Mitochondrial ribosomal proteins involved in tellurite resistance in yeast *Saccharomyces cerevisiae*

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A considerable body of evidence links together mitochondrial dysfunctions, toxic action of metalloid oxyanions, and system and neurodegenerative disorders. In this study we have used the model yeast *Saccharomyces cerevisiae* to investigate the genetic determinants associated with tellurite resistance/sensitivity. Nitrosoguanidine-induced K$_2$TeO$_3$-resistant mutants were isolated, and one of these mutants, named Sc57-Te$_5$, was characterized. Both random spore analysis and tetrad analysis and growth of heterozygous (Te$_5$Te$_5$) diploid from Sc57-Te$_2$ mutant revealed that nuclear and recessive mutation(s) was responsible for the resistance. To get insight into the mechanisms responsible for K$_2$TeO$_3$-resistance, RNA microarray analyses were performed with K$_2$TeO$_3$-treated and untreated Sc57-Te$_5$ cells. A total of 372 differentially expressed loci were identified corresponding to 6.37% of the *S. cerevisiae* transcriptome. Of these, 288 transcripts were up-regulated upon K$_2$TeO$_3$ treatment. About half of up-regulated transcripts were associated with the following molecular functions: oxidoreductase activity, structural constituent of cell wall, transporter activity. Comparative whole-genome sequencing allowed us to identify nucleotide variants distinguishing Sc57-Te$_5$ from parental strain Sc57. We detected 15 CDS-inactivating mutations, and found that 3 of them affected genes coding mitochondrial ribosomal proteins (MRPL44 and NAM9) and mitochondrial ribosomal biogenesis (GEP3) pointing out to alteration of mitochondrial ribosome as main determinant of tellurite resistance.

Tellurium (Te) is a highly toxic metalloid belonging to the chalcogen family, chemically related to selenium and sulfur. Te is a relatively rare on Earth’s crust where it is commonly found in combination with many metals forming metal tellurides. Concentrations of Te compounds may be elevated in soils and waters in proximity of wastewater treatment plants of certain industrial settings. These compounds, and in particular potassium tellurite (K$_2$TeO$_3$), are toxic to most microorganisms. It is believed that tellurite toxicity is mainly due to its ability to act as a strong oxidizer toward a variety of cellular components. It has been recently argued that tellurite could exert its toxic effect through the generation of reactive oxygen species (ROS). Indeed, intracellular ROS such as hydrogen peroxide (H$_2$O$_2$), superoxide anion (O$_2^−$) and hydroxyl radical (•OH) are common by-products of the aerobic metabolism that are produced upon exposure of cells to free radical-generating molecules like metals and metalloids. Nevertheless, naturally occurring tellurite resistant bacteria may be isolated from polluted environments, and they often reduce toxic tellurite to less toxic elemental form Te$_0$ that accumulates inside the cell forming black deposits. On a mechanistic point of view, tellurite enters bacterial cells thorough the phosphate terminal oxidases, glutathione and other thiol-carrying molecules. Other proposed mechanisms mediating...
tellurite resistance involve cysteine-metabolizing enzymes and methyl transferases. Bacterial Te resistance (TeR) genetic determinants may be located on either plasmids or chromosome, and most of these determinants mediate tellurite resistance by unknown mechanisms.

Not only prokaryotes, but also eukaryotes such as fungi and yeasts, in addition to plant and animal tissues, have the ability to reduce tellurite by various reactions that lead to accumulation of black Te0 precipitates. Interestingly, Te toxicity has been related to the etiopathogenesis of several neurodegenerative disorders. In rat, Te causes cerebral lipofuscinosis with cognitive impairment, a pathological picture that is similar to that described in Kuf’s disease that has some clinical and neuropathological traits reminiscent of the Alzheimer’s disease. Te may impair mitochondrial functions resulting in defective energy metabolism and increased oxidative damage, which may be relevant to the pathogenesis of neurodegenerative disorders. Moreover, tellurite resistance and/or its underlying mechanisms have been directly/indirectly implicated in Friedreich’s ataxia, an autosomal recessive neurodegenerative disorder that is caused by mutations affecting frataxin gene (FRDA) coding for a mitochondrial matrix protein. Although this protein has been implicated in mitochondrial iron homeostasis, its function has not yet been completely elucidated. Frataxin homologs were found in Caenorhabditis elegans roundworm and S. cerevisiae yeast. A frataxin homologue (cyaY) is also present in Proteobacteria including purple bacteria, which are believed to be the closest living relatives to mitochondria, suggesting that FRDA evolved from cyaY from the bacterial ancestor of mitochondria. Multiple sequence alignment of frataxin with homologous proteins from eukaryotes and bacteria showed that the conserved core region between frataxin and CyaY proteins share sequence homologies with the N-terminal region of small proteins which confer resistance to Te (TeR) compounds. Although the biochemical function of tellurite resistance proteins is currently unknown, it is a generally accepted hypothesis that TeR results from conversion of tellurite to a less toxic compound. However, since tellurium compounds are very rare in the environment it appears unlikely that primary function of the TeR protein is to detoxify tellurium. On the other hand, the extensive diffusion of TeR proteins among bacteria suggests an important role in cellular life. Hence, the identification of mutations conferring tellurite resistance in yeasts should provide insights into fundamental functions of cellular importance to unveil the molecular bases of certain human diseases.

**Results**

**Te0 precipitation test.** Tellurium precipitation (likely Te0) was observed in the presence of increasing amounts of K2TeO3 in microtiter plate assays (Fig. 1). The amount of Te0 deposited in microtiter wells by respiratory-competent strain Sc57 increased as a function of K2TeO3 concentration. Conversely, Te0 precipitation could not be detected either when Sc57 was cultivated in YEG medium (data not shown), or when respiratory-deficient Sc57- R3, cultured in YED medium, was used (Fig. 1). The different results of tellurium precipitation assays with rho+ and rho0 strains, and the different tolerance to K2TeO3 of rho+ yeast cells in YED with respect to YEG suggested that Te was affecting mitochondrial activities, confirming previous work.

**Transmission electron microscopy.** Images from transmission electron microscopy (TEM) demonstrated that Te0 formed large deposits along the cell wall of yeast cells (Fig. 2A, arrowheads), and smaller deposits in the cytoplasmic matrix (Fig. 2B, arrowheads). In yeast cells mitochondria, Te0 grains were also detected either on the internal membranes (crisatae), or in the interspaces between the internal and external membranes (Fig. 2B, arrowheads). This finding further indicated that mitochondria could play a key role in tellurite toxicity.

**Isolation and physiological characterization of the tellurite resistant mutant.** Since S. cerevisiae rho+ cells were able to grow in glucose medium with increasing concentrations of K2TeO3 causing blackening of either the culture medium (Fig. 1) or the plated colonies, the isolation of tellurite resistant mutants was possible only in YEG medium containing glycerol as non-fermentable carbon source. Growth of the parental S. cerevisiae wild type strain Sc57 is inhibited by 12.5 μg/ml K2TeO3 in YEG broth, whilst is inhibited by 25 μg/ml K2TeO3 in YEG plates. The strain Sc57 was mutagenized with NTG (40 μg/ml; 28°C; 50 min) to yield a surviving fraction of 5–10%. Cells were plated onto glycerol medium (YEG plates) containing 100 μg/ml K2TeO3. Mutants appeared at a frequency of about 5 per 107 cells after 10 days of incubation at 25°C. No spontaneous mutants appeared among 106 cells under the same conditions. Altogether 3400 colonies were screened by replica plating for resistance to K2TeO3. Nine out 3400 colonies examined showed the TeR phenotype. These putative mutants were purified
three times by isolation and only four of them presented a stable TeR phenotype. One of these mutants, named Sc57-Te5R (rho+), was subsequently characterized.

Growth curves of the parental strain Sc57 TeS as well as the mutant Sc57-Te5R in YEG broth in presence and absence of 100 µg/ml K-Te are presented in Fig. 3. As shown, growth of the parental strain Sc57 TeS was completely inhibited, whereas a growth kinetic similar to that of strains in K₂TeO₃ free medium was observed for the mutant Sc57-Te5R. Besides, it was also performed the growth by dropping for controlling the resistance to K₂TeO₃ on solid medium with glycerol (YEG) as shown in Supplementary Fig. S1. It is well evident in Supplementary Fig. S1 the colony growth of both the mutant Sc57-Te5R and the parental Sc57 TeS strains without tellurite, while with tellurite only the mutant Sc57-Te5R was able to grow.

No particular difference were observed by TEM in tellurium deposit along the cell wall and in the cytoplasmic matrix between Sc57 and Sc57-Te5R yeast cells grown in YED whereas a slight reduction of Te0 deposits seemed to be evident in the mitochondria of Sc57-Te5R (data not shown).

Figure 2. TEM micrograph sections of S. cerevisiae Sc57 yeast cells grown in the presence of K₂TeO₃. (A) Sc57 cell. Scale bar: 2.5 µm. Arrowheads indicate elemental Tellurium (Te0) deposits. (B) Details of the image in A showing elemental Te⁰ grains tended to form deposits along the cell wall, in the cytoplasmic matrix, in the mitochondrial cristae and in the interspaces between the mitochondrial internal and external membranes (arrowheads). Scale bar: 1 µm.

Figure 3. Growth curves of S. cerevisiae strains in non-fermentable media (YEG) in presence (closed symbol) or absence (open symbol) of 100 µg/ml K₂TeO₃: ○ Sc 57. △ Sc 101. ■ Sc 57-Te5R.
Genetic analysis of Sc57-Te5R. The mutant Sc57-Te5 was crossed with strain Sc101 (TeR) and diploids selected on minimal medium (YNG supplemented with appropriate nutritional). Approximately 2,000 individual diploid clones were assayed by replica plating on K2TeO3 containing medium. All of them proved to be sensitive to the drug, indicating recessive nuclear mutation. Recombinant diploids from the cross were sporulated and genetic analysis was carried out either by random spore analysis or by tetrad analysis. With reference to random spore analysis, 2,300 spores were analyzed showing a nearly 1:1 segregation of auxotrophic markers, while 47% of the progeny was wild type tellurite sensitive and 53% showed a tellurite resistant phenotype. These data demonstrate that the tellurite resistant phenotype is associated with nuclear mutation(s). The nuclear origin of resistance to tellurite is being confirmed by 12 complete tetrads analyzed. In all cases the tellurite sensitive versus resistant phenotype segregated 2 TeS: 2 TeR. In all tetrads the nuclear auxotrophic markers segregate 2:2.

Cloning and homology analysis of PCR frataxin sequences. The homology between frataxin family to bacterial protein family that are associated to resistance to Te4 phenotype. We thus amplified, cloned in E. coli, and sequenced the frataxin gene from Sc57 and Sc57-Te5. A total of 10 clones were examined from each yeast strain. The alignment of sequences highlighted the almost total correspondence between all the sequences obtained and those deposited in the database, and the absence of differences between Sc57 and Sc57-Te5 frataxin genes. Therefore, the phenotypic differences observed between Sc57 (TeR) and Sc57-Te5 could not be attributed to the nucleotide variants in the frataxin locus.

Gene expression measurement by RNA microarray. In order to measure fluctuations in gene expression, Affymetrix genechips (GeneChip Yeast Genome 2.0 Array) were utilized, providing a comprehensive coverage of both S. cerevisiae (5,841 probe sets) and S. pombe (5,021 probe sets). Total RNA, obtained from Sc57-Te5 strain treated with K2TeO3, and from an untreated control was used to prepare molecular probes and hybridize the yeast microarray. The dataset obtained was analyzed using the FlexArray 1.6.1 software suite (Génome Québec and McGill University). First, raw data were normalized using the PLIER (Probe Logarithmic Intensity Error) algorithm. Subsequently, significant expression differences were identified by means of t-tests. Only differently expressed sequences with p-values < 0.01 and expression ratios > 2.0 between induced and non-induced yeast cells were taken into consideration. In this manner, a total of 370 differentially expressed loci were identified, corresponding to 6.37% of the S. cerevisiae transcriptome. Of these, 286 sequences appeared up-regulated upon tellurite induction (Supplementary Table S1), while 84 sequences resulted down-regulated (Supplementary Table S2). Differentially expressed sequences were analyzed with «The Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.8» (https://david.ncifcrf.gov/), to identify enriched groups of genes with altered expression values associated with the applied treatment. Down-regulated sequences did not, but marginally, combine into larger groups after gene enrichment by molecular function (65% of the sequences did not combine into groups). Two groups with a reasonable number of genes could be identified. A first group, counting 15 sequences, was related with «transferase activity» (GO:0016740), while a second group of 10 sequences was related with «oxidoreductase activity» (GO:0016491). Up-regulated sequences, on the other hand, combined into several groups with distinct molecular functions. Sequences aggregated into 39 groups after GO enrichment analysis for «Biological Processes», while 17 enriched groups were obtained for the «Molecular Function» sub-ontology group (Table 1). Taking into consideration the «Cellular Component» sub-ontology group, a large cluster containing 101 (35.4%) of the differentially expressed sequences could be identified. These sequences were all correlated with GO:0031224 (intrinsic component of membrane). A smaller group of 26 sequences (9.1%), correlating with GO:0016022 (endoplasmic reticulum) could also be identified (data not shown).

Mitochondrial genes were not differently expressed. However, when the expression of nuclear genes coding for 901 “high-confidence” mitochondrial proteins was examined, 20 and 17 sequences appeared, respectively, up- and down-regulated in response to K2TeO3 treatment (Supplementary Tables S1, S2, and Table 2). Among the up-regulated genes there were some involved in carbon and energy metabolism (HXK2, ALD5, CYC1), leucine biosynthesis (LEU9), mitochondrial iron homeostasis (FTR1, MMT1, GGC1), oxidation-reduction processes (OYE2), repair of oxidative damage to mitochondrial DNA (OGG1), and mitochondrial inner membrane protein assembly (TIM12). Among the down-regulated genes we found those involved in 2-methylcitrate pathway (CIT3, PDH1, ICL2), glyoxalase system (GLO4), redox homeostasis (AA1, TRR2), iron-sulfur clusters assembly (ISU1), lipid metabolism (YAT1, YMR210W, TES1), and cytochrome oxidase assembly (COX23).

Comparative genomics of Sc57 and Sc57-Te5 strains. We sought to identify variants introduced in Sc57-Te5 strain by the mutate-and-screen method that determined the tellurite resistance phenotype. We thus sequenced the whole genome of Sc57-Te5 and parental strain Sc57, and compared them with those of reference strain S288C.25 We then identified nucleotide variants distinguishing Sc57-Te5 from parental strain Sc57. Apart from synonymous substitutions and variants occurring in intergenic regions, we counted a total of 270 variants affecting 217 coding sequences (CDS) in Sc57-Te5 (Supplementary Table S3). Among these, 248 were missense mutations, 11 nonsense mutations, 5 sense mutations, 1 frame-shift mutation, and 5 in frame insertions/deletions. Among missense mutations, 128 are conservative and 120 non-conservative based on BLOSUM62 algorithm. All mutations map on nuclear genes consistently with tetrad analysis. We focused on 15 CDS-inactivating mutations (Table 3) and noticed that 3 of them affect single-copy genes coding mitochondrial ribosomal proteins (MRPL44 and NAM9) or a protein that is involved in mitochondrial ribosomal biogenesis (GEP3). The other mutations affect gene encoding ribosomal protein (RPL33A), histone H2A (HTA1), chromatin-silencing protein (SIR3), phosphoglucomutase (PGM2), phosphatidylinositol-3-/phosphoinositide 5-phosphatase (INP53), ferric-chelate reductase (Fre8), transcriptional regulatory proteins (CYC8, TBS1), SEM1 (proteasome regulatory...
## Table 1.
Summary of microarray analysis. GO IDs associated with significantly altered gene expression (p < 0.01) and their corresponding terms are listed. BP – biological process, MF – molecular function, % - percentage of gene sequences included within each group with respect to the total number of sequences identified.

| Term | GO | % | P-Value |
|------|----|----|---------|
| transport | GO:0006810 | 16.35 | 4.80E-04 |
| transmembrane transport | GO:0035085 | 8.34 | 2.00E-06 |
| oxidation-reduction process | GO:0053114 | 7.77 | 5.60E-03 |
| ribosome biogenesis | GO:0042254 | 5.48 | 3.00E-03 |
| rRNA processing | GO:0006364 | 4.91 | 2.00E-02 |
| fungal-type cell wall organization | GO:0031505 | 4.33 | 3.50E-03 |
| ion transport | GO:0006811 | 4.01 | 2.20E-03 |
| iron ion homeostasis | GO:0055072 | 3.43 | 1.80E-08 |
| response to stress | GO:0006950 | 3.19 | 2.00E-05 |
| maturation of SSU-rRNA from tricistronic rRNA transcript | GO:0000462 | 3.19 | 1.10E-03 |
| rRNA methylation | GO:0031167 | 2.62 | 4.20E-03 |
| cellular amino acid biosynthetic process | GO:0006696 | 2.62 | 6.60E-02 |
| cellular iron ion homeostasis | GO:0006879 | 2.29 | 2.20E-03 |
| steroid metabolic process | GO:0006879 | 2.29 | 2.20E-03 |
| steroid biosynthetic process | GO:0015918 | 1.14 | 2.20E-02 |
| fructose transport | GO:0044690 | 0.57 | 8.70E-02 |
| mannose transport | GO:0044319 | 0.57 | 8.70E-02 |
| arseneate ion transmembrane transport | GO:0041684 | 0.57 | 8.70E-02 |
| fructose transmembrane transporter activity | GO:0005353 | 4.35 | 2.60E-02 |
| cyclo-dependent protein serine/threonine kinase regulator activity | GO:0041658 | 4.35 | 8.80E-02 |
| ferric-chelate reductase (NADPH) activity | GO:0032851 | 3.42 | 3.60E-02 |
| iron ion transmembrane transporter activity | GO:0035381 | 3.42 | 4.60E-02 |
| ferric-chelate reductase activity | GO:0000293 | 3.42 | 5.80E-02 |
| monooxygenase activity | GO:0084497 | 3.42 | 5.80E-02 |
| zinc ion transmembrane transporter activity | GO:0085385 | 3.42 | 8.30E-02 |
| alcohol dehydrogenase activity | GO:0008012 | 2.17 | 8.70E-02 |
| methionine adenosyltransferase activity | GO:004478 | 2.17 | 8.70E-02 |
| sugar-phosphatase activity | GO:0050308 | 2.17 | 8.70E-02 |

**Term GO % P-Value**

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- transport GO:0006810 16.35 4.80E-04
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- ion transport GO:0006811 4.01 2.20E-03
- iron ion homeostasis GO:0055072 3.43 1.80E-08
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- maturation of SSU-rRNA from tricistronic rRNA transcript GO:0000462 3.19 1.10E-03
- rRNA methylation GO:0031167 2.62 4.20E-03
- cellular amino acid biosynthetic process GO:0006696 2.62 6.60E-02
- cellular iron ion homeostasis GO:0006879 2.29 2.20E-03
- steroid metabolic process GO:0006879 2.29 2.20E-03
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- sugar-phosphatase activity GO:0050308 2.17 8.70E-02

**MF**
- oxidoreductase activity GO:0016491 27.33 2.30E-03
- structural constituent of cell wall GO:0005199 10.87 1.20E-04
- transporter activity GO:0005215 9.94 3.20E-02
- transcription factor activity GO:0003700 8.70 3.00E-02
- hydrolase activity, acting on glycosyl bonds GO:0016798 6.52 5.20E-02
- mannose transmembrane transporter activity GO:0015578 4.35 2.60E-02
- fructose transmembrane transporter activity GO:0005353 4.35 2.60E-02
- cyclin-dependent protein serine/threonine kinase regulator activity GO:0041658 4.35 8.80E-02
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Tellurite resistance in MRPL44, NAM9 and GEP3 knock-out mutants. To evaluate whether the tellurite resistance phenotype could be associated with the 3 CDS-inactivating mutations affecting the mitochondrial ribosomal proteins or the mitochondrial ribosomal biogenesis, MRPL44, NAM9 and GEP3 knock-out mutants were tested. All three mutants were available as heterozygous diploid strains derived from BY4743. Two of them (MRPL44 and GEP3) were also available as MATa haploid strains derived from BY4741 (Table 4). The results of MIC experiments demonstrated that the two parental strains BY4743 and BY4741 were inhibited by K2TeO3 concentrations equal or higher than 25 μg/ml in YEG broth (Supplementary Fig. S2). In contrast all three heterozygous diploid derivative MRPL44, NAM9 and GEP3 knock-out mutants, and the haploid MRPL44 knock-out mutant were able to grow at this concentration (Supplementary Fig. S2), and were inhibited at K2TeO3 concentrations equal or higher than 100 μg/ml (data not shown). The GEP3 haploid failed to grow in glycerol medium YEG exhibiting a rho- phenotype.

Discussion

The budding yeast has established as a formidable experimental model for the study of the fundamental cell mechanisms and their dysfunctions. In this study the model yeast has been used to investigate the molecular mechanisms underlying tellurite resistance/toxicity, a phenotype that has been associated to several neurodegenerative disorders in humans16–18. We have isolated and characterized at both the genetic and molecular levels an induced mutant of S. cerevisiae yeast, resistant to tellurite by means of nitrosoguanidine treatment. The results described here present as a first experiment the evidence of the deposit of elemental tellurium Te0 during the growth of the parental strain Sc57 (TeS) in YED broth, with glucose as a fermentable carbon source. As demonstrated in Fig. 1, in the growth wells is observed a deposit of elemental tellurium, which increases with increasing of the K2TeO3 dose. Slightly less deposit is observed with mutant Sc57-Te5, strain. The place where elemental tellurium is deposited within the yeast cell was analyzed by TEM. The images show that Te0 formed large deposits along the yeast cell wall (Fig. 2A), and smaller deposits in the cytoplasm and mitochondria (inner membrane cristae and interspace between inner and outer membranes) (Fig. 2B, arrows). With reference to the isolation of the mutant strain named Sc57-Te5, resistant to 100 μg K2TeO3 in glycerol medium YEG, it was isolated from the parental strain Sc57 Te2 after NTG treatment and successively characterized both genetically and molecularly.

The tellurite resistance phenotype of the mutant strain Sc57-Te5 was shown by monitoring the growth of the yeast strains in YEG broth, glycerol complete medium, with and without addition of 100 μg K2TeO3. In fact, as shown in Fig. 3, growth of the parental strain Sc57 Te2 was completely inhibited in presence of 100 μg K2TeO3, whereas growth kinetics similar to that of strains in tellurite free medium was observed for the mutant Sc57-Te5, strain. Besides, the tellurite resistance phenotype was also controlled by dropping the yeast cultures on YEG plates, glycerol complete solid medium, with and without addition of 100 μg K2TeO3, respectively. As shown in Supplementary Fig. S1, both colonies of Sc57 Te2 strain and Sc57-Te5 are growing on YEG without addition of tellurite, while on YEG plate with 100 μg K2TeO3 is observed only growth of the mutant strain Sc57-Te5.

To investigate the molecular mechanisms involved in K2TeO3 resistance/toxicity, the complete transcript profile of Sc57-Te5 cells treated with tellurite and from an untreated Sc57-Te5 control was analyzed by RNA microarray. A total of 372 differentially expressed loci could be identified, corresponding to 6.37% of the S. cerevisiae transcriptome. Of these, 288 sequences appeared up-regulated upon K2TeO3 treatment (Supplementary Table S1), while 84 sequences resulted down-regulated (Supplementary Table S2). As previously mentioned, Te0 deposition in Sc57-Te5 cells, was found predominantly along the cell wall, in the cytoplasmic matrix, and the internal membrane of mitochondrial cristae (Fig. 2). This finding is in good agreement with the obtained expression patterns showing an increased expression of a large group (16.35%) of sequences involved in membrane transport (GO:0006810; GO:00055085; GO:0005199; GO:0005215 in Table 1).

Curiously, while tellurium deposition in bacteria is commonly associated with Te(IV) resistance and in yeast, conversely, with Te(IV) susceptibility, the Sc57-Te5 strain exhibits a resistant phenotype in spite of an evident Te0 accumulation. A common response of S. cerevisiae to metal exposure regards the suppression of genes involved in ribosomal biogenesis and translation20–22. Again, the Sc57-Te5 strain exhibited an opposite response pattern, since several ribosome biogenesis related sequences exhibited an augmented expression upon tellurite induction (GO:0042254; GO:0042273; GO:0042274 in Table 1), an indication of the resistant phenotype of this strain. Finally, the Sc57-Te5 strain exhibited an enhanced expression of sequences involved in general defense functions such as (i) a general stress response (GO:0006950 in Table 1), (ii) metal-specific detoxification strategies such as the chelation of metal ions via siderophores (GO:0005891; GO:0044718 in Table 1), (iii) the sugar/carbohydrate metabolism (GO:0046323; GO:0015755; GO:0015761 in Table 1); and (iv) metal-ion homeostasis and transport (GO:0006811; GO:0005072; GO:0006879; GO:0015677; GO:0006825; GO:0006829; GO:0006826; GO:0071577 in Table 1) as expected during growth under metal exposure23–25.

The expression of yeast frataxin gene (YFH1) was not significantly affected. However, when expression of nuclear genes coding for 901 “high-confidence” mitochondrial proteins26 was analyzed, two sequences dealing with frataxin gene function were found to be down-regulated upon K2TeO3 treatment: ISU1 coding for a protein of the mitochondrial matrix, which plays scaffolding function during iron-sulfur clusters assembly, and physically and functionally interacts with yeast frataxin11–13, and AAT1 coding for mitochondrial aspartate aminotransferase, which contributes to the respiratory deficit of yeast frataxin-deficient cells14 (Table 2). ISU1 down-regulation might represent a defensive response toward tellurite-induced disabling of iron-sulfur clusters that leads to release ferrous ions, which in the presence of hydrogen peroxide may generate DNA-damaging hydroxyl radicals. Iron-sulfur clusters disabling may also explain the observed alterations of the expression of genes involved in

[1] Integral Membrane Protein 1 (INP1), a protein involved in peroxisome retention, CDC40 (pre-mRNA splicing and cell cycle progression factor), and retroviral element (YHR214C-B).
| GeneID  | Symbol | Chromosome | Description |
|--------|--------|------------|-------------|
| 852540 | YBR238C| II         | Mitochondrial membrane protein with similarity to Rmd9p; not required for respiratory growth but causes a synthetic respiratory defect in combination with rmd9 mutations; transcriptionally up-regulated by TOR, deletion increases life span |
| 852639 | HXK2   | VII        | Hexokinase isoenzyme 2 that catalyzes phosphorylation of glucose in the cytosol |
| 852707 | SUA5   | VII        | Single-stranded telomeric DNA-binding protein, required for normal telomere length |
| 856445 | FSH1   | VIII       | Fsh1p, serine-type hydrolase |
| 852817 | PUS2   | VII        | Mitochondrial tRNA-pseudouridine synthase |
| 856804 | ALD5   | V          | Ald5p, acetaldehyde dehydrogenase involved in the biosynthesis of acetate during anaerobic growth on glucose |
| 854942 | OGG1   | XIII       | Mitochondrial glycosylase/lyase that specifically excises 7,8-dihydro-8-oxoguanine residues located opposite cytosine or thymine residues in DNA, repairs oxidative damage to mitochondrial DNA, contributes to UVA resistance |
| 853507 | CYC1   | X          | Cyc1p, iso-1-cytochrome c |
| 852388 | TIM12  | II         | Essential protein of the inner mitochondrial membrane, peripherally localized; component of the TIM22 complex, which is a twin-pore translocase that mediates insertion of numerous multispanning inner membrane proteins |
| 850636 | SSA2   | XII        | ATP binding protein involved in protein folding and vacuolar import of proteins; member of heat shock protein 70 (HSP70) family |
| 852401 | YMC2   | II         | Mitochondrial protein, putative inner membrane transporter with a role in oleate metabolism and glutamate biosynthesis; member of the mitochondrial carrier (MCF) family |
| 851561 | MCD1   | IV         | Essential subunit of the cohesin complex required for sister chromatid cohesion in mitosis and meiosis; apoptosis induces cleavage and translocation of a C-terminal fragment to mitochondria; expression peaks in S phase |
| 856584 | OYE2   | VIII       | Oye2p, NADPH oxidoreductase; involved in oxidative stress response |
| 856888 | FTR1   | V          | Ftr1p, ferric iron permease |
| 855215 | MMT1   | XIII       | Mmt1p; involved in mitochondrial iron export |
| 852060 | UTP6   | IV         | Nuclear protein, component of the small subunit (SSU) processome containing the U3 snRNA that is involved in processing of pre-18s rRNA |
| 856671 | UTR2   | V          | Chitin transglycosylase that functions in the transfer of chitin to beta(1–6) and beta(1–3) glucans in the cell wall |
| 851329 | GGC1   | IV         | Ggc1p, mitochondrial GDP/GTP carrier; has a role in mitochondrial iron transport |
| 146646 | YJL133C-A| X         | Putative protein of unknown function; the authentic, non-tagged protein is detected in highly purified mitochondria in high-throughput studies |
| 854275 | LEB9   | XV         | Alpha-isopropylmalate synthase II (2-isopropylmalate synthase), catalyzes the first step in the leucine biosynthesis pathway |
| 856108 | PDH1   | XVI        | Mitochondrial protein that participates in respiration, induced by diauxic shift; homologous to E. coli PrpD, may take part in the conversion of 2-methylcitrate to 2-methylisocitrate |
| 850844 | PCD1   | XII        | Pcd1p, Nudix hydrolase for oxidized purine nucleoside triphosphates |
| 856114 | ICL2   | XVI        | 2-methylisocitrate lyase of the mitochondrial matrix, functions in the citrate cycle to catalyze the conversion of 2-methylisocitrate to succinate and pyruvate; ICL2 transcription is repressed by glucose and induced by ethanol |
| 856107 | CIT3   | XVI        | Cit3p, citrate/2-methylcitrate synthase |
| 854205 | GLO4   | XV         | Gho4p, mitochondrial glyoxalase II |
| 852179 | BNA4   | II         | Bna4p, kynurenine 3-monoxygenase; involved in tryptophan degradation; involved in Huntington disease in humans |
| 852663 | MIG2   | VII        | Mig2p, transcriptional regulator mediating glucose repression |
| 853145 | BNS1   | VII        | Bns1p; component of FEAR (CDC14 early anaphase) network |
| 853755 | AAT1   | XI         | Aat1p, mitochondrial aspartate aminotransferase; contributes to the respiratory deficit of yeast frataxin-deficient cells |
| 856506 | TRR2   | VIII       | Mitochondrial thoredoxin reductase involved in protection against oxidative stress, required with Glt1p to maintain the redox state of Trx3p |
| 855968 | ISU1   | XVI        | Conserved protein of the mitochondrial matrix, performs a scaffolding function during assembly of iron-sulfur clusters, interacts physically and functionally with yeast frataxin (Yfh1p) |
| 853477 | TES1   | X          | Peroxisomal acyl-CoA thioesterase likely to be involved in fatty acid oxidation rather than fatty acid synthesis |
| 855250 | YMR210W| XIII       | Potassium acyltransferase with similarity to Eeh1p and Eh1p, has a minor role in medium-chain fatty acid ethyl ester biosynthesis; may be involved in lipid metabolism and detoxification |
| 851285 | YAT1   | I          | Outer mitochondrial carnitine acetyltransferase |
| 856516 | COX23  | VIII       | Cox23p; required for cytochrome oxidase assembly |
| 855045 | RSF1   | XIII       | Protein required for respiratory growth; localized to both the nucleus and mitochondria; may interact with transcription factors to mediate the transition to respiratory growth and activate transcription of nuclear and mitochondrial genes |
| 850861 | YLR164W| XII        | Mitochondrial inner membrane of unknown function; similar to Tim18p and Sdh4p; expression induced by nitrogen limitation in a GLN3, GAT1-dependent manner |

Table 2. Genes coding for mitochondrial proteins, which were up- or down-regulated upon K$_2$TeO$_3$ treatment in SC57-Te$_8$$^8$ strain.
mitochondrial carbon and energy metabolism, iron homeostasis, redox balance, and oxidative stress response (Table 2). This view is also consistent with the observed down-regulation of AAT1. Indeed, decreased activity of the mitochondrial aspartate aminotransferase, which functions in tandem with malate dehydrogenase in the malate/aspartate NADH shuttle, was observed in yeasts lacking mitochondrial DNA or mutants deficient in iron-sulfur cluster assembly including frataxin-deficient cells34. Down-regulation of the three key genes of the 2-methylcitrate pathway (CIT3, PDH1, ICL2), which is devoted to utilization of propionyl-CoA that is derived by beta-oxidation of odd-chain fatty acids, degradation of amino acids including isoleucine, methionine and valine is well placed in this context. Indeed, PDH1 encodes a 2-methylcitrate dehydratase that is homolog to PrpD of E. coli, a monomeric iron-sulfur protein with an unstable [2Fe-2S] cluster37.

Both tetrad analysis and random spore analysis confirmed the K_{2}TeO_{3} resistance phenotype of the mutant strain Sc57-Te^{R} to be both a recessive and chromosomally inherited character, not associated with nucleotide variants in the frataxin locus. The results of comparative whole-genome shotgun sequencing allowed us to identify numerous nucleotide variants distinguishing Sc57-Te^{R} from parental strain Sc57. Indeed, apart from synonymous substitutions and variants occurring in intergenic regions, we counted a total of 270 variants affecting 217 coding sequences (CDS) in Sc57-Te^{R} (Supplementary Table S3). Among these, 248 were missense mutations (of which 128 conservative and 120 non-conservative), 11 nonsense mutations, 5 sense mutations, 1 frame-shift mutation, and 5 in frame insertions/deletions. Such a high number of variants were probably due the mutagenic treatment with the potent NTG, and possibly to non-conservative mutation affecting the DNA mismatch-repair gene MSH4 (Supplementary Table S3), which may have generated a mutator phenotype during strain selection. In Sc57-Te^{R} we identified 15 CDS-inactivating mutations. Some of them affect essential genes that are represented in more than one copy on the yeast genome. Three of them, however, inactivate single-copy genes coding mitochondrial ribosomal proteins (MRPL44 and NAM9) or a protein that is involved in mitochondrial ribosomal biogenesis (GEP3). This result points out to alteration of mitochondrial ribosome as a main determinant of tellurite resistance consistently with previous findings32. This finding, which was supported by measurement of tellurite resistance in MRPL44, NAM9 and GEP3 single knock-out mutants (Supplementary Fig. S2), is of great interest also because mitochondrial ribosomal protein genes are candidate genes for many systemic and neurodegenerative diseases in humans including spinocerebellar ataxia with blindness and deafness syndrome (SCABD), autosomal dominant and recessive nonsyndromic sensorineural disease (DFNA and DFNB), multiple mitochondrial dysfunctions (MMDFS), vacuolar neuromyopathy, Moebius syndrome, Leigh syndrome, Usher syndrome, Di George syndrome, Joubert syndrome, Russell-Silver syndrome, Alzheimer disease, Parkinson disease, dilated cardiomyopathy 1E, cataract and diabetes38.

In particular, MRPL44 whose CDS is early interrupted by Q5* nonsense Sc57-Te^{R} (Table 3) encodes a mitochondrial 54S ribosomal protein that is thought to be located in close proximity to the tunnel exit of yeast mitochondrial ribosome39. In human mitochondria MRPL44 protein seems to be involved in the assembly/stability of nascent mitochondrial polypeptides exiting the ribosome40. A role of this protein in regulating the expression of mtDNA-encoded genes has been also postulated41. Reduced levels of this protein mildly affect de novo mitochondrial translation, but lead to large ribosome assembly defect and complex IV deficiency42. MRPL44 mutations cause a slowly progressive multisystem disease with childhood-onset hypertrophic cardiomyopathy and neurological and neuro-ophthalmological impairment that occurs during the second and third decades of life41. NAM9 (also known as MNA6) encodes mitochondrial 37S ribosomal protein that is located to the S4 small subunit ribosomal protein of bacteria, which plays a key role in ribosome assembly and translational fidelity43. However, at variance with the bacterial homologues, Nam9p contains an additional C-terminal extension (CTE) that shows no obvious homology with any known protein. In Sc57-Te^{R} W396* nonsense prematurely interrupts the CTE (Table 3). Disruption of NAM9 perturbs mitochondrial DNA integrity and causes respiratory deficiency44,45, while amino acid substitution in the C-terminal domain causes temperature-dependent loss of the 15S rRNA44. GEP3 (also known as MTG3) is an inner membrane mitochondrial protein that is required for mitochondrial ribosome small subunit biogenesis46. Null mutant is defective in respiration and in maturation of 15S rRNA46. In Sc57-Te^{R} K376* nonsense interrupts the CDS of GEP3.

In conclusion, in Sc57-Te^{R} alteration of mitochondrial ribosome seem to be responsible for tellurite resistance. Based on literature data, common traits of all these mutations are assembly defect, perturbation of mitochondrial DNA integrity and partial respiratory deficiency suggesting that toxicity of tellurite is mainly associated with its reduction into Te^{0} by respiratory chain of yeast mitochondria. Indeed, this process may generate electron leakage leading to release of ROS and induction of cellular oxidative stress. This conclusion is consistent with both RNA microarray data with K_{2}TeO_{3} exposed and control yeast cells (Table 1, Supplementary Tables S1 and S2) and C15* nonsense that inactivate the FRE8 gene coding for a NADPH oxidase. It has been recently demonstrated that FRE8 in Candida albicans produces a burst of ROS during fungal morphogenesis47, and, thus, FRE8 mutation may thus further contribute to tellurite resistance phenotype in Sc57-Te^{R}, strain by further lowering ROS levels in K_{2}TeO_{3} exposed cells. Altogether our findings may help to understand in the yeast model the complex relationships between the mitochondrial biogenesis and functionality, the toxic action of metalloid oxanions such as tellurite, and the cellular induction and response to ROS, thereby shedding light on human pathologies that involve these mechanisms.

Materials and Methods

Microbial strains. A list of S. cerevisiae strains used in this work is given in Table 4. The origin of strains BY4741, BY4742, BY4743, BY4743-20811, BY4743-27248, BY4743-22461, BY4741-811 and BY4741-2461 is the Yeast Knockout Clones and Collection of the Dharmacon™, and were provided by Carlo Erba s.r.l. (Italy). The yeast knock-out mutants were obtained by using a PCR based strategy to replace each ORF with a kanMX cassette48. The yeast strains were propagated and verified following the manufacturer’s instruction. When required G418 (200 μg/ml) was for strain selection.
E. coli JM109 competent cells (Sigma-Aldrich) were used for transformation experiments.

Media for microbial growth. Yeast strains were cultivated in the following media:

- **Yeast-Extract Dextrose-medium (YED):** yeast extract 1% [w/v], glucose (dextrose) 2% [w/v].
- **Glycerol-complete-medium (YEG):** yeast extract 1% [w/v], glycerol 3% [w/v].
- **Minimal medium (YNB):** yeast Difco nitrogen base without amino acids 0.67% [w/v], glucose 2% [w/v].

Supplements were added when required.

To obtain solid media, 2.5% [w/v] Bacto agar was used.

Bacterial strains were grown in L-broth as previously described by Del Giudice49,50.

Tellurium precipitation test. Stock solutions of K$_2$TeO$_3$ were prepared, filter sterilized and stored as described15. The tellurium precipitation assays were carried out with sterile microtiter plates (Falcon). In each microtiter wells 0.2 ml of YED broth containing about $2 \times 10^5$ yeast cells and increasing amounts of K$_2$TeO$_3$ were placed. Microtiter plate cultures were incubated at 28 °C without shaking, and Te$_0$ precipitation was analyzed after 5-6 days.

Electron microscopy. TEM Images were obtained as described15.

**N-methyl-N'-nitro-N-nitrosoguanidine treatment and isolation of Te$^8$ mutants.** N-methyl-N'-nitro-N-nitrosoguanidine treatment and isolation of Te$^8$ mutants was performed according to13. After mutagenesis about $10^8$ cells were plated onto YEG medium supplemented with 100 μg/ml K$_2$TeO$_3$ and incubated at 28 °C. Mutants

| Chromosome | GeneID | Symbol | Description | CDS length | Amino acid variation |
|------------|--------|--------|-------------|------------|---------------------|
| II         | 852410 | CYC8   | transcription regulator CYC8 | 967        | +T|TTG 1211/1212 |
| II         | 852447 | TBS1   | Tbs1p       | 1095       | -G|3233            |
| IV         | 851811 | HTA1   | histone H2A  | 133        | +G|117/118          |
| IV         | 851967 | SEM1   | proteasome regulatory particle lid subunit SEM1 | 90        | +A|61/62           |
| IV         | 851968 | CDC40  | Cdc40p      | 456        | -E|94             |
| VIII       | 856623 | YHR214C-B | gag-pol fusion protein | 1794       | E1782             |
| XII        | 807036 | FRE8   | putative ferric-chelate reductase | 687 | C15             |
| XII        | 851163 | SIR3   | chromatin-silencing protein SIR3 | 979 | --C 2020       |
| XII        | 855133 | PMG2   | phosphoglucomutase PMG2 | 570 | W605             |
| XII        | 855244 | INP1   | Inp1p       | 421        | +T|1085           |
| XII        | 855265 | MRPL44 | mitochondrial 54S ribosomal protein Mrnl44 | 148 | Q5             |
| XIV        | 855585 | NAM9   | mitochondrial 37S ribosomal protein Nam9 | 487 | W396             |
| XV         | 854276 | INP53  | phosphatidylinositol-3-/phosphonosidate 5-phosphatase Inp53 | 1108 | K356             |
| XV         | 854380 | GEP3   | Gep3p – Protein required for mitochondrial small subunit biogenesis | 557 | Y373G; K376*     |
| XVI        | 855969 | RPL33A | ribosomal 60S subunit protein L33A | 283 | W25*            |

| Strain | Nuclear genotype | Mitochondrial genotype | Origin |
|--------|------------------|------------------------|--------|
| Sc57   | a ura3–52 his 3–Δ200 ade2–101 lys2–801 tyr1–501 Te$^7$ | rho$^+$ | M. Johnston |
| Sc57–Te$^8$ | a ura3–52 his 3–Δ200 ade2–101 lys2–801 tyr1–501 Te$^8$ | rho$^+$ | This work |
| Sc57–R5 | a ura3–52 his 3–Δ200 ade2–101 lys2–801 tyr1–501 Te$^8$ | rho$^+$ | L. Del Giudice |
| Sc101–DG1141 | a his3 A200 ura3–167 Ty1his3 AI–242 Gal$^{+}$ Te$^7$ ts | rho$^+$ | M. J. Curcio |
| BY4741 | a his3Δ1 leu2–30 met15Δ0 ura3Δ0 | rho$^+$ | DharmaconTM |
| BY4742 | a his3Δ1 leu2–30 lys2–801 ura3Δ0 | rho$^+$ | DharmaconTM |
| BY4743 | BY4741/BY4742 | rho$^+$ | DharmaconTM |
| BY4743–20811 | BY4743 mrpl44::kanMX | rho$^+$ | DharmaconTM |
| BY4743–27248 | BY4743 nam9::kanMX | rho$^+$ | DharmaconTM |
| BY4743–22461 | BY4743 gep3::kanMX | rho$^+$ | DharmaconTM |
| BY4741–811 | BY4741 mrpl44::kanMX | rho$^+$ | DharmaconTM |
| BY4741–2461 | BY4741 gep3::kanMX | rho$^+$ | DharmaconTM |

| Strain | Nuclear genotype | Mitochondrial genotype | Origin |
|--------|------------------|------------------------|--------|
| Sc57 = YM654 | a ura3–52 his 3–Δ200 ade2–101 lys2–801 tyr1–501 Te$^7$ | rho$^+$ | M. Johnston |
| Sc57–Te$^8$ | a ura3–52 his 3–Δ200 ade2–101 lys2–801 tyr1–501 Te$^8$ | rho$^+$ | This work |
| Sc57–R5 | a ura3–52 his 3–Δ200 ade2–101 lys2–801 tyr1–501 Te$^8$ | rho$^+$ | L. Del Giudice |
| Sc101–DG1141 | a his3 A200 ura3–167 Ty1his3 AI–242 Gal$^{+}$ Te$^7$ ts | rho$^+$ | M. J. Curcio |
| BY4741 | a his3Δ1 leu2–30 met15Δ0 ura3Δ0 | rho$^+$ | DharmaconTM |
| BY4742 | a his3Δ1 leu2–30 lys2–801 ura3Δ0 | rho$^+$ | DharmaconTM |
| BY4743 | BY4741/BY4742 | rho$^+$ | DharmaconTM |
| BY4743–20811 | BY4743 mrpl44::kanMX | rho$^+$ | DharmaconTM |
| BY4743–27248 | BY4743 nam9::kanMX | rho$^+$ | DharmaconTM |
| BY4743–22461 | BY4743 gep3::kanMX | rho$^+$ | DharmaconTM |
| BY4741–811 | BY4741 mrpl44::kanMX | rho$^+$ | DharmaconTM |
| BY4741–2461 | BY4741 gep3::kanMX | rho$^+$ | DharmaconTM |

**Table 3.** Comparative genomics: summary of CDS-inactivating mutations in SC57-Te$^8$.

**Table 4.** List of yeast strains, nuclear and mitochondrial genotypes and origins.
appearing as papillae above background growth were isolated and purified three times. Growth measurements were carried out by using a Klett-Summerson colorimeter.

**Genetic techniques.** Standard genetic techniques were used as described by.

**Nucleic acids.** Total RNA was purified from yeast cells with the use of the MagneSil total RNA mini-Isolation System (Promega). Single preparations were performed on 50 mg of frozen starting material, which was grinded to a fine powder under liquid nitrogen before the extraction.

DNA for PCR analysis was purified from *S. cerevisiae* yeast cultures according to, while the method described by was followed for DNA extraction from *E. coli* cultures.

**DNA manipulations.** General DNA manipulations were carried out by earlier published methods.

In order to verify the yeast frataxin sequence present in the Sc57-Te mutant, gene sequences were amplified from DNA extracted from both wild type and mutant *S. cerevisiae* strains, using two PCR primers situated upstream (fra5: 5′-CGAGAAGATAGTGATGC-3′) and downstream of the frataxin coding sequence (fra3: 5′-GATTGGATGCGTTAACAAGTG-3′), respectively. Amplification products were gel purified, ligated into pBluescript vector, and transformed into JM109 competent cells following standard procedures. DNA from 10 recombinant clones carrying the wild type derived frataxin gene and 10 recombinants carrying the mutant-derived frataxin sequence were, subsequently, subjected to sequence analysis using universal forward and reverse sequence primers. Alignment of the obtained sequence was performed with ClustalV software.

**RNA microarray analysis.** To measure fluctuations in gene expression, Affymetrix genechips (GeneChip Yeast Genome 2.0 Array) were utilized. These Gene Chips provide comprehensive coverage of both *S. cerevisiae* (5,841 probe sets) and *Schizosaccharomyces pombe* (5,021 probe sets). Total RNA extracted from Sc57-Te strain treated with K2TeO3 and from an untreated control was used to prepare molecular probes. Three independent RNA extractions were performed on each single yeast sample. Probe preparation and microarray hybridization were performed in the laboratory of University of Bristol, following manufacturer’s instructions. Microarray data were analyzed using the FlexArray 1.6.1 software suite (Génome Québec and McGill University). Raw data were normalized using the PLIER (Probe Logarithmic Intensity Error) algorithm. This algorithm is the latest in a series of algorithms produced by Affymetrix to analyze their oligonucleotide GeneChips and employs M-estimators to obtain estimates of probe affinity and target signal. Significant expression differences were identified by means of t-tests. Only differently expressed sequences with p-values < 0.01 and expression ratios > 2.0 were taken into consideration. Differential gene expression was assessed using «The Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.8» (https://david.ncifcrf.gov), in order to enrich the data obtained and deduce groups of genes with altered expression values due to the applied treatment.

**Whole-genome sequencing.** Whole-genome sequences of *S. cerevisiae* strains Sc57 and in Sc57-Te were determined by Next Generation Sequencing (NGS). Preparation of indexed libraries and whole-genome shotgun sequencing were performed as a service by GenomiX4Life S.r.l. using an Illumina platform. Samples were assessed after dilution by using Nanodrop 2000c spectrophotometer and Agilent 4200 TapeStation. After sequencing and quality check control of raw sequence data, paired-end reads were aligned against reference genome of strain *S. cerevisiae* S288C to identify single nucleotide variants (SNVs) and small insertions and deletions (InDels) using gold standard procedures. All SNVs and InDels are listed in Supplementary Datasets S1 and S2. Alignments were performed with BWA, and SAMTools and BEDTools were used for filtering steps and file formats conversion. Reference genome of strain *S. cerevisiae* S288C (assembly R64) was used for functional annotation. Mapping of SNVs and InDels on *S. cerevisiae* coding sequences (CDS) and intergenic genome regions, and classification of variants based on their effects on CDSs were obtained by using *ad hoc* developed Python programs.

**Data availability.** All data generated or analysed during this study are included in this published article (and its Supplementary Information files).

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Author Contributions
L.D.G. conceived the study and participated in its design and realization. P.P., D.R.M. and A.D.G. isolated and characterized the mutants at the genetic level. G.P. and R.R. performed growths and prepared samples for T.E.M. observations. M.D.St. performed T.E.M. analyses. J.T. and H.H. carried out R.N.A. microarray analysis. M.D.Sa. and P.A. analyzed S.N.Vs, InDels and determined mutated CDSs in Sc57-Te5R. LDG, HH and PA wrote and revised the manuscript. All authors read and approved the final manuscript.

Additional Information
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