Hydrolysis of cellulose from palms leaflets of Date palm (phoenix dactylifera) using a new strain of Aspergillus iranicus

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Research Article

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Abstract

A new strain of *Aspergillus iranicus* MS-34 isolated from Subaqueous soils of the Djorf Torba dam in Algeria was used for the hydrolysis of palm cellulose from the date palm (phoenix dactylifera), by submerged fermentation on a Mandels medium. Three degrees of pretreatment of the palm using KOH were made: 10%, 20%, 50% and the control with no pretreatment. After ten days of fermentation, a high carbohydrate concentration (197.84 mg/L) was noted in 50% KOH palm pretreatment fermentation. Eventually, the highest enzymatic activity was observed with 20% KOH palm pretreatment hydrolysis, 112.97 IU/mL for endoglucanase activity and 47.62 IU/mL for exoglucanase activity. Besides, glucose and proteins concentration in batch fermentations with 1% CMC medium fluctuated between 86,41 mg/l to 108.85 mg/l. and the protein level was 0.713–0.991 mg/ml. A significant effect of temperature and pH on the enzymatic activity was noticed with an optimal at 50–60°C and at pH 4.0.

Introduction

Algeria is a phoenicultural contry, ranked sixth in the world and first in the Maghreb for its large areas of cultivation with 160 000 ha (Bouguedoura et al. 2010) and 16 million of palm trees in 2021 (APC 2021). Based on the fact that a date palm yields an average of 15 palms per year and that a palm has on average 180 leaflets, each leaflet weighing averagely 5 g (Chehma and Longo 2001). One can estimate the gross weight of dry palms per year as follows: 5g × 180 leaflets × 15 palms × 16 000 000 of palm trees = 216 000 000 kg. That is 216 103 tons of dry palms/year. As a major agriculture Algerian residue, dry palm is an attractive potential low-cost feedstock for biofuel. The value of any particular type of biomass as feedstock for fermentation depends on the ease with which it can be converted into sugars (Demirbas 2008). Before being fermented into ethanol, the cellulose molecule must be hydrolyzed into glucose by the action of cellulase. Conversion efficiencies of cellulose into glucose may depend on chemical and mechanical pretreatments used for the elimination of lignin and hemicellulose layers surrounding the cellulose fibrils (Adeeb 2004; Jayasekara and Ratnayake 2019; Hebal et al 2021). Alkaline pretreatment is a chemical treatment that has been considerably studied and proven to be effective for all types of biomasses. Potassium hydroxide (KOH) received a lot of attention in recent researches due to its dynamism for the delignification of biomass (Jaffar et al. 2016; Zahoor et al. 2021).

A broad range of microorganisms synthesizes the enzyme cellulase, especially fungal. *Aspergillus niger*, *Aspergillus fumigates*, *Alternaria sp.*, *Cladosporium sp.*, H*elminthosporum sp.*, *Penicillium sp.*, *Verticillium sp.*, *Trichoderma reesei and T. harzianum*, are the most studied, produced and industrially important cellulolytic fungi (Roussos and Raimbault 1982; Dashtban et al. 2011; Meena et al. 2018). The enzymatic hydrolysis of cellulose is a complex process, that requires the participation of several enzymes: Endo-ß-1,4-glucanase (EC3.2.1.4), Exo-ß-1,4-glucanase or cellobiohydrolases (EC 3.2.1.91) and ß-1,4-glucosidase (EC 3.2.1.21), (Roussos and Raimbault 1982; Hebal et al 2021). *Aspergillus iranicus* is a little-known species, described quite recently by Arzanlou et al (2016). This Aspergillus is not known to date except in osmophilic environment, precisely hypersaline soils (Papizadeh et al., 2018).

The present study focuses on the ability of a new strain of *Aspergillus iranicus* isolated from subaqueous soil to hydrolyze the cellulose contained in the dry palms of phoenix dactylifera in order to convert it in fermentable carbohydrate.

Materials And Methods

1. Chemical composition of the palm

Dry palm of *Phoenix dactylifera*, Fegousse cultivar was collected from Bechar region, south-western Algeria during February 2020. Five chemical parameters were analyzed in the laboratory of Scientific and Technical Research Center on Arid Regions (CRSTRA). First, dry matter (DM) is obtained by drying the sample in an oven at 105°C for 24 hours. Next, mineral matter (MM) or ash is attained by calcining MS in a muffle furnace at 550°C for 2 hours. Organic matter (OM) is the outcome of separating DM from MM (AOAC method 1990). Then, crude protein (CP) is derived by the conventional Kjeldahl method, which includes a step of mineralization with concentrated sulphuric acid and a distillation step, followed by titration of the distillates. The CP is estimated by applying to the percentage of nitrogen; the coefficient 6.25 is conventionally accepted, and crude fiber (CB) which is obtained by Weende method (AFNOR.,1993). The determination of calcium (Ca) and manganese (Mg) contents was carried out by means of an atomic absorption spectrophotometer (AAS). The amounts of Sodium (Na) and potassium (K) concentration were determined with a flame photometer (Pauwels et al., 1992; Kolsi-Benzina and Zougari 2008).

2. Palm pretreatment

The alkaline treatment using KOH was performed. 25 g of dry palm powder placed in Erlenmeyer flasks containing 250 ml of sterile distilled water. Along with a 0% KOH (control sample), KOH concentrations were carefully added at, 10%, 20% and 50% (g KOH/g dry palm) in separate Erlenmeyer flasks. The samples were mixed until homogenization and then incubated at 20 ° C. After 24 hours of incubation, the mixture was filtered using sterile tissue. The solid material obtained was washed three times with 100 ml of sterile distilled water (Liu et al., 2015). All the treated palm samples were stored at 4 ° C for less than 48 hours for further analysis.

3. Determination of cellulose in the treated palm

To estimate the concentration of liberate cellulose in the medium, the Anthrone method was used (Leng et al 2016). In a series of test tubes, 1g of each palm treated with KOH (0%, 10%, 20% and 50%) was placed in 9 ml of distilled water. Then, 1 ml of each solution was added to 5 ml of the Anthrone reagent. As a control, distilled water treated in the same way as the analyzed solution was used. All tubes were shaken thoroughly and then placed in a water bath at 80 ° C for 20 minutes. Optical density was measured at 620 nm in a spectrophotometer. The Anthrone method was preceded by lead acetate (10%) and bicarbonate (0.1 mg/l) precipitation of samples.

4. Origin of strain

Aspergillus sp. SB 34 was the cellulolytic strain used in this work. Collected on 27/4/2019 and isolated from the Subaqueous soil samples of the Djorf Torba dam (Lat 31°30' 45.8"N Long 2°46'56.8"W) (Fig. 1), which is located in the Province of Bechar Algeria. The strains were maintained in inclined tubes of potato dextrose agar (PDA) and then stored at 4°C.

5. Morphological and molecular identification

Morphological analysis of the strain was carried out by the micro-culture method and single sporing method (Makhloufi et al. 2018; Pitt and Hocking 2009). The isolate wase cultivated for 7 days in potato dextrose agar (PDA), malt extract agar (MEA), Glycerol Nitrate Agar (G25N) at 25°C, while Czapek yeast autolysate agar (CYA) medium was incubated at different temperatures: 5° C, 25° C and 37° C. The molecular identification was carried out by comparative analyses of the ITS sequence (amplification of fragments by the use of the primers ITS1 and ITS4) at Paval Laboratory, university of Grenoble Alpes France. The Aspergillus sequences were supplemented with sequence data from GenBank. After compilation of the sequence data sets, datasets were aligned using the MAFFT multiple sequence alignment software v.7.221 (Katoh et al. 2019). The maximum likelihood analysis was calculated using the MEGA 11 software.

6. Screening of cellulases enzymatic activity

The Endo-1,4-β-D-glucanase, of *A. iranicus straine* MS-34 strains was determined by growing in CMC medium (Carboxymethyl Cellulose 1% w/v) petri dishes, and for the productions of Exo-1,4-β-D-glucanase (Avicelase) the strain was grown in an Avicel medium (Cellulose microcrystalline 1% w/v) petri dishes. The plates were inoculated with a fungal mycelium disc of 1 cm diameter and incubated at 28°C for 5 days (Teather and Wood 1982; Colonia & Chagas Junior, 2014; Bahry, 2021). After the incubation period, the plates were plunged in 10 mL of Congo Red (0.1% w/v) solution for 30 min (Colonia & Chagas Junior, 2014), then the solution was discarded. The crops were washed with 5 mL NaCl (0.5 M) solution for 10 min. The enzymatic index (EI) was calculated to estimate the endo and exo-cellulase, i.e. the relation between the colony growth (Øc) and the enzymatic halo (Øh: indicative of areas of hydrolysis) (Lübeck and Lübeck 2018): EI = Øh / Øc. Experiments was performed in triplicate.

7. Batch fermentation process

a. Using dry palm

The production medium used is a Mandels and Weber, (1969) medium, pH 5.5. Dry palm powder (1%) was used as a unique carbon source in the four experiences. The submerged fermentation is carried out in sterile volumetric flasks of 500 ml at a rate of 250 ml of medium. Production medium was inoculated with 10^6 spores/ml, temperature was kept at 25°C and the agitation was 150 rpm. Air exchanges was insured by sterile Pasteur pipette that were inserted in the cap of flask. The samples were taken by a sterile tubing attached to a syringe. A daily sampling was performed for 10 days for pH measurement and dosage of reducing sugars using the Anthrone method (Leng et al. 2016).

b. Using CMC medium

The fermentation media was the same as the batch fermentation using dry palm, except for the carbon source that is replaced by 1% of CMC. Then, it was sterilized using an autoclave at 121°C for 15 min. The culture parameters (pH, temperature, inoculum concentration, and fermentation period) are the same as the ones used for the previous fermentation.

8. Harvested enzymes

Samples are taken daily, before being centrifuged at 4000g for 10 min at 4°C (Leghlimi et al. 2013). The supernatant constitutes the crude enzyme used for the analysis of the cellulolytic activities and protein concentration.

9. Measurement of cellulase activity

The cellulase activities were determined in a different treated palm batch fermentation and synthetic culture medium (CMC medium). The carboxymethyl cellulase (CMCase) and Filter paper activity (FPase) activities were determined as reported by Ghose (1987):

- Endoglucanase activity (CMCase) was measured in a total volume of 1,5 ml of a reaction mixture containing crude enzyme diluted in 0.1 M citrate buffer, pH 4.8 and a 1% (W/ V) CMC (carboxymethyl-cellulose), incubated at 50°C for 30 min.
- Overall cellulase activity complex (FPase) was determined by the insertion of 0,5 ml of crude enzyme into a test tube containing 50 mg of Whatman No 1 filter paper (1.0 × 6.0 cm) and 1ml of citrate buffer (0.1 M, pH 4.8) and incubated at 50°C for 60 min.

The concentration of reducing sugars released by hydrolysis of the CMC and the filter paper was measured according to the Miller method (1959) by a colorimetric reaction due to the presence of DNS, and the absorbance was determined at 540 nm. The amount of reducing sugar liberated was determined from a glucose standard (50–250 mg/L). One activity unit was defined as the amount of enzyme that liberated 1 µmol of glucose per minute under the assay conditions.

10. Estimation of protein

Protein content was determined by the Bradford method (1976) described under general Materials and methods with crystalline bovine serum albumin (BSA) as standard. It relies on the binding of the dye Coomassie blue (G250) to protein, by thorough mixing and measuring the absorbance at 595 nm.

11. Characterization of cellulase activity

For CMCase and FPAase characterization, the crude enzyme was tested. The optimum temperature was measured by performing the enzyme activity assay at different temperatures (40, 50, 60, 70 and 80°C) in citrate buffer (0.1 M, pH 4.8) and for optimum pH effect, assays were performed at 50°C, over a pH range of

3.0 to 8.0. Three buffers were used: 0.1 M citrate (pH 3.0 and 4.0), 0.1 M citrate phosphate (pH 5.0, 6.0 and 7.0) and 0.2 M phosphate (pH 8.0). Residual activity of each sample was then measured in triplicate using the standard methods. A t-test was used to analyse the results and the significantly different $P < 0.05$.

Results

1. Chemical composition of the palm

The results obtained for the analysis chemical and mineral composition of dry palm are reported in Table 1.

Table 1
Chemical and mineral composition of dry palm of Fegousse cultivar

Unit	**wt %**						**meq/l**			
Analyzed parameter	**HR**	**DM**	**MM**	**OM**	**CB**	**CP**	**Ca**	**Mg**	**Na**	**K**
Palm	1.0733 ± 0.055	98.92 ± 0.055	92.12 ± 0.40	07.87 ± 0.40	37.2 ± 1.249	0.0284 ± 0.0149	09.73 ± 0.305	05.86 ± 0.461	0.933 ± 0.073	0.73 ± 0.017

HR: Relative humidity; DM: dry matter; MM: mineral matter; OM: Organic matter; CB: crude fiber CP: crude protein.

2. Concentration of cellulose in the treated palm

The liberate cellulose in the medium before and after pretreatment is represented in Table 2.

Table 2

Cellulose concentration in the medium of fermentation before and after alkaline pretreatment

Treated dry palm with KOH	**0% (control)**	**10%**	**20%**	**50%**
Cellulose (mg/l)	539.44 ± 0.059	897.22 ± 0.0535	1171.11 ± 0.321	1276.11 ± 0.1635

0% (control): dry palm powder; 10%: dry palm treated with 10% KOH; 20%: dry palm treated with 20% KOH; 50%: dry palm treated with 50% KOH.

In all plates, the colonies present a low to moderately deep white mycelium without sclerotia. The result was a surface texture granular to floccose, conidial mass white to off-white, densely packed and pale or yellow orange color in the revers. Except, for PDA plates, the mycelium have a predominant yellow color in

old culture (Fig. 2). The Colony diameter (mm) after 7 days is: 28–31 at PDA; 18–23 MEA; 18–22 at CYA 25°C; 21–25 at CYA 37°C with no fungal growth at 5°C.

3. Presentation of Aspergillus strain

Figure 2: *Aspergillus iranicus* isolate MS-34. a: young colony after 5 days of incubation in PDA; b: old colony after 10 days of incubation in PDA; c, d: Conidiophores; e: Conidia, Scale bars 10µm

4. Molecular identification and Phylogenetic analyses

Ribosomal DNA primers (ITS1 and ITS 4) were used to amplify the region of the rRNA gene, The level of similarity of *Aspergillus sp.* SB 34 DNA sequence was compared to the isolates in GenBank data shows 99.82% similarity with *Aspergillus iranicus strain* DTO 203-D7 under the accession number KP987077.1 The phylogenetic analyses process was initiated by importing sequences from BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi), with elimination of short and doubling sequences compared to our sequence in BioEdit (Sequence Alignment Editor). The alignment was made using MAFFT v.7.221 (Katoh et al. 2019). Phylogenetic analyses were conducted in MEGA11 (Tamura et al. 2021) by using the Maximum Likelihood method and Tamura 3-parameter model (Tamura 1992). The tree with the highest log likelihood (-788.71) is shown. The percentage of trees in which the associated taxa clustered together is shown below the branches. Initial tree for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura 3 parameter model, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+ G, parameter = 0.1000)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 17 nucleotide sequences. All positions with less than 100% site coverage were eliminated, i.e., fewer than 0% alignment gaps, missing data, and ambiguous bases were allowed at all positions (partial deletion option). There were a total of 526 positions in the final dataset (Fig. 3). The sequences derived from the new strain of specie *Aspergillus iranicus* were deposited in the NCBI database under the following GenBank accession number: OL823064.1

5. Screening

In CMC medium the diameter of the colonies were between 54 to 57 mm and in the Avicel medium was between 50 to 52 mm. The screening performed in CMC medium presented different colorations and enzymatic degradations compared with screening in Avicel medium (Fig. 4). Hydrolysis zone diameters varies from 61 to 51 mm. As a result, the Enzymatic Index Averages (EI) were: 1.073 ± 0.0026 for the endoglucanase activity and 1.0195 ± 0.0005 for the exoglucanase activity.

6. Enzymatic hydrolysis and enzymatic activity

In flasks, experiments were performed with 10^6 spores/ml inoculum of *A.iranicus* strain MS-34. During 10 days of incubation, the carbohydrate amount in all the hydrolysis experiments are ranged between 02.84–

197.84 mg/L. Moreover, 44.04 g/ml was the highest carbohydrates concentrations for the dry palm hydrolysis after 24h (Fig. 5). For treated palm, it was 67.84 mg/L, 104.09 mg/L and 197.84 mg/L respectively to 10, 20 and 50% KOH palm pretreatment hydrolysis, at different times (Fig. 5).

Besides, the highest enzymatic activity was observed with 20% KOH treated palm hydrolysis, we noted 112.97 IU/mL for endoglucanase activity and 47.62 IU/mL for exoglucanase activity, followed by 50% KOH treated palm hydrolysis with 96.32 IU/mL of endoglucanase activity and 36.90 U/ml of exoglucanase activity. The lowest endoglucanase activity was recorded with CMC medium 11.53 U/ml and for exoglucanase it was 21.62 U/ml recorded with 10% KOH treated palm hydrolysis (Fig. 6).

7. Glucose and proteins concentration and pH in CMC fermentation

The concentration of glucose was fluctuated between 86.41 mg/l on day one to 108.85 mg/l on day 10. However, the protein level was 0.713–0.991 mg/ml. The highest concentration of protein 1.17 mg/ml was achieved on day 10. Therefore, there was a negative correlation between the glucose and the protein ($R2 = 0.04783$). The equation of the regression line is $y= -128.2x + 230.7$ (simple linear regression of correlation, XY test) (Fig. 7; (a)). The pH in hydrolysis fermentation of CMC medium was measured. The ranges of pH during the fermentation period were from 5.8 to 6.8 (Fig. 7; (b))

8. Effect of temperature and pH on the enzymatic activity

Two physiological properties of the crude enzymes contained in the supernatants from CMC culture of the *A.iranicus* strain MS-34 were analyzed. Essays at different temperatures revealed that CMCase and FPAase had an optimal activity at 60 and 50°C, respectively (Fig. 8). The temperature effect on the enzymes was significant ($F = 1.855$; $t = 6.051$), $p = 0.0003$. Both enzymatic tests showed an optimal activity at pH 4.0 using citrate buffer. Besides, the endoglucanase and FPA presented a higher value at pH 8.0, with phosphate buffer compared to pH 6.0 and 7.0 with citrate phosphate (Fig. 9). The analysis of these results by t-test tube revealed that the pH affects considerably the two enzymatic activities ($F = 5.712$; $t = 8.232$), $p < 0.0001$.

Discussion

The chemical composition of Fegousse cultivar palm is represented in Table 1 concerning the mineral content, and the amount of calcium, and it can be noticed that sodium and potassium are low compared to bibliographic results. Ranges reported were for azote 0.4 to 1.0%, for potassium 0.57 to 1.04%, for calcium 0.054 to 0.219%, and for sodium 0.005 to 0.39% (El- Shurafa 1984). The study of Kolsi-Benzina (2008) showed that this variation could be due to several factors, such as region factor effect, palm position effect and palm potion effect. Furthermore, mineral content varies depending on the ripening stage, cultivar, environmental conditions and agronomical practices (Dghaim et al. 2021; Tripler et al. 2011)

The contents of brute cellulose, which is the organic matter remaining insoluble after the acid and alkaline treatment were 37.2%. Compared to the result of Almi et al (2015), the percentage of cellulose brute

contents in petiole, rachis and leaflets is 23.97%; 54.02% and 49.00% respectively. This can give an idea about the content of brute cellulose of their palm simples (42,33%). It can be seen that these values are higher than our result. The same is observed with Nasser et al (2016) results which ranged from 32.8–47.5%. However, it was reported that the date palm residues composition contains lower amount of cellulose compared to other lignocellulosic residues (Bledzki et al 1996).

Alkaline pretreatment has been reported to efficiently improve the hydrolysis yield of many biomass materials. The pretreatment used in this study was chosen for samples with a cellulose content between 33–40% w/w (Liu et al. 2015; Talebnia et al. 2010). Therefore, the pretreatment with our dry palm samples with 37.2% cellulose proved effective. This was demonstrated by the analysis cellulose concentration in the treated palm (Table 2). Consequently, the treatment of dry palm with 10% KOH released 357.78 mg/l, with 20% KOH it released 631.67 mg/l and with 50% KOH 736.67 mg/l (Table. 2). It was observed that the color intensity of the separated liquid derived from KOH treatment became darker with pretreatment severity. A study by Liu's et al (2015) demonstrated that the percentage of delignification degree increase in the black liquor with increasing KOH. According to He et al (2008), the pretreatment removed amorphous components like lignin and hemicellulose.

Djorf Torba dam built in 1969 is located in a Saharan zone characterized by increasing aridity from year to year (Kabour 2016). This old dam suffers from many problems (high summer temperatures, high evaporation, high precipitation and a decreased level of restraint), which cause the increases in salinity level in the Subaqueous soil. It signs that this new strain of *Aspergillus iranicus* isolate MS-34 emanates from the same biotope as the ones described by Arzanlou et al (2016).

The strain studied in this article showed potential for production of cellulases. It was observed that the screening method using dye Congo Red as chromogenic indicator for detection of cellulase activity has a good revelation of the contrast between the original color and the clear halo. Further, several researchers have used this dye developer for the detection of extracellular enzymes (Jo et al. 2011; Sharma and Sumbali, 2014, Colonia and Chagas Junior, 2014; Castrillo et al. 2020). This change in the color is due to the fact that the Congo Red dye is absorbed only by long chains of polysaccharides (Percival Zhang et al. 2006). Compared to other results the larger halos of screening performed in CMC medium using Congo Red was 40 mm and in Avicel medium 51 mm using filamentous fungi isolated from soils (Colonia and Changas junior. 2014). Strong activity group shows 2.5- 4 cm of clear zone size using *Fusarium spp* strain in Kwon's et al (2007) study.

The enzymatic hydrolysis performance of dry palm and treated dry palm have showed a fluctuation on the carbohydrate concentrations, which is linked to liberation of glucose in the medium of fermentation resulting from the conversion of cellulose and then, its consumption by the fungal cells. A considerable amount of research also demonstrated that the production of cellulase is regulated at the transcriptional level in fungi, and that the fungus does not begin producing these enzymes until plant polysaccharides such as cellulose are provided as carbon sources (Vázquez-Montoya et al. 2019). However, when using simple carbohydrates sources such as glucose, the production of these enzymes is inhibited (Wang et al. 2020). Moreover, the study of Xiao et al (2004) revealed that the increased glucose content in the

hydrolysate of two types of cellulosic material (Avicel and acetic acid-pretreated Softwood) resulted in a dramatic increase in the degrees of inhibition on cellulase and β-Glucosidase activities.

Furthermore, the inverse correlation between the quantity of reducing sugars and protein concentration during the period of submerged fermentation in the saccharification experiments using Mandel medium supplementing with 1% Carboxyl methylcellulose as unique carbon source, support the previous interpretation.

In hydrolyses process the pH and temperature are two important factors affecting the enzymatic activity. A stability of the endoglucanase activity was noted with two optimal temperatures, 52.41 IU/ml in 50°C, and 52.46 IU/ml in 60°C. In addition, FPA activity was higher at 50°C (24.26 IU/ml). Cellulase activities from mesophilic fungi as *Trichoderma spp*. and *Aspergillus spp*. are at their optimum when assayed at about 50°C (Leghlimi et al. 2013).

The concentration of hydrogen ions in the reaction highly affected cellulase activity. Optimum pH for maximum enzymatic activity of both CMC (46.19 IU/ml) and FPA (18.15 IU/ml) obtained from this study was 4.0. The result obtained from this study agreed with the findings of Sulyman et al (2020) who isolated cellulase from *A.niger* and reported a pH value between 3.0 and 6.0 in a 50 mM citrate buffer.

Conclusion

The study presented shows the efficiency of using new cellulolytic fungi strains for production of significant amounts of fermentable sugars from dry date tree palm, which can be subsequently used to produce biofuel such as ethanol. Also, this research demonstrates the importance of alkaline pretreatment in the hydrolysis process. It was observed that cellulose content of the treated dry palm increased with increase in KOH pretreatment. We can conclude that *A.iranicus* isolate MS-34 is an efficient alternative for the production of cellulases using treated palm as substrate in submerged culture.

Declarations

Ethics approval and Consent to participate

not applicable for that specific section.

Consent for publication

not applicable for that specific section.

Availability of data and materials

Data Sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Competing interests

The authors declare that they have no competing interests in this section

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Authors' contributions

Material preparation, conceiving experiments, carrying out experiments and analytic calculations were performed by Makhloufi Souad. Also, the wrote of the manuscript. The contributed to interpretation of data and manuscript revisions by Makhloufi Ahmed. Chebloune Yahia, carried out the molecular genetic studies and participated in the sequence alignment. All authors discussed the results and contributed to the final manuscript.

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Figures

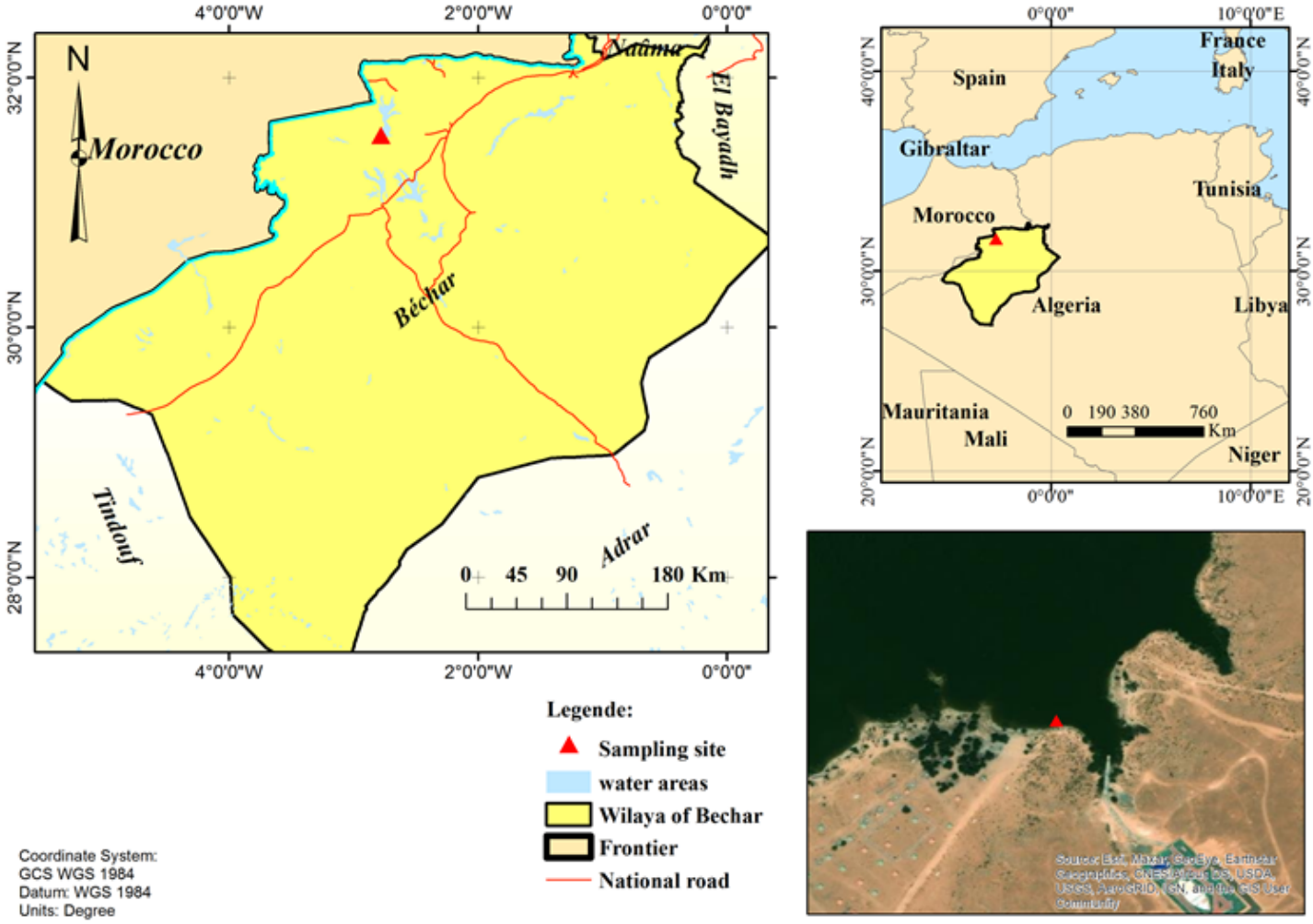

Figure 1

Map of Algeria showing the location of Bechar province and the soil-sampling site in Djorf Torba dam

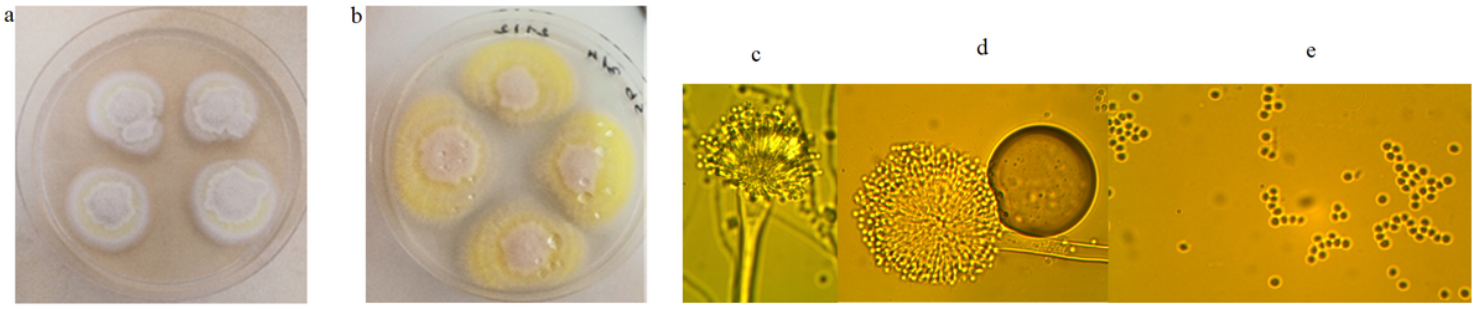

Figure 2

Aspergillus iranicus isolate MS-34. a: young colony after 5 days of incubation in PDA; b: old colony after 10 days of incubation in PDA; c, d: Conidiophores; e: Conidia, Scale bars 10µm

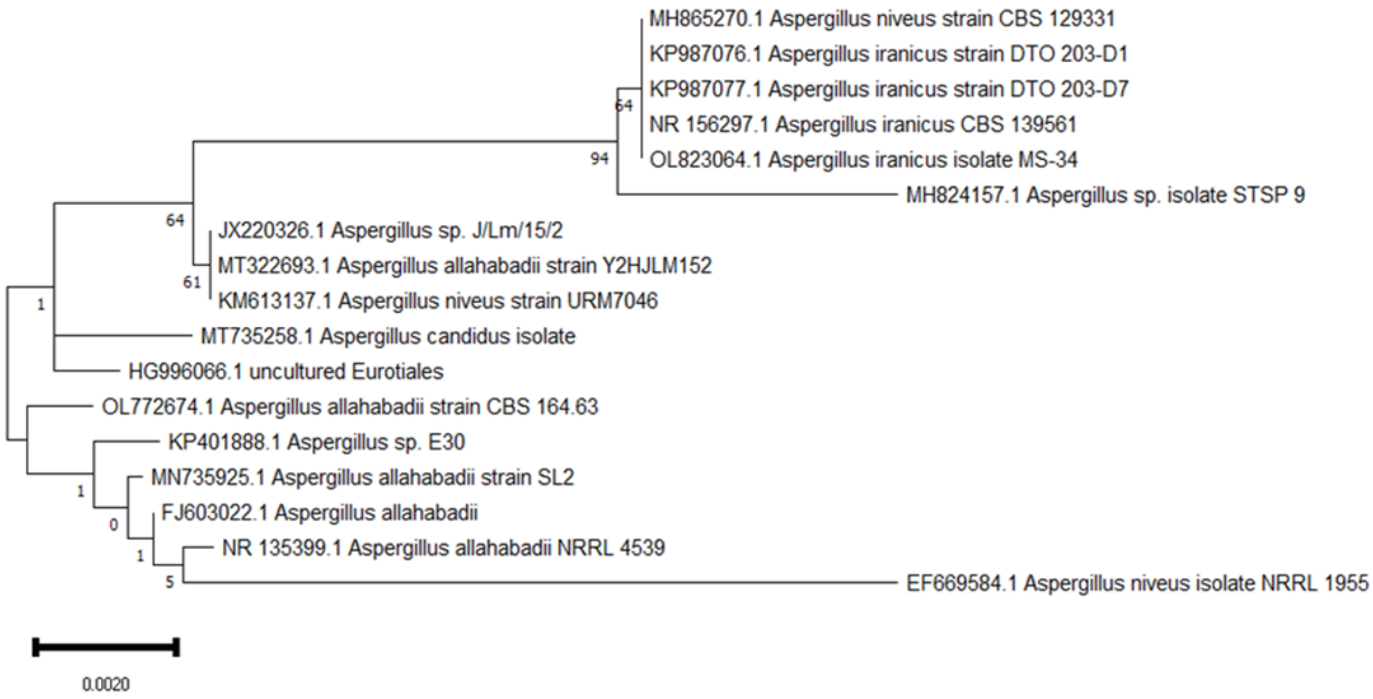

Figure 3

Phylogenic tree of Aspergilus iranicus isolate MS-34 using the Maximum Likelihood method and Tamura 3-parameter model (Tamura 1992)

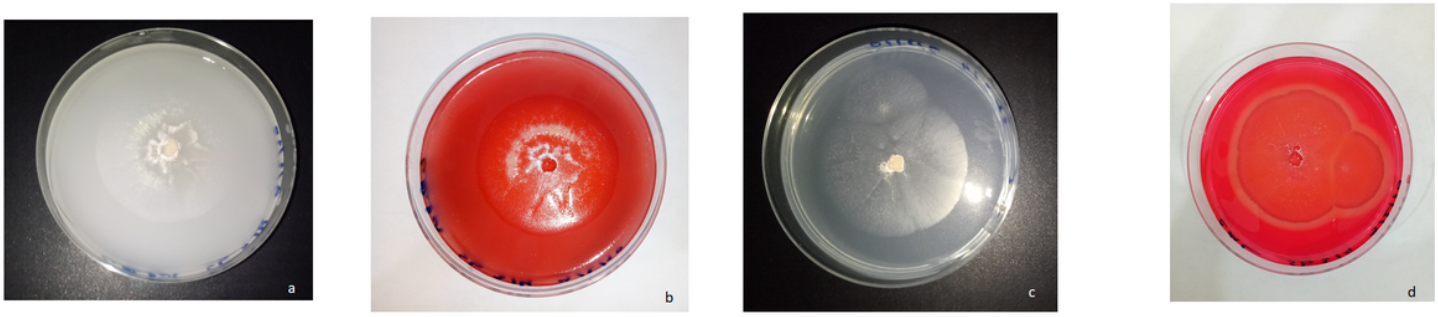

Figure 4

Observation of the clear zone around a colony of *A.iranicus* isolate MS-34 using Congo red dye at 25°C. a: colony after 5 days of incubation in Avicel medium; b: colony after 5 days of incubation in CMC medium; c: coloration of the colony in Avicel medium; d: coloration of the colony in CMC medium

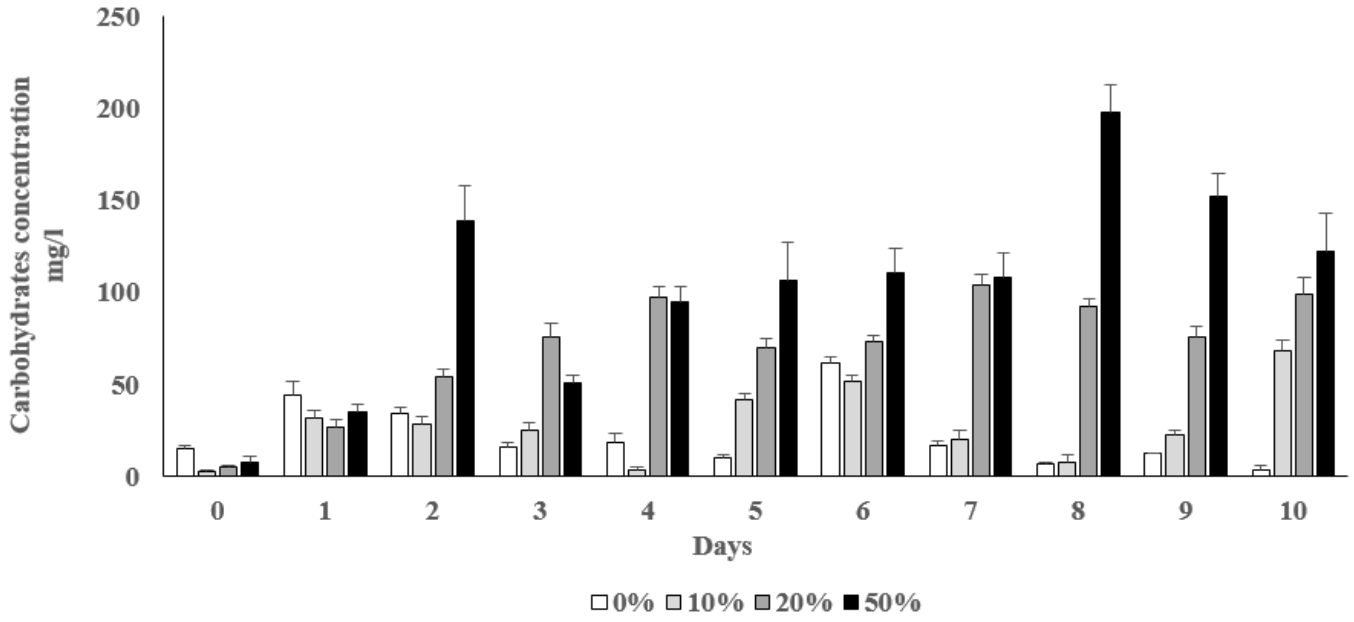

Figure 5

Carbohydrates concentrations in different batch fermentations at 28 °C. ■ fermentation using dry palm without treatment (control), ■ fermentation using treated dry palm with 10% KOH loading, ■ fermentation using treated dry palm with 20% KOH loading, ■ fermentation using treated dry palm with 50% KOH loading.

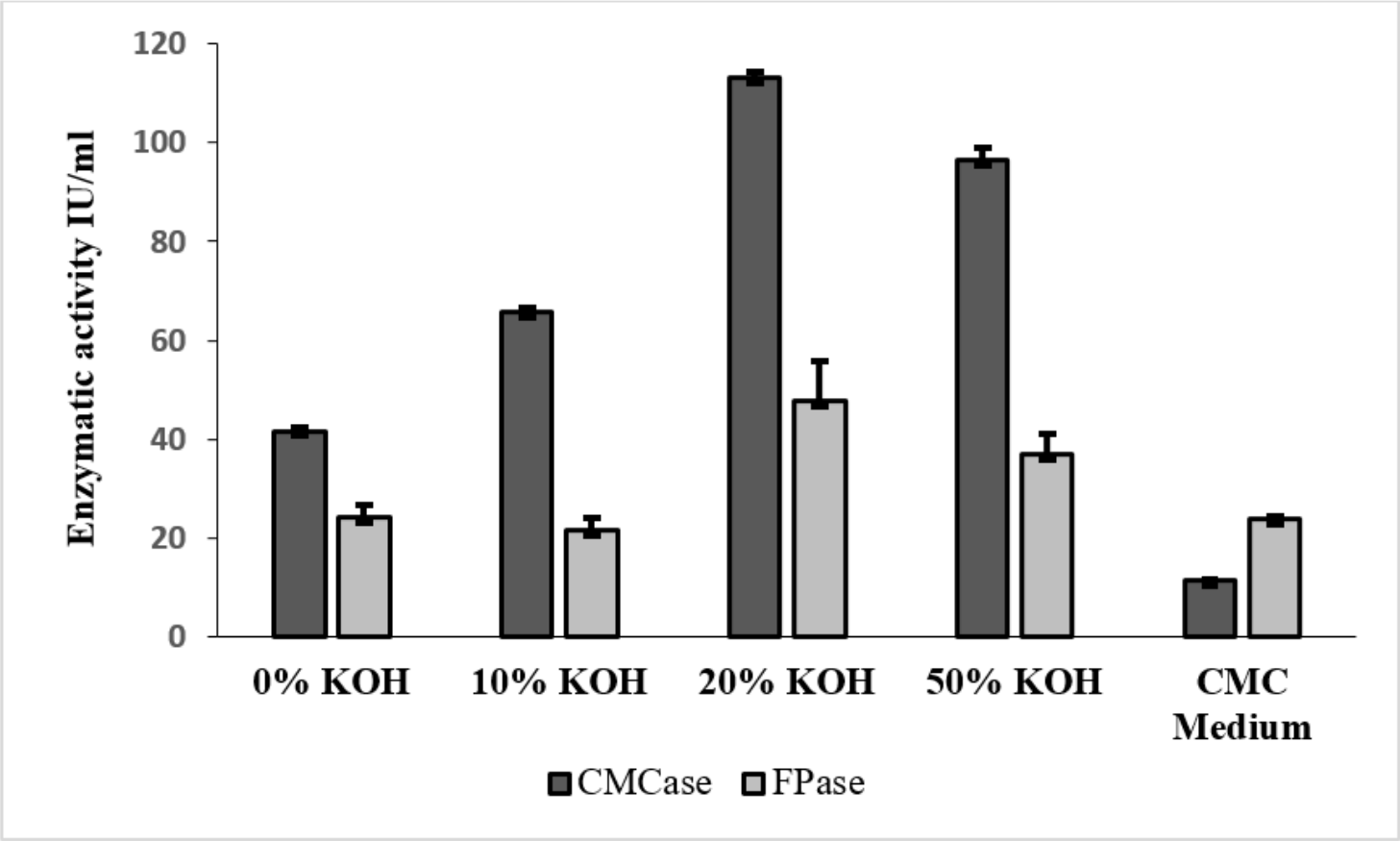

Figure 6

Enzymatic activity of A.iranicus isolate MS-34 grown on treated dry palm medium (0% KOH, 10% KOH, 20% KOH, 50% KOH) and CMC medium at 28°C.

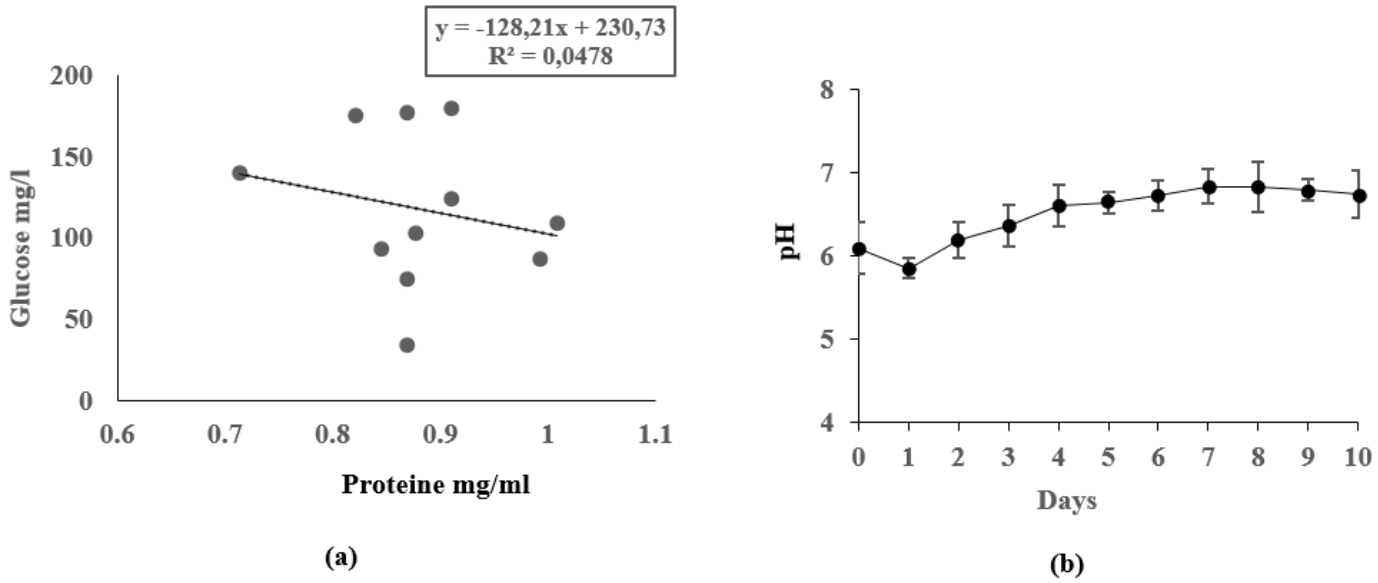

Figure 7

Study of hydrolysis in CMC medium during 10 days of fermentation; (a): Correlation between glucose and protein concentration; (b): pH variation during the hydrolysis period

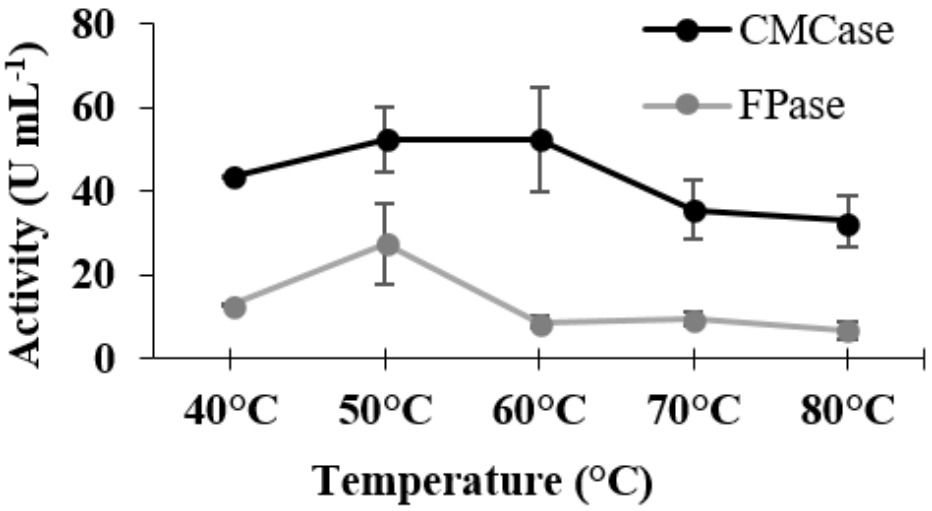

Figure 8

Temperature effect on endoglucanase (CMCase) and Exoglucanase (FPase) activity of A.iranicus streain 34-MS

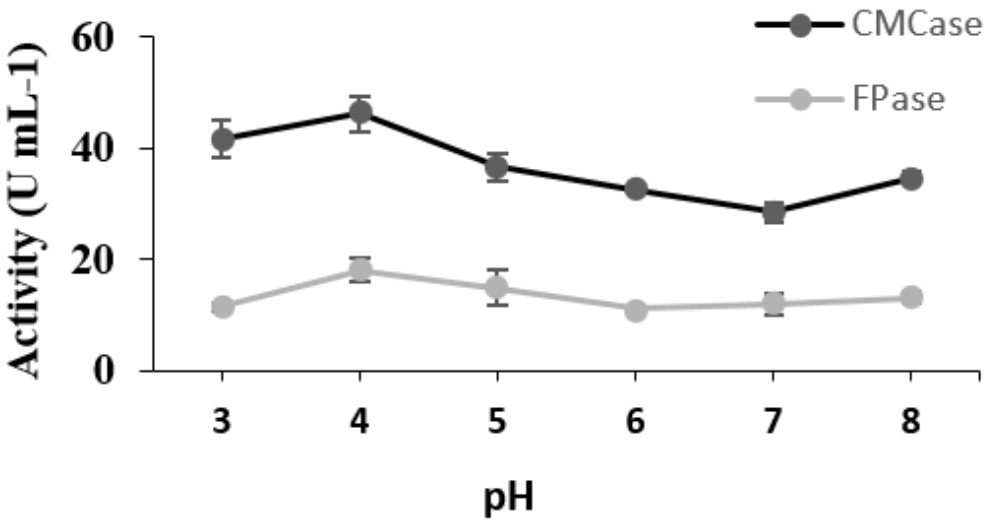

Figure 9

pH effect on endoglucanase (CMCase) and Exoglucanase (FPase) activity of A.iranicus streain 34-MS