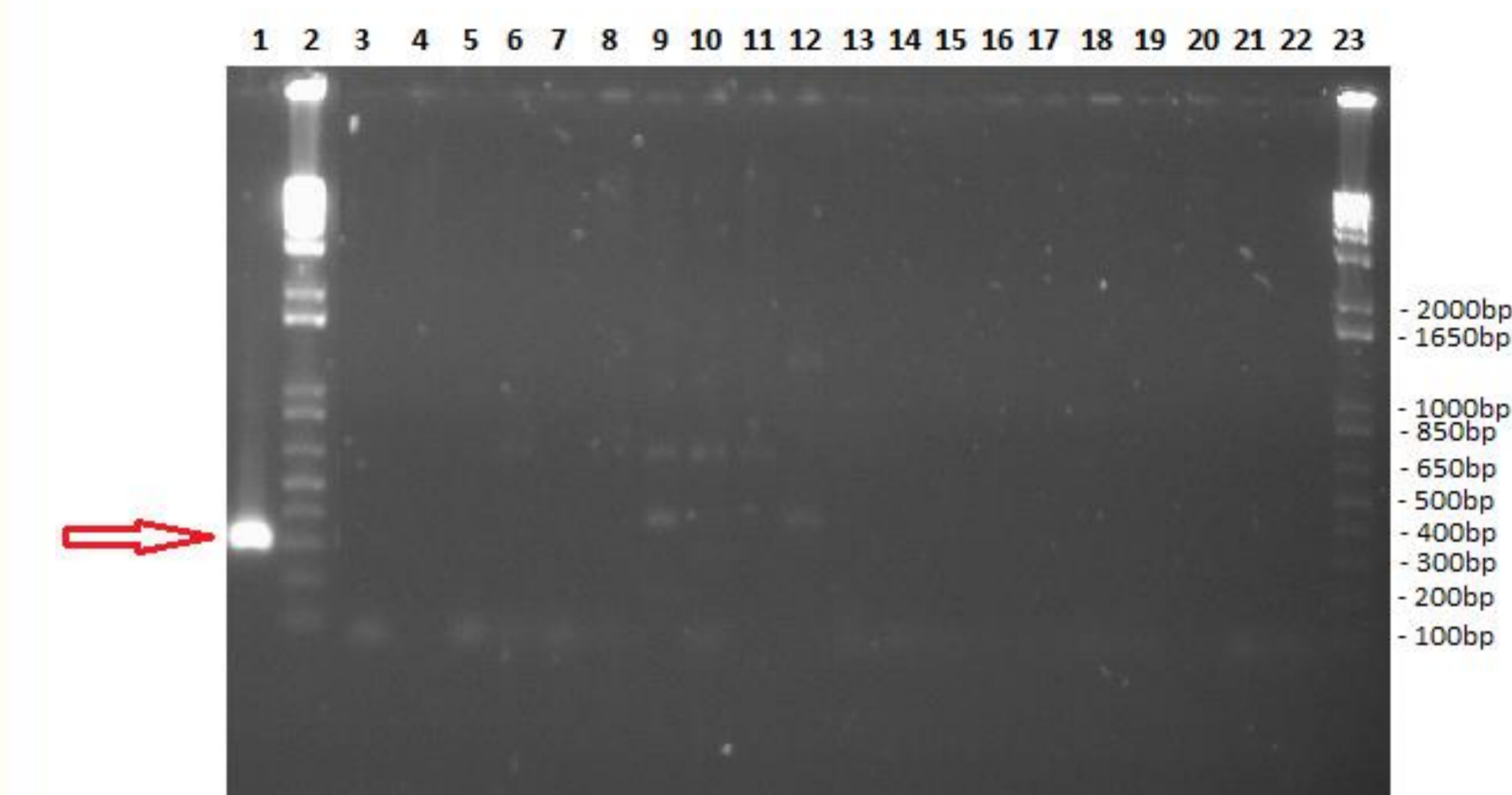


Figure 3b. PCR amplification products on 1% agarose gel. A 300 bp band corresponds to the presence of the TonB-dependent receptor (red arrow). Lane 1: white birch (*Betula papyrifera*), 2 and 23 are loaded with 1kb+ ladder, 3 – 22 are the following species in order: *Quercus rubra*, *Populus tremuloides*, *Acer rubrum*, *Pinus strobus*, *Pinus monticola*, *Pinus nigra*, *Pinus sylvestris*, *Pinus contorta*, *Pinus rigida*, *Pinus banksiana*, *Pinus resinosa*, *Pinus montana*, *Picea glauca*, *Picea sitchensis*, *Picea wilsonii*, *Picea jezoensis*, *Picea orientalis*, *Picea engelmannii*, and *Picea pungens*.

Conclusion

- This is the first study that shows and documents the existence of a TonB-dependent receptor and a TonB-like family protein in plants.
- The TonB-dependent receptor and TonB-like family protein are associated with nickel resistance in white birch.
- The TonB-dependent receptor appears to be exclusive to the *Betula* genus, suggesting that *Betula* species might have acquired the gene via a recent horizontal gene transfer from prokaryotes or fungi.

Acknowledgements

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