ISOLATION AND CHARACTERIZATION OF CELLULOLYTIC BACTERIA FROM UNDERGROWTH SOILS IN THE ADAMAOUA REGION (CAMEROON)

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Abstract

Cellulases are enzymes that hydrolyze the β-1,4-glycosidic bonds of the cellulose polymer into smaller oligosaccharides and glucose. Today, bacterial cellulases are attracting growing interest due to their potential industrial applications in the detergent, textile, pulp and paper, biofuel and compost industries. The aim of the present study was to characterize cellulolytic bacterial strains isolated from the soils of several woodland ecosystems in the Adamaoua region. To this end, twelve (12) soil samples were taken in five (05) departments with forested ecosystems in the Adamaoua region (Vina, Mbéré, Mayo Banyo, Djerem, Faro and Déo). A total of thirty-five (35) strains were obtained from these samples and screened for cellulolytic activity on Carboxymethylcellulose (CMC) agar. After screening, twenty (27) isolates were able to utilize cellulose as the sole source of carbon and energy by revealing Congo Red decolorization halos on CMC medium. The hydrolysis rate of these strains ranged from 1.65 to 6.65. The strains with the best cellulolytic activity were I_2, I_3, A_3, A_4 and H_2, with halos ranging from 3.42 to 6.65 respectively. These strains were obtained from the following districts : Lycée Classique et Moderne in Ngaoundéré (Vina) and the Djoumbal and Pedeng districts in Bagno, Mayo Banyo department. This work leads to the conclusion that the undergrowth soils of the Adamaoua region are sources of cellulase-producing cellulolytic bacteria, and their valorization deserves to be investigated.

Key words: *Cellulolytic bacteria; undergrowth; enzymatic activity ; Adamaoua*

INTRODUCTION

Green residues are biodegradable wastes derived from the remains of lignocellulosic plants from the cutting and maintenance of public and private green spaces, hedge pruning and vegetation in general [6]. They include, in particular, dead leaves, grass, grass clippings and tree and shrub trimmings. It is reported that the maintenance of public and private green spaces in the Ile de France region generates a total quantity of waste of around 900,000 tonnes per year [7]. The large quantity of green waste produced in cities is clogging up the world's ecosystems and posing a major problem in terms of treatment. While they are essentially burnt in many underdeveloped and developed countries, they have the advantage of being the raw material for industries in various sectors such as agriculture, animal feed, biorefinery, etc. [11]. The utilization of green residues involves its degradation, and in this context microorganisms have a major role to play. Cellulose being the main constituent of

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green residues [3], cellulolytic microorganisms are the best colonizers of green residues. Bacteria represent a group that, thanks to their multiple biotechnological applications, rapid growth and easy genetic manipulation [1], would be good candidates for the degradation of green residues [14]. Soils in which lignocellulosic biomass decomposes are promising sources of microorganisms that carry out this degradation [13]. As a result, cellulolytic bacteria can be isolated from the environments hosting these green wastes, so their effectiveness varies according to the ecosystems that host them. Our study revolves around the following central research question : what are the properties of cellulolytic bacteria colonizing the undergrowth of the Adamaoua region?

Investigations into the isolation and characterization of cellulase-producing bacteria have already been carried out by several researchers around the world. Notably, the work of [4] in India, [12] in Brazil, [20] in China, Irène *et al*. [10] in Kenya. To the best of our knowledge, no such study has yet been carried out in Cameroon. Given that the effectiveness of cellulolytic microorganisms depends on the ecosystems in which they are isolated, as well as on culture conditions, the search for new cellulolytic microbial strains remains a challenge, in order to provide strains that can withstand the most extreme conditions.

The general objective of the present work is to isolate and screen cellulolytic bacteria from woodland ecosystems in the Adamaoua region.

MATERIAL AND METHODS

Twelve soil samples were taken in different environments containing undergrowth in the Adamaoua region, namely: Vina (03 soil samples), Mbéré (02 soil samples), Djerem (02 soil samples), Mayo-Banyo (03 soil samples), Faro and Déo (02 soil samples). The code assigned to each soil sample was recorded in Table 1.

Soil characterization

The pH of the various soil samples was measured using the method described by [15].. 20 g of soil was weighed into a 100 mL beaker, then 50 mL of demineralized water was added using a test tube, and the suspension was stirred on a magnetic stirrer. The electrodes were then carefully immersed in the stirred suspension of the first sample, and the pH meter was allowed to reach its stable value before taking the reading.

Isolation and screening of cellulolytic bacterial strains

Microorganisms were isolated from the samples using the method described by [2]. Twenty-five grams of soil samples were suspended separately in 225 milliliters of Carboxymethylcellulose broth containing (in g/L): 5 CMC; 2.2 K2HPO4; 1.5 KH2PO4; 1.3 (NH4)2SO4; 0.1 MgCl2; 0.02 CaCl2 and 0.001 FeSO4. 7H2O. Incubation was carried out at 37°C for 7 days with shaking at 150 rpm. Streak isolation by the release method was then carried out on CMC (Carboxymethylcellulose) agar medium containing 1.5% agar and 0.1% yeast extract, and incubated at 37°C for 48 hrs.

Isolated bacterial colonies were purified by successive subculturing, then inoculated into tubes containing 7 mL of chancelled CMC broth and incubated at 37°C for 48 h. The broth was then centrifuged at 4000 rpm. Screening for cellulolytic activity was carried out by introducing 50 µL of a suspension containing10^8 into Petri dish wells containing 0.1% sterile Congo Red Carboxymethylcellulose (CMC) agar. Petri dishes were sealed with cling film to limit the risk of contamination, then incubated at 37°C for 72 hours. The presence of Congo Red discoloration halos around the wells reflects the cellulolytic activity of the bacteria present. The enzymatic activity of the cellulolytic bacteria obtained was determined by measuring the hydrolysis rate using the formula :

$$Hydrolys\ rate = \frac{halo\ diameter}{well\ diameter}$$

Statistical analysis of results

Results were expressed as mean ± standard deviation. The DUNCAN test was used to compare means with significant differences, using STATGRAPHICS Centurion XVI software.

RESULTS AND DISCUSSION

Soil physico-chemical characteristics

The pH values of undergrowth soil samples from the Adamaoua region are recorded in Table 2. All soil samples showed a range of pH values from 5.22 to 7.12. This variation is in line with the literature, which mentions that cellulolytic bacteria are able to grow over neutral, slightly basic and acidic pH ranges in the environment [4]. The present study shows similarities with that presented by [17], where cellulolytic bacteria had shown excellent cellulase production at pH values between 6.2-7.45. In addition, there was a significant difference between the soils of the Djerem department and those of the other departments, where the average pH obtained was 5.32, whereas the average value for the other sites hovered around 6. This could be explained by higher biological activity in this area compared with the others, as microorganisms, by degrading organic matter, release acids that could lower the pH. The work of [16], demonstrated that their cellulolytic bacteria produced a maximum of cellulase at pH values between 5 and 6.

Isolation and screening of cellulolytic bacteria

Isolation of cellulolytic bacteria on medium enriched with CMC (Carboxymethylcellulose) yielded 35 strains in the Adamaoua region, including 09 strains in the Vina department, 06 strains in the Mbéré department, 06 strains in the Djerem department, 08 strains in the Mayo-Banyo department and 06 strains in the Faro et Déo department, after 7 days incubation at 37°C (Table 3). A similar study carried out on tea field soil in India by [4], isolated 25 cellulolytic strains. The CMC used for isolation served as the sole source of carbon and energy. The choice of CMC as the sole source of carbon and energy is very important for the growth of cellulolytic bacterial strains.

Demonstration of cellulolytic activity

The clear zone around the wells in the Petri dishes testifies to the ability of the selected strains to hydrolyze cellulose (Figure1). These results are similar to those found by [17].

Table 4 shows that strainsA_2, B_2, D_2, D_3, E_1, E2, F_1, F_2, F_4, G_1, G_2, H_1, J_1, J_2, J_3 do not possess cellulolytic activity; they showed no transparent halo around their respective wells. However, 20 strains showed their ability to hydrolyze cellulose as CMC, proving cellulase production. These results concur with those of Fatima and Zineb (2002), who reported that the presence of clear zones on 0.1% Congo red CMC agar medium confirmed the cellulolytic nature of their strains. 20 out of 35 strains, representing 57.14%, were cellulolytic. These results concur with those of [19], who showed that over 50 or 70.92% of bacteria isolated from soils in the Pangi Valley region of the Chamba district in the Himalayas of India were cellulolytic in nature.In addition, of the 20 isolates showing a halo of cellulolytic activity, 5 attracted our attention by producing the highest rates of hydrolysis; namely, strains I_2, I_3,A_3,A_4 and H_2 (table 5). In particular, strain I2 presented the highest rate with a cellulolytic activity ratio of 6.65. This ratio is higher than that of [8] who found a ratio of 5.5 for their strain isolated from Iranian soils; as well as that of [9] when isolating and identifying cellulolytic bacteria, who found a ratio ranging from 1 to 5.

Table 1 Soil sample codes

Department / City	Sampling site	Geographical coordinates	Sample code
VINA (Ngaoundéré)	Lycée Classique et Moderne	7°18'55'' N 13°34'32'' E	A
	High Plateau Nursery	7°17'44'' N 13°35'13'' E	B
	Dang sub-prefecture	7°25'0'' N 13°33'39'' E	C
MBERE (Meiganga)	Razel	6°30'2'' N 14°16'24'' E	D
	Nbakoungué	6°29'44'' N 14°16'28'' E	E
DJEREM (Tibati)	Wourtababal	6°28'21'' N 12°37'14'' E	F
	Sawa	6°28'51'' N 12°37'4'' E	G
Mayo-Banyo (Banyo)	Djoumbal	6°44'19'' N 11°48'49'' E	H
	pedeng	6°44'53'' N 11°48'58'' E	I
		6°44'59'' N 11°48'56'' E	J
Faro et Déo	Sabongari	7°37'18'' N 12°65'36'' E	K
	Bakassi	7°22'10'' N 12°38'52'' E	L

Table 2 pH of Adamaoua understorey soil samples

	VINA			MBERE		DJEREM		MAYO-BANYO			FARO ET DEO		Mean ± Standard deviation
	A	B	C	D	E	F	G	H	I	J	K	L	
pH	6,35	6,15	6,58	6,65	6,85	5,22	5,42	6,40	6,21	7,12	6,82	6,65	6,36± 0,56
Mean ± Standard deviation	6,36 ± 0,2[a]			6,75 ± 0,14[a]		5,32 ± 0,14[b]		6,57 ± 0,48[a]			6,73± 0,12[a]		

With P= 0,009<0,05

Table 3 Summary of the coding of isolated cellulolytic bacterial strains

Sampling site	Département	Number of strains	Isolated strain codes
Lycée classique	VINA	04	A_1, A_2, A_3, A_4
High Plateau Nursery		03	B_1, B_2, B_3
Dang sub-prefecture		02	C_1, C_2
Razel	MBERE	03	D_1, D_2, D_3
Nbakoungué		03	E_1, E_2, E_3
Wourtababal	DJEREM	04	F_1, F_2, F_3, F_4
Sawa		02	G_1, G_2
Djoumbal	MAYO BANYO	02	H_1, H_2
Pedeng		03	I_1, I_2, I_3
		03	J_1, J_2, J_3
Sabongari	FARO ET DEO	03	K_1, K_2, K_3
Bakassi		03	L_1, L_2, L_3
TOTAL		35	

Sampling code Xijr, with :
X= (A ; L) representing the twelve samples taken from the different sampling sites.
i= (1 ; 4) representing the number of strains isolated at each site.

Table 4 Diameters of cellulose hydrolysis zones by isolated cellulolytic bacteria

Isolats	Halo diameter (mm)				Well diameter (mm)				Hydrolysis zone
	1	2	3	M ± E	1	2	3	M ± E	
A_1	12	15	13	13.33±1.52	6	7	6	6.66±0.57	2
A_2	/	/	/	/	/	/	/	/	/
A_3	35	32	30	32.33±2.51	7	7	7	7 ± 0	4.61
A_4	24	36	/	30 ± 8.48	10	6	/	8 ± 2.82	3.75
B_1	12	13	12	12.33±0.57	7	7	7	7 ± 0	1.76
B_2	/	/	/	/	/	/	/	/	/
B_3	12	/	13	12.5 ± 0.70	7	/	7	7 ± 0	1.78
C_1	15	/	13	14 ± 1.41	7	6	/	6.5±0.70	2.15
C_2	18	15	/	16.5±2.12	7	7	/	7 ± 0	2.35
D_1	24	19	/	21.5±3.53	8	6	/	7 ± 1.41	3.07
D_2	/	/	/	/	/	/	/	/	/
D_3	/	/	/	/	/	/	/	/	/
E_1	/	/	/	/	/	/	/	/	/
E_2	/	/	/	/	/	/	/	/	/
E_3	20	/	21	20.5 ± 0.70	6	/	6	6 ± 0	3.41
F_1	/	/	/	/	/	/	/	/	/
F_2	/	/	/	/	/	/	/	/	/
F_3	12	13		12.5 ± 0.70	6	6	/	6 ± 0	2.08
F_4	/	/	/	/	/	/	/	/	/
G_1	/	/	/	/	/	/	/	/	/
G_2	/	/	/	/	/	/	/	/	/
H_1	/	/	/	/	/	/	/	/	/
H_2	22	22	21	21.66 ± 0.57	6	7	6	6.33±0.57	3.42
I_1	20	22	20	20.66±1.15	6	6	6	6 ± 0	3.44
I_2	28	53	52	44.33±14.15	6	7	7	6.66±0.57	6.65
I_3	48	37	/	42.5±7.77	7	7	/	7 ± 0	6.07
J_1	/	/	/	/	/	/	/	/	/
J_2	/	/	/	/	/	/	/	/	/
J_3	/	/	/	/	/	/	/	/	/
K_1	12	11	10	11 ± 1	7	7	6	6.66±0.57	1.65
K_2	16	/	17	16.5 ± 0.70	6	/	7	6.5±0.70	2.53
K_3	18	18	/	18 ± 0	6	7	/	6.5±0.70	2.76
L_1	22	/	23	22.5 ± 0.70	6.5	/	6	6.25 ± 0.30	3.6
L_2	12	20	24	18.66±6.11	6	7	6	6.33±0.57	2.94
L_3	12	12	/	12 ± 0	6	6	/	6 ± 0	2

Code : X= (A1 to L3) representing the code of strains isolated from different sites.
i= (1 ; 2 ; 3) representing the number of repeats.

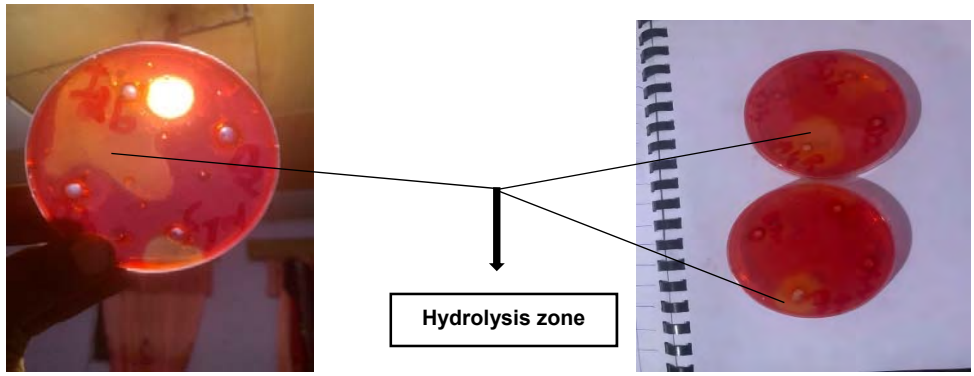

Fig. 1 Demonstration of cellulolytic activity (I_{2b} =I_3 et A_{4R} = A_4)

CONCLUSION

The undergrowth of Adamaoua is a source of bacteria, almost all of which are cellulolytic. Activities are highly varied, with the $_{12}$ strain showing the greatest potential. The pH of soils colonized by these bacteria is neutral to acidic (6.36).

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