

Original Research Article

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Study of Metabolic Diversity (Enzymatic Diversity) of *Pantoea dispersa*

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ABSTRACT

Pantoea belong to a large group of organisms of the Enterobacteriaceae family, also called enteric bacilli. Enzymes are biocleaning agent without harmfully affecting environment. *Pantoea dispersa* are found with these potential which are metabolically diverse and produce different kinds of enzymes likes chitinase, CGTase, Pectinase, xylanase, Protease, L-Glutaminase etc. Extensive uses of agrochemicals create environmental pollution and severely affect living organisms. *Pantoea* has potential degrade harmful environmental pollutants. In present study nine different enzymes are selected for enzymatic profiling of *Pantoea dispersa*. Antimicrobial activity with different antibiogram was performed to check the sensitivity for the isolates. Qualitative assessment of these enzymes is carried out by standard protocol in which particular substrate of enzymes is used. Enzymatic activity was observed with the zone of utilization. Result of the study indicates that *Pantoea dispersa* is metabolically versatile as it produce all the enzymes viz., chitinase, xylanase, pectinase, CGTase, L-Glutaminase, protease, glycosidase and cellulase. Result of antibiotic sensitivity show that *Pantoea dispersa* was sensitive against all the beta lactum group of antibiotic and some of the macrolide and glycoside group of antibiotics at given concentration.

Keywords

Chitinase, CGTase,
Xylanase, Pectinase

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Introduction

The genus *Pantoea* is a diverse group of yellow-pigmented, rod-shaped Gram-negative bacteria in the *Enterobacteriaceae*. Some of the first members were recognized as plant pathogens causing galls, wilting, soft rot and necrosis in a variety of agriculturally relevant plants, but since then, *Pantoea* strains have

been frequently isolated from many aquatic and terrestrial environments, as well as in association with insects, animals and humans (Dutta *et al.*, 2015; Adinarayana *et al.*, 2003). Some *Pantoea* isolates produce antimicrobials, and have been developed into commercial biocontrol products, such as BlightBan C9-1 and Bloomtime Biological, to help control fire blight of apple and pear trees

(Gohel *et al.*, 2004), while others have bioremediation potential, with the capacity to degrade herbicides without the generation of toxic by-products. As well, some isolates have been harnessed as immunopotentiators for the development of supportive drugs for melanoma, infections, allergies and the reversal of immunosuppression. The ubiquity, versatility and genetic tractability of *Pantoea* isolates makes it an ideal group for not only exploring niche-specific adaptation and opportunism, but also for the development of commercially relevant medical, agricultural and environmental products (Kumar *et al.*, 2012).

Materials and Methods

Chitinase

The medium used for the chitinase production plate assay was (g/l) chitin, 5.0; $(\text{NH}_4)_2\text{SO}_4$ 1.0; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5; KH_2PO_4 , 1.36; The pH of the medium was adjusted to 7.2. The medium was sterilized by autoclaving at 121 °C for 15 min. Then, *Pantoea dispersa* was streaked on the chitin agar plates containing calcofluor white M2R. The plates were incubated at 30 °C and examined under UV for the formation of clear Zone (CZ) around colonies up to 7 days. The size of clear zone and colony size were measured and the colony were transferred to chitin agar slant.

Preparation of acid swollen chitin

Phosphoric acid swollen chitin was prepared according to the method given by Hackman. Phosphoric acid-swollen chitin was prepared according to the method described by Hackman (1962). 10g chitin powder was taken for the preparation. While shaking at 4°C - 8°C add slowly 88% phosphoric acid then crush and mix chitin with H_3PO_4 for 2 hrs. Add this chitin with H_3PO_4 in chilled distilled water shake properly get swollen chitin. After

several time wash with cold distilled water add the wash with 1% NaHCO_3 till acidity removed. Suspended this in sterile chilled distilled water. Blend to make homogenizing suspension.

Chitinase activity is checked by preparing minimal salt medium with acid swollen chitin and Calcofluor M2R white. Chitinase activity is checked by observing plate under UV light.

Xylanase

Soil is rich and diverse source of microorganism. Microbes isolated from the soil which is dumped with fruit and vegetables have potential to degrade varied biopolymer. *Pantoea* spp. isolated from the vegetable market soil is screened for xylanase activity. Nutrient agar plates are prepared with 1% xylan area used as screening of xylanase activity. These xylan containing N-agar plates are streaked with young culture of *Pantoea* and incubated at 30 °C for 48 hrs. After the incubation plates are examined for growth. Xylanase activity is checked by adding 0.1% aqueous congo red solution. Plates were flood with congo red and incubated for 15 mins. Excess congo-red drained and plate are destained with 1M NaCl solution. This removed unbound congo-red. Congo red has good affinity for xylan. Surrounding the colonies where xylan is utilised will give clear halos. While in rest of the part of plate congo red is bind with xylan and give red color.

Pectinase

Soil is major platform for the growth of diverse group of microorganism. These microbes have different kind of activity. The pectinase producing microbes isolated from the soil which is dumped with fruit and vegetables. *Pantoea* spp. Isolated from the vegetable market soil is screened for pectinase activity.

Pectin agar plate are streaked with young culture of *Pantoea* spp. and incubated at 30 °C for 48 hours. After the incubation plates are examined for growth. Pectinase activity is checked by adding 1% cetyl -tri methyl ammonium bromide (CTAB) solution and incubated for 15 minute and observe for clear halo surrounding colony.

CGTase

CGTase activity was checked by method given by Illias *et al.*, 2002. Screening of CGTase producer on Basal Horikoshi (II) medium consists of (% w/v): soluble starch (1%), peptone (0.5%), yeast extract (0.5%), MgSO₄ (0.002%), K₂HPO₄ (0.1%), Phenolphathalein (0.002%), Agar (2.5%) and Na₂CO₃ (1%) (autoclaved separately), pH 7.5. *Pantoea dispersa* streak on this plate and incubate it at 28 °C. After incubation clear halos surrounding colonies indicates CGTase activity.

L-Glutaminase

Pantoea dispersa was tested for L-glutaminase activity. The L- glutamine containing media were supplemented with 0.135µl of 2.5 % of phenol red as an indicator. The two control plates were also prepared for glutamine – one was without dye while the other was without glutamine (using NaNO₃ as a nitrogen source). L-glutaminase activity was identified by formation of a pink zone around colonies.

Protease

Protease activity was checked by using milk agar plate. The diluted samples were streaked onto skim milk agar plates containing peptone (0.1% wt/vol), NaCl (0.5% wt/vol), agar (2.0% wt/vol), and skim milk (10% vol/vol). Plates were incubated at 37°C for 24 hours. A clear halos surrounding the colonies indicates

hydrolysis of milk by *Pantoea dispersa* gave an indication of protease activity.

Lipase

Lipase activity was checked on tributyrin agar medium. Lipase producing microorganisms produced a zone of clearance (hydrolysis) when diluted samples were spread on the TBA medium containing per liter of peptone, 5g; beef extract, 3g; tributyrin, 10ml and agar-agar, 20g. The zone of tributyrin hydrolysis was checked after 24 and 48 h of incubation at 37°C.

β-Glucosidase

Screening of β-Glucosidase activity was carried out on MRS agar that is supplemented with esculin (3 g/l) and ferric ammonium citrate (0.2 g/l). The plates were incubated at 37°C for about 48 h and colonies producing browning or blackening of the medium were noted as positive for β-Glucosidase. Young and active culture of *Pantoea dispersa* was streaked over the plate and plate was kept for 48 hrs at room temperature.

Cellulase

For screening of Cellulase activity carboxy methyl cellulose substrate containing minimal medium are prepared and active culture *Pantoea dispersa* was used to check the cellulose activity. After sufficient growth 0.5% congo red solution was applied and keep it for 15 minutes and excess congo red is drain away. This plate then destained with the help of 1M NaCl solution. Presence of clear halos surrounding the colonies indicates cellulose activity.

Antibiotic Sensitivity

Pantoea spp. was studied for sensitivity to different antibiotic. This was carried out with

respect to various multidisc having different antibiotics. On N-agar plate culture was spreaded with the help of sterile spreader and multidisc is kept on their aseptically.

After incubation of 48 hrs results are note down. For the antibiotic sensitivity multidisc with the different antibiotics are used.

Results and Discussion

Chitinase

Chitin agar plates containing calcofluor white M2R was streaked the plate inoculated with *Pantoea dispersa* and after incubation the plates are examined under UV for the formation of clear Zone (CZ) around colonies up to 7 days. Results indicate that colonies of *Pantoea dispersa* produces clear halos surrounding the colonies. *Pantoea dispersa* is good producer of chitinase enzyme (Fig. 1).

Xylanase

Addition of 0.1% congo red and subsequent destain with 1M NaCl indicates the *Pantoea dispersa* has xylanase activity. Results showed that addition of congo red bind to the xylan molecule and whole plate looks red color. Subsequent addition of destain remove unbind congo red stain and surrounding the colonies clear halos are seen this indicate xylanase activity of *Pantoea dispersa*.

Pectinase

Pectin agar medium containing plates are inoculated with *Pantoea dispersa* and after growth plates were flooded with 1% Cetyl trimethyl ammonium bromide solution surrounding colonies pectin was not precipitated that indicated pectinase activity. CTAB precipitate the pectin molecule if organism produces pectinase surrounding the colonies pectin is utilised and clear halos are

produced that indicate pectinase activity by *Pantoea dispersa*.

CGTase

Screening of the CGTase activity was carried out on the medium having starch. If organism produce CGTase enzyme it will convert starch into β - cyclodextrin.

After incubation period of 48 hrs plate is flood with Phenolphthalein solution. This reacts with starch molecule and produces pink to yellow color. Surrounding the colonies of *Pantoea dispersa* there were no color (clear halos) indicates CGTase activity.

L-Glutaminase

L-Glutaminase activity was checked on plate having L-Glutamine. Actively growing culture of *Pantoea dispersa* was streaked on this plate and incubated at 48hrs at room temperature. After incubation no pink colored colonies are produced that indicates absence of L-glutaminase activity by *Pantoea dispersa*.

Protease

Activated culture of *Pantoea dispersa* was streaked on milk agar plate and plates are incubated at 37 °C for 48 hrs. After incubation no clear halos are produced which indicates no protease activity by *Pantoea dispersa*.

Lipase

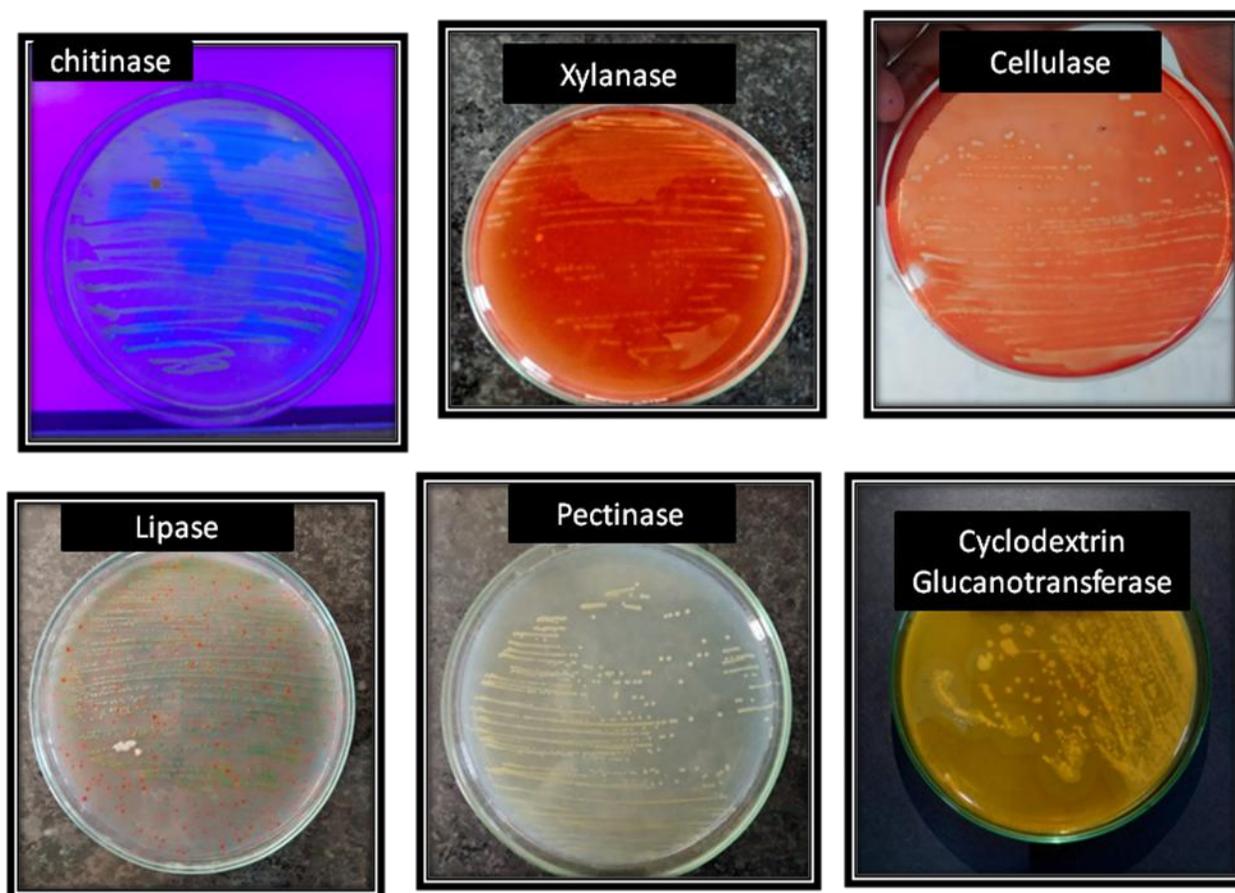
Twenty four hrs actively growing culture of *Pantoea dispersa* was streaked on Tributyrin agar plate and plated incubated at room temperature for 48hrs. After incubation plates are observed for colonies of *Pantoea dispersa* have greening color that indicates the lipase production by *Pantoea dispersa*.

Table.1

Antibiotic	Concentration	Sensitivity
COMBI 69		
Ciprofloxacin	5 µg	Yes
Oflaxacin	5 µg	Yes
Sparfloxacin	5 µg	Yes
Gatifloxacin	5 µg	Yes
Aztreonam	30 µg	Yes
Azithromycin	15 µg	Yes
Vancomycin	30 µg	Yes
Doxycycline	30 µg	Yes
COMBI 95		
Ceftizoxime (CZX)	30µg	Yes
Ceftriaxone (CTR)	30µg	Yes
Cefuroxime (CXM)	30µg	Yes
Cefadroxil (CFR)	30µg	Yes
Co-Trimoxazole (COT)	25µg	Yes
Doxycycline HCl (DO)	30µg	Yes
Gatifloxacin (GAT)	5µg	Yes
Gentamicin (GEN)	10µg	Yes
COMBI 516		
Imipenem (IPM)	10µg	Yes
Meropenem (MRP)	10µg	Yes
Amoxyclav (AMC)	30µg	Yes
Ampicillin/Sulbactam (A/S)	10/10µg	Yes
Azithromycin (AZM)	15µg	Yes
Vancomycin (VA)	30µg	Yes
Linezolid (LZ)	30µg	Yes
Nitrofurantoin (NIT)	300µg	Yes
G- III Plus		
Amikacin (AK)	10µg	Yes
Amoxycillin (AMX)	10µg	No
Bacitracin (B)	10Unit	No
Cephalothin (CEP)	30µg	Yes
Erythromycin (E)	15µg	Yes
Novobiocin (NV)	30µg	Yes
Oxytetracycline (O)	30µg	Yes
Vancomycin (VA)	30µg	Yes
COMBI 61		
Imipenem (IPM)	10µg	Yes

Meropenem (MRP)	10µg	Yes
Ciprofloxacin (CIP)	5µg	Yes
Tobramycin (TOB)	10µg	Yes
Moxifloxacin (MO)	5µg	Yes
Ofloxacin (OF)	5µg	Yes
Sparfloxacin (SPX)	5µg	Yes
Levofloxacin (LE)	5µg	Yes
COMBI VII		
Amoxicillin (AMX)	10µg	No
Cloxacillin (COX)	5µg	No
Erythromycin (E)	15µg	Yes
Tetracycline (TE)	10µg	Yes
Penicillin (P)	2Unit	No
Co-Trimoxazole (COT)	25µg	Yes
Penicillin-V (PV)	3µg	Yes
Cefalexin (CN)	30µg	Yes

Fig.1



β- Glucosidase

After incubation of 48 hrs plates was observed for the color change. No development of brown or black colored indicates *Pantoea dispersa* is not producing β- Glucosidase.

Cellulase

Carboxy methyl cellulose containing medium are inoculated with young and active culture of *Pantoea dispersa*. These plates are incubated at room temperature for 72 hrs. These plates are then flood with agar are then flood with congo red solution and then it is destain with 1M NaCl solution. Results indicates that the *P. dispersa* has ability to degrade cellulose.

Antibiotic Sensitivity

Pantoea spp. found to be sensitive for almost all the antibiotics used in experiment. It was remain unaffected with almost all the antibiotics used in experiment. Results of antibiotic sensitivity are shown in Table 1. *Pantoea dispersa* found to be sensitive for Ciprofloxacin, Ofloxacin, Sparfloxacin, Gatifloxacin, Aztreonam, Azithromycin, Vancomycin, Doxycycline used in experiment.

It was remain unaffected with Bacitracin, Cephalothin, Amoxycillin, Cloxacillin and Penicillin. Result of this study indicates the *Pantoea dispersa* is metabolically diverse and produce very important enzymes chitinase, xylanase, CGTase, Lipase, Pectinase and cellulose.

They lack to produce L- glutaminase, β- Glucosidase and protease. This indicates role of *Pantoea dispersa* as a Biocontrol agent as a future scope.

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