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Research Article

Antifungal Activity of Mangrove Rhizobacterium *Pseudomonas aeruginosa* against Certain Phytopathogenic Fungi and its Growth Characterization

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Abstract: Antimicrobial substances are widespread and they are likely to play an important protective role. Marine bacterium has been recognized as producer of important antimicrobial substances which has an exceedingly bright future in the discovery of life saving drugs. The present study was carried out to screen the antifungal activity of mangrove rhizobacteria against certain phyto pathogens from Manakudi estuary, Kanyakumari District, Tamilnadu. Around 20 colonies obtained in Zobell marine agar plates were screened for antifungal traits. Among the 20 isolates, the candidate bacterial isolate exhibited good anti fungal ability. Identification of strains was carried out and confirmed by cultural, biochemical and 16S rDNA sequences. The potent strain was identified as *Pseudomonas aeruginosa*. Various process factors such as different pH, temperature, carbon and nitrogen sources and NaCl were tested for the bacterial growth in static and shaking conditions. The isolated *Pseudomonas aeruginosa* possesed a variety of promising properties that favoured as a better biocontrol agent. In the present investigation antifungal activity of the mangrove isolate was tested against common pathogens like *Penicillium* sp., *Candida* sp., *Aspergillus* sp., *Aspergillus fumigatus*, *Aspergillus flavus*, *Pescalotionbsis* sp., *Fusarium oxysporum* and *Glomerella cinculata*. The candidate bacterium showed inhibitory action to the tested fungal pathogens except *Fusarium oxysporum* and *Glomerella cinculata.*

Keywords: *Pseudomonas aeruginosa*; fungal pathogens; antifungal activity; Mangrove Rhizobacteria
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Introduction

Nature has been a source of medicinal agents for thousands of years. An impressive number of modern drugs have been isolated from microorganisms, mainly based on their use in traditional medicine. Microorganisms have been the study of importance in recent years because of the production of novel metabolites, which exhibits antibacterial, antiviral, anti tumour as well as anticoagulant properties. Most of the current antimicrobial drugs are the derivatives of the earlier generation and microbial resistance against them further intensify the need for new drug discovery. Acceptable options available are the metabolites of plants or animal origin, which are biocompatible, biodegradable and non-toxic in nature. These metabolites are widely studied and are produced by various groups of microorganisms like *Pseudomonas* [1] and *Streptomyces* [2] which are studied for their secondary metabolites.

Pseudomonas is Gram-negative, strictly aerobic, polarly flagellated rods. They are aggressive colonizers of the rhizosphere of various crop plants, and have a broad spectrum antagonistic activity against plant pathogens, such as antibiosis (the production of inhibitory compounds) [3], [4] siderophores production (iron-sequestering compounds) [5] and nutrition or site competition [6]. Some species of *Pseudomonas* can also produce levels of HCN that are toxic to certain pathogenic microorganisms [7].

These characteristics make *Pseudomonas* species good candidates for used as seed inoculants, root dips for biological control of soil-borne plant pathogen and also as antibacterial agents. This study aims at the examination of the antifungal activity of mangrove rhizobacterium Pseudomonas aeruginosa against the target fungal pathogens *Penicillium* sp., *Candida* sp., *Aspergillus* sp., *Aspergillus fumigatus, Aspergillus flavus, Pescalotionbsis* sp., *Fusarium oxysporum* and *Glomerella cinculata.*

Materials and Methods

Isolation of *Pseudomonas aeruginosa*

1gm of mangrove rhizosphere sample was suspended in 99 ml of sterile distilled water. Samples were serially diluted and 0.1 ml of sample was spread on Zobell marine agar plates and incubated at 37 ℃ for 24 hrs. Colonies were counted and the results were expressed as CFU/g. The isolated bacteria was confirmed by morphological (staining and motility), cultural (Nutrient agar, Cetrimide agar), biochemical tests (IMVIC test, triple sugar iron test, nitrate reduction test, catalase test, oxidase test, starch hydrolysis, casein hydrolysis, lipid hydrolysis, gelatin liquefaction and carbohydrate tests).

Screening of antifungal activity of *Pseudomonas aeruginosa* isolated from mangrove rhizosphere soil sample was done by *in vitro* techniques using Muller Hinton agar plates (MHA) at 37 ℃ for 24 hrs.

A fresh colony of potential antifungal mangrove rhizobacterium *Pseudomonas aeruginosa* obtained from *Rhizophora mucoroneta* was inoculated individually in Zobell marine broth and incubated at 37 ℃ for 24 hrs. After swabbing the isolate on the sterile Muller Hinton agar plates, wells of 6mm were punched for agar well diffusion assay method.

Overnight culture of (50 µl) potential antifungal isolate of *Pseudomonas aeruginosa* was added individually in to the wells of Muller Hinton agar plates. They were incubated at 37 ℃ for 24 hrs, where the inhibitory activity was observed as a zone of clearing around the wells. The diameter of the clearing zones was measured in mm using the ruler scale.

Optimization of media components for the growth and characterization of the candidate bacterium

Effect of pH on growth

The effect of pH on the candidate bacterium was determined by culturing the bacterium with different pH (4-11) and incubated at 37^{o}C for 24 hours. After incubation, growth was observed by measuring the absorbance at 405 nm.

Effect of temperature on growth

The candidate bacterium was inoculated in Zobell marine broth and incubated at different temperatures such as 20^{0}C to 70^{0}C for 4 days. Growth of the culture was measured recording the absorbance in spectrometer at 405 nm.

Effect of various carbon sources on growth

Different carbon sources such as sucrose, glucose, galactose, maltose, lactose, fructose and arabinose were added in the Pikovskaya's broth at 1% concentration. Culture broth of the candidate bacterium was inoculated in to the culture media and incubated at 35^{0}C.

Effect of various organic nitrogen sources on growth

Different organic nitrogen sources such as soymeal, beef extract, peptone, yeast extract, skim milk powder and casein were added in the Pikovskaya's broth separately at 1% concentration. Growth of culture was measured by using absorbance in spectrometer at 405 nm.

Effect of various inorganic nitrogen sources on growth

Different nitrogen sources such as ammonium sulphate, ammonium chloride, sodium nitrate, ammonium nitrate and potassium nitrate were added in the Zobell marine broth separately at 1% concentration. Growth of the culture was measured by using absorbance in spectrometer at 405 nm.

Effect of sodium chloride on growth of the candidate bacterium

The impact of different concentrations of sodium chloride on the growth of mangrove rhizobacteria was studied. Different concentrations such as 0.5% to 4.5% were added in the Zobell marine broth separately at 1% concentration. Growth of culture was measured by using absorbance in spectrometer at 405 nm.

Results

Antifungal activity of mangrove rhizobacterium *Pseudomonas aeruginosa* against certain phytopathogenic fungi

In the present study, mangrove rhizosphere soil sample was plated on Zobell marine agar by serial dilution technique. From the sample, isolates I and II of *Pseudomonas aeruginosa* were isolated and identified based on morphological, cultural and biochemical characteristics (Table 1) including carbohydrate fermentation test. The culture filtrate showed antifungal activity against the target fungal pathogens (Table 2; Fig. 1). Measurement of inhibition of zone (expressed in mm) was observed in agar well by diffusion assay method.

Optimization of media components for the growth of mangrove rhizobacteria

Effect of carbon sources on growth of the candidate bacterium

In the present investigation, the effect of various carbon sources on growth of mangrove rhizobacteria was studied. The candidate bacterium utilized various carbon sources for their growth (Fig. 2). Among the tested carbon sources, glucose showed the maximum influence.

Effect of inorganic nitrogen sources on growth of the candidate bacterium

The influence of various inorganic nitrogen sources on the growth of mangrove rhizobacterium was investigated. Ammonium sulphate was the apt substrate for the optimum growth of the candidate bacterium among the used inorganic nitrogen sources (Fig. 3), while, the minimum growth was recorded in potassium nitrate added medium.

Effect of organic sources on growth of the candidate bacterium

Influence of various organic nitrogen sources on the growth of mangrove rhizobacterium was studied. Among the tested organic nitrogen sources, tryptone was the suitable substrate for maximizing the growth of the candidate bacterium (Fig. 4). At the same time, the least growth was recorded in beef extract supplemented medium.

Effect of sodium chloride on growth the candidate bacterium

Among the tested concentrations, the maximum growth was recorded in 3% supplemented medium (Fig. 5), after which there was no considerable improvement.

Effect of pH and temperature on growth of the candidate bacterium

The highest growth was noticed in medium adjusted with pH 9 (Fig. 6). Afterwards there was no considerable improvement. In the present study, we found that the preferential temperature for the optimum growth of mangrove rhizobacterial strain as 40 ℃ (Fig. 7). At the same time the growth was automatically retarded at higher and lower levels.

Table 1 Biochemical characterization of the isolate *Pseudomonas aeruginosa*

Pseudomonas aeruginosa	Characters
Gram negative, rod	Gram's staining
Negative	Indole production
Positive	Methyl red
Negative	Voges Proskauer
Positive	Citrate utilization
Positive	Catalase & Oxidase
Negative	Gelatin hydrolysis
Negative	Urea hydrolysis
Alkaline butt / Alkaline slant	Triple sugar iron agar
Positive	Casein hydrolysis
Positive	Starch hydrolysis
Positive	Lipid hydrolysis
Positive	Phosphate solubilisation

Table 2 Evaluation of anti-fungal activity of the isolate

Pseudomonas aeruginosa	Fungi
17.7	*Penicillium* sp.
28	*Candida* sp.
21.5	*Aspergillus* sp.
24.7	*Aspergillus fumigatus*
153	*Aspergillus flavus*
33	*Pescalotionbsis* sp.
-	*Fusarium oxysporum*
-	*Glomerella cinculata*

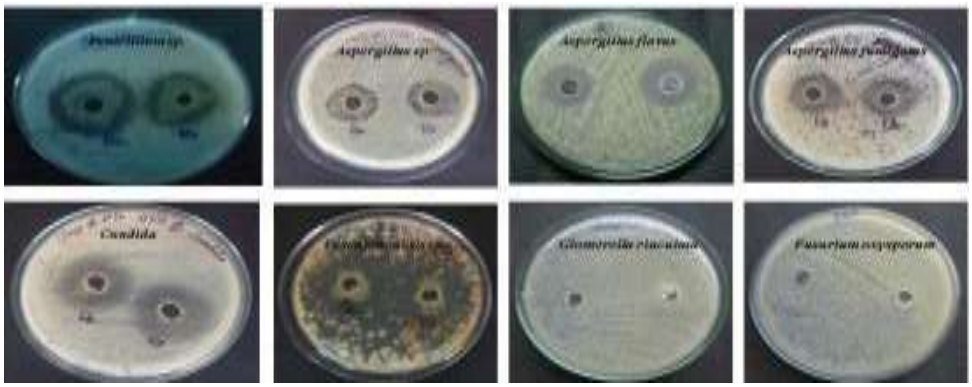

Fig.1 Antifungal activity of the isolate Pseudomonas aeruginosa

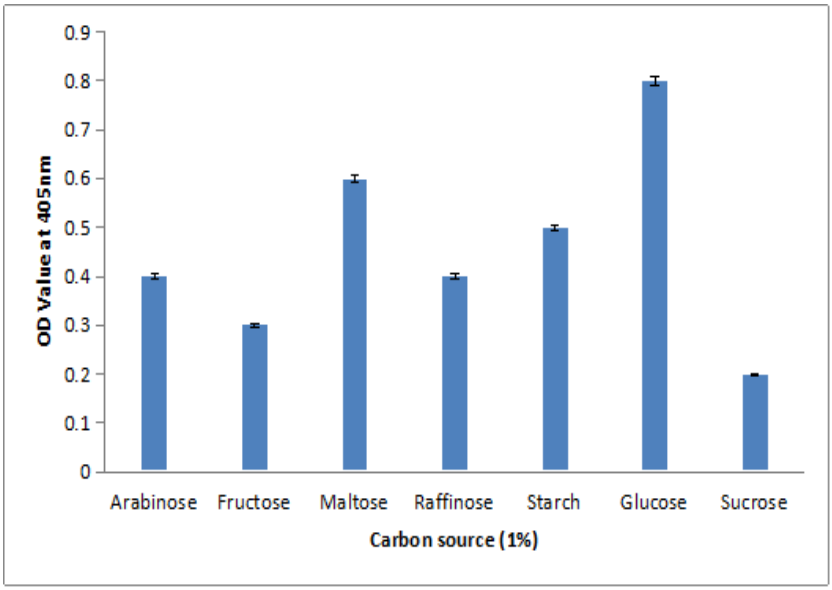

Fig. 2 Growth profile of mangrove rhizobacterium *Pseudomonas aeruginosa* in media supplemented with various carbon sources

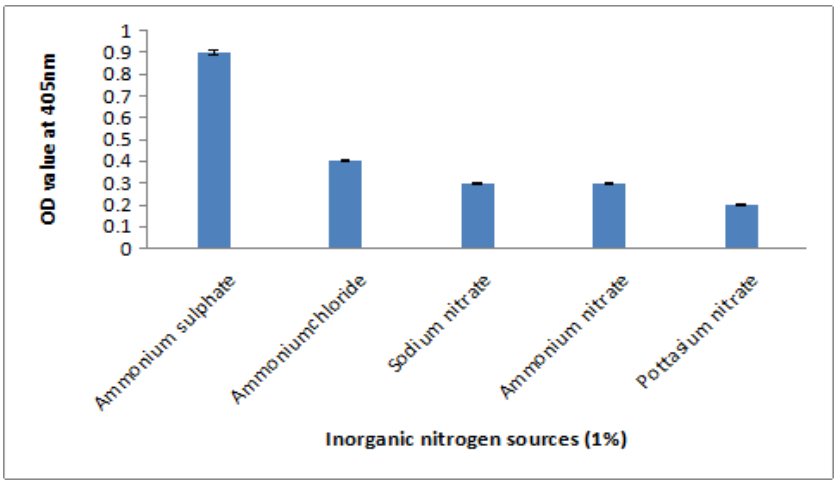

Fig. 3 Effect of inorganic nitrogen sources on growth of mangrove rhizobacteria

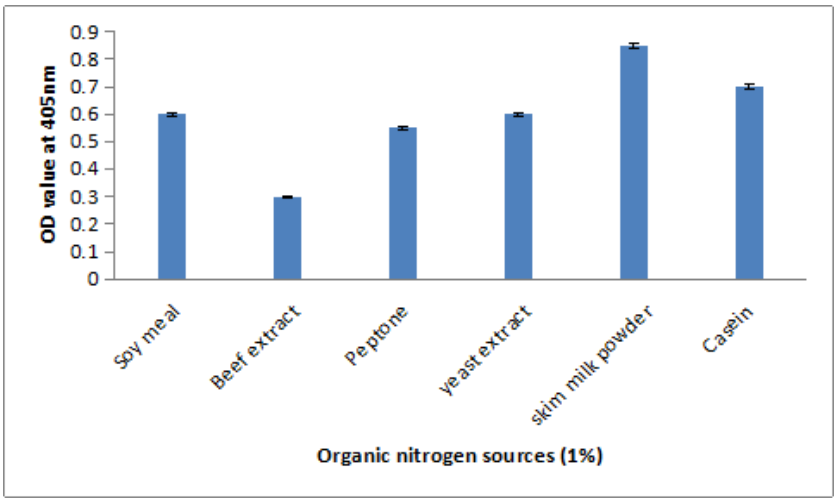

Fig. 4 Effect of organic nitrogen sources on growth of mangrove rhizobacteria

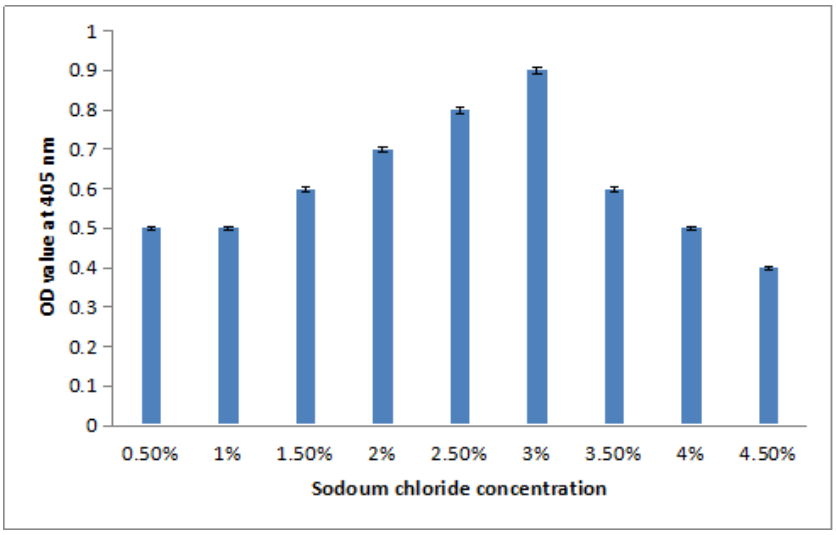

Fig. 5 Effect of sodium chloride on growth the candidate bacterium

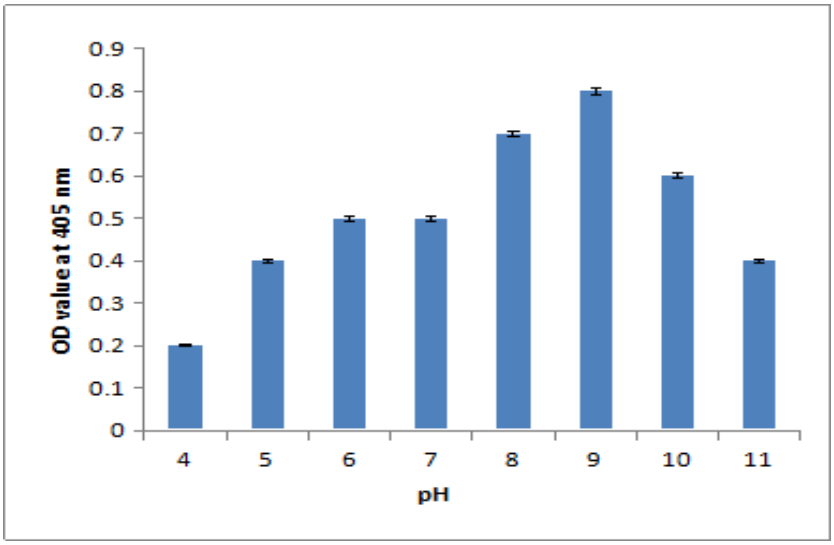

Fig. 6 Effect of pH on growth the candidate bacterium

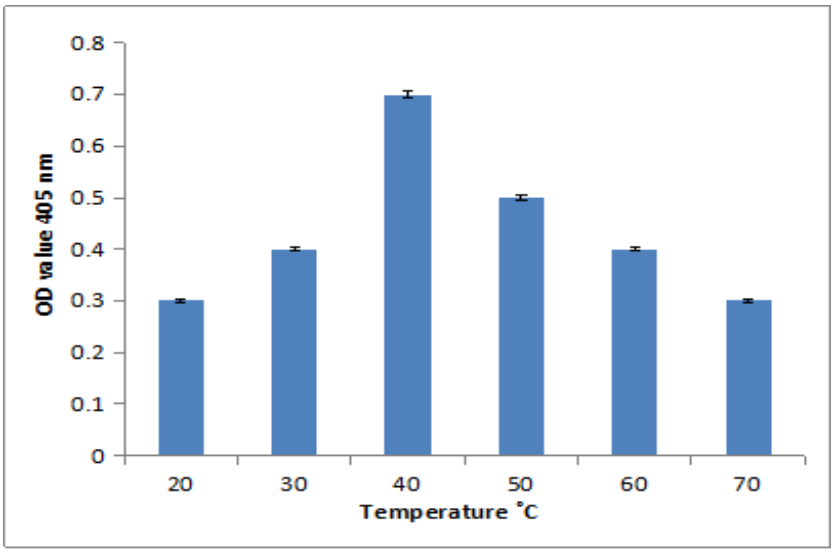

Fig. 7 Effect of temperature on growth the candidate bacterium

Discussion

The genus Pseudomonas is an aggressive colonizer of the rhizosphere of various crop plants, and has a broad spectrum antagonistic activity against plant pathogens, such as antibiosis (the production of inhibitory compounds) [3] [8], siderophores production (iron-sequestering compounds) [5] and nutrition or site competition [6]. Some species of Pseudomonas can also produce levels of HCN that are toxic to certain pathogenic fungi [8].

These characteristics make *Pseudomonas* species as good candidate for use as seed inoculant and root tips for biological control of soil-borne plant pathogen. In the present investigation the isolate I and II were tested against common pathogens like, *Penicillium* sp., *Candida* sp., *Aspergillus* sp., *Aspergillus fumigatus, Aspergillus flavus, Pescalotionbsis* sp., *Fusarium oxysporum and Glomerella*

cinculata. Both isolates I and II showed inhibitory action over all the above said fungal pathogens except *Fusarium oxysporum and Glomerella cinculata.*

Saprophytic organisms play an important part in reducing the incidence of foliar diseases from fungi and bacteria on crops in the field [9], [10]. Fungicide requirements are excellent potency against a variety of plant pathogens and safety, not only for humans, animals, and host plants but also for the ecosystems. Fungicides of microbial origin, which are synthesized biologically, have been demonstrated to be not only specifically effective on the target [11]. Modern medicine obtained began a fruitful adventure since the beginning of twentieth century associated with discover and development of antibiotics. Many antifungal, antimicrobial, insecticidal and herbicidal products which are being used in crop production have been obtained from microorganisms especially bacteria.

Strains of *Pseudomonas aeruginosa* showed known biological control activity against certain soil-borne phytopathogenic fungi and have the potential to produce known secondary metabolites such as siderophore, HCN and protease that showed antagonistic activity against *Macrophomina phaseolina*, *Rhizoctonia solani*, *Phytophthora nicotianae* var. *parasitica*, *Pythium* sp. and *Fusarium* sp. [12]. These characteristics make *Pseudomonas* species good candidates for used as seed inoculants and root dips for biological control of soil-borne plant pathogen. *Pseudomonas* has been recognized as antagonists of plant fungal pathogens and antibiotic producers [13]. This is probably due to the abundance of this diverse group of bacteria and their obvious importance in the soils. *Pseudomonas* plasmids confer resistance to many antibiotics and antibacterial agents [14]. Antagonistic activity was also observed for *Pseudomonas* sp. in the rhizosphere has been recognizers as major factor in the suppression of many phytopathogens. Several antibiotics like substance have been identified, including bacteriocins and phenazine antibiotics [15].

In the present study, the effect of different carbon sources on growth of the *Pseudomonas aeruginosa* was studied. The candidate bacterium utilized various compounds as carbon source for their growth (Fig. 5). But the maximum growth was found in medium supplemented with glucose. [16] also reported that the PGPR potential of *Rhizobium* sp. was utilized glucose for their optimum growth.

In the present study, the effect of various inorganic nitrogen sources on the growth of the mangrove rhizobacterium was studied. Among the tested organic nitrogen sources, the optimum growth of the candidate bacterium was recorded in ammonium sulphate added medium. In the present study is identical to the earlier report of [17], who have reported that the inorganic nitrogen sources proved better than organic one for the growth and phosphate solubilization of *Bacillus subtilis*.

In the present study, the highest growth was noticed in medium adjusted with pH 9. Afterwards considerable reduction was obtained in medium. This result is identical with the earlier report of [18]. They have reported that the optimum pH of fermentation parameters of *Streptomyces* isolates was found to be at pH 8.0 – 9.0.

In the present study, we found that the preferential temperature for the growth of the candidate bacterium was 40 ℃. At the same time the growth was automatically retarded at higher and lower levels. This result is identical with the earlier report of [19]. They have reported that the optimum temperature of fermentation parameters of *Pseudomonas aeruginosa* exhibited in 40°C.

In the present study, the optimum growth of the candidate bacterium was recorded in 3% sodium chloride supplemented medium. In this report is similar to earlier report of [16], who have reported that the optimum growth of *Rhizobium* sp. was recorded in 3% sodium chloride supplemented medium.

Conclusion

The presented data exhibit the antifungal activity of *Pseudomonas aeruginosa* strain from mangrove rhizosphere soil sample and it indicated the possibility of using *Pseudomonas aeruginosa* as a biological control agent of some plant pathogenic fungi. However, this requires further screening of a large number of *Pseudomonas* strains from different regions of India. The antimicrobial activity of *Pseudomonas* may be attributed to the various phytochemical constituents have even more potency with respect to the inhibition of microbes.

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