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Optimization of Cellulase Production Conditions in Bacteria Isolated from Soils in Brazzaville

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Abstract

Objective: The objective of the present study is to optimize cellulase production in five strains: *Pantoea dispersa* MLTBY6 (MT646430.1); *Pseudomonas aeruginosa* MLTBM2 (MT646431.1); *Pseudomonas monteilii* MLTBC10 (MT674682.1); *Bacillus subtilis* MLTBC5 (MT674681.1) and *Lysinibacillus fusiformis* MLTBB7 selected cellulase producers isolated from soils in Brazzaville, Republic of the Congo. **Materials and Methods:** The cellulolytic activity of the selected cellulase-producing strains was determined by transferring the strains to a petri dish containing CMC culture medium with the following composition: cellulose 1%, K_2HPO_4 0.2%, $MgSO_4$ 0.03%, peptone 1%, $(NH_4)_2SO_4$ 0.2% adjusted to a pH value of 7, previously poured and then frozen. The dishes were incubated in an oven at 37˚C for 48 hours. The petri dishes were then flooded with 1% lugol for 15 minutes. A positive reading is indicated by the formation of a hydrolysis zone, the diameters of the hydrolysis zone were measured with a ruler. Strains with a broad lysis spectrum were selected. Optimisation of cellulase production by five bacterial strains isolated from the soil was done using the following factors: temperature and pH. **Results:** The production of cellulase showed that these strains showed a high production of cellulase at pH values between 5.6 and 9 with an optimum of pH = 8 and temperature values between 35˚C and 40˚C with an optimum at temperature t = 40˚C. Of the carbon sources used, two sources, namely glu-

cose and galactose, showed a high production of cellulase compared to the other carbon sources. However, the two nitrogen sources, ammonium sulphate and urea, were favourable for cellulase production by all five strains. Fe^{2+}, CO^{2+}, Zn^{2+} ions are favourable for cellulase production by these strains, with a referendum for Fe^{2+}. **Conclusion:** From these results, we conclude that the sources of carbon (glucose and galactose), nitrogen (ammonium sulphate and urea) and ions (Fe^{2+}, CO^{2+}, Zn^{2+}) added to these five strains were the elements favouring the good production of cellulase.

Keywords

Optimization, Condition, Production, Cellulase, Soil Bacteria

1. Introduction

Cellulose is the most abundant molecule on earth. It has a basic polysaccharide structure in plant cell walls and accounts for about 50% of the total mass of organic matter (Deguchi *et al.*, 2006) [1]. Cellulose $[C_6H_{10}O_5]_n$ is a long-chain polyholoside of cellobiose units, consisting of anhydroglucoses linked by β-1,4 bridge. It occurs in amorphous or crystalline form, generated through the establishment of hydrogen bonds between the different chains (Oh *et al.*, 2005) [2]. In plants, cellulose is present in the form of microfibrils and has a diameter of 20 - 30 nanometres (Zhao *et al.*, 2007) [3] with a length of 100 - 40,000 nanometres. Each cellulose molecule contains 2000 to more than 15,000 cellobiose subunits.

In nature, cellulose is broken down by a group of enzymes of fungal or bacterial origin called cellulases. Bacteria synthesise cellulases which are a group of enzymes acting together and hydrolysis cellulose into simple sugar (Korish, 2003) [4]. It is one of the main members of the glycoside hydrolase family, which is a complex enzymatic system composed of three main types of enzymes playing different roles in the hydrolysis of cellulose into simple sugar. These groups are: Endo β (1 - 4) - glucanase or endo cellulase (EC 3.2.1.4) which is a group of cellulases that degrade internal bonds for the disruption of the crystal structure of cellulose and exposure of different polysaccharide chains of cellulose, by rapid decrease of the degree of polymerisation of the substrate (Xu *et al.*, 2000) [5]. This group of enzymes cuts cellulose randomly in the amorphous areas of the cellulose, thus generating new chain ends. Exoglucanase or exo-cellulase (EC 3.2.1.91) on the other hand is a group of enzymes (cellulase) that attack the β (1 - 4) glycosidic bond of the chains of non-reducing ends and exclusively releases cellobiose (Xu, 2002) [6]. Cellobiase (EC 3.2.1.21) which are a final group of cellulases, which hydrolyse the β (1 - 4) glycosidic bonds of cellobiose and yield two glucose molecules (Onsori *et al.*, 2005) [7].

Cellulose is a molecule produced abundantly by plants with which fungi and

some bacteria synthesise cellulase. Moreover, cellulase is a group of enzymes that are of great interest in several fields for the progress of scientific research. These enzymes can be used:

In industry: cellulases have a wide spectrum of applications in various industries, they are applied in the food and brewing industry (Juturu *et al.*, 2014) [8], but also in the production and processing of animal feed, detergent production, laundry, textile and pulp manufacturing (Zhang & Zhang, 2013) [9].

In the food industry: Cellulases can be used to facilitate the filtration of various suspension rich in cellulosic fibres handled by the food industry (Scriban, 1993) [10]. Cellulases can be used with other enzymes to degrade plant walls in fruit and vegetable processing and also in the beverage industry (Leghlimi, 2013) [11].

In animal nutrition: Cellulosic enzymes can be used as feed additives for livestock and poultry. The addition of cellulases to poultry or pig feed improves the digestibility of the cellulosic fractions, and thus reduces both the consumption of energy sources such as starch and the excretion of undigested cellulose. (Gusakov *et al.*, 2000) [12].

In bioethanol production: Lignocellulosic biomass is a source of fermentable sugars that are used for the production of liquid fuels such as bioethanol. These polymerised sugars, which are cellulose and hemicellulose, are used to produce bioethanol by fermenting glucose after several physical, chemical and biological hydrolysis treatment stages known as biorefining. Bio-refining is a process of transforming biomass into bio-based products used in food, chemicals and materials. Bioethanol has been used to substitute gasoline in many countries (Mood *et al.*, 2013) [13].

Finally, in the production of biofuels (oil and coal) are the main sources of energy, but these have many adverse effects on the environment such as air pollution, global warming and emission of greenhouse gases. In order to reduce these negative environmental effects, alternative energy sources are being developed. The energy yield of ethanol obtained from plant biomass is almost equal to that of petrol, it is obtained from fermentable substrates such as sugar cane, sugar beet, maize, barley, wheat, …, this biofuel is called first generation fuel, and the second generation fuel is obtained from the cellulose of agricultural residues such as straw or maize cane or forestry residues.

In this study, the optimization of physicochemical and nutritional parameters related to the production of cellulase of five strains isolated from soil in Brazzaville selected as cellulase producers were carried out. These strains have already been identified by molecular biology techniques and their accession numbers in GenBank are *Pantoea dispersa* MLTBY6 (MT646430.1); *Pseudomonas aeruginosa* MLTBM2 (MT646431.1); *Pseudomonas monteilii* MLTBC10 (MT674682.1); *Bacillus subtilis* MLTBC5 (MT674681.1) and *Lysinibacillus fusiformis* MLTBB7. The optimised factors are: temperature, pH, carbon sources and nitrogen sources.

2. Materials and Methods

2.1. Screening of Cellulase Producing Strains

The screening of the selected cellulase producing strains was carried out by subculturing the strains on the petri dish containing CMC culture medium with the following composition: cellulose 1%, K_2HPO_4 0.2%, $MgSO_4$ 0.03%, peptone 1%, $(NH_4)_2SO_4$ 0.2% adjusted to a pH value of 7, previously poured and then solidified. The plates were incubated in an oven at 37˚C for 24 hours. One colony of each strain was grown in a 50 ml Erlenmeyer flask containing 20 ml of LB culture medium. The mixture was incubated under agitation at 37˚C for 18 hours. The supernatant was recovered by centrifugation at 6000 rpm for 20 minutes at 4˚C. After centrifugation, 100 µl of the supernatant was placed in the wells previously prepared. The petri dishes were incubated at 37˚C for 48 hours. Development was done by flooding with 1% lugol for 15 minutes. A positive reading is indicated by the formation of a hydrolysis zone, the diameters of the hydrolysis zone were measured with a ruler (Andro *et al.*, 1984) [14].

2.2. Bacterial Identification

The bacterial strains that were the subject of this study were presumptively identified by means of phenotypic examination and some biochemical characterisations. The parameters studied included colonial morphology, microscopy, Gram reactions, endospore formation, catalase production. In addition to the microbiological identification, a molecular identification was carried out following molecular biology techniques, based on the analysis of the rRNA16S gene.

2.3. Cellulase Production

The cellulase production medium is a medium containing (g/L) glucose 0.5 g, peptone 0.75 g, $FeSO_4$ 0.01 g, KH_2PO_4 0.5 g and $MgSO_4$ 0.5 g. Ten millilitres of medium was taken in a 50 ml Erlenmeyer flask. The flasks containing the medium were autoclaved at 121˚C for 15 min, and after cooling, the flask was inoculated with a bacterial culture grown overnight. The seeded medium was incubated in an oven at 37˚C for 24 h. At the end of the fermentation period, the culture medium was centrifuged at 6000 rpm for 20 min to obtain the crude extract, which served as the enzyme source.

2.4. Enzyme Assay

Cellulase activity was measured according to the method of Miller (1959) [15]. Indeed, a reaction mixture consisting of 0.5 ml crude enzyme solution plus 1 ml of 1% carboxymethylcellulose (CMC) in 0.05 mM phosphate buffer (pH 7.4) was incubated at 50˚C in a water bath for 30 min. The reaction was stopped by adding 3 ml of dinitrosalysilic reagent (DNS). The colour was then developed by boiling the mixture for 5 min. The OD of the samples was measured at 575 nm against a blank containing all reagents minus the crude enzyme.

2.5. Study of the Monitoring of Cellulase Kinetics

The catalytic potential of cellulase in the selected strains (*Bacillus subtilis*, *Pantoea dispersa*, *Pseudomonas aeruginosa*, *Pseudomonas monteilii* and *Lysinibacillus fusiformis*) was evaluated. These strains were inoculated into a CMC culture medium containing 1% cellulose and incubated at 37˚C for 24 hours. The supernatant was recovered by centrifugation at 6000 rpm at 4˚C for 20 minutes and the crude cellulase activity was assessed by the DNS (Dinitrosalicylic Acid) method. A volume of 0.5 ml of the supernatant was added to a volume of 1 ml of 0.05 M phosphate buffer solution (pH 7.4) containing 1% cellulose, incubated in a water bath at a temperature of 50˚C at time intervals of (1, 2, 5, 10, 20, 30 minutes) and the amount of reduced glucose was measured by the Dinitrosalicylic Acid method.

2.6. Optimisation of the Physical Parameters Involved in the Maximum Production of Crude Cellulase

2.6.1. Effect of pH on Cellulase Production

The optimum pH value for cellulase production of the five selected cellulase producing strains was determined by inoculating a 0.5 ml volume of the supernatant containing crude cellulase with 0.05 M phosphate buffer solution at 0.5% cellulose, adjusted to different pH values (5.0; 6.0; 7.0; 8.0; 9.0; 10.0 and 11.0). The reaction was initiated by incubating the reaction mixture consisting of 0.5 ml volume of the supernatant with 1 ml volume of 0.05 M Phosphate Buffer pH = 7 containing 1% concentration of cellulose at a temperature of 50˚C in the water bath for 30 minutes. Crude cellulase activity was measured by the DNS method (Ifran *et al.*, 2012) [16].

2.6.2. Effect of Temperature on Cellulase Production

The optimal temperature for cellulase production of the five selected strains was determined by inoculating a 0.5 ml volume of the cellulase-containing supernatant with a 0.05 M phosphate buffer solution with 1% cellulose, adjusted to a pH value of 7. The reaction was initiated by incubating the reaction mixture at different temperatures (25˚C, 30˚C, 35˚C, 40˚C, 45˚C, 50˚C, 55˚C and 60˚C) for 24 hours. The cellulase activity was measured by DNS method (Ifran *et al.*, 2012) [16].

2.6.3. Optimisation of the Nutritional Conditions Involved in Cellulase Production

1) Effect of Carbon and Nitrogen Sources on Cellulase Production

In order to optimise the nutritional conditions for cellulase production, strains isolated from selected cellulase-producing soils were inoculated at 37˚C for 24 hours in CMC culture medium containing different carbon sources at a concentration of 0.5% such as starch, glucose, sucrose, galactose, fructose, mannose and nitrogen sources at a concentration of 0.5% such as peptone, yeast extract and ammonium sulphate and urea were demonstrated. Cellulase activity was measured by the DNS method (Ifran *et al.*, 2012) [16].

2) Effect of Ions on Crude Cellulase Production

In order to explore the effect of ions on crude cellulase activity, the selected cellulase-producing strains isolated from the soils were inoculated into 250 ml Erlenmeyer flasks containing a volume of 100 ml of sterile CMC production culture medium, supplemented with ion source: Fe^{2+}; K^{+}; CO^{2+}; Zn^{2+} and Mg^{2+} at a concentration of 1%, the mixture was incubated at 37˚C under shaking at 100 rpm for 48 hours and cellulase activity was measured by the dinitrosalycilic acid (DNS) method (Ifran *et al.*, 2012) [16].

3. Results

Five selected cellulase-producing strains isolated from the soils of Brazzaville were characterised morphologically, biochemically and identified by modern molecular biology techniques. These are: *Pantoea dispersa* MLTBY6 (MT646430.1); *Pseudomonas aeruginosa* MLTBM2 (MT646431.1); *Pseudomonas monteilii* MLTBC10 (MT674682.1); *Bacillus subtilis* MLTBC5 (MT674681.1) and *Lysinibacillus fusiformis* MLTBB7. From these strains, a study of the monitoring of cellulase kinetics, an optimisation of the physical parameters and nutritional conditions involved in the maximum production of crude cellulase was carried out.

3.1. Study of the Monitoring of Cellulase Kinetics

The study of the monitoring of cellulase kinetics in selected strains presumed to produce cellulase (*Bacillus subtilis*, *Pantoea dispersa*, *Pseudomonas aeruginosa*, *Pseudomonas monteilii* and *Lysinibacillus fusiformis*) was tested. The results obtained are presented in **Figure 1**. This figure shows that the more the product is consumed (amorphous region of the cellulose) the more difficult the degradation of the remaining cellulose (crystalline region) becomes. This figure shows

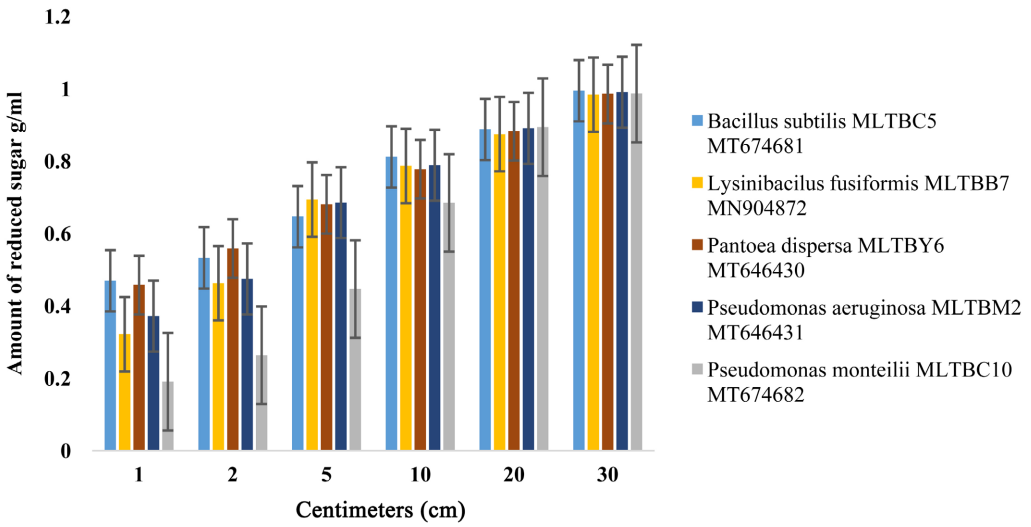

Figure 1. Cellulose hydrolysis monitoring study (1%) in *Pantoea dispersa* MLTBY6 (MT646430.1); *Pseudomonas aeruginosa* MLTBM2 (MT646431.1); *Pseudomonas monteilii* MLTBC10 (MT674682.1); *Bacillus subtilis* MLTBC5 (MT674681.1) and *Lysinibacillus fusiformis* MLTBB7.

that the growth of cellulase production is proportional to time. There is an increase as a function of time: from t = 1 minute to t = 30 minutes.

3.2. Optimisation of the Physical Parameters Involved in the Maximum Production of Crude Cellulase

3.2.1. Effect of pH on Crude Cellulase Production

The effect of pH value on cellulase production in selected strains presumed to produce cellulase (*Bacillus subtilis*, *Pantoea dispersa*, *Pseudomonas aeruginosa*, *Pseudomonas monteilii* and *Lysinibacillus fusiformis*) was tested. The results obtained are presented in **Figure 2**, which shows that in *Bacillus subtilis* MLTBC5, *Lysinibacillus fusiformis* MLTBB7 and *Pseudomonas monteilii* MLTBC10, cellulase production is proportional to the increase in pH up to pH = 8, however, above pH = 8, cellulase production decreases rapidly up to pH = 9, and then continues to decrease slowly. The optimum value for cellulase production in these strains is at pH = 8.

3.2.2. Effect of Temperature on Crude Cellulase Production

The effect of temperature value on cellulase production in selected strains presumed to produce cellulase (*Bacillus subtilis*, *Pantoea dispersa*, *Pseudomonas aeruginosa*, *Pseudomonas monteilii* and *Lysinibacillus fusiformis*) was tested. The results obtained are presented in **Figure 3**. This figure shows that in Bacillus subtilis MLTBC5, *Lysinibacillus fusiformis* MLTBB7 and *Pantoea dispersa* MLTBY6, the production of cellulase is proportional to the increase in temperature up to a temperature of T˚ = 40˚C, however, above 40˚C, the production of cellulase decreases rapidly until it becomes null at 60˚C. The optimal temperature for the production of cellulase in these strains is 40˚C.

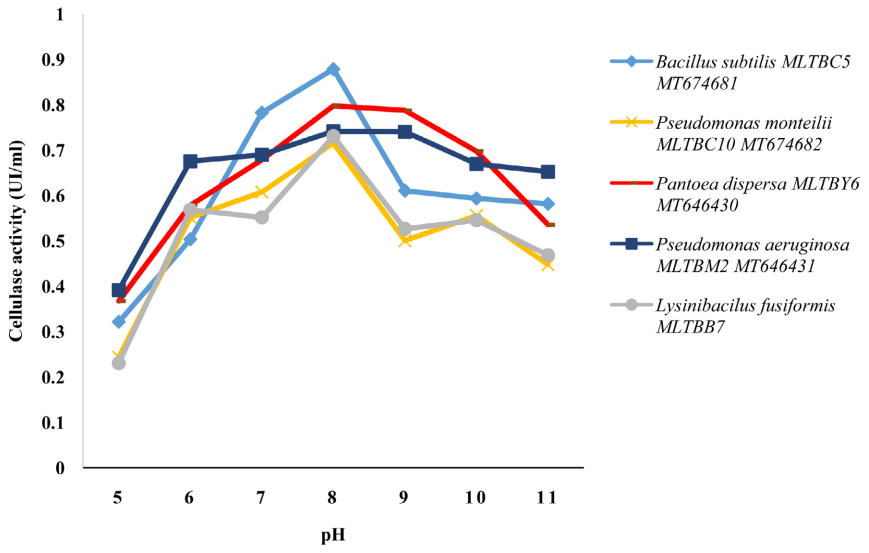

Figure 2. Profile of cellulase production as a function of pH in *Pantoea dispersa* MLTBY6 (MT646430.1); *Pseudomonas aeruginosa* MLTBM2 (MT646431.1); *Pseudomonas monteilii* MLTBC10 (MT674682.1); *Bacillus subtilis* MLTBC5 (MT674681.1) and *Lysinibacillus fusiformis* MLTBB7.

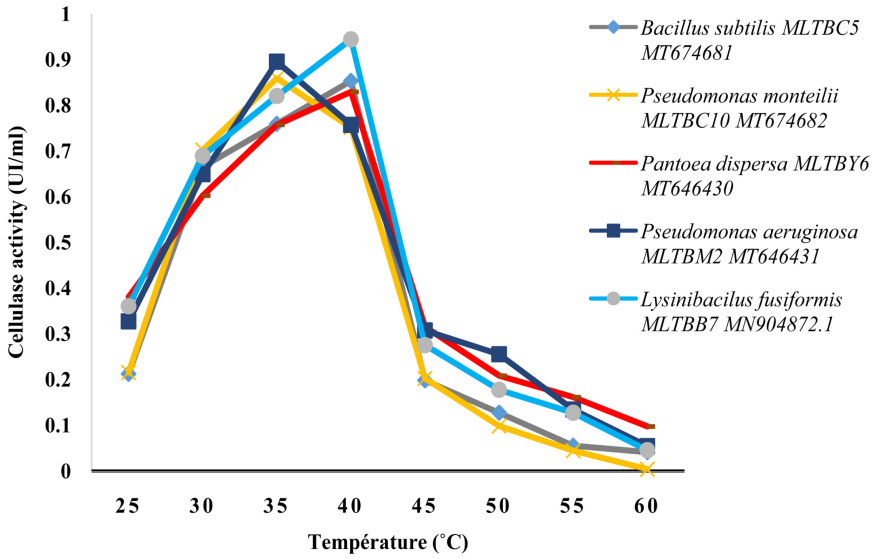

Figure 3. Temperature-dependent profile of cellulase production in *Pantoea dispersa* MLTBY6 (MT646430.1); *Pseudomonas aeruginosa* MLTBM2 (MT646431.1); *Pseudomonas monteilii* MLTBC10 (MT674682.1); *Bacillus subtilis* MLTBC5 (MT674681.1); and Lysinibacillus fusiformis MLTBB7.

3.3. Optimisation of Nutritional Conditions for the Production of Crude Cellulase

3.3.1. Effect of Carbon Sources on Cellulase Production

The effect of carbon sources on cellulase production in selected strains presumed to produce cellulase (*Bacillus subtilis*, *Pantoea dispersa*, *Pseudomonas aeruginosa*, *Pseudomonas monteilii* and *Lysinibacillus fusiformis*) was tested. The results obtained are presented in **Figure 4**, which shows that glucose and galactose are two favourable carbon sources for *Pantoea dispersa* MLTBY6 and *Pseudomonas aeruginosa* MLTBM2 for maximum cellulase production.

3.3.2. Effect of Nitrogen Sources on Cellulase Production

The effect of carbon sources on cellulase production in selected strains presumed to produce cellulase (*Bacillus subtilis*, *Pantoea dispersa*, *Pseudomonas aeruginosa*, *Pseudomonas monteilii* and *Lysinibacillus fusiformis*) was tested. The results obtained are presented in **Figure 5**, which shows that ammonium sulphate and urea are the favourable nitrogen sources for *Pantoea dispersa* MLTBY6 and *Pseudomonas monteilii* MLTBC10.

3.4. Effect of Ions on Crude Cellulase Production

The effect of ions (CO^{2+}, K^{+}, Fe^{2+}, Mg^{2+}) on cellulase production in selected cellulase-producing strains (Bacillus subtilis, Pantoea dispersa, Pseudomonas aeruginosa, Pseudomonas monteilii and Lysinibacillus fusiformis) was tested. The results obtained are presented in **Figure 6**. This figure shows that Fe^{2+} and CO^{2+} ions are two sources of ions favourable to these five strains for maximum cellulase production.

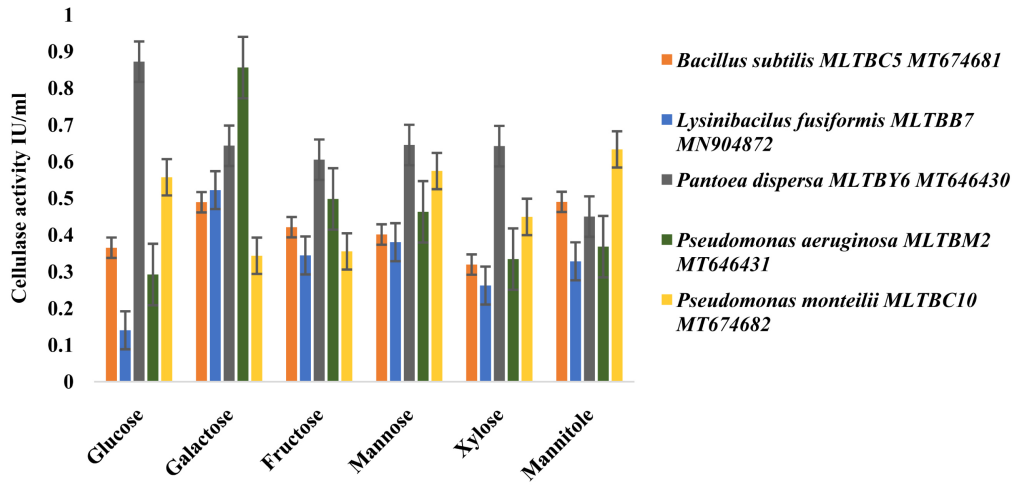

Figure 4. Effect of carbon sources on cellulase production in *Pantoea dispersa* MLTBY6 (MT646430.1); *Pseudomonas aeruginosa* MLTBM2 (MT646431.1); *Pseudomonas monteilii* MLTBC10 (MT674682.1); *Bacillus subtilis* MLTBC5 (MT674681.1) and *Lysinibacillus fusiformis* MLTBB7.

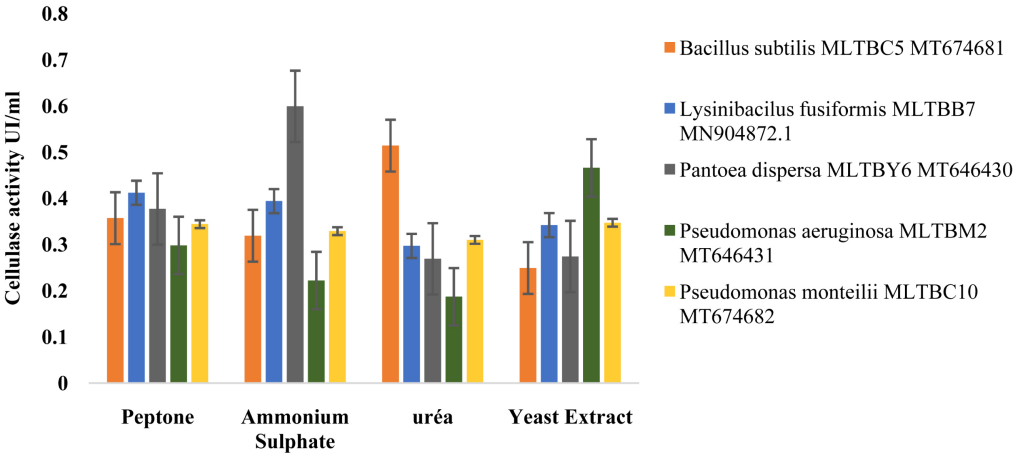

Figure 5. Effect of nitrogen sources on cellulase production in *Pantoea dispersa* MLTBY6 (MT646430.1); *Pseudomonas aeruginosa* MLTBM2 (MT646431.1); *Pseudomonas monteilii* MLTBC10 (MT674682.1); *Bacillus subtilis* MLTBC5 (MT674681.1) and *Lysinibacillus fusiformis* MLTBB7.

4. Discussion

Cellulose is the main constituent of plants and has the major fraction of organic carbon in the soil. Microorganisms, which live in the soil, are responsible for recycling this organic carbon into the environment Wang *et al.* 2008 [17]. The degradation of cellulosic materials is a complex process and requires the participation of microbial cellulolytic enzymes. Habitats where these substrates are present are the best sources for the isolation of cellulolytic microorganisms Das *et al.* 2010 [18]. About one fifth of freshwater and soil samples contain cellulose degrading bacteria after enrichment, but some samples do not contain this type of bacteria Ivanen *et al.* 2009 [19]. This is due to the existence of microenvironments in which different conditions for the cultivation of cellulose-degrading bacteria are present. These bacteria are generally present in pit manure soils

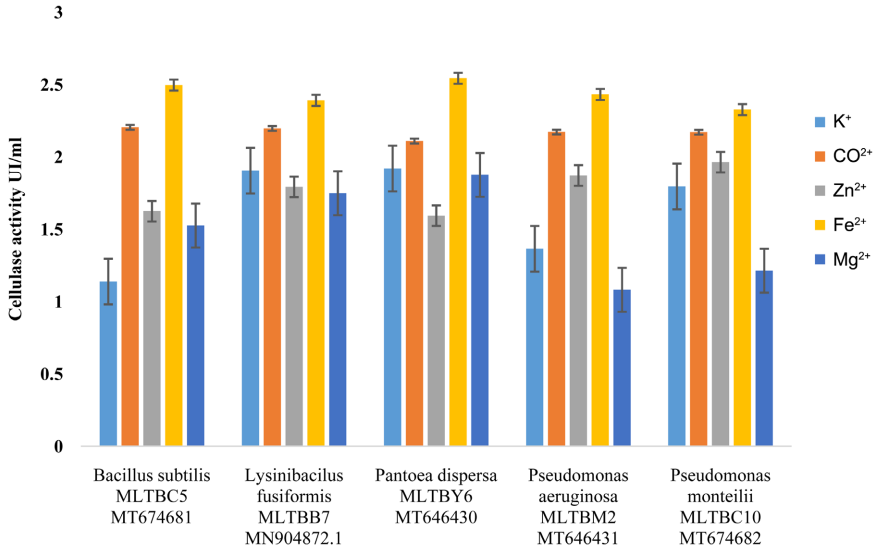

Figure 6. Effect of ions on cellulase production in *Pantoea dispersa* MLTBY6 (MT646430.1); *Pseudomonas aeruginosa* MLTBM2 (MT646431.1); *Pseudomonas monteilii* MLTBC10 (MT674682.1); *Bacillus subtilis* MLTBC5 (MT674681.1) and *Lysinibacillus fusiformis* MLTBB7.

Morris *et al.* 2008 [20]. Several microorganisms have been discovered over the decades that have the ability to convert cellulose into simple sugars Perez *et al.* 2002 [21]. Among these microorganisms with cellulolytic potential is *Bacillus subtilis* which is a microorganism capable of synthesising significant amounts of cellulase at temperatures ranging from 35˚C to 45˚C Nguimbi *et al.* 2014 [22].

The results of the effect of pH on crude cellulase production showed that the selected cellulase producing strains (*Bacillus subtilis*, *Pantoea dispersa*, *Pseudomonas aeruginosa*, *Pseudomonas monteilii* and *Lysinibacillus fusiformis*) generally exhibited high productions of cellulase at pH values between 5.6 and 9. Indeed, high cellulase production was observed in *Bacillus subtilis*, *Pseudomonas monteilii* and *Lysinibacillus fusiformis* at an optimum value of pH = 8. Consequently, in *Pantoea dispersa*, *Pseudomonas aeruginosa* and *Lysinibacillus fusiformis*, a high production of cellulase was observed at an optimum value of pH = 9. These results are close to those found by (Reffas, 2017) [23] who, working on the isolation and characterization of cellulase producing bacteria proved that selected cellulase producing bacteria showed excellent cellulase activity at pH values between 6.2 to 7.45 and those found by (Mawadza *et al.*, 2000) [24]; (Kim *et al.*, 2009) [25] who demonstrated that the optimal pH for cellulase activity is between 5 and 6.5 for strains belonging to the genus Bacillus, 6.5 for Bacillus subtilis. Our results are also in line with those found by the study conducted by (Lynd *et al.*, 2002) [26] who reported that most of the cellulases produced by different microorganisms studied possess activities at pH values of 3 to 7. Our results are also close to those found by (Sonia Sethi *et al.*, 2013) [27] who, working on the optimisation of cellulase producing bacteria isolated from soils found optimal pH values for cellulase production between 9 and 11 in *E. coli*, *Pseudo-*

monas fluorescens, *Bacillus subtilis*, and *Serratia marscens*. Based on these results, we argue that the optimal pH values for cellulase production vary from one bacterial species to another.

The results of the effect of temperature on cellulase production showed that the selected cellulase-producing strains (*Bacillus subtilis*, *Pantoea dispersa*, *Pseudomonas aeruginosa*, *Pseudomonas monteilii* and *Lysinibacillus fusiformis*) exhibited high cellulase production at optimum temperatures of 35˚C - 40˚C. High cellulase production was observed in *Pseudomonas aeruginosa* and *Pseudomonas monteilii* at the optimum temperature of 35˚C. In addition, at an optimum temperature of 40˚C, high cellulase production was observed in *Bacillus subtilis*, *Pantoea dispersa* and *Lysinibacilus fusiformis*. Above these temperatures (from 45˚C onwards), cellulase production decreased in all strains and became inactive at 60˚C. In this study, we found that our selected cellulase-producing strains have an optimal temperature of 35˚C to 40˚C for maximum cellulase production. Indeed, the amount of enzyme produced depends from one strain to another, but especially from one temperature to another. These results are not similar to those found by (Ifran *et al*., 2012) [16]; (Yin *et al*., 2010) [28]; (Mawadza *et al*., 2000) [24]; (Mahjabeen *et al*., 2016) [29] who, working respectively on Isolation and screening of cellulolytic bacteria from soil and optimization of cellulase production and activity, on Isolation of Cellulase-Producing Bacteria and Characterization of the Cellulase from the Isolated Bacterium *Cellulomonas* sp. YJ5. J Agric Food Chem, on the Purification and characterization of cellulases produced by two Bacillus strains and on Isolation of Cellulolytic Bacteria from Soil, Identification by 16S rRNA Gene Sequencing and Characterization of Cellulase. Have shown that the optimal temperature for cellulase production is 60˚C and 65˚C in *Bacillus subtilis*. Our results are similar to those found by (Sonia *et al*., 2013) [27] who, working on the optimisation of cellulase producing bacteria isolated from soils proved that the optimal temperature for cellulase production is 40˚C in bacteria of the Bacillus group, in *E. coli*, *Pseudomonas aeruginosa* and *Sarratia*. Our results also confirm those found by (Ray *et al*., 2007) [30] who found optimal cellulase production temperatures of 30˚C - 35˚C in *Bacillus subtilis* and Pseudomonas fluorescence. Following these results, we find that not all selected strains have the same optimal cellulase production temperature, so that the amount of enzyme produced varies from strain to strain.

The results of the effect of carbon sources on cellulase production showed that *Bacillus subtilis* and *Pseudomonas monteilii* showed better cellulase production in the presence of starch as carbon source with production rates of (0.49 IU/ml; 0.633 IU/ml) respectively; *Pantoea dispersa* showed better cellulase production in the presence of Glucose as carbon source with a rate of 0.872 IU/ml; *Pseudomonas aeruginosa* and *Lysinibacillus fusiformis* showed better cellulase production in the presence of galactose as carbon source with production rates of (0.856 IU/ml; 0.522 IU/ml) respectively. These results prove that not all bacterial strains have the same carbon source requirements. Indeed, we note in this work that the source of carbon depends from one strain to another. In sum, we conclude that

glucose and galactose are the carbon sources that gave the best cellulase production respectively in *Pantoea dispersa* and *Pseudomonas aeruginosa* with production rates reaching 0.872 IU/ml and 0.856 IU/ml. These two elements are therefore preferential carbon sources for these strains. Therefore, glucose is not a good source of carbon in *lysinibacillus fusiformis* because in the presence of carbon, this strain showed the lowest production rate of cellulase with a value of 0.14 IU/ml. Our results are similar to those found by (Reffas, 2017) [23]; (Shabeb *et al.*, 2010) [31]; (Robson *et al.*, 1984) [32] which demonstrated the variability and preferences of carbon source from one strain to another with production rates of (0.93; 1.43; 1.61 IU/ml) on cellulase production in different bacterial species such as *Cellulomonas* sp., *Clostridium*, *Bacillus* sp.

Our results of the effect of nitrogen source shows that, the selected cellulase producing strains showed excellent cellulase production in the presence of ammonium sulphate as nitrogen source in *Pantoea dispersa* with a production rate of 0.599 IU/ml, in the presence of urea in *Bacillus subtilis* with a production rate of 0.514 IU/ml, in the presence of yeast extract in *Pseudomonas aeruginosa* with a production rate of 0.466 IU/ml, in the presence of peptone in *Lysinibacillus fusiformis* with a production rate of 0.412 IU/ml This variability is sufficient evidence that the choice and preference of nitrogen sources differs from strain to strain in this study. Our results are in perfect agreement with those found by (Reffas, 2017) [23] who found an excellent production of cellulase in the presence of ammonium sulphate in strains EC4', EC3', EC3" with rates of (8.66; 1.86; 0.92 IU/ml) respectively and in the presence of peptone with a production rate of 1 IU/ml in strain EC2'. Our results are similar to those found by (Jaradat *et al.*, 2008) [33] who obtained a maximum production of cellulase in the presence of ammonium sulphate. Our results are also in perfect similarity with those found by (Vyas *et al.*, 2005) [34] who confirm that ammonium sulphate is the best source of nitrogen for good endoglucanase and exoglucanase activity.

Our results of the effect of ions on cellulase production show that, the selected cellulase producing strains (*Bacillus subtilis*, *Pantoea dispersa*, *Pseudomonas aeruginosa*, *Pseudomonas monteilii* and *Lysinibacillus fusiformis*), exhibited high cellulase activity in the presence of Fe^{2+} ions with production rates of (2.544; 2.490, 2.432; 2.391; 2.327 IU/ml) respectively in *Pantoea dispersa*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Lysinibacillus fusiformis*, and *Pseudomonas monteilii*, in the presence of CO^{2+} ions with production rates of (2, 205, 2.197, 2.172, 2.171, 2.11 IU/ml) in *Bacillus subtilis*, *Lysinibacillus fusiformis*, *Pseudomonas aeruginosa*, *Pseudomonas monteilii*, *Pantoea dispersa*, in the presence of K^+, CO^{2+} and Zn^{2+} ions respectively. In contrast, a low rate of cellulase production (1.082 IU/ml) was observed in Pseudomonas aeruginosa in the presence of Mg^{2+} ions. Our results are similar to those found by (Reffas, 2017) [23]; (Ifran *et al.*, 2012) [16]; (Saha *et al.*, 2004) [35] who found excellent cellulase production in the presence of K^+ ions. Our results are close to those found by (Irfan *et al.*, 2012) [16] who, working on Isolation and screening of cellulolytic bacteria from soil and optimization of cellulase production and activity found excellent cellulase

production in the presence of CO^{2+}, Mg^{2+} ions. However, in the presence of Fe^{2+} ions, our results do not collaborate with those found by (Irfan *et al.*, 2012) [16]. In sum, the need and preference for ion source differs from strain to strain in this study. Indeed, Fe^{2+} in this study is the preferred ion source for all the selected strains, as it is in the presence of Fe^{2+} that good cellulase productions were observed in these strains.

5. Conclusion

The objective of the present work was to optimise the physical and nutritional parameters involved in the maximum production of cellulase in five strains isolated from soils. From the results obtained, we can conclude that our strains (*Bacillus subtilis*, *Pantoea dispersa*, *Pseudomonas aeruginosa*, *Pseudomonas monteilii* and *Lysinibacillus fusiformis*) produced maximum cellulase yield. The optimum temperature and pH were determined to be 35˚C - 40˚C and 5.6 - 9. The best sources of carbon, nitrogen and ions were glucose, galactose, ammonium sulphate, urea, Fe^{2+}, CO^{2+}, and Zn^{2+}. This information allowed us to draw the conclusion that: the choice and preference of carbon, nitrogen and ion sources differ from strain to strain in this work. Finally, due to its production at high temperatures, crude cellulase produced in this study may be beneficial for industrial applications.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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