Remediation potential of a soil bacterium, *Planomicrobium flavidum* (S5-TSA-19) for 2,4,6-trinitrotoluene (TNT) i.e., a nitro-aromatic explosive in aquatic medium.

Jyoti Lamba

Panjab University

Shalini Anand (✉ shalini@cfees.drdo.in)

CFEES: DRDO Centre for Fire Explosive and Environment Safety

Jayanti Dutta

Panjab University

Pramod Kumar Rai

CFEES: DRDO Centre for Fire Explosive and Environment Safety

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Abstract

2,4,6-Trinitrotoluene (TNT), a nitro-aromatic explosive commonly used for defence and several non-violent applications is contributing to serious environmental pollution problems including human health. The current study investigated the remediation potential of a native soil isolate i.e., *Planomicrobium flavidum* (strain S5-TSA-19) against TNT. At a TNT concentration of 120 mg/L with a specified environment, the isolate remained incubated for 30 days in a minimal salt medium (MSM). Regular growth of the isolate, the concentration of TNT, nitrate, nitrite and ammonium ion were evaluated at a particular time during the experiment. Within 168 hours (i.e., 7 days) of incubation, *P. flavidum* co-metabolically degraded 100% TNT. The biodegradation procedure succeeded the first order kinetics mechanism. Formation of additional metabolites like 2,4-dinitrotoluene (DNT), 2,4-diamino-6-nitrotoluene (2-DANT) and 2-amino-4,6-dinitrotoluene (2-ADNT), were also witnessed. TNT seems to be non-toxic for the isolate, as it reproduced magnificently in explosive presence. For TNT degradation, the nitro-reductase enzyme was observed to be responsible. This is so far the first reporting of *Planomicrobium flavidum*, efficiently bioremediating TNT i.e., a nitro-aromatic compound via different degradation pathways, leading to the production of simpler as well as less harmful end products. Further, in the near future, at the field scale application *Planomicrobium flavidum* may be explored for the bioremediation of TNT (i.e., a nitroaromatic-compound) contaminated effluents.

Highlights

- Evolution of bacteria is supported by aerobic conditions in aqueous medium.
- Gram-positive isolate breaks down TNT efficiently.
- Co-metabolism confirmation through isolate for TNT degradation.
- Degradation pattern of explosive follows First-order kinetics.
- Bioremediation technique as an economical future potential for degrading TNT.

1. Introduction

Numerous applications of military and at supplementary civil areas such as engineering, construction, extracting, mining and rocket propellants explosives i.e., organic energetic compounds (nitroamines and nitroaromatic) are being used (Chatterjee et al. 2017; Nagar et al. 2018, 2021). 2,4,6-trinitrotoluene (TNT), is a reported universally utilized secondary explosive (Ryu et al. 2007; Stenuit and Agathos 2010; Ayoub et al. 2010; Kao et al. 2016). As per Alothman et al. 2020, TNT, being a teratogenic, cytotoxic nitroaromatic compound, may affect cellular mutations in humans, animals, plants, and microorganisms (Juhász and Naidu 2007; Fahrenfeld et al. 2013; Kao et al. 2016). Throughout the two World Wars, because of the immense usage of TNT, ecosystem of soil and water has turn out to be utterly polluted (Fuller et al. 2004; Lewis et al. 2004). Around 10,000 mg per kg and 100 mg per litre of TNT was spotted in nitroaromatic contaminated soil and water respectively. The lethal and mutagenic effect of TNT is being exhibited on eukaryotes and prokaryotes (Honeycutt et al. 1996; Khan, Lee, and Park. 2013; Kao et

al. 2016). Subsequently, TNT's detrimental impacts in soil and groundwater is a critical issue with respect to environmental protection which required to be controlled or minimize as per demand of the hour.

Plentiful reports have been recorded with numerous remediating methods for TNT polluted spots (Ayoub et al. 2010; Gümüşcü and Tekinay 2013; Kao et al. 2016). Conventional treatment involves physical and chemical approaches, like incineration, adsorption, and advanced oxidation procedures, which are expensive and result in lethal by-products (Rodgers and Bunce 2001; Gümüşcü and Tekinay 2013; Kao et al. 2016). On the other hand, biological-based remediation approaches are promising both ways; i.e., economically and organically (Chien et al. 2014; Kao et al. 2016). Bioremediation explores the potential characteristics of microorganisms to profitably bio-transform or biodegrade contaminants (Fahrenfeld et al. 2013; Kao et al. 2016). Bioremediation can be studied in situ and is frequently a cheap, less expertise practice satisfactorily to the general public (Vidali 2001; Kao et al. 2016). Microbial biotransformation can be approached for TNT biodegradation (Chien et al. 2014; Kao et al. 2016). Hence, TNT bioremediation through bacteriological degradation is a very optimistic practice.

Quite a lot of reviews concise widespread exploration of TNT biodegradation by fungi and bacteria (Hawari et al. 2000; Van Aken and Agathos 2001; Rosser et al. 2001; Heiss and Knackmus 2002; Zhao et al. 2004; Serrano- González et al. 2018). In TNT, the existence of three electron-withdrawing nitro groups announces steric constraints accompanying elevation in electron shortage to aromatic ring. Thus, the molecule resists oxidative degradation by microbes, nitro groups of TNT are used as source of nitrogen by microbes (Claus et al. 2007). Anaerobic and aerobic degradation of TNT through diversity of microorganisms has been testified, alongwith some anticipated metallic pathway (Esteve-Nunez, Caballero, and Ramos 2001; Kao et al. 2016). These comprised of rigid anaerobic bacteria (such as *Clostridium* and *Desulfovibrio*), aerobic bacteria (such as *Achromobacter*, *Bacillus*, *Citrobacter* and *Pseudomonas*), and fungi (*Phanerochaete*) (Duque et al. 1993; Michels and Gottschalk 1994; Montpas et al. 1997; Kalafut et al. 1998; Oh and Kim 1998; Esteve-Nunez, Caballero, and Ramos 2001; Lee et al. 2002; Kao et al. 2016).

Until now rare studies of xenobiotic compounds degradation through *P. flavidum* have been recorded. Potential of *P. flavidum* against TNT and PETN was evaluated in presence of anaerobic atmosphere comprising an augmentation source i.e., sewage sludge (Amin et al. 2017). Mohamed and Mostafa., 2018 analyzed *Planomicrobium flavidum* aptitudes for fungicide (captan) degradation and determined it as an effectual isolate. *Planomicrobium flavidum* was also reported as a potential isolate for degrading nitro-amine based explosive (Nagar et al. 2018). Recently, strain WH2-56, belonging to *Planomicrobium* genus was examined to have the capacity to degrade cellulose in presence of additional agricultural wastes compounds (Zhuge et al. 2020). Though, degradation ability of *P. flavidum* against nitro-aromatic based explosives under aerobic conditions has not yet been explored. In the present study, for the first-time native soil bacteria i.e., *Planomicrobium flavidum* (strain S5-TSA-19) was isolated and analyzed with an objective to get characterized for its ability to bio-transform TNT through bioremediation technique at laboratory-scale under aerobic conditions.

2. Materials And Methods

2.a. Samples

Extracted soil samples from an explosive manufacturing site located in India were sent to Institute of Microbial Technology (IMTECH), Chandigarh for the purification and characterization of microbial strains.

2.b. Chemical and reagents

TNT (> 99% purity) was from a reliable manufacturing unit in India obtained only for the study purpose. During the current study, all analytical and gradient grade chemicals together with standard of 2-ADNT were from Sigma-Aldrich.

2.c. Media

Initially, Tryptone Soya Agar (TSA) plates were used to maintain the primary cultures of *Planomicrobium flavidum*. Throughout the aqueous phase study, Minimal salt medium (MSM) for microbial proliferation and associated experimentations was favored (Cook and Huetter 1981; Thompson et al. 2005). Essential media enhancement was done through 40 mM potassium phosphate buffer (pH 7.2), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (61.61 mg), NH_4Cl (50 mg) and trace elements. Trace element mixture supplementation was done with a composition (per liter) as reported earlier (Nagar et al. 2018, 2020; Lamba et al. 2021) with an aim of studying the proliferation and degrading potential of *P. flavidum* proliferation, TNT (120 mg/L) solution was prepared in MSM medium.

2.d. Characteristics of the microbial strains

Strain S5-TSA-19 (Microbial type culture collection number 12608) with GenBank accession number LT631687.2 was received in lyophilized form and was characterized through 16S ribosomal DNA sequence practice. Optical density (OD) of the bacterial biomass was observed at 600 nm of absorbance with UV-Vis spectrophotometer from Perkin Elmer, Model Lambda 650S. For obtaining culture, bacteria was initially nurtured in minimal salt medium till the third generation. For the degradation study, the cell suspension in log phase through an OD 0.8 ($\approx 10^8$ cells/mL) was used.

2.e. Tolerance assessment

The isolated strain was assessed regarding its tolerance level against TNT by growing it in minimal salt media comprising different concentrations of TNT (60, 80, 100 and 120 ppm) and also with additional nitrogen source in 2 sets along with pH and temperature variations.

2.f. Aqueous phase experiment preparation

Under sterile conditions, the isolate *P. flavidum* (in its log phase) was inoculated in MSM medium in 250-mL Erlenmeyer flasks already spiked with 120 mg/L of TNT along with 3 replications per study. 5% (v/v) of the bacterium was inoculated in TNT containing MSM. Two sets of controls, viz., MSM containing TNT

but excluding bacterial strain and MSM with isolate excluding TNT were also prepared. Flasks were nurtured for 30 days duration (at 30°C and 120 rpm) in an orbital incubator shaker. Throughout the incubation period, on a regular basis (i.e., at each alternative day) aliquots (2 mL) were drawn off from individual experimental arrangement and were further centrifuged (10,000 rpm; 10 minutes). Further, supernatant solution was filtered with a 0.45- µm Teflon filter prior to quantification of TNT and intermediates concentrations (USEPA 2007) with a photodiode array detector of model e2695, Perkin Elmer Inc., high-performance liquid chromatography (HPLC). C18 reversed-phase column (4.6 mm × 150 mm) was employed with acetonitrile and water (at a ratio 1:1, v/v) as mobile phase and a flow rate of 1 mL/min.

2.g. TNT and converted compounds analysis

Further the conversion, disappearance and tolerance against TNT was evaluated and quantified. To get brief evidence about the disappearance and conversion of TNT along with the formation of secondary metabolites Liquid Chromatography Mass Spectrophotometric (LCMS) analysis was carried out. LCMS analyses were accomplished via Waters, Micro mass Q-TOF micro system (Waters Alliance 2795) comprising of Waters X Bridge Column C18 with negative ion (ES⁻). 20 µL injection volume through a flow rate of 0.8 mL/min was utilized to analyze individual TNT peak and its degradation by-products. For solvent purpose, acetonitrile, formic acid (0.1%) and methanol were utilized. Apart from spectrophotometric analyses, strain's tolerance towards explosive was also viewed using Scanning Electron Microscopy (SEM) detailed earlier by Prasad et al., 2016. Au-Pd layering was done through Sputter Coater (Quorum SC7620 Mini), and detected via ZEISS EV^o18 scanning electron microscope.

2.h. Evaluation of Nitrite (NO₂⁻) and Nitrate (NO₃⁻)

Nitrate and Nitrite, being important metabolites of TNT degradation were analyzed regularly (every alternate day). Amount of nitrite ion was measured at OD 540 nm and nitrate ion was analyzed at OD 220 & 275 nm via UV-VIS Spectrophotometer (Mercimek et al. 2013; Lamba et al. 2021).

2.i. Evaluation of Ammonia (NH₄⁺)

Using UV-VIS Spectrophotometer at 490 nm of absorbance, concentration of ammonium ion was evaluated at regular interval of time. Quantification of ammonium ion was done using Nessler's reagent. Supernatant (0.5 mL) was incubated at 37°C designed for 3 hours, succeeding with Nessler's reagent (0.1 mL) addition (Mackie and MacCartney 1989; Lamba et al. 2021).

2.j. Nitrate Reductase Activity Test

Sensitivity of nitrate reductase in the broth was tested colorimetrically to examine its role in TNT degradation. It is active in converting nitrate (NO₃) to nitrite (NO₂). Its activity was hampered by adding respective inhibitor (Rahal and Lobna 2011).

2.k. Statistical Analysis

Through OriginPro 8.5 software, One-way Analysis of Variance (ANOVA) was taken out to significantly examine the differences between sample data's mean obtained from MSM comprising *P. flavidum* with and without the explosive. At 0.05, significant level descriptive statistical normality test of the sample data was evaluated. Shapiro-Wilk data specified that samples were drawn from normally distributed population because the significant value was greater than 0.05. Further, at 0.05 significance level, means of the data were compared employing Tukey's post hoc test and homogeneity of variance was derived with Levene's test.

3. Results And Discussion

Key emphasis of current study was to explore efficiency of a native bacterial isolate in remediating nitroaromatic explosive i.e., TNT aerobically. The strain S5-TSA-19 is a soil bacterium named *Planomicrobium flavidum* isolated from the sample collected from an explosive manufacturing site containing up to a concentration of 900-1000 mg/Kg TNT. *P. flavidum* can sustain in temperature between 4-37°C.

3.a. Tolerance level of *P. flavidum*

The tolerance or sustaining capacity of the isolate with the explosive was evaluated in MSM at various concentrations of TNT (60, 80, 100, 120 mg/L), temperature (20 to 35°C) and pH (5 to 9). Satisfactory results were detected upto higher concentration of TNT i.e., 120 mg/L at 30°C ($\pm 5^\circ\text{C}$) and neutral pH i.e., 7.

3.b. Isolate's morphology

SEM images of S5-TSA-19 isolate (Fig- 1) with and without the nitroaromatic explosive depicted that (a) strain is rod shaped which is comparable to the morphology of gram positive *Planomicrobium flavidum* strain CCUG 56756T belonging to *Bacilli* class (Jung et al. 2009), (b) no variation was observed in the isolate's shape neither in presence or absence of TNT, this confirms that *P. flavidum* thrives flexibly well in minimal medium comprising TNT and (c) 120 mg/L TNT was non-lethal to the strain.

3.c. Co-metabolism factor

Cells of *P. flavidum* exhibited alike growth pattern in MSM with and without TNT (Fig- 2) and proliferate very well in both the circumstances. This depicts the unaffected behavior of isolate S5-TSA-19 against the nitro-aromatic explosive and co-metabolic pathway for degrading TNT as mentioned elsewhere (Dalton et al. 1982; Nagar et al. 2018, 2020; Lamba et al. 2021). It attained extreme proliferation in 12 days as is confirmed with the rise in absorbance at 600 nm from 0.086 initially to 1.073 on 12th day (Fig. 2). Levene's test for homogeneity of variance of growth of S5-TSA-19 depicted that there is insignificant difference in the variances in isolate's population with and without TNT at 0.05 level since p

value is 0.7857 which means $p < 0.05$. Similarly, no significant difference among the pair of means was detected in Tukey's test for paired mean comparison at 0.05 level.

3.d. Degradation kinetics

To evaluate the degradation rate of TNT with bacterial strain S5-TSA-19, the kinetics model, with first order was exercised against residual TNT concentrations (in treatment sets). Eq. (1) depicted the "First order rate equation".

$$\ln A = -kt + \ln A_0$$

1

where, A_0 are the nitroaromatic explosive concentration initially, A is TNT concentration at specified incubation duration, ' t ' and ' k ' represents rate constant for degradation. $t_{0.5}$ is represented as follows:

$$t_{0.5} = 0.693 k^{-1} \quad (2)$$

Through the rate of reaction, value of $k = 0.027$ per day and $t_{0.5} = 25.67$ hours.

Within the first 7 days of incubation period (30 days), 120ppm TNT was observed to be 100% degraded by isolate S5-TSA-19. In the control samples containing 120mg/L TNT in MSM but no bacterial strain, no explosive degradation was noticed (Fig. 3).

3.e. Correlation between Nitrite, Nitrate, Ammonium and TNT degradation

End product of TNT degradation comprised of ammonium ions, thus detection and analyzation of concentration of nitrite, nitrate and ammonium ions respectively and further correlating them along is important in TNT degradation. According to Mercimek et al. 2015, TNT's demineralization in the culture with the advances of bacterial growth corresponds to the liberation of nitrite ions. In the present experiment too, assemblage of nitrite ions was detected with the rise of S5-TSA-19 strain in the media which corresponds to TNT degradation escalation till complete explosive degradation (Fig. 4). Likewise, consistent pattern was observed in the case of ammonium ion concentration., i.e., after incubation originally concentration unswervingly amplified through the first 3 days, which subsequently becomes persistent (Fig. 4). Earlier reports support the observation made for nitrite, nitrate, and ammonium ions production along with the pattern of nitro-aromatic explosive degradation (Lotufo and Lydy 2005; Mercimek et al. 2015; Lamba et al. 2021).

A reductive pathway is responsible for TNT degradation where electron withdrawing nitro groups (03) of TNT expedites the nucleophilic attack of hydride ion. This leads in the development of a changeable Meisenheimer complex among TNT and the hydride ion (Esteve-Nunez et al. 2001; Conder et al. 2004). Further the formation of DNT is being confirmed with the release of nitrite ions. Within 7 days of TNT degradation through *P. flavidum* (Fig- 3), formation of 2ADNT was being depicted in HPLC

chromatogram. Furthermore, LC-MS study confirms TNT degradation pathway along with the transitional compounds formation (Fig. 5). As per Lachance et al. 2004, nitro group conversion to an amino group during TNT degradation leads to the formation of 2-amino-4,6-dinitrotoluene (2-ADNT) and same was evident in the current study (Fig. 4). Steady rise in nitrite concentration is indicative of emergence of DNT as the initial intermediate detected at the preliminary phase of the catabolism of TNT (Lamba et al. 2021) but at the concluding phase no DNT was noticed that put forward the increasing metabolism and bacterium accelerating potential in degrading the compound ultimately producing toluene. Earlier, diverse researches recorded transformation of DNT to toluene continuing to TCA cycle via toluene cis-dihydrodiol formation (Esteve-Nunez et al. 2001; Serrano-González et al. 2018) and here in this study also LC-MS peaks confirmed the same (Fig. 5).

Analysis of Nitrate ions concentration showed an escalation at the early phase of the study followed with a gradual decrease which might be because of the primary transformation of NO_2^- ions to NO_3^- . But due to the instability of NO_3^- ions, they further get converted to NH_4^+ ions.

3.f. Nitroreductase role in TNT degradation

Sodium azide, a specific inhibitor was employed to study the role of nitroreductase enzyme in TNT degradation (Villanueva 1959; Roldán et al. 2008). Sodium azide with a concentrations viz., 0.1, 0.5, 0.7 and 1 mM was added in the reaction mixture containing resting cells of *P. flavidum* in MSM having TNT (10 mg/L TNT). Quantification of TNT degradation was carried out with HPLC. Fig. 7. illustrates the degradation of TNT at various concentrations of inhibitor. In 3 days, 100% TNT degradation was observed with no inhibitor. Results concluded that TNT degradation decreased with the increasing sodium azide concentration. This depicts that, nitroreductase enzyme plays a role in degradation pathway of TNT explosive by *P. flavidum*. Fig. 8. showed the anticipated TNT degradation pathway.

3.g. Disappearance of TNT concurrently through different pathways

TNT mineralization by pure bacterial system as compared to mixed or undefined bacterial cultures under aerobic or anaerobic conditions is missing in literature (Kalderis et al. 2011; Serrano- González et al. 2018). Degradation rate of TNT in soil bacteria through nitrate is best in contrast with other electron acceptors such as sulfate and carbon (Boopathy et al.1993). TNT is transforming through mutually occurring aerobic and anaerobic circumstances reducing to amino-derivatives via non-specific NAD(P)H-dependent nitro-reductase (Kalderis et al. 2011). Although most of the bacteria follows lone initial metabolic pathway for TNT transformation; but some of them follows two- different pathways at the same time. (Vorbeck et al. 2016; Haïdour and Ramos 1996).

3.g.a. Direct reduction of two-electrons pathway:

With the increase of time in the incubation period, noticeable variation in the color of TNT enriched medium was detected. Explosive enriched medium changed its color from white to reddish brown (darkened gradually till the end). The conversion of culture's color from red to brown is a validation for

dihydride-Meisenheimer complex formation (Pak et al. 2000; Nyanhongo et al. 2009; Gn Gk et al. 2019; Lamba et al. 2021). The product of direct reduction of two-electrons of the aromatic ring via hydride addition reaction forms dihydride Meisenheimer complex, hydride Meisenheimer complex and protonated tautomers (Serrano-Gonzlez et al. 2018). This confirms the release of nitro groups during TNT degradation from the nitro-aromatic structural ring of the explosive. The unstable nature of dihydride Meisenheimer complex contributes to the release of intermediate products like nitrite ions and DNT during TNT degradation (Mackie and Mac Cartney 1981). The consecutive reduction in TNT concentration was in synergy with Meisenheimer complex formation and isolate's activity which steadily led to darkening of MSM color comprising TNT (Lamba et al. 2021).

3.g.b. Consecutive reduction of two-electron pathway:

This metabolic pathway of TNT degradation initiates via successive reduction of two electrons of the nitro groups of the aromatic ring. Removal of electrons in the nitro- groups produces reduced intermediates like Di-amino-nitrotoluene (DANT), 2-Amino-4,6-dinitrotoluene (2-ADNT), 4- Amino-2,6-dinitrotoluene (4-A-2,6-DNT), isomers, and azo and azoxy dimers (Lewis et al. 1997; Serrano- Gonzlez et al. 2018).

3.g.c. Collective Aerobic and Anaerobic degradation of TNT:

Aerobic degradation followed two initial biochemical routes. During the initial reduction process, TNT transforms to 2-Hydroxylamino-4,6-dinitrotoluene or 2-hydroxylamino-2,6-dinitrotoluene. This is intervened by nitroreductase/ nitrobenzene nitroreductase (Serrano- Gonzlez et al. 2018) and the establishment of this led to the formation of 4-Amino-2,6-dinitrotoluene or 2-Amino-4,6-dinitrotoluene via nitroreductase along with tetranitro-azoxybenzene compound. Aerobic route leads to the production of 2-Amino-4-nitrosotoluene and 2,6-diamino-4-nitrotoluene as a progress, further continuing with anaerobic degradation producing 4-amino-2,6-dinitrotoluene and 2-ADNT to conclude the process. By means of NAD(P)H nitroreductase (non-specific) conversion of both 4-amino-2,6-dinitrotoluene and 2-ADNT to DANT takes place with anaerobic bacteria. By the involvement of hydrogenase or carbon monoxide dehydrogenase, DANT converts to 2,4-diamino-6-hydroxyl-aminotoluene (DAHAT), along with TAT formation by dissimilatory sulfite reductase. On the other hand, DANT get directly converted to TAT as also reported earlier in presence of *C. sordelli*, *C. bifermentans* and *C. sporogenes* (Serrano- Gonzlez et al. 2018). TAT sequentially reduced to toluene and then to toluene cis-dihydrodiol. Toluene cis-dihydrodiol is an transitional compound within the pathway of toluene degradation. Further, toluene cis-dihydrodiol gets transformed into 4-Hydroxybenzaldehyde as confirmed through LC-MS chromatogram. Finally, 4-Hydroxybenzaldehyde followed the pathway resulting to trichloroacetate cycle i.e., TCA cycle (Serrano- Gonzlez et al. 2018).

4. Conclusion

Present study depicts the first reporting of both aerobic and anaerobic degradation of TNT in presence of a novel soil isolate *P. flavidum*, strain S5-TSA-19 in an aerobic environment. Throughout the study,

satisfactory multiplication of the bacterium was observed in the growth medium containing TNT (120 mg/L). TNT, as known to be xenobiotic compound, showed complete degradation after *P. flavidum* action. Irrespective of the fact that the study was planned for 30 days, complete disappearance of TNT (120 mg/L) was observed on 7th day of incubation. Through the elucidation of degradation pathway, DNT and 2-ADNT were observed to be the two key metabolites of this bioconversion process. Hindrance of nitroreductase led to the hampering of degradation process of TNT, thus demonstrating the role of nitroreductase enzyme in TNT degradation. SEM results confirmed the adaptable nature of the isolates, as the cell morphology did not change in presence of toxic compound TNT. Admirable outcomes of the study prove the outstanding efficiency of *P. flavidum* for TNT degradation. Therefore, in the near future, we can use the bacterium on a large field scale along with suitable organic and inorganic amendments.

Declarations

Author contribution JL: Conceptualization, Investigation, Methodology, Software, Formal analysis, Writing – original draft. SA: Conceptualization, Methodology, Writing – review & editing, Supervision. JD: Writing – review & editing, Supervision. PKR: Resources, Head of the Team.

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Data availability The datasets generated and/or analyzed during the current study are not publicly available as they also form part of an ongoing study but are available from the corresponding author on reasonable request.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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Figures

Figure 1

Scanning electron micrographs of isolate S5-TSA-19 in culture media a) in absence of TNT b) in presence of TNT.

Figure 2

Growth comparison of *P. flavidum* with and without TNT during incubation in MSM. Error bars specifies the standard deviation at specified time.

Figure 3

TNT degradation in treated medium with the absence and presence of *P. flavidum*. Error bars specifies the standard deviation at specified time.

Figure 4

Pattern of nitrite release, nitrate transformation and ammonium formation during the incubation period of *P. flavidum* (data represented in of triplicate). Error bars specifies the standard deviation at specified time.

Figure 5

HPLC chromatographs of supernatant of culture media all through the degradation period along with the standard of metabolite (2ADNT).

Figure 6

LC-MS spectra of TNT and its transformation products during the growth of isolate *P. flavidum* in MSM supplemented with TNT.

Figure 7

TNT degradation vs concentrations of sodium azide. Error bars specifies the standard deviation at specified time.

Figure 8

Projected Pathway for TNT degradation.

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