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Antimicrobial Activity of *Pseudomonas Spp* under Different pH, Temperature and Nutritional Values

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Abstract: ***Antimicrobial activity is ability to destroy and inhibit the growth of other microorganisms. Effect of stress conditions on antimicrobial activity is variable. Antimicrobial activities of the isolates were checked against two species of Bacillus and two species of E.coli. The microbes exhibiting the antimicrobial activity can affect their host in different ways; this effect may be stimulatory or inhibitory under different environmental factors such as pH, temperature and minimal media. Cell morphology and gram staining behavior of these strains were also studied after different intervals, at different pH and temperature conditions both in broth and agar. It can be concluded that antimicrobial activity was manifested by the bacterial strains mostly at pH 7 and 37°C on L-agar. On minimal media antimicrobial activity was not expressed. When different concentrations of glucose, fructose and peptone supplements were added to the minimal media antimicrobial activity was retained by the bacterial strains. Cell morphology sporulation, cell size and staining behavior were affected with higher temperatures and pH. In L-broth effects of stress were more pronounced than in L-agar. Overall environmental affects either physical or chemical have important role in manifesting and inhibiting antimicrobial activity. The microorganisms showed prominent results can be analyzed further in the field of agriculture.***

Keywords: Antimicrobial, Bacteria, Pseudomonas, pH, Temperature, Nutrients

1. Introduction

The ability of microorganisms to secrete certain metabolites that inhibit the growth of other microbes is termed as antimicrobial activity; this property is reported in different microorganism such as *Penicillium* and *Cephalosporium* produce β lactum antibiotics, Penicillin, Cephalosporin and their relatives. Most microbiologists distinguish two groups of antimicrobial agents "Antibiotics" which are natural substances produced by certain groups of organisms [1], [2] and chemotherapeutic agents which are chemically synthesized also referred to as "synthetic antibiotics" [3].

A naturally occurring Gram negative non obligate predator bacterial strain (679-2) exhibits a broad spectrum activity that is due to the production of three extracellular compounds. These compounds are isolated and identified as Pyrrolnitrin, Maculosin and a new compound named Banegasine [4].

Many bacteria secrete ribosomally synthesized antimicrobial peptides, termed as Bacteriocins. They exert their lethal activity through absorption to specific receptors located on extreme surface of sensitive bacteria, followed by metabolic and morphological changes leading to the killing of such bacteria [5]. Bacteriocins are also known to be active against food borne pathogens and other closely related Gram-positive bacteria. A novel antimicrobial protein, designated as Enterolysin A, was purified from an *Enterococcus faecalis* LMG 2333 culture that inhibited the growth of selected *Enterococci*, *Pedicocci*, *Lactococci* and *Lactobacilli* [6]. Most antimicrobial peptides (AMPs) impair the viability of target bacteria by permeabilizing bacterial membranes. However, the proline-rich AMP has been shown to kill susceptible organisms without causing significant membrane perturbations and may not by inhibiting the activity of bacterial target. Antimicrobial peptides are a source of novel agents that can be useful for the treatment of chronic lung infections and are active against *Pseudomonas aeurginosa; Staphylococcus aureus* and *Haemophilus influenza*. Doripenem, a novel carbapenem confers β lactamase stability and greater bactericidal action, is tested against a worldwide organism collection. It shows promising results against isolates resistant to other β lactams [7].

However negative effects of *Pseudomonas sp.* on the incubated plants. Inoculation of *Pseudomonas putida* caused the subsequent reduction in root biomass and root hair deformation of pea seedling. Tomato seedling were protected from infection from *Pseudomonas syringae* pv by inoculating with growth promoting bacterium *Azospirillum bransilense*. This is because of antimicrobial activity of *Azospirillum brasilense* against *Pseudomonas syringae* [8].

Biological control, or the use of microorganisms to prevent disease, offers an attractive alternative or supplement to pesticidic and genetic resistance for the management of plant diseases. A *Burkholderia* strain, isolated from soil, have capablity of inhibiting the growth of bacteria, plant pathogenic fungi, yeast and protozoa by excretion of extracellular compounds [9].

Antimicrobial activity is affected by environmental conditions such as pH and temperature because under unfavorable environment i.e. under different pH bacterial strains showed minimum of the antimicrobial activity. Many species of LAB (lactic acid bacteria) under unfavorable environmental conditions produce exopolysaccharide (EPS) which protect them against dessication, bacteriophage and protozoan attack [10]. Temperature and pH also affects the stability of anitimicrobial peptides. Antimicrobial activity of bacteriocin EJ97 isolated from *Enterococcus faecalis* EJ97 against strains of *Bacillus macroides* and *B. maroccans* was also studied under the influence of several factors like

bacteriocin concentration, incubation, temperature, pH, growth media and chemical preservatives and it was found that Enteriocin has a marked bactericidal effect on strain INRA P53-2 after 4 hours of incubation at 37 °C, 24 h at 15 °C or 48 h at 4 °C. Activity was markedly reduced at pH values 5.0 and 9.0. *Bacillus sp.* is also affected by different environmental factors such as extreme temperature, pH, starvation, radiations and enzyme [11], [12]. pH is one of the most important environmental factor which affects the microbial physiological activities and reactivities etc. A broad range antimicrobial substance, isolated from *Bacillus cereus*, was purified by reverse phase HPLC and characterized as a bacteriocin like inhibitory substance was stable in the pH range of 2-9.

2. Materials and Methods

2.1 Bacterial Strains

Total thirty six strains isolated by Ahmad (2000)[13] (table-1) and Zaidi (2001)[14] (table-2) were used to check the antimicrobial activity against strains of *Bacillus* PY79 and *Bacillus* J_2, *E.coli*C600(M. Rosenberg, S.K. and F Laboratories USA) and *E.coli*DH5α(FOCUS,1986) (table-3). All the thirty six bacterial isolates belong to genus *Pseudomonas.*

Table 1: Bacterial isolates from hisoloplane and rhizoplane of *Trianthema partulacastrum, Rumex dentatus* and *Coronopus didymus* by Ahmad (2000)[13]

Plant's Name	Rhizoplane	Histoplane
Trianthema Partulacastrum		Tp_{-1}, Tp_{-2}, Tp_{-3}, Tp_{-4}, Tp_{-5}, Tp_{-6}, Tp_{-7}, Tp_{-8}, Tp_{-9}, Tp_{-11}, Tp_{-13}, Tp_{-14}, Tp_{-15}, Tp_{-17}
Rumex dentatus		Rd_{-1}, Rd_{-2}, Rd_{-3}, Rd_{-4}
Coronopus didymus	Rcd-1, Rcd-2, Rcd-3	

Table 2: Bacterial isolates from hisoloplane and Soil of *Trianthema partulacastrum* and *Rumex dentatus* by Zaidi (2001)[14]

Plant's Name	Soil	Histoplane
Trianthema Partulacastrum	STp_{-1}, STp_{-2}, STp_{-3}, STp_{-4}, STp_{-5}, STp_{-13}, STp_{-14}	Tp_{-18}, Tp_{-20}, Tp_{-21}, Tp_{-22}, Tp_{-25}, Tp_{-26}, Tp_{-27}, Tp_{-28},
Rumex dentatus		Rd_{-1}, Rd_{-2}, Rd_{-3}, Rd_{-4}

Table 3: List of *Escherichia coli* and *Bacillus subtilis* against which the antimicrobial activity of bacterial strains were checked

Strains	Description	Reference
E.coli C600	gal E^+ T^+ K^-lac the leu	M. Rosenberg, S.K. and F Laboratories USA
DH5α	Fϕ8od lac ZΔM15Δ (lac ZYA-argF) U169 end A1 recA1 hsd R17 (r_k – m_k) deo R thi-1 phoA sup E44λ gyrA96rel A1	FOCUS(1986)
Bacillus subtilis PY79	Prototroph	Youngman *et al.*, 1983[15]
J_2	Isolated from phytoplan of *Jatropha sp.*	Ijaz (2003)[16]

2.2 Antimicrobial Activity

Antimicrobial activity of strains was checked by disk absorption method. 10 ul of suspended inoculum (with 10^{-8} dilution factor) was absorbed on a disk of filter paper and placed on a set of media plates spreaded of *E. coli* and *B. subtilis* strains separately. Overnight incubated and next day antimicrobial activity was measured by measuring the clear zone formed around disk [17].

2.3 Cell Morphology

Cell morphology was checked by Gram staining, after different intervals (4, 8, 24 and 48 hours) of incubation.

2.4 Stress Conditions

Antimicrobial activity was checked under varying conditions of pH (4-11), temperature (4 °C, 28 °C, 37 °C, 42 °C) and nutritional requirements (MM2 and M9 media supplemented without and with (0.1 % and1 % concentration) of glucose, fructose and peptone). Different media plates of variable nutrients (glucose, fructose and peptone with 0 %, 0.1 % and1 % concentration separately) adjusted with pH ranges from 4-11were prepared. Plates were plated with *E. coli* and *B. subtilis* strains separately. After placement of inoculated disk with isolates, inocubated at different temperatures (4 °C, 28 °C, 37 °C, 42 °C) for different intervals (4, 8, 24 and 48 hours)

3. Results and Discussion

Nearly all of the currently used antibiotics are version of weapons long yielded by microbes and fungi. Different microorganisms such as *Penicillium* and *Cephalosporium* produce beta lactum antibiotics, Penicillin and Cephalosporin and their relatives. Bacteria have evolved a lot of dynamic systems to cope with fluctuation within environment [18].

Thirty six isolates isolated by Ahmad (2000)[13] (table-1) and Zaidi [14] (table-2) were screened for the antimicrobial activities against strains of *Bacillus* (PY79 and J_2) and *E.coli* (C600 and DH5α). Eight of them (Tp_{-9}, Tp_{-13}, Tp_{-21}, Tp_{-22}, Tp_{-28}, STp_{-14}, Rcd_{-3}, and Rd_{-3}) gave positive results (table-4).

Table 4: Extent of inhibitory zone exhibited by bacterial strains against strains of *Bacillus sp.* and *E-coli*

Strains	*E.coli*		*Bacillus*	
	C600	DH5α	PY79	J_2
TP_{-9}	-	-	0.5	-
Tp_{-13}	-	3	1.5	-
Tp_{-21}	-	1	-	-
Tp_{-22}	-	1	-	-
Tp_{-28}	1	-	0.5	-

STp-14	1	-	-	-
Rcd-3	2	0.5	-	1
Rd-3	-	2	-	-

inhibition zone in millimeters: - absence, +minute

Explaining in the terms of zone size the maximum antimicrobial activity was exhibited by the bacterial isolate Rcd-$_3$ against *E.coli* strain C600 and DH5α and *Bacillus* strain J_2 while the minimum antimicrobial activity was exhibited by the isolate Tp-$_{13}$ against *Bacillus* strain PY79 (table-4).

Lactobacillus reuteus LTH 2584 exhibited antimicrobial behaviour against *Bacillus subtilis*, *Bacillus cereus*, *Enterococus* and *Staphyllococus.* Spore germination of *Bacillus* was also inhibited. Inhibition of *E.coli* was also reported by many workers [19]. Vanillin, the principle flavour component of Vanilla exhibits antimicrobial activity against *Escherischia coli*, *Lactobacillus plantarum*, and *Listeria innocua*. Bacteria exhibiting antimicrobial activity must produce certain antibiotics but are resistant to the action of their own antibiotic, although the organisms are affected by other antibiotics and their antibiotic may be effective against closely related strains [20].

3.1 Effect of varying pHs on antimicrobial activity

To observe the effect of pH on antimicorbial activity strain of *Bacillus* (PY79 and J_2) and *E.coli* were spread on L-agar plates of varying pH (4-11). Eight isolates were stabbed on these plates and were incubated at 37 ˚C. None of the isolates exhibited antimicrobial activity at low pH, except strain STp-$_{14}$ and Rcd-$_3$, which formed very minor inhibition zones at pH 4. Similarly, no prominent antimicrobial activity was observed at alkaline pH, except strain Tp-$_{13}$, Tp-$_{28}$ and STp-$_{14}$ formed very small inhibition zone against *E.coli* strain C600 at pH 10. Most of the strains exhibited maximum antimicrobial activity at neutral pH as evident by the size of inhibition zone (table-5).

Table 5: Results of antimicrobial activity under varying pHs

Strain	Test Strain	pHs							
		4	5	6	7	8	9	10	11
Tp-9	C600	-	-	-	-	-	-	-	-
	DH5α	-	-	-	-	-	-	-	-
	PY79	-	-	-	0.5	-	-	-	-
	J_2	-	-	-	-	-	-	-	-
Tp-13	C600	-	-	-	-	2	1.5	0.5	-
	DH5α	-	-	1	2.5	2	-	-	-
	PY79	-	-	-	2	-	-	-	-
	J_2	-	-	-	-	+	-	-	-
Tp-21	C600	-	-	-	-	-	-	-	-
	DH5α	-	-	1	1	-	-	-	-
	PY79	-	-	+	-	0.5	-	-	-
	J_2	-	-	-	-	+	-	-	-
Tp-22	C600	-	-	-	-	-	0.5	-	-
	DH5α	-	-	1	1	-	-	-	-
	PY79	-	-	+	-	0.5	-	-	-
	J_2	-	-	-	-	0.5	-	-	-
Tp-28	C600	-	-	-	1	-	-	+	-
	DH5α		-	-	-	-	-	-	-
	PY79	-	-	-	0.5	-	-	-	-
	J_2	-	-	-	-	-	-	-	-
STp-14	C600	-	-	-	1	-	-	+	+
	DH5α	+	-	-	-	-	-	-	-
	PY79	-	-	-	-	-	-	-	-
	J_2	-	-	-	-	-	-	-	-
Rcd-3	C600	-	-	-	2	-	0.5	-	-
	DH5α	-	-	0.5	0.5	-	-	-	-
	PY79	+	-	-	-	-	-	-	-
	J_2	-	-	-	1	-	0.5	-	-
Rd-3	C600	-	-	-	-	-	-	-	-
	DH5α	-	-	-	2	-	-	-	-
	PY79	-	-	+	-	-	-	-	-
	J_2	-	-	-	-	-	-	-	-

inhibition zone in millimeters: - absence, +minute

Strain Tp-$_9$ exhibited antimicrobial activity only at pH 7. Strain Tp-$_{13}$ exhibited antimicrobial at a broader range of pH from 6-10. Strains Tp-$_{21}$, Tp-$_{22}$, Rcd-$_3$ and Rd-$_3$ exhibited antimicrobial activity at pH 6 to 9. pH is one of the important environmental factor which affect microbial physiological activity, toxicitiy and reactivity. *Schwanniomyces occidentails* was identified due to the production of a killer protein that was formed to be lethal to the sensitive strains of *Saccharomyces cervisia* the killer protein was chromosomally encoded and its maximum killer activity was between pH 4.2 and 4.8 [21](table-5).

3.2 Effect of varying temperatures on anitmicrobial activity

To observe the effects of varying temperature on antimicorbial activity and the extent of inhibition zones, strains of *Bacillus* (PY79 and J2) and *E.coli* (C600 and DH5α) were spread on L-agar plates and the eight strains (Tp-$_9$, Tp-$_{13}$, Tp-$_{21}$, Tp-$_{22}$, Tp-$_{28}$, STp-$_{14}$, Rcd-$_3$, and Rd-$_3$) were stabbed on these plates and incubated at different temperatures (4 ˚C, 28 ˚C, 37 ˚C, and 45 ˚C).

Relatively less antimicrobial activity was observed at 4 ˚C than that of higher temperatures. Strain Tp-$_{13}$ showed maximum antimicrobial activity, in terms of inhibitory zone size, at 37 ˚C against *E.coli* strain DH5α while against other strains relatively small inhibitory zones were observed. At lower temperatures strain a Tp-$_{28}$ formed an inhibitory zone at 4 ˚C against *Bacillus strain* J_2. Strain Tp-$_{21}$, Tp-$_{22}$ and Tp-$_{28}$ formed maximum inhibitory zone at 28 ˚C against strains of *E.coli* C600. Strain Tp-$_{13}$ formed maximum inhibitory zone at 45 ˚C against both the strains of *E.coli* (C600 and DH5α). While the rest of the strain

exhibited maximum antimicrobial activity at 37 ˚C (table-6).

Table 6: Results of antimicrobial activity under varying temperatures

Strain	Test Strain	Temperatures (˚C)			
		4	28	37	45
Tp-$_9$	C600	-	2	+	2
	DH5α	-	1	-	1
	PY79	-	+	0.5	1
	J_2	+	-	-	-
Tp-$_{13}$	C600	-	1	-	2
	DH5α	-	1	2.5	2
	PY79	-	0.5	2	1
	J_2	-	-	-	2
Tp-$_{21}$	C600	-	2	-	1
	DH5α	-	+	1	1
	PY79	-	+	+	1.5
	J_2	-	+	-	1.5
Tp-$_{22}$	C600	-	2	-	0.5
	DH5α	-	-	1	0.5
	PY79	-	-	-	1
	J_2	-	+	-	1.5
Tp-$_{28}$	C600	-	2	1	+
	DH5α		+	-	+
	PY79	-	-	0.5	0.5
	J_2	1	1	-	+
STp-$_{14}$	C600	-	0.5	1	+
	DH5α	-	+	-	-
	PY79	-	+	-	+
	J_2	-	+	-	+
Rcd-$_3$	C600	-	1	2	+
	DH5α	-	+	0.5	1
	PY79	-	-	-	1
	J_2	+	+	1	+
Rd-$_3$	C600	-	1	-	+
	DH5α	-	+	2	1
	PY79	-	+	-	1
	J_2	-	+	-	-

inhibition zone in millimeters: - absence, +minute

Bacterial activity of Enteriocin EJ97 against strain P53-2 was higher at 37 ˚C and neutural pH. 30 ˚C is the optimum temperature for the toxin production. The inhibitory effect of camel's milk was studied on strains of *Escherischia coli* and *Listria monocytogenes* and it was found that inhibitory effect of camel milk was reduced by heat treatment [22].

3.3 Antimicrobial activity on MM2 and supplemented media

To observe antimicrobial activity on MM2 media and the extent of the inhibitory zones, strains of *Bacillus* (PY79 and J2) and *E.coli* (C600 and DH5α) were spread on MM2 plates and the eight strains were stabbed on these plates. In MM2

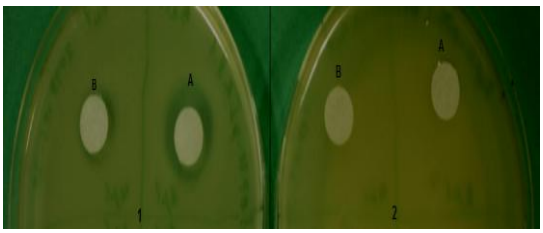

Figure 1: Zone of inhibition on MM2 media (1 % fructose) by Tp-$_{13}$ (A) and Stp-$_{14}$ (B) against *Bacillus C600* before (1) and after (2) 24 hours.

Medium maximum antimicrobial activity in terms of maximum inhibitory zone size was observed (Figure-1), for all the strains except Rd-$_3$, when the media was supplemented with different concentration of Fructose which formed (table-7)

Table 7: Result of antimicrobial activity on MM2 and supplemented media

Strains	Test Strain	MM2	Glucose		Fructose		Peptone	
			0.1%	1%	0.1%	1%	0.1%	1%
Tp-9	C600	-	-	-	-	1.5	2	0.5
	DH5α	-	-	-	-	+	-	1
	PY79	-	-	1	2	2	-	0.5
	J_2	-	-	-	-	-	-	1
Tp-13	C600	-	-	-	+	3	-	1.5
	DH5α	-	-	-	-	-	2.5	1.5
	PY79	-	-	1	-	1	-	1
	J_2	-	-	1	+	2	-	0.5
Tp-21	C600	-	-	-	-	-	2	+
	DH5α	-	-	-	-	-	-	1
	PY79	-	-	-	-	-	-	-
	J_2	-	-	1	2.5	0.5	+	1.5
Tp-22	C600	-	-	-	1	-	+	-
	DH5α	-	-	-	-	-	1	1
	PY79	-	-	-	-	-	-	0.5
	J_2	-	-	1	-	1	-	-
Tp-28	C600	-	-	-	-	-	1	-
	DH5α	-	-	-	+	-	-	-
	PY79	-	-	-	-	-	-	-
	J_2	-	-	-	-	2.5	-	-
STp-14	C600	-	-	-	+	+	1	0.5
	DH5α	-	-	-	+	-	+	-
	PY79	-	-	-	3	+	-	-
	J_2	-	-	1	-	1.5	-	-
Rcd-3	C600	-	-	-	2.5	2	2	0.5
	DH5α	-	-	-	3.5	-	+	-
	PY79	-	-	-	-	-	-	-
	J_2	-	-	-	2	2	-	-
Rd-3	C600	-	-	2	2	2	2	2
	DH5α	-	-	2	-	-	-	-
	PY79	-	-	-	-	-	-	-
	J_2	-	-	-	-	2	-	-

inhibition zone in millimeters: - absence, +minute

Maximum inhibitory zone against *E.coli* strain C600, when the media was supplemented with 1 % Glucose and 1 % peptone.

3.4 Antimicrobial activity on M9 and supplemented media

To observe antimicrobial activity on M9 media and the extent of the inhibitory zones, strain of *Bacillus* (PY79 and J2) and *E.coli* (C600 and DH5α) were spread on M9 plates and the eight strains Tp-21, Tp-22, Tp-28, STp-14, Rcd-3, Rd-3) were stabbed on these plates and were incubated at 37 ˚C for 24 hours. On M9 medium, majority of the strains formed maximum inhibitory zones when the media was supplemented with different concentrations of fructose. Strain Tp-13 formed an inhibitory zone (size of 1.5 mm) against *E.coli* strains DH5α, when M9 media was supplemented with 1 % peptone. Strain Rd-3 formed inhibitory zones, size of about 2-3 mm against *E.coli* strain C600, when the media was supplemented with 0.1% and 1% Glucose (table-8).

Table 8: Result antimicrobial activity on M9 and supplemented media

Strain	Test Strain	M9	Glucose		Fructose		Peptone	
			0.1%	1%	0.1%	1%	0.1%	1%
Tp-9	C600	-	-	-	-	+	-	-
	DH5α1	-	-	-	-	+	-	-
	PY79	-	-	-	-	+	-	+
	J_2	-	-	-	-	-	-	+
Tp-13	C600	-	-	-	0.5	+	-	-
	DH5α	-	-	-	+	-	-	1.5
	PY79	-	-	1	+	0.5	+	1
	J_2	-	-	-	+	-	-	-
Tp-21	C600	-		-	0.5	-	-	-
	DH5α	-	-	-	0.5	-	+	-
	PY79	-	-	-	-	-	-	-
	J_2	-	-	-	-	-	-	-
Tp-22	C600	-	-		+	+	-	-
	DH5α	-	-	-	2	+	-	-
	PY79	-	-	-	+	-	-	-
	J_2	-	-	-	+	0.5	-	-
Tp-28	C600	-	-	-	-	-	-	-
	DH5α	-	-	-	+	-	-	-
	PY79	-	-	-	-	-	-	-
	J_2	-	-	1	1	-	-	+
STp-14	C600	-	-	-	0.5	+	+	-
	DH5α	-	-	-	+	+	-	-
	PY79	-	-	-	-	+	-	-
	J_2	-	-	0.5	-	-	-	-
Rcd-3	C600	-	-	-	1	-	-	+
	DH5α	-	-	-	+	-	-	-
	PY79	-	-	-	1	-	-	-
	J_2	-	-	-	-	-	-	-
Rd-3	C600	-	2	3	-	-	-	-
	DH5α	-	-	-	-	-	-	-
	PY79	-	-	-	1	-	-	-
	J_2	-	-	-	-	-	-	-

inhibition zone in millimeters: - absence, +minute

Free living thermotolerant Amoeba and *Acanthamoeba* are known to harbour a wide range of pathogens like *Legionellae* sequestering them from antimicrobial activity environmental stress [20], [23].

3.5 Effects of varying pHs on cell morphology

Cell morphology of antimicrobial activity strains (Tp-9, Tp-13, Tp-21, Tp-22, Tp-28, STp-14, Rcd-3 and Rd-3) was determined under different pH. The bacterial strains were cultured both on L-agar and L-broth at different pH (5, 7, 9 and 11) at 37 ˚C. Sample were drawn periodically after different intervals (4, 8, 24 and 48 hours of incubation), gram staining was performed and variation in cell morphology was studied. Majority of strains exhibit reduced cell size under alkaline pH, Gram-variable straining behavior was observed and with the increase in incubation time sporulation increased. Alkaline pH has more severe effects on cell morphology and sporulation as compared to acidic pH. Gram staining and resolution were affected with the increase in incubation time i.e., 48 hours. Degenerating cells were also observed under alkaline pH. While for the strains Tp-3, Tp-21, Tp-22, Rd-3 both acidic and alkaline pH proved to be lethal for the cells as staining, resolution and cell size was highly affected ,

some abnormal cell types were formed by the strain Tp-$_{22}$ under alkaline conditions while most of the strains formed short filaments under alkaline pH. Strain TP-$_{21}$ and ST-$_{14}$ formed bipolars at acidic pH. More drastic effects were observed in L-broth as compared to L-agar medium.

Staining behaviour and cell morphology was affected by different environmental factors which include media composition, time period of growth and stress conditions. Sabri and Hasnain. (1996) [24] reported filamentation in *Bacillus subtilus* due to nutrient / environmental stress. Change in intracellular pH regulates important cellular functions [25].

3.6 Effects of varying temperature on cell morphology

Cell morphology of antimicrobial activity exhibiting strain (Tp-$_9$, Tp-$_{13}$, Tp-$_{21}$, Tp-$_{22}$, Tp-$_{28}$, STp-$_{14}$, Rcd-$_3$ and Rd-$_3$) was determined under different conditions of temperature. The bacterial strains were both cultured both on L-agar and L-broth at pH 7 at different temperatures (4 ˚C, 28 ˚C, 37 ˚C and 42 ˚C) samples were drawn periodically after different intervals (4, 8, 24 and 48 hours of incubation), Gram staining and variation in cell morphology was studied.

Sporulation was also very frequent at higher temperatures. In strains Tp-$_{21}$ abnormal cell types (round cells) were observed at 28 ˚C and for Tp-$_{28}$ and Rcd-$_3$ at 45 ˚C. With the increase in incubation time i.e. 24 hours degenerating rods were very common. Cell with a reduced size were observed at low temperatures and bipolar were observed for some of the strains at 4 ˚C. Strain Rcd-$_3$ form long filaments at normal temperatures as well as high temperature. Gram negative cell became Gram-variable under high temperatures. Gram staining property and cell morphology of bacteria can be altered under extreme conditions. This may be due to the reason that extreme pH alter cell wall composition. The influence of adaptation to pH 5.0 to 9.0 alters membrane lipid composition in *E.coli*. According to Ahmed and Sabri (2004)[26] staining behaviour of cells as well morphology were affected by higher temperature. Rise in temperature also causes thermal denaturation and also cause cell lysis, death and loss of viability.

On the basis of above discussion it can be concluded that antimicrobial activity was manifested by the bacterial strains mostly at pH 7 and 37 ˚C on L-agar. On minimal media antimicrobial activity was not expressed. When different concentrations of glucose, fructose and peptone supplements were added to the minimal media antimicrobial activity was retained by the bacterial strains. Cell morphology sporulation, cell size and staining behaviour were affected with higher temperatures and pH. In L-broth effects of stress were more pronounced than in L-agar. The strains which exhibited pronounced antimicrobial activity will be further used to analyse at molecular level. The plant microbe interaction of these strains, in presence of different hazardous strains, will be checked for the betterment of crops.

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