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## Qualitative and quantitative screening of coprophilous fungi for cellulase production

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### ABSTRACT

Filamentous fungi, especially the herbivore dung-inhabiting (coprophilous), are widely explored for cellulase production owing to their ability to secrete abundant extracellular enzymes, rapid growth, and adaptability to diverse substrates. Microbial enzymes are favoured over plant and animal-derived enzymes due to their ease of production, cost-effectiveness, and genetic manipulation potential. This study isolated, identified, and determined cellulase activity of fungal species from cow dung. Three composite cow dung samples were collected from Ikare-Akoko, Oka-Akoko, and Supare-Akoko. The macroscopic and microscopic features of fungal isolates were used to identify them. Screening for cellulase-producing fungi was assessed using the carboxymethyl cellulose (CMC) agar plate screening method. Cellulase is produced by submerged fermentation, quantified by dinitrosalicylic acid (DNSA) enzyme assay methods, and all experiments were performed in triplicate. The results revealed that the sample from Supare (SUP) had the highest fungal counts of  $2.9 \times 10^5$  CFU/g, followed by Oka-Akoko ( $2.7 \times 10^5$  CFU/g), and the lowest fungal count of  $1.8 \times 10^5$  CFU/g was obtained from Ikare-Akoko. A total of nineteen (19) fungal species belonging to 12 genera (*Acremonium*, *Alternaria*, *Aspergillus*, *Byssoschlamys*, *Candida*, *Curvularia*, *Eurotium*, *Fusarium*, *Geomyces*, *Penicillium*, *Rhizopus*, and *Trichoderma*) were identified. Thirteen (13) fungal species demonstrated cellulolytic activity with varying efficiencies. *Alternaria tenuissima* had the highest cellulase activity of 5.79 U/mL, followed by *Aspergillus fumigatus* (5.31 U/mL) and *Penicillium* sp (5.14 U/mL). Moderate activity was observed in *Trichoderma harzianum*, *Curvularia geniculata*, and *Byssoschlamys nivea*, while *Aspergillus glaucus* showed the least activity (0.88 U/mL). This study revealed that cow dung harbours diverse cellulolytic fungi with cellulase-producing capacity. Therefore, these fungi are promising candidates for sustainable cellulase production in biofuel generation, waste management, and related biotechnological applications.

### INTRODUCTION

The potential application of cellulases for bioconversion of cellulosic biomass has drawn attention to their use on a worldwide scale (Bhardwaj et al., 2021; Ejaz et al., 2021; Ilic et al., 2023). Cellulose biodegradation by cellulases are preferred over chemical degradation (Sari et al., 2017). A class of enzymes known as cellulases can work together to hydrolyze cellulose (Maravi and Kumar, 2020). Applications of cellulases span from food, brewery and wine, animal feed, pollution treatment, textile and laundry, detergent, pulp and

paper industries to biofuel production (Asmare, 2018; Bayer et al., 2019; Jayasekara et al., 2019; Araujo et al., 2021; Rani et al., 2024; Khan et al., 2025; Hussain et al., 2025).

Cellulase is made up of three main parts. First, the enzyme endo- $\beta$ -glucanase produces glucose and cello-oligosaccharides by rupturing internal glycosidic bonds in the amorphous portion of cellulose. Second, an exo- $\beta$ -glucanase that catalyzes the conversion of the reducing and non-reducing ends of the cellulosic fibril into either cellobiose or glucose units. Lastly,  $\beta$ -glucosidase which

hydrolyzes cellobiose into glucose. Together, the three enzymes can break down cellulose to create glucose units (Bhatia et al., 2024). Cellulose is among the most common biomaterials on earth (Fatema et al., 2022), yet a significant portion of its annual production (estimated at  $1.5 \times 10^{12}$  tons) remains unutilized or wasted (Al-Kharousi et al., 2015).

Numerous investigations have concentrated on investigating different microorganisms for the production of cellulase, especially in biomass conversion and biofuel production (Saroj & Narasimhulu, 2021; Singh et al., 2021). Fungi are known as the best cellulose decomposers in nature (Corbu et al., 2023). More than 80% of cellulose degradation in nature is performed by fungi. The Ascomycota and basidiomycota groups of fungi are made up of several fungal species that degrade cellulose (Hernandez et al., 2018). Fungi can secrete abundant extracellular cellulose, and this makes them desirable candidates for industrial use. Cellulases have been produced from various fungal species such as *Aspergillus*, *Penicillium* (Prasanna et al., 2016), and *Trichoderma* (Ellilä et al., 2017; Naher et al., 2021).

The discovery and utilization of microorganisms for industrial enzyme production have revolutionized various industries such as food, textiles, biofuels, pharmaceuticals, and waste management (Maravi & Kumar, 2020). Microbial enzymes are favoured over plant- and animal-derived enzymes due to their ease of production, cost-effectiveness, and genetic manipulation potential (Gat et al., 2022; Bautista-Cruz et al., 2024; Nazir et al., 2024). Cellulases are vital for converting lignocellulosic biomass into valuable products (Chaudhari et al., 2023), reducing environmental waste, and supporting sustainable industrial processes (Chaudhari et al., 2023; Bhatia et al., 2024).

Herbivore dung-inhabiting (Coprophilous) fungi can develop on new dung in a few weeks and at any time of year (Holter, 2016; Pauline et al., 2022; Calaça et al., 2024). Herbivore dung provides a habitat for coprophilic fungi (Calaça et al., 2020; Melo et al., 2020). Herbivore dung usually creates an environment that permits fungal growth, for example, nutrient and pH of the dung is above 6.5. These nutrients and pH have positive effects on both the sporulation and growth of fungi (Thilagam

et al., 2015; Frank et al., 2017). The investigation of fungi from the dung of some herbivores, such as those from cow dung, may aid in the production of economical and effective cellulases, which would be advantageous to several applications. Therefore, this study identified and evaluated cellulolytic fungi from cow dung for enzyme production.

## METHODS

### Sample collection

Three (3) composite fresh cow dung samples per location were collected as early as 7:30 a.m. from Ikare-Akoko, Supare-Akoko, and Oka-Akoko. Each sample was placed in a zip lock bag, labeled, and immediately conveyed to the laboratory of Department of Microbiology Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria. The samples were stored at 4 °C throughout the study period.

### Isolation and enumeration of fungal species

Potato Dextrose Agar (PDA) medium was used to isolate and enumerate of fungi. A PDA powder (39 g/L) was prepared in an Erlenmeyer flask, corked with cotton. This medium was then sterilized at 121 °C for 15 minutes in a bucket autoclave. The PDA medium was left cool to 45 °C after sterilization and poured into sterile Petri plates and allowed to set. Serial dilution of each cow dung sample was performed and 100 µL of dilution 10<sup>3</sup> was cultured onto a potato dextrose agar plate and incubated for 7 days at 30°C. Discrete fungal colonies were picked and sub-cultured to obtain a pure isolate. Stock cultures were maintained on potato dextrose agar slants at 4 °C (Helal et al., 2022; Pahnwar et al., 2023; Pepe et al., 2025).

### Macroscopic characteristics of the isolated fungi

The fungal cultural characteristics were studied by considering their colonial features such as colour, shape, size, and hyphae of each fungus (Fapohunda et al., 2020; Kichu et al., 2020).

### Fungal staining and Microscopic characteristics

The morphological traits of fungi are examined using the lactophenol cotton blue (LPCB) staining procedure. A fragment of each fungus was taken from the edge of a 5 days old fungal colony using an inoculating needle and placed on a sterile glass slide. A drop of LPCB stain was applied, and it was carefully teased-out to form a thin smear. The preparation was carefully covered with a coverslip. The slide was then examined under a microscope at

both low ( $\times 10$ ) and high ( $\times 40$ ) magnifications. The morphology of each fungus, including its hyphae, conidia, and spores were analyzed and compared with those of Pictorial Atlas of Soil and Seed Fungi, Compendium of Soil Fungi (Knoll et al., 2023).

#### Qualitative screening of cellulolytic activity from fungal species

Cellulase-producing fungi were screened on CMC agar plates containing (g/L):  $\text{NaNO}_3$ , 2.0;  $\text{KH}_2\text{PO}_4$ , 1.0;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.5; KCl, 0.5; CMC, 10.0; peptone, 0.2; agar, 15.0. Each plate was spot inoculated with a 6 mm mycelial mat of fungal pure culture in triplicate and incubated at 28 °C for 5 days. After incubation, plates were flooded with 1% Congo red solution for 15 minutes then destained with 1 M NaCl solution for 15 minutes. The diameter of the zone of decolonization around each colony was measured and the enzymatic index was determined using the following formula:

$$\text{Enzymatic index} = \frac{\text{Clear zone diameter} - \text{Colony diameter} \times 100}{\text{Colony diameter}}$$

#### Quantitative screening of cellulolytic activity from fungal species

One gram of carboxymethyl cellulose (CMC) was placed in 250 mL Erlenmeyer flask with 100 mL basal medium of (g/L):  $\text{KH}_2\text{PO}_4$ , 2g;  $(\text{NH}_4)_2\text{SO}_4$ , 1.4 g; Urea, 0.3g;  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 0.3g;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.3g; peptone, 1.0g; Tween 80, 0.2%;  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ , 2.0 mg;  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 5.0 mg;  $\text{MnSO}_4 \cdot 2\text{H}_2\text{O}$ , 1.6 mg;  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 1.4 mg. A 6 mm mycelial mat of each fungus was placed in each 100 ml of the production medium (pH 5.5). The inoculated flasks were placed in a rotary incubator with a shaker, set at 28 °C and agitated at 150 rpm for 7 days. The cellulase produced was filtered through Whatman No. 1 filter paper and filtrate was centrifuged at 6000 rpm for 15 min to obtain crude enzyme (Islam & Roy, 2018; Marđetko et al., 2021).

#### Assay for cellulase-producing fungal species

Dinitrosalicylic acid (DNSA) enzyme assay method as described by Islam & Roy (2018) was used. One gram of CMC was added to 100 mL of distilled water (substrate). A 1 mL of substrate and 1 mL of the enzyme were mixed and incubated for 30 minutes at 30 °C. The reaction was terminated by the addition of 2 mL of 3, 5-dinitrosalicylic acid reagent. The mixture was boiled for 5 minutes and placed on ice for another 5 minutes. A

spectrophotometer was used to measure the absorbance at 540 nm. A unit/milliliter (U/mL) of cellulase is expressed as the amount of enzyme that catalyzes the conversion of 1  $\mu\text{mol}$  of substrate per minute per milliliter.

## RESULTS AND DISCUSSION

The study successfully isolated and screened cellulolytic fungi from cow dung samples collected from Iwaro-Akoko, Supare-Akoko, and Ikare Akoko. Nineteen (19) fungi were obtained from cow dung samples, out of which thirteen showed cellulolytic activity. The highest number of cellulolytic fungi was obtained from samples collected from Supare-Akoko and Ikare-Akoko. Fungal counts of cow dung samples collected in selected areas in Ikare-Akoko, Oka-Akoko, and Supare-Akoko (Table 1). The results revealed that the sample from Supare (SUP) had the highest fungal count of  $2.9 \times 10^5$  CFU/g, followed by OKA, which is a sample collected from Oka-Akoko with a count of  $2.7 \times 10^5$  CFU/g, while the least fungal count of  $1.8 \times 10^5$  CFU/g was observed in IKA (sample from Ikare-Akoko). A total of 19 fungal species belonging to 12 genera were identified, and they include *Acremonium*, *Alternaria*, *Aspergillus*, *Byssochlamys*, *Candida*, *Curvularia*, *Eurotium*, *Fusarium*, *Geomyces*, *Penicillium*, *Rhizopus*, and *Trichoderma* (Table 2).

Table 1. Fungal counts of fungi

No	Sample/Location	Fungal Count (CFU/g)
1.	IKA/Ikare-Akoko	$1.8 \times 10^5$
2.	OKA/Oka-Akoko	$2.7 \times 10^5$
3.	SUP/Supare-Akoko	$2.9 \times 10^5$

#### Screening for cellulase-producing fungal species

Table 3 shows the screening of all the fungal species for cellulase activity. *Byssochlamys nivea* (IKA 17) had the highest enzymatic index of 0.316, followed by *Aspergillus acidus* (IKA 1) with enzymatic index of 0.292 (Figures 1 and 2), *Aspergillus niger*, OKA B6 (0.129), *Alternaria tenuissima*, SUP 17 (0.123), *Trichoderma harzianum* (0.068), *Aspergillus glaucus*, SUP A (0.063), *Aspergillus fumigatus*, IKA 22 (0.055), *Alternaria arborescens*, IKA 32 (0.054), *Penicillium* sp (0.042), *Curvularia geniculata* (0.041), *Eurotium rubrum* (0.036), *Rhizopus stolonifer* (0.035), while *Aspergillus* sp (SUP 15)

had the least enzymatic index of 0.027. Other fungi had no noticeable enzymatic activity (Table 3).

Table 4 shows the cellulase activity of the fungal species extrapolated from the maltose standard curve (Figure 3). The results revealed that the fungi varied significantly in their enzyme production capacities. *Alternaria tenuissima* had the highest cellulase activity with  $5.79 \pm 0.01$  U/mL, followed closely by *Aspergillus fumigatus* ( $5.31 \pm 0.00$  U/mL) and *Penicillium* sp ( $5.14 \pm 0.12$  U/mL). These species, therefore, represented the most efficient cellulase producers among the isolates. Moderately, cellulase activities were

observed in *Trichoderma harzianum* ( $4.77 \pm 0.17$  U/mL), *Curvularia geniculata* ( $4.04 \pm 0.21$  U/mL), and *Byssoschlamys nivea* ( $3.98 \pm 0.18$  U/mL). Also, *Rhizopus stolonifer* ( $2.18 \pm 0.07$  U/mL) showed moderate activity compared to the top producers. The lowest cellulase activities were found in *Alternaria arborescens* ( $1.68 \pm 0.01$  U/mL), *Aspergillus* sp ( $1.69 \pm 0.19$  U/mL), *Aspergillus niger* ( $1.54 \pm 0.01$  U/mL), *Eurotium rubrum* ( $1.43 \pm 0.34$  U/mL), *Aspergillus acidus* ( $1.19 \pm 0.01$  U/mL), and *Aspergillus glaucus* ( $0.88 \pm 0.04$  U/mL), which was the least cellulase producer.

Table 2. Morphological and microscopic characteristics of fungal species

No.	Sample code	Colony Colour	Nature of hyphae	Spore-producing structure	Probable organism
1	IKA 14	White powdery that floccose with age	Septate	Simple, long awl-shaped phialides (cells that produce conidia with inconspicuous collarettes)	<i>Acremonium</i> sp
2	IKA 32	Dark gray	Septate	Conidiophores with chains of muriform conidia	<i>Alternaria arborescens</i>
3	IKA 13	Blackish-brown	Septate	Conidiophore with a muriform conidium each	<i>Alternaria</i> sp
4	SUP 17	Brown	Septate	Each conidiophore bears a single long, unbranched chain of conidia	<i>Alternaria tenuissima</i>
5	IKA 1	Brown	Septate	Conidiophore bearing vesicles with a layer of phialides that produce long chains of conidia	<i>Aspergillus acidus</i>
6	SUP 15	Grayish yellow	Septate	Vesicle with biseriate phialides producing white conidia	<i>Aspergillus</i> sp.□
7	IKA 22	Grayish-green	Septate	Vesicle with uniseriate phialides producing conidia	<i>Aspergillus fumigatus</i>
8	SUP A	Green with yellow patches	Septate	Conidiophore bearing vesicle with biseriate phialides producing conidia	<i>Aspergillus glaucus</i>
9	OKA B6	Dark brown	Septate	Vesicle with phialides producing conidia	<i>Aspergillus niger</i>
10	IKA 17	White	Septate	Conidia with an ellipsoidal shape borne on short cylindrical phialides. Asci with 8 ascospores	<i>Byssoschlamys nivea</i>
11	IKA 35	Creamy	Septate	Buds formed ovoid yeast cells with blastoconidia which elongated to form pseudohyphae	<i>Candida tropicalis</i>
12	IKA 23	Dark brown	Septate	Conidiophores are unbranched and geniculate (bent-like shape), bearing conidia	<i>Curvularia geniculata</i>
13	SUP 25	Reddish brown	Septate	Cleistothecia containing asci with yellowish-brown disciform ascospores.	<i>Eurotium rubrum</i>

14	IKA 26	White	Septate	Flask-shaped phialides bearing sickle-shaped macroconidia (formed on sporodochia) and microconidia	<i>Fusarium oxysporum</i>
15	IKA 28	Yellowish-brown	Septate	Arthroconidia (formed directly on tips or sides of branched conidiophores)	<i>Geomyces pannorum</i>
16	IKA 9	Gray	Septate	Brush-like conidiophores with phialides producing chains of conidia	<i>Penicillium sp</i>
17	SUP 13	Grayish black	Non-Septate	Sporangium bearing sporangiospores with a columella	<i>Rhizopus stolonifer</i>
18	SUP 18	Yellowish green	Septate	Phialides bearing conidia in a clustered manner on branched conidiophores	<i>Trichoderma harzianum</i>
19	IKA 6	Greenish brown	Septate	Highly branched conidiophores with multiple phialides clustered at the tips	<i>Trichoderma sp</i>

Table 3. Screening of all fungi from cow dung for cellulolytic activity

No	Organism	Enz. index
1	<i>Acremonium sp</i> (IKA 14)	0
2	<i>Alternaria arborescens</i> (IKA 32)	0.054
3	<i>Alternaria sp</i> (IKA 13)	0
4	<i>Alternaria tenuissima</i> (SUP 17)	0.123
5	<i>Aspergillus acidus</i> (IKA 1)	0.292
6	<i>Aspergillus sp</i> (SUP 15)	0.027
7	<i>Aspergillus fumigatus</i> (IKA 22)	0.055
8	<i>Aspergillus glaucus</i> (SUP A)	0.063
9	<i>Aspergillus niger</i> (OKA B6)	0.129
10	<i>Byssoschlamys nivea</i> (IKA 17)	0.316
11	<i>Candida tropicalis</i> (IKA 35)	0
12	<i>Curvularia geniculata</i> (IKA 23)	0.041
13	<i>Eurotium rubrum</i> (SUP 25)	0.036
14	<i>Fusarium oxysporum</i> (IKA 26)	0
15	<i>Geomyces pannorum</i> (IKA 28)	0
16	<i>Penicillium sp</i> (IKA 9)	0.042
17	<i>Rhizopus stolonifer</i> (SUP 13)	0.035
18	<i>Trichoderma harzianum</i> (SUP 18)	0.068
19	<i>Trichoderma sp</i> (IKA 6)	0



Figure 1. Screening for cellulolytic activity in *Byssoschlamys nivea* (IKA 17)

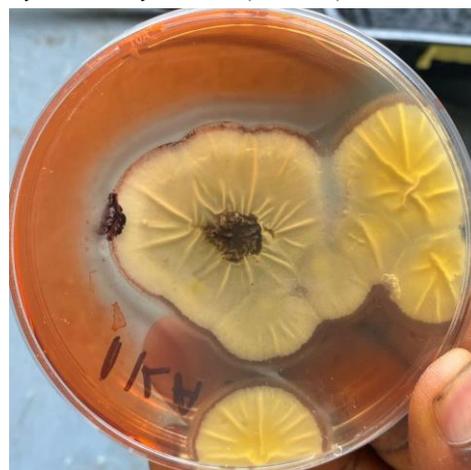


Figure 2. Screening for cellulolytic activity in *Aspergillus acidus* (IKA 1)

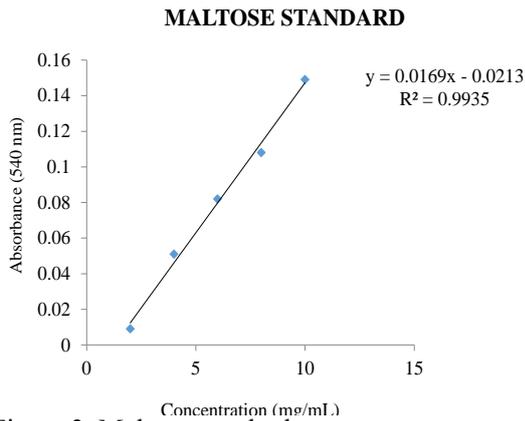


Figure 3. Maltose standard curve

Table 4. Quantification of cellulase from cow dung

No	Organism	Cellulase Activity (U/mL)
1	<i>Alternaria arborescens</i> (IKA 32)	1.68±0.01
2	<i>Alternaria tenuissima</i> (SUP 17)	5.79±0.01
3	<i>Aspergillus acidus</i> (IKA 1)	1.19±0.01
4	<i>Aspergillus</i> sp (SUP 15)	1.69±0.19
5	<i>Aspergillus fumigatus</i> (IKA 22)	5.31±0.00
6	<i>Aspergillus glaucus</i> (SUP A)	0.88±0.04
7	<i>Aspergillus niger</i> (OKA B6)	1.54±0.01
8	<i>Byssoschlamys nivea</i> (IKA 17)	3.98±0.18
9	<i>Curvularia geniculata</i> (IKA 23)	4.04±0.21
10	<i>Eurotium rubrum</i> (SUP 25)	1.43±0.38
11	<i>Penicillium</i> sp (IKA 9)	5.14±0.12
12	<i>Rhizopus stolonifer</i> (SUP 13)	2.18±0.07
13	<i>Trichoderma harzianum</i> (SUP 18)	4.77±0.17

Key: ±SEM = Standard error of the mean

*Aspergillus*, *Alternaria*, *Eurotium*, and *Trichoderma* were the most common among the fungi identified. This agrees with earlier studies, which reported that these fungi were among the most efficient producers of cellulases used in industries. This aligns with the earlier report of Behera et al. (2016), who also identified cellulolytic fungi from agricultural and organic waste sources.

Screening for cellulolytic activity showed that some isolates such as *B. nivea*, IKA 17 (Figure 1) had the highest enzymatic index of 0.316, followed by *A. acidus* (0.292), IKA 1 (Figure 2), *A. niger*, OKA B6 (0.129), *A. tenuissima*, SUP 17 (0.123), while *Aspergillus* sp (SUP 15) had the least enzymatic index of 0.027. In contrast, some isolates, such as IKA 28 (*G. pannorum*), showed little or no activity. This variation may be due to differences in species, growth conditions, and enzyme secretion ability. This is consistent with the reports by Prasanna et al. (2016), who demonstrated

that *Penicillium* species were effective cellulase producers, and Ellilä et al. (2017), who emphasized the cellulolytic potential of *Trichoderma* species.

The quantitative assay confirmed that certain isolates produced higher cellulase activities. This is comparable with cellulase activity values reported in other studies involving *Aspergillus niger* and *Trichoderma* species. The high enzyme activity showed that cow dung (coprophilous) fungi can be good candidates for cellulase production, especially fungal species such as *A. tenuissima*, *A. fumigatus*, and *Penicillium* sp were good cellulase producers. This result corroborates the reports of Prasanna et al. (2016), who demonstrated that *Penicillium* species were effective cellulase producers, and Ellilä et al. (2017), who emphasized the cellulolytic potential of *Trichoderma* species. Similarly, Eder et al. (2018) and Baig & Saleem (2021) reported significant cellulase activity from *A. niger* grown on rice husk and sawdust substrates. The implication of these findings is important because cellulases are widely used in industries such as paper and pulp, textiles, animal feed, detergents, and waste management. Using fungi from cow dung could provide a cheaper and more sustainable source of cellulases compared to commercial strains.

## CONCLUSION

This study shows that cow dung is a rich source of cellulolytic fungi with great capacity to produce cellulases for the bio-economy. The best isolates produced enzyme activity of up to 5.79 U/mL, indicating strong potential for use in industries. Overall, this study concludes that cow dung cellulolytic fungi can be explored as cost-effective and environmentally friendly sources of cellulase enzymes. For future work, optimization of fermentation conditions and molecular identification of the high-performing strains are recommended to enhance large-scale cellulase production.

## CONFLICTS OF INTEREST

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

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