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Investigation on Aerobic Cellulolytic Bacteria in Some of North Forest and Farming Soils

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Abstract: This study was conducted for investigation on number of cellulolytic bacteria and their ability in the decomposition of cellulose. 30 surface soil samples were collected from forest and farming soils in the north of Iran. After analyzing some physical and chemical properties of soil samples, then total number of bacteria in nutrient-agar and aerobic cellulose decomposing bacteria in cellulose-agar were counted. Assessment of bacteria ability in cellulose decomposition was performed via measurement of clear zone around of colony. Results showed that the total number of bacteria, number and percent of cellulolytic bacteria in forest soil samples were more than those of farming soil samples. Mean ratio of clear zone diameter to colony were 1.6 and 2.1 for forest soil and farming soil, respectively. Higher ability of the cellulolytic bacteria in farming soils probably is due to great microorganism diversity in the two soils.

Key words: Cellulolytic bacteria % Cellulose % Forest soils % Farming soils % Clear zone

INTRODUCTION

The plants produce a large amount of plant mass throughout photosynthesis process annually, which cellulose comprises most of them. The cellulose which is produced due to photosynthesis every year is estimated to be approximately 40 billion tons [1].

Cellulose is a linear polysaccharide which is constructed from monomers of Glucose bound together whit $1-4 glucosidal linkage [2].

The cellulose net of plant tissues is not solely constructed from polysaccharide glucose. Other polysaccharides are found as complex with pure cellulose. These polysaccharides are called as cellulosan. This compound is 20-30% plant cellulotical structure [3].

Cellulose is so insoluble than cellulosan, therefore the later compounds are more available than net cellulose and microorganisms prefer to decompose them. The availability of cellulosan leads to rapid increasing of decomposing microbial population. Cellulose decomposition is an indispensable process for carbon cycle in nature [4]. After harvesting agricultural products (such as grains and beans) a great amount of plant residues are left in each hectare. To conduct agricultural processes for the second farming in many agricultural systems, there is not enough time to decompose the residues so to manage the process of soil tillage and prepare an appropriate bed for the second farming. The residues generally are burned. However, most of soils have not more than 0.5% organic matter. Cellulose hydrolysis is the key process for biological conversion of cellulosic materials [5]. Decomposition of cellulose bearing compounds in soils is a useful and beneficial biological process that different microorganisms (bacteria and fungi) are involved in it. Each factor which affects the number and activity of decomposing microorganisms will have influence the cellulose decomposing intensity. Soil moisture and aeration also influence cellulose decomposition [6,7]. Cellulose decomposition will be occurred in a vast range of temperature and different aeration conditions that aerobic, micro aerobic, facultative and obligate anaerobic microorganisms (which maybe psychrophilic, thermophilic or mesophilic) are involved in it. The maximum rate of decomposition has been occurred by mesophilic microorganisms in aerobic condition [1]. Other factors affecting on cellulose decomposition are the ambient pH and the level of available N [8,9]. Cellulose decomposition will progress more rapidly in neutral pH and abundance of N. Ljungdahl *et al*., [10] showed that cellulose decomposing microorganisms utilize cellulose as carbon and energy resource. To assess cellulase enzyme activity, Kluepfe [11] mixed the agar gel with filter paper or microcrystalline cellulose called as Avicel. A clear zone was observed around the colonies after a

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definite time. The clear zone diameter was related to bacterial activity in cellulose decomposition [1,12,13].

Bobbie *et al*., [2] isolated Cellulomonas bacterium from the soil and cultured it on media contained amorphous cellulose called as (CMC). The rate of cellulose hydrolization by the bacteria was assessed with clear zone formation in cellulose agar media.

Fuller *et al*., [3] studied the activity of cellulolytic aerobic bacteria population on filter paper and grain stems. It has been observed that each organism decomposes more grains stem cellulose than filter paper cellulose in the same time and it has been attributed to the presence of xylan in cellulosan which is present in grains stem cellulose.

Wood *et al*. [14] used Congo red since the intracellular enzymes of *Cytophaga* don't make a clear zone in cellulose agar, so Congo red make a visible change of the red color.

The objective of this study was investigation the distribution of cellulolytic aerobic bacteria in forest and farming soils and also assessing their potential to cellulose decomposition.

MATERIALS AND METHODS

Soils and analysis: Thirty surface (0-30) complex samples from soils of wheat cultivated farms and forest were collected in Guillan province. Samples were sieved (2 mm) and stored field moist in polyethylene bags at 5 C until analyzed.

Soil subsamples were analyzed for organic carbon with Walky and Black method [15], particle size distribution [16] and pH in a 1:1 soil to water suspension.

The number of cellulolytic aerobic bacteria and total bacteria in the various soils were determined by plating an appropriate dilution of the soil sample on cellulose agar (James G. Cappuccino and Natalie Sherman) and incubating the plates at 30C for 2 weeks. The ingredient of the cellulose agar medium which was applied to isolate the cellulolytic aerobic bacteria was as follow:

CMC (Carboxy Metyl Cellulose), 5g ; K_2HPO_4, 1g ; $MgSO_4$, 0.2g ; $CaCl_2$, 2-5 mg ; $Fe_2\ (SO_4)_3$, 15-20 g per litter. The PH was adjusted to 7 - 7.5.

The colony formation and microbial growth were being controlled daily. After 2 weeks the number of visible colony in the plates were recorded. To recognize the cellulolytic colonies from the others, 5 ml of Hexa Decyl Trimetyl Amonium Bromide (1%) was used in each plate. After about 20 minutes, the clear zones were observed around the colonies which were able to decompose cellulose; also the ratios of clear zone diameter to colony diameter were measured. Fresh suspension of bacteria which were able to decompose cellulose was used for mobility and Gram test (James G. Cappuccino and Natalie Sherman).

RESULTS AND DISCUSSION

Some properties of soils which used in this experiment were presented in Table 1. In general the pH of farming soils was more than that of forest soils. The pH of saturated extract of farming soils was about 8 and in forest soils were about 7.2. While organic carbon content of forest soils was ten times more than that of farming soils.

Some characteristics of cellulolytic bacteria in forest and farming soil are comprised in Table 2.

Table 1: Some physical and chemical properties of farming and forest soils used in this experiment

	Farming soil			Forest soil		
No. of soil	soil texture	OC (%)	pHs (1:1)	soil texture	OC (%)	pHs (1:1)
1	Loam	1.36	8.5	Silty clay loam	6.818	6.96
2	Clay loam	1.30	8.3	Clay loam	6.8	6.9
3	Clay loam	0.98	7.95	Clay loam	8.8	6.5
4	Loam	0.974	7.8	Silty clay loam	12.44	6.55
5	Sandy loam	0.75	8.1	Clay loam	12	6.61
6	Sandy loam	0.88	8.3	Clay loam	13.24	6.66
7	Clay loam	0.487	7.90	Clay loam	12.7	4.43
8	Clay loam	1.46	7.93	Clay loam	11.3	7.3
9	Loam	1.30	8.20	Silty clay loam	12.68	7.3
10	Loam	1.82	7.90	Silty clay loam	18.5	7.6
11	Loam	2.529	8.20	Clay loam	20.5	7.73
12	Clay loam	2.622	7.80	Silty clay loam	4.15	7.86
13	Loam	0.87	7.93	Silty clay loam	6.42	7.88
14	Loam	0.879	8.50	Clay loam	5.75	7.62
15	Clay loam	0.788	7.97	Silty clay loam	5.95	7.6

Table 2: G comparison of cellulolytic bacteria characteristics isolated from forest and farming soils

Bacterial characteristics	Forest soils	Farming soils
Morphology	*Cococci - cocobacil*	*Cococci - cocobacil*
Gram reaction	G^-	G^-
Mobility	variable	variable
Mean of total number	326.5	145.2
mean of cellulolytic bacteria	138.66	37.4
The ratio of clear zone diameter to colony diameter	1.6	2.1

Table 3: Total number of bacteria and the number and percentage of cellulolytic bacteria in farming and forest soil samples

	Farming soils			Forest soils		
Soil No.	Total no. of bacteria (cfu g^{-1})	No. of cellulolytic (cfu g^{-1})	Percentage of cellulolytic bacteria	Total no. of bacteria (cfu g^{-1})	No. of cellulolytic (cfu g^{-1})	Percentage of cellulolytic bacteria
1	306	69	22.5	50	30	60.0
2	9	3	33.0	1058	420	40.0
3	53	16	30.0	1033	415	40.0
4	66	40	60.0	175	119	68.0
5	300	116	39.0	198	92	46.0
6	696	49	7.0	50	25	50.0
7	145	68	46.0	16	5	29.0
8	165	34	20.0	637	252	39.0
9	40	15	37.5	769	401	52.0
10	2	1	50.0	8	5	62.0
11	94	40	42.5	74	50	67.0
12	39	11	28.0	50	44	88.0
13	91	43	47.0	120	77	64.0
14	135	47	35.0	599	105	17.0
15	37	9	24.0	61	40	64.0
Total mean	145.2	37.4	34.7	326.5	138.66	52.4

*Low numbers of total bacteria in some samples were recorded in farming and forest soils due to their low ability to grow on the selective media of cellulose agar

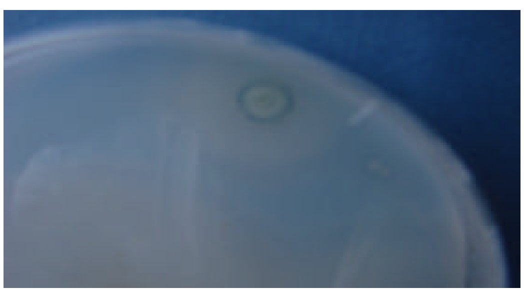

Fig. 1: using 1% solution of Hexa Decyl Trimetyl Ammonium Bromide and Recognition of cellulolytic bacteria by clear zones formation

The results of enumeration of bacteria, the number of cellulolytic bacteria and the percentage of these bacteria in two soil samples were illustrated in Table 3.

Total number of bacteria and the number and percentage of cellulolytic bacteria in forest soil samples were more than farming soil samples. Soil organic matter is utilized as carbon and energy resources by heterotrophic bacteria. Higher number of total bacteria and cellulolytic bacteria in forest soil can be attributed to higher organic carbon content in forest soils [17] (Table 1).

Total number of bacteria, the number and percentage of cellulolytic bacteria in forest soil samples is more than farm soils due to the type of organic matter in forest soils.

Table 4: The ratio of clear zone diameter to colony diameter in forest and farming soils isolated bacteria

Soil number	The ratio of clear zone diameter to colony diameter in forest soils	The ratio of clear zone diameter to colony diameter in farming soils
1	0.15	1.38
2	0.89	0.42
3	0.00	1.83
4	1.99	2.00
5	1.25	2.79
6	0.00	1.08
7	0.00	0.00
8	2.20	1.99
9	2.80	0.00
10	0.00	0.00
11	1.65	1.10
12	1.47	0.00
13	1.49	2.52
14	1.25	4.00
15	1.37	2.33
Total mean	1.60	2.10

Recognition of cellulolytic bacteria on plates surface was evaluated by clears zones formation and using 1% solution of Hexa Decyl Trimetyl Ammonium Bromide (Fig. 1).

The ratio of clear zone diameter to colony diameter in forest soils was 1.6 and in farming soils 2.1 (Table 4).

The reason for greater clear zone diameter to colony diameter in farming soils which can express higher potential to decompose cellulose is probably due to the variety of the microorganisms in farming and forest soils. Cellulolytic bacteria present in farm soils have more potential than forest soil bacteria to decompose cellulose due to the type of organic matter in these soils. In order to the type of organic matter in farm soils, superior strains with more ability will survive and activate.

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