

Antifungal Activity and Molecular Docking of Phenol, 2, 4-Bis (1, 1-Dimethylethyl) - Produced By Plant Growth-Promoting Mangrove Actinobacteria; Kutzneria Sp. Strain TSII

Thangarasu Suganya Devi

Alagappa University

Karuppiah Vijay

Alagappa University

Vidyavathi RM

Alagappa University

Ponnuchamy Kumar

Alagappa University

Muthusamy Govarthan

Kyungpook National University <https://orcid.org/0000-0001-8725-3059>

Thangavel Kavitha (✉ kavikmicro@gmail.com)

Alagappa University

Research Article

Keywords: Kutzneria sp. strain TS II, Plant growth promotion, Antagonism, Phenol, 2, 4-Bis (1, 1-Dimethylethyl)-, ATP synthase and Pithomyces atro-olivaceous

Posted Date: April 5th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-291369/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

The present study reveals the plant growth-promoting (PGP) potentials and characterizes the antifungal metabolites of *Kutzneria* sp. strain TSII isolated from mangrove sediment soil through *in vitro* and *in silico* studies. In this study, *Kutzneria* sp. strain TSII was screened for PGP activities and the antifungal activities against *Pithomyces atro-olivaceous*, a leaf spot associated pathogen in groundnut plants. The ethyl acetate extract of *Kutzneria* sp. strain TSII was purified using column chromatography, and the presence of various antimicrobial compounds was studied by Gas Chromatography-Mass Spectrometry (GC-MS) analysis. *In silico* modeling and docking were carried out to evaluate the antifungal potent of bioactive compound. *Kutzneria* sp. strain TSII produced Proteases, Phosphatases, Ammonia, Siderophores, Cellulases, Indole Acetic Acid (IAA), Lipases, and Amylases, indicating its ability to enhance the growth of plants. The ethyl acetate extract of *Kutzneria* sp strain TSII was found to be a potent inhibitor of fungal mycelial growth in the potato dextrose agar (PDA) plates. The GC-MS spectral study showed 24 antimicrobial compounds belonging to five chemical groups: Phenolics, Phthalates, Fatty acid methyl esters (FAME), Spiro, and Fatty alcohols. *In silico* docking studies showed that Phenol, 2, 4-Bis (1, 1-Dimethylethyl) – effectively attaches with the active site of mitochondrial F_1F_0 Adenosine triphosphate synthase enzymes of *Pithomyces atro-olivaceous*. Hence, it is clear that these antifungal compounds shall be formulated shortly to treat many plant fungal diseases in an eco-friendly manner.

Introduction

Groundnut (*Arachis hypogaea* L.) is one among the most important oilseed crops. Groundnut is ranked 13th among the most critical food crops. Leaf spot disease is almost co-existent with the plants and contributes to a significant yield loss throughout the world (Meena 2010). Massive application of chemical fungicides adversely affects the resident microbiota and also causes severe health hazards to society. Besides, systemic use of these pesticides influences the fungicide resistance in phytopathogens (Mishra et al. 2008). Hence, the effective alternative method suggested was biocontrol agents such as bacteria, fungus, and actinobacteria. These beneficial microbes control the soil-borne pathogens either directly by inducing the plants' immune system or indirectly by producing ammonia, HCN, cell wall degrading enzymes, siderophores, and antibiotics (Kohl et al. 2019).

Actinomycetes are gram positive, filamentous spore-forming bacteria belonging to the order Actinomycetales. They are isolated from the terrestrial and aquatic ecosystem, mainly involved in the decomposition of organic matter, there by facilitating the soil's nutrient recycling process. Actinobacteria, especially *Streptomyces* species, are reported for the massive array of bioactive compounds like antibiotics, bio-pesticides, enzymes, which could find applications in agricultural and pharmaceutical industries (Genilloud 2017). Recently, researchers report the plant-growth-promoting and antagonistic traits in some actinobacteria species; their inoculation improved the biomass yield and influenced the synthesis of defense-related compounds in legume plants (Sathya et al. 2017). However, the formulation and commercialization of the bioactive metabolites from actinobacteria with relevance to the biocontrol

Loading [MathJax]/jax/output/CommonHTML/jax.js actinobacteria, owing to its slow growth. Mangrove is the

outstanding muddy ecosystem support the growth of microbes, including actinobacteria. These microbes are well adapted to this salty habitat to produce a wide range of extracellular metabolites for their survival. Hence, we described here the antifungal metabolites of a rare unexplored mangrove actinobacteria *Kutzneria* sp. strain TSII for the biocontrol of *Pithomyces atro-olivaceus*, a leaf spot disease-causing novel fungus in groundnut crops identified in this study.

In a previous study by Vijay et al. (2020), GC-MS/MS identified antifungal metabolites from tomato-associated rhizobacteria were docked with short-chain dehydrogenase/reductase enzymes in *Fusarium oxysporum* and regarded to be the potential target for the observed antifungal effects. Similarly, we address the mitochondrial ATP synthase subunit 'A' found in the F₀ complex as the potential target for the fungicide leads from actinobacteria identified in the study using GC-MS/MS analysis. Because this subunit plays a crucial role in proton channeling across the inner mitochondrial membrane, which offers ATP synthesis in the mitochondrial matrix of *Pithomyces atro-olivaceus* (Carbajo et al. 2005), we performed an *in silico* targeting of subunit 'A' with mangrove sediment-associated actinobacterial ligands. Hence these compounds would probably attenuate ATP synthesis and substantiate the observed *in vitro* growth inhibitory effects on the novel leaf spot disease-causing pathogen *Pithomyces atro-olivaceus* in groundnuts.

Materials And Methods

2.1. Isolation and identification of actinobacteria

Mangrove soil sediments (approx. 50g) were collected from Muthupet mangrove ecosystem from the South east Coast (10° 20'N, 79° 35'E), Tamilnadu, India. Yeast Malt Agar plates supplemented with Nalidixic acid (100 mg/l) (Himedia) and Nystatin (100U/L) (Himedia) to suppress the growth of gram negative bacterial and fungi. Plate was incubated at 28°C for 7 days (Thawai et al. 2004). Biochemical characterization was carried out to identify the isolate at the genus level by following Gerencser and Slack (1969) and Tille (2015). Scanning Electron Microscopy (SEM) was carried out to visualize the arrangements of highly potent actinobacterial isolate at a maximum magnification (5.0 kx). 16S rDNA sequencing of the potential isolate TSII was carried out to identify based on signature sequence homology and constructed 16S rDNA phylogeny (Kumar et al. 2018).

2.2. PGP traits of Actinobacteria

2.2.1. Tube assay

IAA synthesis of the actinobacterial isolate was confirmed by following the method of (Bric et al. 1991). Presence of ammonia was identified by the development of brown to yellow colour upon the addition of Nessler's reagent Cappucino and Sherman (1992). HCN production was assessed on Trypticase Soy Agar tubes according to the method of Alstrom (1987).

2.2.2. Plate assay

Loading [MathJax]/jax/output/CommonHTML/jax.js

Utilization of rock phosphate was evidenced by noticing the appearance of clear zone around the colony on Pikovskaya plates (Kapur et al. 2018). A qualitative analysis of cellulolytic, proteolytic, lipolytic and starch hydrolytic properties was evaluated as zone of hydrolysis around the colony on Carboxymethyl cellulose agar (Bettache et al. 2018), Skim milk agar (Lamilla et al. 2017) and Tributyrin agar (Aly et al. 2012), respectively.

2.3. Characterization of indirect biocontrol molecules, the siderophores

A Fluorescent yellow to orange halos around the actinobacterial colony on modified CAS (Chrome Azural S)-Succinate blue agar plate incorporated with insufficient concentrations of iron (0.01M $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) is suggestive of siderophore secretion by the bioactive actinobacterial isolates (Louden et al. 2011). The ability of the siderophores secreted from actinobacteria to act at distantly localized insufficient concentrations of iron was also comparatively studied among the siderophore producing actinobacterial isolates on CAS -Succinate + YMA Duo agar plate, which is a slight modification of the dual agar plate devised by Dimkpa (2016).

Siderophore producing actinobacteria were grown in succinate broth for 48 h. The siderophores released in the cultured supernatants of actinobacteria were detected using the standard chemical characterization tests such as Tetrazolium salt test (Baakza et al. 2004) for hydroxylamine groups, Arnow's test for catecholate groups (Perez-Miranda et al. 2007), Vogel's test (Vogel 1987) for carboxylate groups, UV-Visible radiation absorbance properties at 420 - 450 nm for hydroxamates; at 495 nm for catecholates; and at 190 – 280 nm for carboxylates.

2.4. Antifungal activity

Agar well diffusion (Silambarasan et al. 2012) assay was adapted to evaluate the antagonistic nature of actinobacteria against the leaf spot pathogen which was previously isolated and stored on potato dextrose agar medium (Kumari et al. 2013).

2.5. Characterization of direct biocontrol molecules, the antifungals

Ethyl acetate extract of bioactive actinobacterium was fractionated through silica gel GF254 thin layer chromatography (TLC) followed by active spot identification in an autobiography assay on PDA (Vijay et al. 2020). Active fractions were collected using silica gel column chromatography with mesh size between 200 and 400 at a linear gradient (% v/v) of Dichloromethane and Benzene. The pooled active fraction was again subjected to the linear gradients of chloroform and ethyl acetate. Purified active fraction was further separated in High-performance liquid chromatography (HPLC) equipped with ODS/C18 column using the mobile phase of chloroform and ethyl acetate (95% / 5%, V/V) at flow rate of 0.5mL/min wherein Kirby Bauer disc diffusion assay was used to monitor the antifungal activity. In GC-MS, the active fraction was injected into capillary type column with injector port at a temperature of 250° C, temperature of detector port at 280° C, column oven at 50° C with constant increment of 6° C per min,

and holding time of 2 min. The entire procedure lasted for 40.33 minutes obtaining a Gas chromatogram and corresponding mass spectrum for each compounds using Shimadzu GCMS/QP 2020.

2.6. Sequence retrieval, homology modeling and docking

The ATPase sequence in related *Pithomyces* sp. was retrieved from National Center for Biotechnology Information (NCBI) and modeled using ATP synthase subunit A of *Saccharomyces cerevisiae* used as a template (PDB: 6B2ZM). The ATPase model in related *Pithomyces* sp was constructed by homology modeling through the prime module of Schrodinger 2018-4. The constructed ATPase model was docked with twenty-four ligands profiled in GCMS analysis as antifungals using Schrodinger 2018-4.

Results And Discussion

3.1. Isolation and identification of actinobacteria

The mangrove actinobacteria was identified as *Kutzneria* sp strain TSII based on Morphological, Biochemical, and 16S rRNA sequencing (Table 1). The 16S rRNA sequence was submitted to the Genbank database of NCBI and obtained the accession number MN 565961. The identified strain TSII was found to be pale yellow colored, stable, branched, cottony aerial mycelium. Scanning Electron Microscopy observation showed the arrangements of rod-shaped cells of *Kutzneria* sp. strain TSII at the maximum resolution of 5000 x in the field view of 10 µm with energy of electrons at 10.0 keV (Fig. 1). Evolutionary analysis (using MEGA X software) showed the isolate's phylogenetic relationship based on 16S gene sequence homology of 100 and 99.91% with *Kutzneria chonburiensis* strain SMC 256 and *Kutzneria buriramensis* A-T 1846, respectively (Fig. 2).

3.2. PGP traits of Actinobacteria

3.2.1. Tube Assay

More recently, researchers are disclosing the direct or indirect mechanism of plant growth-promoting potentials of actinobacteria. Interestingly these bacteria secretes the phytohormones such as indole-3-acetic acid (IAA), cytokinins, and gibberellins, solubilize the minerals such as phosphorus (P) and produce iron chelators- siderophores and 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase. They indirectly benefit the plants by protecting them from pathogens by synthesizing antagonistic substances and fungal cell wall degrading enzymes. Though several previous reports evidenced the PGP properties of actinobacteria, to the best of our knowledge, this is the first attempt revealing the PGP traits and antagonistic potential of *Kutzneria* sp. strain TSII against *P. atro-olivaceous* isolated from the infected groundnut leaves (Table 1). IAA plays a vital role in plant development and physiological processes, including embryogenesis, organogenesis, vascular differentiation, root and shoot development, trophic growth, and fruit development. Examination for the production of IAA indicates that the strain TS II was a strong producer and hence undoubtedly the growth and development of the crop plant can be achieved through direct inoculation of this actinobacteria. A previous investigation by Myo et al. (2019)

demonstrated that the IAA produced by *Streptomyces fradiae* NKZ-259 increases the biomass of tomato seedlings in *in vivo* conditions. The role of HCN in controlling the plant pathogen was proved by several researchers (Etminani et al. 2018; Kumar et al. 2012). In addition to biocontrol, HCN makes phosphorous and iron available, which enables the growth of bacteria and plants (Rijavec et al. 2016). In our experiment, the strain TS II was found to produce HCN in a moderate amount.

3.2.2. Plate Assay

Along with these growth regulating substances, TS II produced some extracellular enzymes such as protease, lipase, cellulase, and lipase. The role of these hydrolytic enzymes in biocontrol has already been proved (Jadhav et al. 2017) and strongly supports our findings. In contrast, the chitinase enzyme test showed that TS II did not produce chitinase that is needed for the cleavage of the cell wall of fungi. However, ethyl acetate extract effectively inhibited fungal growth. The possible reason might be due to the synthesis of various other antimicrobial compounds.

3.3. Characterization of indirect biocontrol molecules, the siderophores

Siderophores are produced by microbes to chelate ferric iron from the surrounding environment. Though these chelators has wider applications it will be more useful for the development of sustainable agriculture (Venkat Kumar et al. 2019). This study indicated that the strain TS II produces siderophores with distantly acting nature when compared to other siderophore producing actinobacteria studied, in an iron starved medium (Fig. 3A), thus able to accumulate iron in root proximity and thereby may improve the plant growth and yield. Further characterization showed the chemical nature of siderophores that belongs to Hydroxamate and Catecholates types based on Tetrazolium salt test and Arnow's test, respectively (Table 2).

3.4. Antifungal activity

The pathogenic fungi were observed with microscopic characteristics of grey to the dark brownish colony (Fig. 3B). An expert taxonomist further confirmed the fungal isolate at Indian Agricultural Research Institute - Indian Type Culture Collection (ITCC) with identification no. 11,167.19. Cell-free culture supernatant of strain TSII has effectively inhibited the growth of *P. atro-olivaceous*, a pathogenic fungal strain, by agar well diffusion assay (Fig. 3C). The reason might be due to the diffusion of antimicrobial metabolites produced by the actinobacteria (Qi et al. 2019).

3.5. Characterization of direct biocontrol molecules, the antifungals

The antimicrobial compound present in ethyl acetate extracts of strain TSII exhibiting excellent antifungal activity was fractionated through thin layer chromatography (TLC) silica gel GF254 using Benzene: Dichloromethane: Methanol (6:2:2) as the solvent system and UV/iodine vapor as detection system and the active spot (R_f value: 0.83) was identified by persistent antifungal activity in an autobiography assay on PDA (Fig. 3D). An antifungal fraction collected using a silica gel column with a linear gradient of Loading [MathJax]/jax/output/CommonHTML/jax.js poled and further eluted in chloroform: ethyl acetate at 9:1.

This partially purified fraction was also separated into its isomeric forms in HPLC equipped with an ODS/C18 column with linear gradients of chloroform and ethyl acetate. GC-MS profiling of HPLC purified active fractions of strain TSII was performed in Shimadzu GCMS/QP2020 and resulted in 24 bioactive metabolites whose m/z ratio, retention time, and molecular formula were matched with compounds available with Wiley Library (Version 8.0) (Table 3 and Fig. 4). These bioactive compounds are categorized into five major groups: Phenolics, Phthalates, Fatty acid methyl esters (FAME), Spiro, and Fatty alcohols. Eicosane (C₂₀H₄₂) and dibutyl phthalate (C₁₆H₂₂O₄) were shown for their antifungal property against *Rhizoctonia solani* AG-3 strain KX85246 (Ashan et al. 2017). The previous study by Qi et al. (2019) demonstrated that phenolics, pyrrolizidine, hydrocarbons, esters, and acids in the crude extract of *Streptomyces* sp. SCA3-4 was responsible for antimicrobial activity against phytopathogens. Similarly, Pyrrolo (1, 2-a) pyrazine -1, 4-dione and aminocoumacin, fungichromin, N- acetyl-D, L- Phenylalanine, and rampamycin from *Streptomyces* sp. UPMRS4 inhibited the growth of *Pyricularia oryzae*, a causative agent of blast disease in rice (Awla et al. 2016).

3.6. Sequence retrieval, homology modeling and docking

Conclusion

Many researchers spoke about the antimicrobial activity of these biologically synthesized compounds against the human pathogen. Only a few were attempted to demonstrate their efficiency against plant pathogens. Most chemical fungicides causes severe health issues to humans and beneficial microbes, ultimately leading to severe environmental pollution. Given this, we paid more attention to find out an alternate method to replace and reduce the use of chemical fungicides. Besides being eco-friendly, microbial fungicides are more economical when compared to the former. At the outset of this study, we conclude that the *Kutzneria* sp. strain TSII alone or its product phenol, 2, 4-bis (1, 1-dimethylethyl) - may act as a promising biocontrol agent. This finding shall improve the crop productivity as well as the economic status of the farmer.

Declarations

Conflict Of Interest: None

References

1. Adamu M, Bagla VP, Eloff JN (2016) Fractionation of *Heteromorpha arborescens* var *abyssinica* (Apiaceae) leaf extracts based on polarity leads to a marked change in cytotoxicity that may yield a commercially useful product. S Afr J Bot 103:36–40
2. Ahsan T, Chen J, Zhao X, Irfan M, Wu Y (2017) Extraction and identification of bioactive compounds (eicosane and dibutyl phthalate) produced by *Streptomyces* strain KX852460 for the biological control of *Rhizoctonia solani* AG-3 strain KX852461 to control target spot disease in tobacco leaf.

3. Ajayi GO, Olagunju JA, Ademuyiwa O, Martins OC (2011) Gas chromatography-mass spectrometry analysis and phytochemical screening of ethanolic root extract of *Plumbago zeylanica*, Linn. *J Med Plant Res* 5(9):1756–1761
4. Alstrom S (1987) Factors associated with detrimental effects of rhizobacteria on plant growth. *Plant Soil* 102(1):3–9
5. Aly MM, Tork S, Al-Garni SM, Nawar L (2012) Production of lipase from genetically improved *Streptomyces exfoliates* LP10 isolated from oil-contaminated soil. *Afr J Microbiol Res* 6(6):1125–1137
6. Awla HK, Kadir J, Othman R, Rashid TS, Wong MY (2016) Bioactive compounds produced by *Streptomyces* sp. isolate UPMRS4 and antifungal activity against *Pyricularia oryzae*. *Am J Plant Sci* 7(07):1077
7. Baakza A, Vala AK, Dave BP, Dube HC (2004) A comparative study of siderophore production by fungi from marine and terrestrial habitats. *J Exp Mar Biol Ecol* 311(1):1–9
8. Balamurugan M, Selvam GG, Thinakaran T, Sivakumar K (2013) Biochemical study and GC-MS analysis of *Hypnea musciformis* (Wulf.) Lamouroux. *AEJSR* 8(3): 117–123
9. Bettache A, Azzouz Z, Boucherba N, Bouiche C, Hamma S (2018) Lignocellulosic biomass and cellulolytic enzymes of Actinobacteria. *SAJ Biotechnol* 5:1–9
10. Bric JM, Bostock RM, Silverstone SE (1991) Rapid in situ assay for indole acetic acid production by bacteria immobilized on a nitrocellulose membrane. *Appl Environ Microbiol* 57(2):535–538
11. Cappucina JC, Sherman N (1992) *Microbiology: A laboratory Manual*, 3rd edn. Benjamin/ Cumming Pub. Co, New York, pp 125–179
12. Carbajo RJ, Kellas FA, Runswick MJ, Montgomery MG, Walker JE, Neuhaus D (2005) Structure of the F₁-binding domain of the stator of bovine F₁F₀-ATPase and how it binds an α-subunit. *J Mol Biol* 351(4):824–838
13. Devi NN, Singh MS (2013) GC-MS Analysis of metabolites from endophytic fungus *Colletotrichum gloeosporioides* isolated from *Phlogacanthus thyrsoiflorus* Nees. *Int J Pharm* 23(2):392–395
14. Dilika F, Bremner PD, Meyer JJM (2000) Antibacterial activity of linoleic and oleic acids isolated from *Helichrysum pedunculatum*: a plant used during circumcision rites. *Fitoterapia* 71(4):450–452
15. Dimkpa C (2016) Microbial siderophores: Production, detection and application in agriculture and environment. *Endocyt Cell Res* 27(2)
16. Etmnani F, Harighi B (2018) Isolation and identification of endophytic bacteria with plant growth promoting activity and biocontrol potential from wild pistachio trees. *Plant Pathol J* 34(3):208
17. Gallo MB, Sarachine MJ (2009) Biological activities of lupeol. *Int J Biomed Pharmaceut Sci* 3(1):46–66
18. Genilloud O (2017) Actinomycetes: still a source of novel antibiotics. *Nat prod rep* 34(10):1203–1232
19. Gerencser MA, Slack JM (1969) Identification of human strains of *Actinomyces viscosus*. *Appl Environ Microbiol* 19(1):80–87

20. Jadhav HP, Shaikh SS, Sayyed RZ (2017) Role of hydrolytic enzymes of rhizoflora in biocontrol of fungal phytopathogens: An overview. In: *Rhizotrophs: Plant growth promotion to bioremediation*. Springer, Singapore, pp 183–203
21. Kapur MK, Das P, Kumar P, Kumar M, Solanki R (2018) Role of microbial extracellular enzymes in the biodegradation of wastes. *J Pharm Chem Biol Sci* 6:237–249
22. Kohl PA, Brossard D, Scheufele DA, Xenos MA (2019) Public views about editing genes in wildlife for conservation. *Conserv Biol* 33(6):1286–1295
23. Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol* 35(6):1547–1549
24. Kumar A, Devi S, Patil S, Payal C, Negi S (2012) Isolation, screening and characterization of bacteria from Rhizospheric soils for different plant growth promotion (PGP) activities: an in vitro study. *Recent res sci technol* 4(1):1–5
25. Kumari A, Kumar R, Maurya S, Choudhary JS, Kumar S (2013) Antifungal efficacy of aqueous extracts of neem cake, karanj cake and vermicompost against some phytopathogenic fungi. *The Bioscan* 8(2):671–674
26. Kuppuswamy KM, Jonnalagadda B, Arockiasamy S (2013) Gc-ms analysis of chloroform extract of *Croton bonplandianum*. *Int J Pharma Bio Sci* 4(4)
27. Lamilla C, Pavez M, Santos A, Hermosilla A, Llanquinao V, Barrientos L (2017) Bioprospecting for extracellular enzymes from culturable Actinobacteria from the South Shetland Islands, Antarctica. *Polar Biol* 40(3):719–726
28. Lay-Jing S, Hooi-Kheng B, Pazillah I, Amirin S, Mohd ZA (2012) Antimicrobial activity of *Gynura segetum's* leaf extracts and its active fractions. *Int J Gen Med* 2:1–5
29. Lichtenstein AH, Appel LJ, Brands M, Carnethon M, Daniels S, Franch HA, Karanja N (2006) Diet and lifestyle recommendations revision 2006: a scientific statement from the American Heart Association Nutrition Committee. *Circ J* 114(1):82–96
30. Loudon BC, Haarmann D, Lynne AM (2011) Use of blue agar CAS assay for siderophore detection. *J Microbiol Biol Educ* 12(1):51
31. Madhuvanathi C, Santhosh Kumar K, Antony Ceasar S, Valivittan K, Srinivasan K, Tamilselvi A (2014) Antibacterial, antioxidant and antiproliferative activities of solvent extracts of *Tiliacora acuminata*. *Int J pharm sci* 6(9):398–403
32. Meena B (2010) Survival and effect of *Pseudomonas fluorescens* formulation developed with various carrier materials in the management of late leaf spot of groundnut. *Int J plant prot* 3(2):200–202
33. Mishra S, Behera N (2008) Amylase activity of a starch degrading bacteria isolated from soil receiving kitchen wastes. *Afr J Biotechnol* 7(18)
34. Mukherjee K, Tribedi P, Mukhopadhyay B, Sil AK (2012) Antibacterial activity of long-chain fatty alcohols against mycobacteria. *FEMS Microbiol Lett* 338(2):177–183

35. Myo EM, Ge B, Ma J, Cui H, Liu B, Shi L, Zhang K (2019) Indole-3-acetic acid production by *Streptomyces fradiae* NKZ-259 and its formulation to enhance plant growth. BMC Microbiol 19(1):155
36. Naragani M, Muvva P, Munaganti RK (2016) Antimicrobial potential of *Streptomyces cheonanensis* VUK-a from mangrove origin. Int J Pharm Pharm Sci 8(3):53–57
37. Niazi J, Gupta V, Chakarborty P, Kumar P (2010) Antiinflammatory and antipyretic activity of *Aleuritis moluccana* leaves. Asian J Pharm Clin Res 3(1):35–37
38. Padmavathi AR, Pandian SK (2014) Antibiofilm activity of biosurfactant producing coral associated bacteria isolated from Gulf of Mannar. Indian J Microbiol 54:376–382
39. Perez-Miranda S, Cabirol N, George-Tellez R, Zamudio-Rivera LS, Fernandez FJ (2007) O-CAS, a fast and universal method for siderophore detection. J Microbiol methods 70(1):127–131
40. Pratik P, Mishra PM (2018) Study of Isolation, Characterization and Antimicrobial Activity of High Value Bioactive Compounds from Methanolic Extract of Leaves of Tilkor (*Momordica monodelpha*). Orient J Chem 34(6):3145
41. Qi D, Zou L, Zhou D, Chen Y, Gao Z, Feng R, Wang W (2019) Taxonomy and Broad-Spectrum Antifungal Activity of *Streptomyces* sp. SCA3-4 Isolated From Rhizosphere Soil of *Opuntia stricta*. Front Microbiol 10
42. Qiu R, Lin M, Ruan J, Fu Y, Hu J, Deng M, Qiu R (2019) Recovering full metallic resources from waste printed circuit boards: a refined review. J Clean Prod 118690
43. Raj AA, Raghunathan R, SrideviKumari MR, Raman N (2003) Synthesis, antimicrobial and antifungal activity of a new class of spiro pyrrolidines. Bioorg Med Chem 11(3):407–419
44. Ren J, Wang J, Karthikeyan S, Liu H, Cai J (2019) Natural anti-phytopathogenic fungi compound Phenol, 2, 4-bis (1, 1-dimethylethyl) from *Pseudomonas fluorescens* TL-1
45. Rijavec T, Lapanje A (2016) Hydrogen cyanide in the rhizosphere: not suppressing plant pathogens, but rather regulating availability of phosphate. Front Microbiol 7:1785
46. Ramala SK, Alagumanivasagam G (2019) GC-MS analysis of phyto constituents presents in ethanolic extract of plant *Cocculus pendulus* (J. R. & G. Forst) Diels Int Res J Pharm 10(3):233–236
47. Sathya A, Vijayabharathi R, Gopalakrishnan S (2017) Plant growth-promoting actinobacteria: a new strategy for enhancing sustainable production and protection of grain legumes. 3 Biotech 7(2):1–10
48. Save SA, Lokhande RS, Chowdhary AS (2015) Determination of 1, 2-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester from the twigs of *Thevetia peruviana* as a Colwell Biomarker. J innov pharm biol sci 2:349–362
49. Silambarasan S, Murugan T, Saravanan D, Balagurunathan R (2012) Antibacterial and antifungal activities of Actinobacteria isolated from Rathnagiri hills. J Appl Pharm Sci 2(10):99
50. Suriyachadkun C, Ngaemthao W, Chunhametha S, Tamura T, Sanglier JJ (2013) *Kutzneria buriramensis* sp. nov., isolated from soil, and emended description of the genus *Kutzneria*. Int J Syst Evol Microbiol 63(1):47–52

51. Swamy MK, Greetha A, Ravinder K, Ali G, Mazina Mohd Y, Uma Rani S (2017) GC-MS Based Metabolite Profiling, Antioxidant and Antimicrobial Properties of Different Solvent Extracts of Malaysian *Plectranthus amboinicus* Leaves. Evid Based Complement Alternat Med Volume 2017 Article ID 1517683:10 pages
52. Tan Y, Leonhard M, Moser D, Ma S, Schneider-Stickler B (2019) Antibiofilm efficacy of curcumin in combination with 2-aminobenzimidazole against single-and mixed-species biofilms of *Candida albicans* and *Staphylococcus aureus*. Colloids Surf B Biointerfaces 174:28–34
53. Thawai C, Tanasupawat S, Itoh T, Suwanborirux K, Kudo T (2004) *Micromonospora aurantionigra* sp. nov., isolated from a peat swamp forest in Thailand. Actinomycetologica 18(1):8–14
54. Tille P (2015) Bailey & Scott's diagnostic microbiology-E-Book. Elsevier Health Sciences
55. Venkatkumar D, Ravindran D (2019) Effect of boundary conditions on residual stresses and distortion in 316 stainless steel butt welded plate. High Temp Mater Processes (London) 38:827–836
56. Vijay K, Devi TS, Sree KK, Elgorban AM, Kumar P, Govarthanan M, Kavitha T (2020) *In vitro* screening and *In silico* prediction of antifungal metabolites from rhizobacterium *Achromobacter kerstersii* JKP9. Arch Microbiol 202(10): 2855–2864
57. Vijay K, Sree KK, Devi TS, Soundarapandian S, Ramasamy V, Thangavel K (2020) Computational Biology Approaches Revealing Novel target in Vascular Wilt Pathogen *Fusarium oxysporum* f. sp. lycopersici for the Ligands of Marine Actinobacterial Origin. J Pure Appl Microbiol 14(1):363–373
58. Vogel AE (1987) Class reactions (reactions for functional groups). In: Elementary Practical Organic Chemistry. CBS Publishing, New Delhi, pp 190–194

Tables

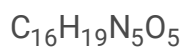
Table 1 Morphological, Biochemical and plant growth promoting characteristics of *Kutzneria* sp. strain TSII

Biochemical characterization	
Gram's reaction	+
Endospore	+
Casein hydrolysis	+
Gelatin hydrolysis	-
Growth at 42°C	-
Nitrate	-
Catalase	+
Oxidase	-
Growth on water agar	-
Starch hydrolysis	+
Urease	-
Plant growth promoting activities	
Indole acetic acid	+++
Siderophore	+
Phosphate solubilization	+
HCN	+
Cellulase	+++
Protease	+
Lipase	+++
Amylase	+++
Chitinase	-
Antifungal activity against the virulent strain <i>Pithomyces atro-olivaceus</i> (ZFGI in mm)	
<i>Kutzneria</i> sp strain TSII	40
Note: + indicates > 90% positive reaction; - indicates > 90% negative reaction; Symbol “+++” represents strong production; ZFGI – zone of fungal growth inhibition	

Table 2 Chemical characterization of siderophores and its mode of action					
Isolate ID	Hydroxamates	Catecholates		Mode of action	
	λ_{max} between 420 nm - 450 nm	TST	λ_{max} at 495 nm	AT	Long Distance (LD) or Short distance (SD)
TSII	-	+	+	+	LD
Note: TST- Tetrazolium salt Test; AT- Arnow's Test; "+" refers to presence of respective siderophore types					

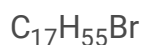
Table 3 GC-MS profile of antifungal compound produced by mangrove actinobacterial strain TS- II				
S.No	Compound Names (Ligands)	Molecular Formula	Structures	Biological activity
1.	Tetracosane	$C_{24}H_{50}$		Antimicrobial activity (Lay-Jing et al. 2012)
2.	Hexatriacontane	$C_{36}H_{74}$		Antibacterial, antioxidant (Madhuvanathi et al. 2014)
3.	Tetrapentacontane	$C_{54}H_{110}$		antioxidant and antimicrobial activities (Mallappa kumara swamy et al. 2017)
4.	Eicosane	$C_{20}H_{42}$		Antifungal (Lichtenstein et al. 2006)
5.	1,2-Benzene Dicarboxylic acid	$C_8H_6O_4$		Antibacterial (Adamu et al.2016)
6.	1,4-Benzenedicarboxylic acid, Bis(2-ethylhexyl) ester	$C_{24}H_{38}O_4$		Anticancer (Save et al. 2015)

7.	Hexadecane	$C_{16}H_{34}$	Antibacterial, antifungal and antioxidant (Balamurugan et al. 2013)
8.	Sulfurous acid, hexyl octyl ester	$C_{14}H_{30}O_3S$	No activity (Sampath Kumar Ramala and Alagumanivasagam 2019)
9.	Sulfurous acid, pentadecyl 2-propyl ester	$C_{18}H_{38}O_3S$	No activity (Sampath Kumar Ramala and Alagumanivasagam 2019)
10.	1,7-Dioxaspiro[5.5]undecane-4,5-diol, 2-ethyl-3-methyl-10-(phenylmethoxy)-8-[(phenylmethoxy)methyl]-, 5-acetate	$C_{29}H_{38}O_7$	anti-inflammatory, antitussive, antifungal and antibacterial (Niazi et al. 2010)
11.	Spiro[cyclopentane-1,2'(1'h)-quinoxaline],3'-(4-morpholinyl)-6',8'-dinitro-		



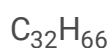
Antifungal and anticandidal (Raj et al . 2003)

12. 1-Bromo-8-Methylhexacosane



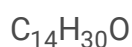
—

13. Dotriacontane



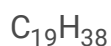
Antioxidant, antimicrobial, antitumor, and antiprotozoal activities (Gallo and Sarachine 2009)

14. 1-Tetradecanol



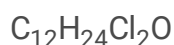
Antibacterial and antifungal (Dilika et al. 2000)

15. Trans-2-Nonadecene



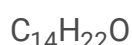
Antifungal (Devi and Singh 2013)

16. 2-Dodecanol, 1,1-Dichloro-



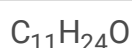
—

17. Phenol, 2,4-Bis(1,1-Dimethylethyl)-



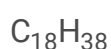
antibacterial, antifungal, anticancer and antioxidant properties (Ajayi et al. 2011)

18. 1-Undecanol



Antibacterial (Mukherjee 2012)

19. Octadecane



Antibacterial and antifungal (Naragani et al. 2016)

20.	1-Nonadecene	$C_{19}H_{38}$	Antifungal (Kuppuswamy et al. 2013)
21.	Trtriacontane	$C_{33}H_{68}$	Antimicrobial activity (Panshu Pratik and Prem Mohan Mishra 2018)
22.	Decanedioic acid, Didecyl ester	$C_{30}H_{58}O_4$	---
23.	Pentane, 2,3,3-Trimethyl-	C_8H_{18}	---
24.	Hexane, 2,3,4-Trimethyl-	C_9H_{20}	---

Table 4 Molecular Docking and Binding free energy of lead compound

Compound name	Docking score (Kcal/mol)	Glide emodel (Kcal/mol)	Glide Energy (Kcal/mol)	Interacting aminoacid residues	ΔG_{bind} (Kcal/mol)
Phenol, 2,4-Bis (1,1-Dimethylethyl)-	-5.355	-27.390	-22.546	SER ⁶⁶ , TYR ⁹⁷	-40.11

Figures

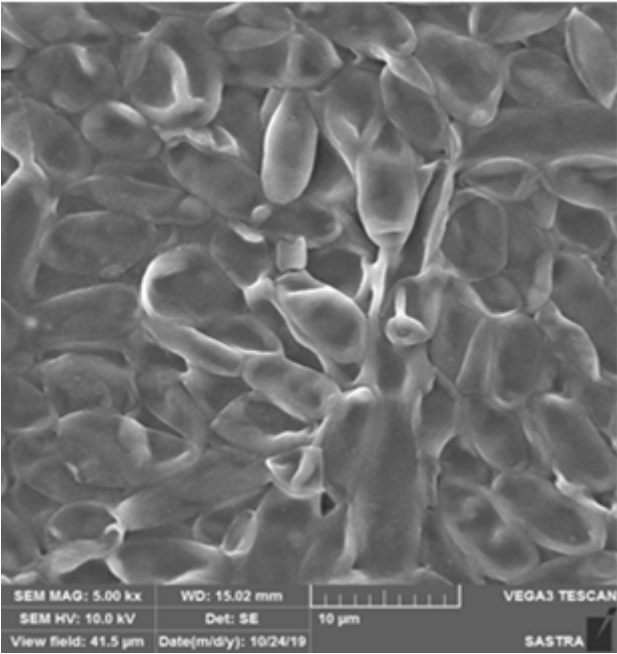


Figure 1

SEM image of *Kutzneria* sp. strian TSII showing globose sporangia emerging from sporangiophores.



Figure 2

Phylogenetic relationship among the ten actinobacterial strains based on 16S rDNA gene sequence in a tree constructed using Maximum Composite Likelihood (MCL) algorithm of the MEGA software version X. Scale bar represents branch length as number of nucleotide substitutions per site.

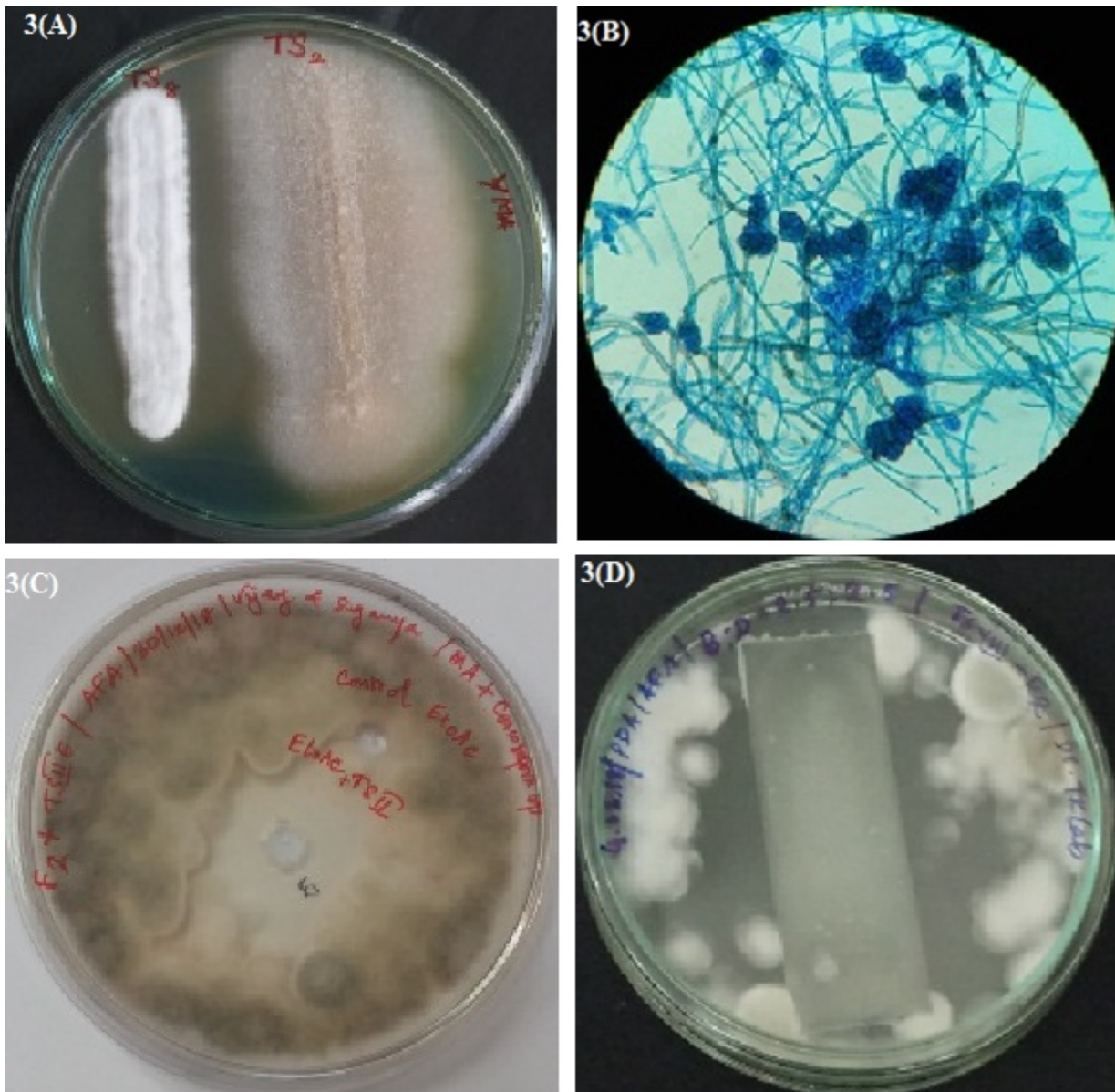


Figure 3

Antagonistic traits of strain TSII. Slides (A), (B), (C), (D), represent distantly acting siderophore production, lactophenol cotton blue stained mycelia and spores of *Pithomyces atro-olivaceus* agar well diffusion assay, and autobiography assay, respectively.

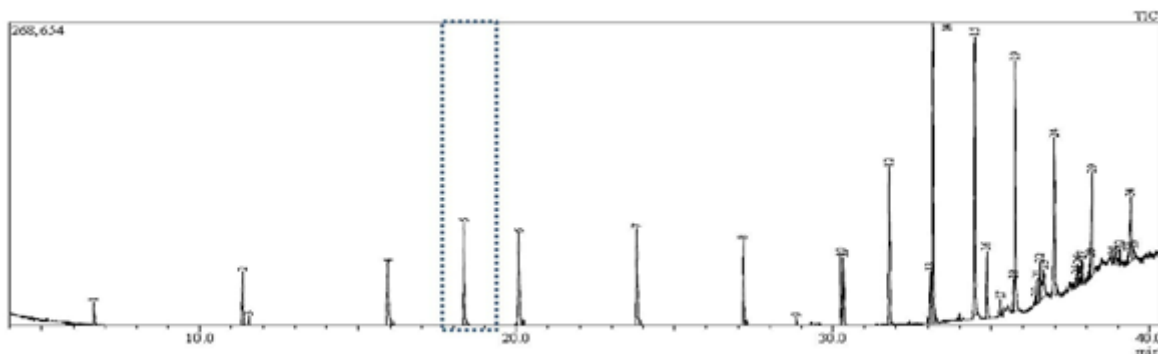


Figure 4

Phenol, 2,4-bis (1,1-dimethylethyl)-. GC-MS chromatogram representing elution of Phenol, 2,4-Bis(1,1-Dimethylethyl)- at Rt (retention time) of 18.33 min and peak area (%) of 4.32. The rectangular dotted lined box indicates the characteristic elution peak of Phenol, 2,4-Bis(1,1-Dimethylethyl)-

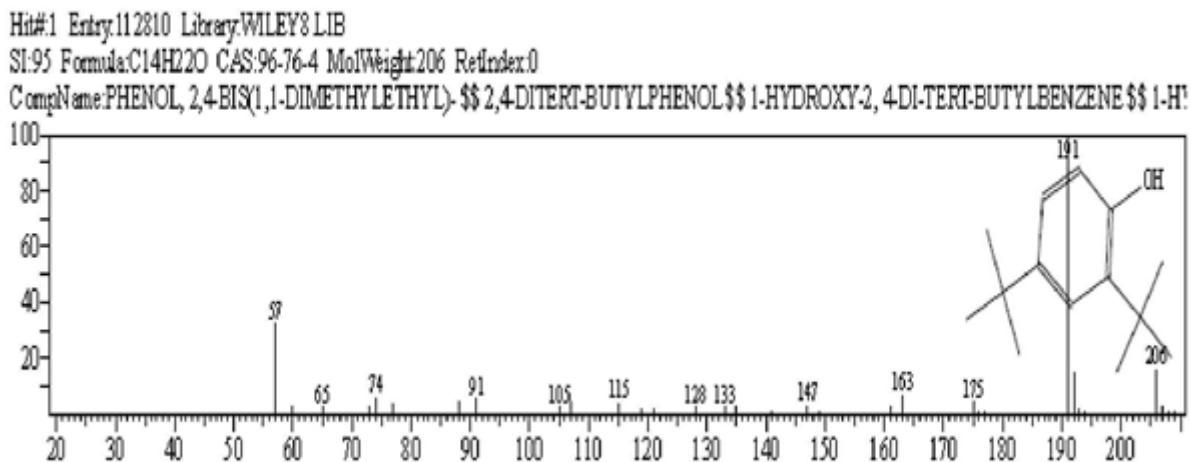


Figure 5

Shows the Gas Chromatographic Mass Spectrum of Phenol, 2,4-Bis (1,1-Dimethylethyl)- produced by *Kutzneria* sp. strain TSII.

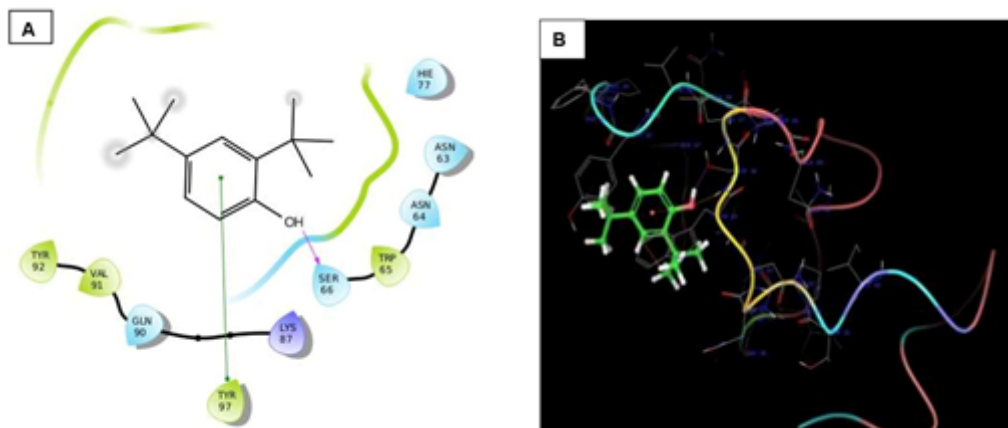


Figure 6

Molecular docking of Phenol, 2,4-Bis(1,1-Dimethylethyl)- origin of *Kutzneria* sp with modeled ATP synthase in *Pithomyces atro-olivaceus*. (A) 2D structure of ligand and target interaction; (B) 3D structure of Phenol, 2,4-Bis(1,1-Dimethylethyl) – ligand complex showing interaction of active site of modeled ATP synthase.

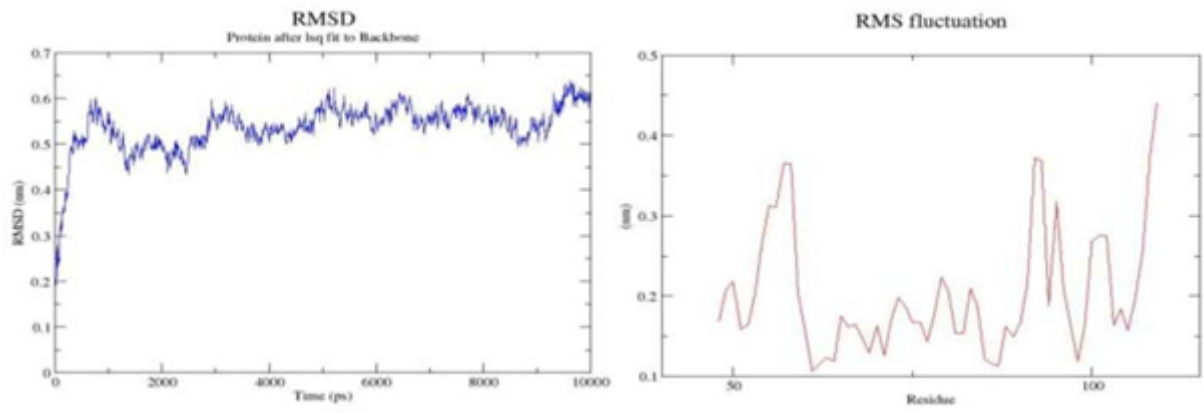


Figure 7

Shows the Root Mean Square Deviation and Fluctuation stability of interacted ligand receptor complex.