Journal of Advanced Laboratory Research in Biology
E-ISSN: 0976-7614
Volume 8, Issue 4, 2017
PP 85-88
https://e-journal.sospublication.co.in

Research Article

Temperature effects on growth of the biocontrol agent *Pantoea agglomerans* (An oval isolate from Iraqi soils)

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Abstract: The growth response of the biocontrol agent *Pantoea agglomerans* to changes in temperature was determined *in vitro* in nutrient yeast extract-sucrose medium. The minimum temperature at which *P. agglomerans* was able to grow was 4°C and the maximum temperature was 42°C. This study defines the range of environmental condition (Temperature) over which the bacteria may be developed for biocontrol of postharvest diseases.

Keywords: Biocontrol agent, *Pantoea agglomerans*, Iraqi soils.

1. Introduction

Pantoea species belongs to the family Enterobacteriaceae of fermentative gram-negative rods and is widely distributed in plants and in soil (1). Although members of the genus *Pantoea* are primarily plant pathogens, they occur in many ecological habitats, including in association with soil, water, dairy products, meat, fish, human and animals (2). This bacterium is commonly isolated from plant surface, seeds, fruit and human feces (3).

Pantoea infections are uncommon in human, within the genus *Pantoea*, the most prominent species and most common hospital isolate is *P. agglomerans* (formerly named *Enterobacter agglomerans*) (4).

This bacterium is known to be opportunistic pathogen in the immunocompromised causing wound, blood and urinary tract infection (2). The prevalence of *Pantoea* spp. on potatoes grown in soil amended with a pathogen-rich wastewater sludge was investigated by Chale-Matsau & Snyman (5), Yamakawa (6) were isolated 42 bacterial strains from various fruit and soil samples nine of them were *Pantoea* species.

It is well known that rhizosphere and soil microorganisms play an important role in maintaining crop and soil health through versatile mechanisms (7), *Pantoea agglomerans* is saprophytic strain originally isolated from an apple surface and widespread in nature as an epiphytic bacterium (8).

Microbial biocontrol agents are emerging as effective alternatives to chemicals in controlling postharvest diseases of fruit and vegetables (9). The relationship between bacterial growth rate and environmental conditions involves different response mechanisms and detailed studies are necessary to understand them and determine the critical conditions under which the microorganism is not able to grow. Microbial responses to stressful conditions may constitute a drain of energy resources of the cell as it attempts to maintain cytoplasmic haemostasis (10).

This paper refers to novel demonstration and isolation of *Pantoea agglomerans* from Iraqi soil and discussion the means of temperature on the growth of this species.

2. Material and Methods

2.1 Sample collection

Soil samples were collected randomly from an agricultural farm in Al-Twaitha in Baghdad using a sterile metal cylindrical tool at a depth of 5 to 20cm below the soil surface. Samples were put in a sterile glass container and delivered to the laboratory within 20-30 min of collection. The samples were homogenized and sieved through a sterile 2mm mesh. The sieved samples (2.0 g) was dissolved in 18ml of sterile physiological saline and serially diluted up to 10-5 dilution.

One-tenth of a milliliter (0.1ml) of the 10-2 to 10-5 dilution was inoculated separately onto nutrient yeast extract-sucrose agar plates. The plates were incubated for 24 h at 28°C.

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Bacterial populations in the soil samples (colony forming units per gram of soil) were determined by enumerating the colonies that formed on nutrient agar (11).

2.2 Identification of *Pantoea* species

After incubation period, the plates of nutrient yeast extract-sucrose agar were examined for typical colonies of *Pantoea*. The colonies bearing typical *Pantoea* spp. Morphology were purified and subcultured on nutrient yeast extract-sucrose agar plates and stored for further assay.

Taxonomic properties (morphology, cultural, physiological and biochemical characteristics of the isolate was determined according to the method and media of the (12) and by API-20E system according to (13).

2.3 Determination of optimum temperature for *Pantoea* growth

The optimum temperature was determined by inoculating 100 microliters of the bacterial inoculum 105 CFU/ml on nutrient yeast extract-sucrose agar. The plates were incubated at different temperatures (4, 28, 42°C) for 24 hours.

3. Results and discussion

The soil contains numerous genera of bacteria, many of which not only have important roles in nutrient cycling, but also protect crops against diseases (14).

Pantoea agglomerans is a common soil bacterium used in the biocontrol of fungi and bacteria, but is also an opportunistic human pathogen (15).

Acknowledge of the environmental niche in which the biocontrol agent is able to grow is essential to establish the growth conditions for the antagonist to be mass-produced and also be highly efficacious parameters are temperature, water and pH of the plant tissue or soil and these factors are directly influencing the capability for growth and establishment of the biocontrol agent on plant surfaces (14).

The data presented in this study indicated that the newly isolated bacteria from Iraqi soil samples, which was characterized according to Bergey's Manual of Systematic Bacteriology (12) as well as other characters reported by (16).

3.1 Identification of the isolates

Cultural, morphological and biochemical characteristics, revealed that this isolate being *Pantoea agglomerans*. The preliminary cultural diagnosis for the isolate exhibited that this isolate grew on nutrient yeast extract-sucrose agar after 24 hrs at 28°C. The colonies obtained had the following macroscopic characteristics: round, smooth, regular edge, raised, glistening, translucent with yellow pigment production.

Microscopic examination revealed right bacilli, Gram-negative with rounded ends.

The results of the conventional biochemical test (Table 1) compared with the characteristics of *P. agglomerans* documented by (12) and (16). The bacterium was sufficient for identification of *P. agglomerans.*

Table 1. Biochemical test for *Pantoea agglomerans* isolated from Iraqi soil.

Test	Result
Indole production	-
Acetone production (VP)	+
Methyl red test (MR)	-
Citrate utilization	+
Gelatin hydrolysis	+
Oxidase	-
Catalase	-
Motility	-
Pigment production	Yellow pigment

Biochemical characteristics confirmed by the API-20E system show that the isolate was mobile catalase positive, oxidase negative, lysine decarboxylase negative, ornithine decarboxylase negative, B-galactosidase positive, ferment glucose without gas production, urease negative, citrate utilization positive and indole production negative as mentioned in Table 2. These results have determined the species: *Pantoea agglomerans*.

3.2 Determination of the optimum temperature

Temperature was one of the most important factors governing the physiology and growth of organisms as reported by Rahman *et al*., (17).

The measure *of P. agglomerans* growth indicated growth of this isolate at different temperature. However, this rate varied according to temperature. It was significantly higher at 28°C but reduced to 37°C. At 4°C temperature, the isolate retained significantly, but low growth ability.

In this study the optimum growth temperature was 28°C, indicating that *P. agglomerans* was mesophilic bacterium (Fig. 1). Jung *et al.,* (15) mentioned the highest growth rate *of P. agglomerans* isolates were 30°C, while Costa *et al.,* (14) showed that the minimum temperature at which *P. agglomerans* able to grow was -6 to -1.

Table 2. API-20E system used for diagnosis of *Pantoea agglomerans* isolated from Iraqi soil.

Test	Substrate	Reaction\enzymes	Result
ONPG	Ortho-Nitrophenyl-galactopyranoside	β-galactosidase	Yellow (+)
ADH	Arginine	Arginine dihydrolase	Red\orange (+)
LCD	Lysine	Lysine decarboxylase	Yellow (-)
ODC	Ornithine	Ornithine decarboxylase	Yellow (-)
CIT	Sodium citrate	Citrate utilization	Blue green (+)
H_2S	Sodium thiosulphate	H_2S production	Colorless (-)
URE	Urea	Urease	Yellow (-)
TDA	Tryptophan	Tryptophan deaminase	Yellow (-)
IND	Tryptophan	Indole production	Colorless (-)
VP	Sodium pyruvate	Acetone production	Pink/red (+)
GEL	Kuhn's gelatin	Gelatinase	Black pigment (+)
GLU	Glucose	Fermentation/oxidation	Yellow (+)
MAN	Mannitol	Fermentation/oxidation	Yellow (+)
INO	Inositol	Fermentation/oxidation	Yellow (+)
SOR	Sorbitol	Fermentation/oxidation	Yellow (+)
RHA	Rhamnose	Fermentation/oxidation	Yellow (+)
SAC	Sucrose	Fermentation/oxidation	Yellow (+)
MEL	Melibiose	Fermentation/oxidation	Blue (-)
AMY	Amygdalin	Fermentation/oxidation	Yellow (+)
ARA	Arabinose	Fermentation/oxidation	Yellow (+)

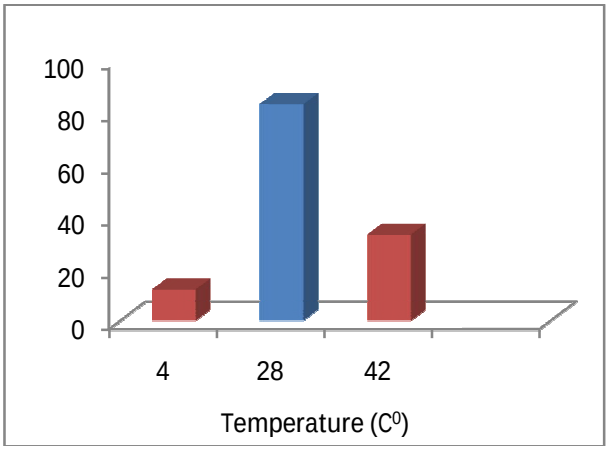

Fig. 1. Temperature effect on the growth of *Pantoea agglomerans* isolated from Iraqi soil.

This study clearly demonstrated that the environmental condition (Temperature) is effective in the growth of the species in the range of the temperature between 4°C to 42°C. Minimum temperature (T min) for bacterial growth was 4°C. T min is an intrinsic property of the organism when growth conditions other than temperature are nonlimiting (18), and it provides information about mesophilic–thermophilic characteristics of bacteria. Temperature indicated that *P. agglomerans* is a mesophilic bacterium (19).

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