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Characterization of Cellulolytic Bacteria from Waste Dumping Sites of Kashmir Himalaya

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A B S T R A C T

Twenty five biodegradable waste samples were collected from different waste dumping sites of district Baramulla from five locations viz., Wadura, Sopore, Baramulla town, Bomai and Pattan. The cellulolytic bacteria were isolated on Carboxyl Methyl Cellulose Agar Medium (CMC) by following serial dilution pour plate method. The cellulolytic bacterial isolates were screened qualitatively as well as quantitatively at three different temperatures (10, 15 & 20^{o}C) and three different pHs (5, 7 and 9). Out of twenty, CBW3 isolate from Wadura showed highest solubilization zone (4 mm) with solubilization efficiency (285.7%) and cellulase activity (2.917 U/ml) followed by CBB3 from Baramulla town (3.4 mm) with solubilization efficiency (261.5%) and cellulase activity (2.566 U/ml). All the 20 cellulolytic bacterial isolates were morphologically, biochemically characterized. Although these microbes showed the cellulose activity under variable pH and temperature combinations but pH 7 and 20 ^{o}C temperature was the most ideal standardized condition for the better performance by the microbes under investigation.

Introduction

Microorganisms represent the major source of genetic diversity on earth. The prestige of microorganisms is due to their high metabolic versatility, which allows the inference about its potential for biotechnological applications, including enzyme production for industrial and environmental uses. Unscientific disposal causes an adverse impact on all components of the environment and human health. Microorganism performs their metabolic processes that rapidly catalyzed complex substrates like cellulose by their diverse enzyme-mediated reactions. Cellulase catalyses hydrolysis of cellulose to D-glucose (Hussain *et al.*, 2009). Cellulose is the most abundant structural polysaccharide of plant

cell walls with β-1, 4 - glucosidic linkages and represents almost 50% of the biomass synthesized by photosynthetic fixation of CO_2 (Eriksson *et al.,* 1990). The majority of cellulose molecules consist of 8000-12000 glucose molecules.

An enzyme alternative to harsh chemical technologies has led to intensive exploration of natural microbial biodiversity for waste management.

The cellulolytic enzyme consists of at least three enzymes(Joachim and Patrick, 2008). Cellulases are a consortium of free enzymes comprised of endoglucanases (β-1,4-D-glucan-4-glucanohydrolase, EC 3.2.1.4, carboxymethyl cellulase, EC), exoglucanases (β-1,4-D-glucan-4-glucohydrolase, EC 3.2.1.91, cellobiohydrolase, CBH), and cellobiases (β-D-glucoside glucohydrolase, EC 3.2.1.21, β-1,4-D-glucosidase) are found in many of the 57 glycosyl hydrolase families (Siddiqui *et al.,* 2000). Cellulase enzymes are produced by both aerobic and anaerobic bacteria like (*Acinetobacter junii, Bacillus subtilis, Cellulomonas biazotea, Pseudomonas cellulose*) and anaerobic (*Acetivibrio cellulolyticus, Butyrivibrio fibrisolvens, Clostridium thermocellum*) (Sukumaran *et al.,* 2005 and Sadhu *et al.,* 2013).

Materials and Methods

Study area

Baramulla district is largest in the entire valley both with reference to the population and area. Baramulla district is bounded by Kupwara district in the north, Budgam and poonch in the south, parts of Srinagar and ladakah in the east.

Baramulla district has severe cold in winter and pleasant whether in summer. Annual rain fall in the district is usually registered 1270 mm. Soil in hilly areas is poor but in the plain areas it is fertile about 83.05% of the population lives in villages and 16.94% in urban areas. Crops like paddy maize pulses grow in abundance. In addition to this the district is also rich in fruit growing,

The district is spread from Srinagar district and Ganderbal district in the east to the line of controlling the west and from Kupwara district in the north and Bandipore district in the northwest to Poonch district in the south and Budgam district in the southwest. Baramulla city is located on the banks of Jhelum river at the highest point of the river. The old town lies on the north (right) bank of the river and the new town lies on the south (left) bank. They are connected by five bridges including a suspension bridge connecting Gulnar park with Dewan Bagh. The district is located between 33 degree to -44 North latitude and 75 degree to 96 E Longitude.

Isolation of cellulose degrading bacteria

The cellulose degrading bacteria was isolated from the waste dumping site by serial dilution plate method using cellulose agar medium. The serial dilutions of the waste samples were made up to 10^{-5} and 0.5 ml of diluted waste suspension was plated Carboxy Methyl Cellulose Agar medium (1gm cellulose, 1gm peptone, 0.2gm ammonium sulphate ($NH_4SO_{4)}$, 0.2gm di-potassium hydrogen phosphate (KH_2PO_4), 0.003gm magnesium sulphate ($MgSO_4$) and 2gm agar. The plates were incubated at 28 ± 2 °C in biological oxygen demand (BOD) incubator for 24-48 hrs. Detection of cellulolytic bacteria solubilization by different cellulolytic bacterial isolates was based upon the ability of solubilization zone formation. The cellulolytic bacterial isolates were maintained by transfer on Cellulose agar medium slants. These bacterial cultures were stored at 4 ℃ in refrigerator for further use.

Screening of cellulolytic bacteria from different waste dumping sites for cellulose solubilization and cellulase enzyme activity

After proper purification, the pure cultures were screened for cellulose solubilization and cellulase enzyme activity by plate assay.

Characterization of cellulolytic bacterial isolates by Morphological, cultural and biochemical features

Morphological characterization

All the cellulolytic bacterial isolates were studied for the colony features like morphological characteristics and pigmentation. The cell shape and gram reaction was also recorded as per the standard procedures given by Barthalomew and Mittewar (1950) and Anonymous (1957).

Colony morphological characteristics and pigmentation

Morphological characteristics of the colony of each isolate were examined on Cellulose agar medium. Cultural characterization of isolates observed by different characteristics of colonies such as size, shape, elevation, surface, margin, color, pigmentation, etc were recorded.

Gram's staining

Biochemical and Physiological Characterization of cellulolytic bacterial isolates

Different biochemical tests were performed like Catalase Test (Blazevic and Ederer, 1975), Starch Hydrolysis (Eckford, 1927), Urease test (James and Natalie Sherman, 1992), Gelatin liquefaction test (Blazevic and Ederer, 1975), Hydrogen Sulfide test (Cowan and Steel, 1970) respectively.

Results and Discussion

Isolation of Cellulolytic bacteria from waste dumping sites

The different cellulolytic bacterial isolates were isolated from the waste dumping sites of district Baramulla from various locations viz: Wadura, Sopore, Baramulla town, Bomai and Pattan. Almost all samples contained the cellulose degrading bacteria. It was interesting to note that bacterial isolates from waste dumping sites were able to grow and solubilize the medium containing complex cellulosic material in the form of carboxyl methyl cellulase (CMC). Out of 155 isolates, 20 most outstanding isolates were retained for further screening and characterization.

These isolates were able to solubilize the Carboxy Methyl Cellulose agar media and produced efficient solubilization zone of more than 3.6mm with a solubilization efficiency of more than 327.2% revealing activity and their ability to metabolise cellulose. Our results are in conformity with the findings of Khatiwada *et al.,* (2016) who also isolated three strains of *Bacillus* sp. *Pseudomonas* sp. and *Serratia* sp. from municipal solid waste and rice straw waste for cellulose hydrolysis. The results also support the work of Sopid Sawangjit (2017) who also isolated cellulose degrading bacteria from soil of waste disposal site and identified four different species of bacteria: *Pseudomonas aeruginosa, Pseudomonas stutzeri, Enterobacter cloacae* and *Bacillus anthracis.*

The present results reveals that cellulolysis is basically the biological process controlled and processed by the enzymes of cellulase system. With the help of cellulolytic system, cellulose can be converted to glucose which is a multiutility product, in a much cheaper and biologically favourable process (Fig. 1–9 and Table 1–9).

Table.1 Isolation of cellulose degrading bacteria at pH 7 and temperature 28°C

S. No.	Isolate	Zone of solubilisation (mm)	Colony diameter (mm)	Solubilisation efficiency (%)
1.	CBW1	3.2	2.0	160.0
2.	CBWA	3.0	2.0	150.0
3.	CBW2	3.1	2.0	156.6
4.	CBWB	2.8	1.8	155.5
5.	CBW3	3.6	1.1	327.2
6.	CBW4	2.2	2.06	106.0
7.	CBS1	2.5	2.3	108.0
8.	CBS2	3.0	2.0	150.0
9.	CBS3	2.5	1.4	178.5
10.	CBS4	2.6	2.2	118.1
11.	CBS5	2.8	1.1	254.5
12.	CBB3	3.3	1.2	275.0
13.	CBB4	2.4	1.4	171.4
14.	CBB5	3.1	1.8	172.2
15.	CBBo3	2.7	1.9	142.1
16.	CBBo4	2.8	2.8	100.0
17.	CBBo5	2.3	1.7	135.2
18.	CBP2	2.0	1.7	117.6
19.	CBP3	3.1	1.7	182.3
20.	CBP4	2.4	1.9	126.3
CD $_{(P\leq0.05)}$		**0.294**	**0.093**	-
SE (m)		**0.104**	**0.033**	-

Table.2 Qualitative screening of Cellulose degrading bacterial isolates at pH 5 and 10°C, 15°C, 20°C temperature after 2 DAI

Isolate	pH5								
	10°C			15°C			20°C		
	Hallow zone (mm)	Colony Diameter (mm)	Solubilisation efficiency (%)	Hallow zone (mm)	Colony Diameter (mm)	Solubilisation efficiency (%)	Hallow zone (mm)	Colony diameter (mm)	Solubilisation efficiency (%)
Control	0	0	0	0	0	0	0	0	0
CB W1	1.5	1.1	136.3	2	1.3	153.8	1.4	1.2	116.6
CB WA	1.3	1.2	108.3	1.8	1.1	163.6	1.9	1.3	146.1
CB W2	1.2	1.2	100	1.9	1.2	158.3	1.8	1.1	163.6
CB WB	1.1	1.1	100	1.6	1.1	145.4	1.6	1.3	123
CBW3	**1.9**	**1.1**	**172.7**	**2.2**	**1.2**	**183.3**	**2.1**	**1.1**	**190.9**
CB W4	1.2	1.2	100	1.9	1.3	146.1	1.8	1.2	150
CB S1	1.1	1.1	100	2.1	1.2	175	2	1.2	166.6
CB S2	1.1	1.1	100	1.9	1.1	172.7	1.5	1.2	125
CB S3	1.4	1.1	127.2	1.4	1.1	127.7	1.3	1.2	108.3
CB S4	1.2	1.3	92.3	2.1	1.2	175	2	1.3	153.8
CB S5	1.8	1.3	138.4	2.2	1.4	157.1	1.8	1.2	150
CB B3	**1.9**	**1.2**	**158.3**	**2**	**1.1**	**181.8**	**1.9**	**1.1**	**172.7**
CB B4	1.4	1.1	127.2	2	1.2	166.6	2	1.2	166.6
CB B5	1.2	1.1	109	1.8	1.3	138.4	1.7	1.1	154.5
CBBo3	1.6	1.1	145.4	2.3	1.3	176.9	1.8	1.1	163.6
CBBo4	1.3	1.2	108.3	1.7	1.3	130.7	1.6	1.2	133.3
CBBo5	1.4	1.2	116.6	2	1.3	153.8	1.7	1.3	153.8
CBP2	1.2	1.1	109	1.7	1.3	130.7	1.8	1.2	150
CB P3	1.7	1.2	141.6	1.7	1.1	154.5	1.7	1.1	154.5
CB P4	1.6	1.2	133.3	2.1	1.2	175	2	1.3	153.8
CD (P≤0.05)	**0.114**	**0.035**		**0.197**	**0.061**		**0.126**	**0.039**	

Table.3 Qualitative screening of Cellulose degrading bacterial isolates at pH7 and 10°C, 15°C, 20°C temperature after 2DAI

Isolate	pH5								
	10°C			15°C			20°C		
	Hallow zone (mm)	Colony diameter (mm)	Solubilisation efficiency (%)	Hallow zone (mm)	Colony diameter (mm)	Solubilisation efficiency (%)	Hallow zone (mm)	Colony diameter (mm)	Solubilisation efficiency (%)
Control	0	0	0	0	0	0	0	0	0
CB W1	2.2	1.3	169.2	2.2	1.2	183.3	3.2	1.6	200.0
CB WA	2.4	1.2	200.0	2.7	1.4	192.8	3	1.5	200.0
CB W2	2.3	1.3	176.9	2.1	1.2	175.0	3.1	1.5	206.6
CB WB	2.6	1.3	200.0	2.8	1.4	200.0	2.8	1.4	200.0
CBW3	**2.8**	**1.2**	**233.3**	**2.9**	**1.2**	**241.6**	**4**	**1.4**	**285.7**
CB W4	2.4	1.2	200.0	2.7	1.4	192.8	2.5	1.3	192.3
CB S1	2.6	1.2	216.6	2.7	1.4	192.8	2.5	1.2	208.3
CB S2	2.3	1.1	209.0	2.6	1.4	185.7	3	1.3	230.7
CB S3	2.2	1.3	169.2	2.5	1.4	178.5	2.5	1.4	178.5
CB S4	2.2	1.2	183.3	2.5	1.5	166.6	2.6	1.1	236.3
CB S5	1.7	1.2	141.6	2.6	1.5	173.3	2.9	1.3	223.0
CB B3	**2.4**	**1.1**	**218.1**	**2.7**	**1.3**	**207.6**	**3.4**	**1.3**	**261.5**
CB B4	2	1.2	166.6	2.5	1.5	166.6	2.4	1.3	184.6
CB B5	2	1.2	166.6	2.6	1.5	173.3	2.5	1.3	192.3
CBBo3	1.9	1.2	158.3	2.6	1.5	173.3	2.7	1.3	207.6
CBBo4	2	1.3	153.8	2.6	1.7	152.9	2.8	1.2	233.3
CBBo5	2.1	1.4	150.0	2.7	1.6	168.7	2.3	1.1	209.0
CBP2	2	1.3	153.8	2.7	2	135	2.2	1.2	183.3
CB P3	2	1.4	142.8	2.7	1.6	168.7	2.7	1.2	225.0
CB P4	1.9	1.3	146.1	2.6	1.5	173.3	2.5	1.3	192.3
CD (P≤0.05)	**0.125**	**0.039**		**0.115**	**0.035**		**0.222**	**0.068**	

Table.4 Qualitative screening of Cellulose degrading bacterial isolates at pH 9 and 10°C, 15°C, 20°C temperature after 2 DAI

Isolate	pH5								
	10°C			15°C			20°C		
	Hallow zone (mm)	Colony diameter (mm)	Solubilisation efficiency (%)	Hallow zone (mm)	Colony diameter (mm)	Solubilisation efficiency (%)	Hallow zone (mm)	Colony diameter (mm)	Solubilisation efficiency (%)
Control	0	0	0	0	0	0	0	0	0
CB W1	1.2	1.2	100.0	1.7	1.2	141.6	1.8	1.2	150.0
CB WA	1.4	1.1	127.2	1.8	1.2	150.0	1.6	1.2	133.3
CB W2	1.6	1.2	133.3	1.6	1.2	133.3	1.9	1.2	158.3
CB WB	1.3	1.1	118.1	1.7	1.1	154.5	1.8	1.1	163.6
CBW3	**1.5**	**1.1**	**136.3**	**1.9**	**1.2**	**158.3**	**2**	**1.1**	**181.1**
CB W4	1	1.2	83.3	1.6	1.3	123.0	2	1.2	166.6
CB S1	1.4	1.2	116.6	1.8	1.2	150.0	1.8	1.2	150.0
CB S2	1.5	1.3	115.3	1.6	1.2	133.3	1.6	1.2	133.3
CB S3	1.2	1.1	109.0	1.8	1.2	150.0	1.4	1.1	127.2
CB S4	1.4	1.3	107.6	1.8	1.3	138.4	1.6	1.3	123.0
CB S5	1.3	1.1	118.1	1.4	1.3	107.6	1.4	1.3	107.6
CB B3	**1.6**	**1.3**	**123.0**	**1.7**	**1.2**	**141.6**	**1.9**	**1.1**	**172.7**
CB B4	1.3	1.1	118.1	1.8	1.3	138.4	1.8	1.1	163.6
CB B5	1.5	1.3	115.3	1.6	1.4	114.2	1.6	1.1	145.4
CBBo3	1.3	1.2	108.3	1.6	1.3	123.0	1.9	1.2	158.3
CBBo4	1.2	1.2	100.0	1.4	1.3	107.6	1.9	1.2	158.3
CBBo5	1.6	1.4	114.2	1.6	1.2	133.3	2	1.3	153.8
CBP2	1.4	1.3	107.6	1.6	1.2	133.3	1.6	1.2	133.3
CB P3	1.5	1.4	107.1	1.6	1.2	133.3	1.7	1.4	121.4
CB P4	1.4	1.3	107.6	1.5	1.3	115.3	1.8	1.3	138.4
CD (P≤0.05)	**0.113**	**0.035**		**0.14**	**0.045**		**0.14**	**0.044**	

Table.5 Cellulase activity (IU/ml) by cellulose degrading bacterial isolates at pH5 and 10°C, 15°C, 20°C temperature

	Isolates	**Temperatures**		
S. No.	**Isolate**	**10°C**	**15°C**	**20°C**
1.	Control	0.024	0.026	0.027
2.	CB W1	0.926	0.997	1.024
3.	CB WA	0.796	0.866	1.024
4.	CB W2	0.993	0.996	1.025
5.	CB WB	0.997	0.997	1.027
6.	**CBW3**	**1.001**	**1.025**	**1.146**
7.	CBW4	0.765	0.996	1.014
8.	CBS1	0.887	0.997	1.027
9.	CBS2	0.775	0.992	1.008
10.	CBS3	0.997	0.996	1.023
11.	CBS4	0.997	0.667	1.036
12.	CB S5	0.337	0.555	1.002
13.	**CB B3**	**0.998**	**1.013**	**1.138**
14.	CB B4	0.878	0.777	1.027
15.	CB B5	0.995	0.998	1.028
16.	CB Bo3	0.228	0.336	1.003
17.	CB Bo4	0.996	0.998	1.004
18.	CB Bo5	0.225	0.375	1.000
19.	CB P2	0.887	0.986	1.124
20.	CB P3	0.774	0.875	1.028
21.	CB P4	0.891	0.993	1.027
	CD (P≤0.05)	0.020	0.005	0.002
	SE (m)	0.007	0.002	0.001
	SE (d)	0.010	0.002	0.001
	C.V	1.599	0.367	0.135

Table.6 Cellulase activity by cellulose degrading bacterial isolates at pH7 and 10°C, 15°C, 20°C temperature

	Isolates	**Temperatures**		
S. No.	**Isolate**	**10°C**	**15°C**	**20°C**
1.	Control	0.028	0.029	0.030
2.	CB W1	1.241	1.741	2.502
3.	CB W1	1.249	1.713	2.402
4.	CB W2	1.236	1.679	2.445
5.	CB W2	1.257	1.606	2.507
6.	**CBW3**	**1.568**	**1.907**	**2.917**
7.	CBW4	1.168	1.663	2.203
8.	CBS1	1.265	1.806	2.201
9.	CBS2	1.233	1.666	2.224
10.	CBS3	1.239	1.593	2.214
11.	CBS4	1.218	1.564	2.043
12.	CB S5	1.213	1.224	2.001
13.	**CB B3**	**1.277**	**1.841**	**2.566**
14.	CB B4	1.213	1.665	2.444
15.	CB B5	1.224	1.668	2.006
16.	CB Bo3	1.227	1.326	2.227
17.	CB Bo4	1.121	1.324	2.005
18.	CB Bo5	1.168	1.187	2.001
19.	CB P2	1.223	1.554	2.224
20.	CB P3	1.221	1.667	2.444
21.	CB P4	1.267	1.557	2.338
	CD (P≤0.05)	0.005	0.046	0.063
	SE (m)	0.002	0.016	0.022
	SE (d)	0.003	0.023	0.031
	C.V	0.264	1.833	1.735

Table.7 Cellulase activity by cellulose degrading bacterial isolates at pH 9 and 10°C, 15°C, 20°C temperature

	Isolates	**Temperatures**		
S. No.	**Isolate**	**10°C**	**15°C**	**20°C**
1.	Control	0.024	0.026	0.027
2.	CB W1	0.347	0.815	0.902
3.	CB WA	0.777	0.774	0.993
4.	CB W2	0.776	0.881	0.886
5.	CB WB	0.666	0.882	0.957
6.	**CBW3**	**0.886**	**0.888**	**0.995**
7.	CBW4	0.771	0.774	0.886
8.	CBS1	0.776	0.779	0.883
9.	CBS2	0.773	0.775	0.882
10.	CBS3	0.557	0.667	0.777
11.	CBS4	0.447	0.678	0.772
12.	CB S5	0.227	0.337	0.443
13.	**CB B3**	**0.813**	**0.884**	**0.994**
14.	CB B4	0.357	0.460	0.667
15.	CB B5	0.669	0.777	0.813
16.	CB Bo3	0.774	0.877	0.888
17.	CB Bo4	0.774	0.775	0.992
18.	CB Bo5	0.228	0.332	0.337
19.	CB P2	0.774	0.864	0.984
20.	CB P3	0.228	0.397	0.668
21.	CB P4	0.561	0.667	0.775
	CD (P≤0.05)	0.022	0.026	0.021
	SE (m)	0.008	0.009	0.007
	SE (d)	0.011	0.013	0.011
	C.V	2.258	2.326	1.650

Table.8 Morphological characterization of cellulose degrading bacterial isolates

Isolate		Colony Features	Cell Features	Gram Reaction	Shape
		Colour of Colony	Nature of Colony		
CB W3		Creamy	Smooth, raised	Gram Positive	Bacilli
CB W4		Creamy	Smooth, irregular, transparent	Gram Positive	Bacilli
CB S2		Whitish	Smooth, raised, transparent	Gram Positive	Bacilli
CB S3		Creamy	Smooth, raised	Gram Positive	Bacilli
CB S4		Whitish	Raised, irregular, transparent	Gram Positive	Bacilli
CB B4		Whitish	Smooth, raised, transparent	Gram Positive	Bacilli
CB Bo5		Creamy	Raised, irregular, transparent	Gram Positive	Bacilli
CB P2		Creamy	Smooth, raised	Gram Positive	Bacilli
CB P3		Creamy	Smooth, raised	Gram Positive	Bacilli
CB P4		Creamy	Smooth, raised, transparent, irregular	Gram Positive	Bacilli
CB W1		Creamy	Smooth, raised, transparent	Gram Positive	Cocci
CB WA		Creamy	Smooth, raised, transparent	Gram Positive	Cocci
CB W2		Whitish	Raised, irregular, transparent	Gram Positive	Cocci
CB WB		Whitish	Raised, transparent, smooth	Gram Positive	Cocci
CB S1		Creamy	Smooth, raised, transparent	Gram Positive	Cocci
CB S5		Creamy	Raised, transparent, irregular	Gram Positive	Cocci
CB B4		Creamy	Smooth, raised, transparent	Gram Positive	Cocci
CB B5		Creamy	Smooth, raised, irregular	Gram Positive	Cocci
CB Bo3		Whitish	Raised, irregular, smooth	Gram Positive	Cocci
CB Bo4		Whitish	Raised, irregular, smooth	Gram Positive	Cocci

Table.9 Biochemical characterization of cellulose degrading bacterial isolates

Isolate	Gram's Reaction	Catalase test	Starch Hydrolysis test	Urease test	Gelatin Hydrolysis test	Hydrogen Sulphide test
CBW1	+	+	+	+	+	+
CBWA	+	+	+	+	+	+
CBW2	+	+	+	+	+	+
CBWB	+	+	+	+	+	+
CBW3	+	+	+	+	+	+
CBW4	+	+	+	+	+	+
CBS1	+	+	+	+	+	+
CBS2	+	+	+	+	+	+
CBS3	+	+	+	+	+	+
CBS4	+	+	+	+	+	+
CBS5	+	+	+	+	+	+
CBB3	+	+	+	+	+	+
CBB4	+	+	+	+	+	+
CBB5	+	+	+	+	+	+
CBBo3	+	+	+	+	+	+
CBBo4	+	+	+	+	+	+
CBBo5	+	+	+	+	+	+
CBP2	+	+	+	+	+	+
CBP3	+	+	+	+	+	+
CBP4	+	+	+	+	+	+

Fig.1 Survey of cellulolytic bacteria from different selected locations of district Baramulla

Fig.2 Isolation of cellulose degrading bacteria

Fig.3 Solubilization zone by cellulose degrading bacteria

Fig.4&5 Gram staining and catalase test

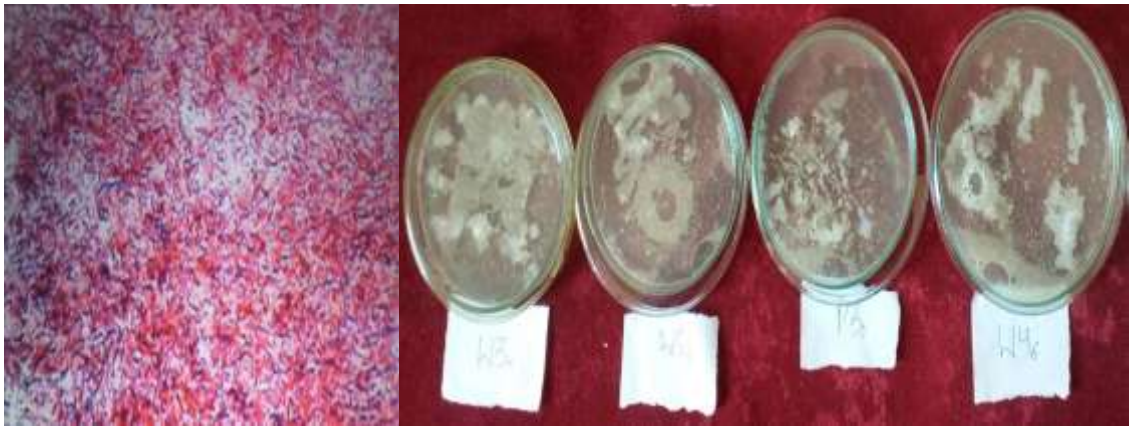

Fig.6&7 Starch hydrolysis test and urease test

Fig.8&9 Gelatin test and hydrogen sulphide test

Screening and characterization of cellulolytic bacterial isolates

After isolation the cellulolytic bacterial isolates were screened both qualitatively and quantitatively for the estimation of cellulase enzyme activity.

The isolates that showed a hallow zone diameter of 2.0mm or more were maintained for further estimation of quantity of cellulase activity. The highest solubilization hallow of 3.6mm with 327.2% SE on CM agar plate after 48 hrs of incubation was shown by the isolate CBW3. This is the reflection of production of higher quantity of cellulase enzymes by this isolate.

However, the solubilization was also observed at very low and high pH values. It is evident here that the isolates showed greater tolerance towards fluctuating environmental conditions. Further the variable performance by cellulolytic bacteria with respect to cellulose degradation under different pH values and temperatures may be due to the reason that pH and temperature significantly influence the growth and enzyme activities of microorganisms. The temperature plays a major role in affecting the activity of bacterial enzymes. The enzymes are most active and enzymatic reactions proceed at the maximum speed and efficiency at an optimum temperature. Beyond the maximum and minimum extremes of temperature for the microorganisms, the enzymes become inactive. Low temperatures are less damaging than high temperatures, which denature proteins causing irreversible changes and total enzyme destruction. The pH of an organism's

environment has the maximum influence on the bacterial growth. It limits the synthesis of enzymes responsible for synthesising the new protoplasm. The increase or decrease in hydrogen in concentration of the medium slows down the rate of chemical reactions because of the destruction of cellular enzymes. These findings are supported by the observations of Pinky and Sheila (2018) who reported that the physical and chemical conditions significantly affect the cellulolytic potential of microorganisms.

The twenty selected cellulolytic bacterial strains were critically examined for their micro morphology colony features, gram reaction and cell shape studies revealed that colonies were creamy to whitish in colour, smooth, raised to irregular and transparent. All strains were gram positive. Biochemical characterization revealed that all the isolates were positive for catalase, starch, urease, gelatin and hydrogen sulphide tests. Preliminary investigation on morphological and biochemical characteristics suggested that the isolates resembled to genera Bacillus and Streptococcus.

The qualitative and quantitative screening of cellulolytic bacterial isolates for the enzyme production was eventually high in the two particular strains viz., CBW3 isolated from Wadura and CBB3 isolated from Baramulla town with solubilisation zone: 4mm with solubilisation efficiency (285.7%) followed by 3.4mm with solubilisation efficiency (261.5%) and the cellulase activity was also found to be highest in both the strains viz: 2.917 U/ml and 2.566 U/ml respectively. The present study findings are in conformity with the findings of Gopinath *et al.,* (2014) who also reported cellulolytic bacterial strains with high solubilization zone and high cellulase enzyme production. The biochemical characterization of cellulolytic bacterial isolates in which cellulolytic bacterial isolates were examined for Gram's reaction, Catalase test, Starch hydrolysis, Urease test, Gelatin test, Hydrogen sulphide test. All the isolates show positive results regarding these tests. The results are in agreement with the work of Dubey *et al.,* (2014) who also performed these biochemical tests to the cellulolytic bacterial isolates.

From the current study it could be concluded that the cellulolytic bacteria were present in all the waste samples, showed cellulose degrading capability under varying pH and temperatures, however, the optimum activity was shown by the isolate CBW3 under pH7 and 20°C temperature. This isolate can be used in future for rapid decomposition of cellulose rich substrates and can be further tested for other beneficial properties like mineral solubilization and biocontrol activity.

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