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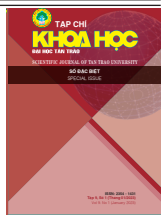

ISOLATION AND SCREENING OF INDIGENOUS MICROORGANISMS CAPABLE OF DEGRADING CELLULOSE TO TREAT HEMP HURD IN THANH HOA PROVINCE

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Abstract:

Hemp Hurd has not yet found an optimal treatment method, therefore, in this paper, we isolated and screened native microorganisms with high cellulose-degradation capability for potentially effective treatment. Native microorganisms were isolated on Gauze I and Hans, media then CMC-as activity was determined. Next, the species names of screening microorganisms were determined by total DNA isolation, PCR and Sequencing. The results showed that 02 bacterial strains and 06 actinomycete strains were selected from 32 isolates. Then, we optimized the growing conditions of the screened microorganisms. Among actinomycetes strains PU1.1, PU2.1 gave the highest weight loss results, therefore they were selected for further study. Our results showed that TG2.1 and CM4.1 are Bacillus subtilis. PU1.1 is Streptomyces lilacs, while PU1.2 is Streptomyces Misiones. The selected microorganisms belong to risk group level 1 (safety group). Thus, these strains can be used for further experiments in the research and production of microorganism-derived bio-products to treat hemp hurd in Thanh Hoa province.

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PHÂN LẬP, TUYỂN CHỌN VI SINH VẬT BẢN ĐỊA PHÂN GIẢI XENLULO CAO ĐỂ SẢN XUẤT CHẾ PHẨM VI SINH VẬT XỬ LÝ BÃ THẢI CÂY GAI TẠI THANH HÓA

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Từ khóa:

Cây gai dầu
Sự cô lập
*S*àng lọc
Phân hủy cellulose
Vi sinh vật

Tóm tắt

Bã thải cây Gai cho đến giờ vẫn chưa tìm ra một phương pháp xử lý tối ưu, vì vậy, trong bài báo này, chúng tôi đã phân lập và tuyển chọn vi sinh vật bản địa phân giải xenlulo cao để có thể xử lý hiệu quả hơn. Nghiên cứu tiến hành phân lập trên môi trường GauzeI và Hans sau đó xác định hoạt tính CMC-aza, hoạt tính của vi sinh vật để tuyển chọn. Tiếp theo, xác định tên loài của các chủng được tuyển chọn bằng phương pháp tách DNA tổng số, PCR và Sequence. Kết quả cho thấy, đã chọn được 02 chủng vi khuẩn và 06 chủng xạ khuẩn từ 32 isolates để tiến hành các nghiên cứu tiếp theo. Các chủng này đã được xác định các điều kiện nuôi cấy phù hợp cho sinh trưởng, phát triển. Đánh giá hoạt tính sinh học của vi sinh vật dựa trên kết quả hoạt tính CMC-aza và tỷ lệ giảm khối lượng của mẫu thí nghiệm so với mẫu đối chứng, Kết quả đã chọn được TG2.1 và CM4.1 là Bacillus subtilis, PU1.1 là Streptomyces lilaceus, PU1.2

Kết quả phân loại TG2.1 và CM4.1 là Bacillus subtilis, PU1.1 là Streptomyces lilaceus, PU1.2 là Streptomyces misionensis và các chủng vi sinh vật tuyển chọn trên thuộc nhóm rủi ro cấp độ 1 (Nhóm an toàn). Vì vậy, có thể sử dụng các chủng này cho các thí nghiệm tiếp theo trong nghiên cứu sản xuất chế phẩm sinh học xử lý bã thải cây Gai tại Thanh Hóa

1. Introduction

An Phuoc import-export and production development investment JSC has invested in a textile yarn factory and developed a raw material area in Cam Tu commune, Cam Thuy district, Thanh Hoa province with a capacity of 10,000 spindles/year. The demand for fresh hemp stems is 693,000 tons. The hemp bark is collected for making fibers, and the remaining biomass such as stems and leaves should be discarded. On average, each hemp consists of 15% bark, and 85% of stem and leaves. This is equivalent to a factory emitting 594 tons of waste per year. Proteins, lipids, minerals, and vitamins in the residue after some time will ferment to create odors, polluting the air and water source. However, at present, there is no appropriate and effective method to treat hemp waste. The waste residue is mainly composted by time-consuming traditional

methods, and the quality of the compost is not high. Therefore, it is necessary to conduct research and apply microbial inoculants to accelerate the composting process of hemp waste into organic fertilizer reducing the environmental pollution.

In the past years, numerous studies have reported treating and reusing by-products and wastes in the horticulture industry, including technologies for composting and processing them into microbial organic fertilizers. Microbial organic fertilizers play important roles in retaining moisture, increasing porosity, and soil texture, limiting leaching, and being relatively safe for humans [2]. Using extracellular cellulases and peroxidases from microorganisms to treat agricultural by-products has brought undeniable benefits to the agricultural industry [1,3]. The used microbial species are relatively abundant in nature [4]. Many microorganisms have been reported to be capable of decomposing cellulose and can be applied to convert agro-forestry by-products into organic fertilizers [4,5,6,7].

However, there is virtually no ideal group of such microorganisms. Thus, this study aims to further research and select microorganisms capable of decomposing agricultural by-products such as hemp waste, and converting substrates for organic fertilizer production may address the aforementioned issues.

2. Research object, content, and method

2.1. Research subjects

Microbial strains with high cellulose-degradation activity were isolated from soil samples collected in Thanh Hoa province.

2.2. Research location

- Hemp production areas in Thanh Hoa province.

- Soils and Fertilizers Research Institute.

2.3. Research content, materials, and methods

2.3.1. research content

- Isolation and selection of microbial strains with high cellulose-degradation ability from soil samples collected in Thanh Hoa.

- Characterizing biological properties of the selected microbial strains.

- Identification of species and biosecurity assessment of the selected microbial strains.

2.3.2. Research Materials

- Soil samples from cultivating hemp areas and soil samples from crop fields were collected in several districts in Thanh Hoa.

- Chemicals and necessary tools used in the experiment.

Content of the microbial culture media:

- Hans medium: $(NH_4)_2SO_4$: 1g; $MgSO_4.7H_2O$: 0,1g; $CaCl_2$: 0,1g; K_2HPO_4: 0,5g; KH_2PO_4: 0,5g; NaCl: 6g; Cao men: 0,1g; CMC: 0,1g; agar: 12g; H_2O: 1.000ml; pH=7.

- Gauze I medium: K_2HPO_4: 0,5g; KH_2PO_4: 0,5g; $MgSO_4.7H_2O$; NaCl: 0,5g; KNO_3: 1g; $FeSO_4.7H_2O$: 0,01g; H_2O: 1.000ml; pH=7; starch: 20g; agar: 12g;

- CMC medium: CMC: 1g; agar: 12g; H_2O: 1.000ml.

- Physiological saline solution: NaCl: 0,85g; distilled water: 1000 ml.

Main buffers:

- CTAB buffer: 100 mM Tris HCl, pH = 9,0; 20 mM NaEDTA, pH = 8,0; 1,4M NaCl; 2% Cetyl Trimethyl Ammonium Bromide (CTAB); 0,2% β- mercapto ethanol.

- Lysis buffer: 50 mM EDTA; 50 mM Tris - HCl;; 3% SDS; 1% 2 - mercapto ethanol; chloroform; TE : saturated phenol (1/1 phenol saturated in TE); TE: 10 mM Tris - HCl (pH = 8,0); 1 mM EDTA; 3M NaOAc (pH = 8,0); isopropanol, ethanol 70%.

- Original solution:

+ Saturated phenol TE.

+ Chloroform: isomylacohol (24/1)

+ TE (10 mM Tris - HCl pH = 8.0; 1 mM EDTA).

- Electrophoresis buffer 10xTBE: Tris Base: 108g; boric acid 55g; 0.5M EDTA (pH = 8.0) 40 ml; 1000 ml water.

2.4. Methods

2.4.1. Isolation and determination of biological activities of indigenous microorganisms capable of degrading cellulose

- Isolation of microorganisms capable of degrading cellulose:

The isolation process is carried out as follows. An amount of 10g sample was taken into a mortar and crushed, then the crush was placed into a flask containing 90 ml of sterile physiological saline. The flask was put on a shaker for 30 min, then incubated for several min to collect 10-1 times diluted sample solution. Next, serial dilution is performed. Specifically, we pipetted 1ml of the suspension (10-1 dilution) and put it into a test tube containing 9ml of sterile distilled water, then mix well to get 10-2 times dilution; We pipetted 0.1 ml (100 µl) of the 10-2 dilution then released it into an Eppendorf tube containing 0.9 ml of sterile distilled water, and mix well to obtain a 10-3 dilution; Finally, we took 0.1 ml of solution at concentrations from 10-4 to 10-6, spread evenly on Petri dishes containing GauzeI and Hans medium then incubated at 28°C and 37°C for 24 to 48 hours. The colonies of different shapes and colors were separated, purified, and kept in test tubes for later use.

- Determination of CMC-use activity [8, 9]:

Biomass of microbial strains after 48 h of culture was centrifuged to remove the residue, then 1ml of the suspension was put into the prepared agar holes on Petri dishes containing solid CMC medium (CMC: 1g; Agar: 12g; H_2O: 1,000 ml). The agar plates were kept in the incubator for 24 h, then the agar plates were coated with Lugol solution.

The biological activity of CMC-use was determined based on the formation of the size of the clearing zone, and the clearing zone surrounding the agar hole (the difference between the diameter of the transparent circle (D) and the diameter of the agar hole (d).

- Assessing the mass reduction ratio of samples compared to control:

Step 1: Post-harvest remains of hemp are moistened (45-50% humidity). We weighed 100g of the remains and then put them into a 500ml conical flask.

Step 2: Add 10 ml of microbial culture solution to each sample flask.

Step 3: Place the flasks at room temperature for 30 days to monitor. Next, wash several times with distilled water then remove dissolved impurities, and the undissolved part. The weight loss ratio was calculated by the formula:

$$\mathbf{X}(\%) = \frac{mo-mt}{mo} x100 \quad (1)$$

X(%): Ratio of weight loss of the tested sample

mt: Remaining dry weight of the tested sample

mo: Remaining dry weight of control sample

2.4.2 Assessing biological characteristics of selected microbial strains

Gram staining, aerobic, anaerobic, growing on suitable culture medium, heat, and salt tolerance was conducted by following the methods of N.W. Schaad – 2002 [10]. Including the following reactions: Gram staining, aerobic, anaerobic, growing on suitable culture medium, heat, and salt tolerance [14].

2.4.3. Identification of selected microbial strains

- Classification of microorganisms was carried out as previously described [10, 11]. Briefly, total DNA was isolated according to the method described by Sambrook and Russell (1989). The 16S rRNA gene was amplified by PCR using primers GF1 5'-TAACACATGCAAGTCGAACG-3' and GR1 5'-GGTGTGACGGGCGGTGTGTA-3'. PCR products were checked on 1% agarose gel, purified using the PureLinkTM – DNA Purification kit (Invitrogen) and sequenced on an ABI PRISM®3100-Avant Genetic Analyzer (Applied Biosystems, Foster City, CA). USA) at the Institute of Biotechnology, Vietnam Academy of Science and Technology, comparing the corresponding sequences on the GenBank database.

- Biosafety assessment of selected microorganisms

The selected strains were evaluated for safety by the safety rating list of microbial strains issued by the Scientific Institute of Public Health, Division of Biosafety and Biotechnology, Belgium.

3. Results and discussion

3.1. Isolation and selection of microbial strains with high cellulose-degradation ability from soil samples collected in Thanh Hoa.

3.1.1. Isolation

Isolation of cellulose-degrading microorganisms from 20 soil samples from the topsoil of a crop field, 25 soil samples from hemp production areas in Thanh Hoa, and 2 samples of hemp-based organic fertilizer in Ngoc Lac district, the results showed that:

On hemp-cultivated soil, 04 strains of bacteria and 06 strains of actinomycetes with low cellulose-

degradation capability were isolated from 20 soil samples (zone of clearance ranged from 0.7 to 2.7 cm).

On soil taken from crop fields, 07 strains of bacteria and 13 strains of actinomycetes with low cellulose-degradation capability were isolated from 20 soil samples (zone of clearance ranged from 1.5 to 2.7 cm).

From there, 2 strains of cellulose-degrading actinomycetes with high activity (zone of clearance from 3.0 to 3.5 cm) were selected for further study.

Table 1. Isolated microorganisms capable of degrading cellulose

No	Sample name	Isolation source	Bacteria		Actinomycetes	
			Strain	Clearing zone (D-d, cm)	Strain notation	Clearing zone (D-d, cm)
1	TG1	Hemp cultivated soil				
2	TG2	Hemp cultivated soil	TG2.1	2,5	TG2.2	0,9
3	TG3	Hemp cultivated soil				
4	TG4	Hemp cultivated soil				
5	TG5	Hemp cultivated soil	TG5.1	2,0	TG5.2	0,7
6	TG6	Hemp cultivated soil				
7	TG7	Hemp cultivated soil				
8	TG8	Hemp cultivated soil			TG8.1	2,7
9	TG9	Hemp cultivated soil	TG9.1	0,8		
10	TG10	Hemp cultivated soil				
11	TG11	Hemp cultivated soil			TG11.1	0,9
12	TG12	Hemp cultivated soil	TG12.1	2,0		
13	TG13	Hemp cultivated soil				
14	TG14	Hemp cultivated soil				
15	TG15	Hemp cultivated soil			TG15.1	1,2
16	TG16	Hemp cultivated soil				
17	TG17	Hemp cultivated soil				
18	TG18	Hemp cultivated soil			TG15.1	2,5
19	TG19	Hemp cultivated soil				
20	TG20	Hemp cultivated soil				
21	TG21	Hemp cultivated soil				
22	TG22	Hemp cultivated soil				
23	TG23	Hemp cultivated soil				
24	TG24	Hemp cultivated soil				
25	TG25	Hemp cultivated soil				
26	CM1	Topsoil	CM 1.1 CM 1.2	1,7 2,0	CM1.1	2,2
27	CM2	Topsoil				
28	CM3	Topsoil			CM 3.1	2,7
29	CM4	Topsoil	CM4.1	2,7	CM4.2 CM4.3	2,0 2,5
30	CM5	Topsoil			CM5.1	2,2
31	CM6	Topsoil				
32	CM7	Topsoil	CM7.1	1,9		
33	CM8	Topsoil			CM8.1	1,7
34	CM9	Topsoil			CM 9.1 CM 9.2	2,0 2,7
35	CM10	Topsoil			CM10.1	1,5

No	Sample name	Isolation source	Bacteria		Actinomycetes	
			Strain	Clearing zone (D-d, cm)	Strain notation	Clearing zone (D-d, cm)
36	CM11	Topsoil	CM 11.1	2,3		
37	CM12	Topsoil				
38	CM13	Topsoil				
39	CM14	Topsoil			CM 14.1	2,3
40	CM15	Topsoil				
41	CM16	Topsoil	CM 16.1	1,5	CM16.2	2,0
42	CM17	Topsoil				
43	CM18	Topsoil	CM 18.1	1,6	CM 18.2	1,5
44	CM19	Topsoil			CM19.1	2,2
45	CM20	Topsoil				
46	PU1	Compost			PU1.1	3,0
47	PU2	Compost			PU2.1	3,5

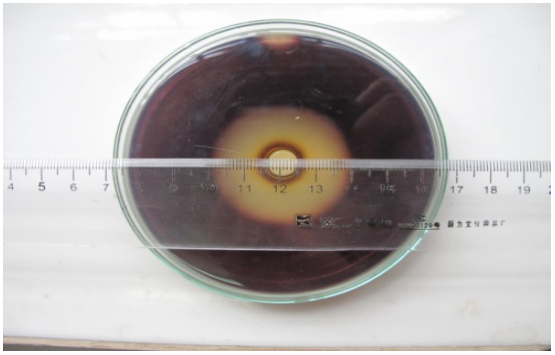

Figure 1. Evaluation of the cellulose-degrading activity of the isolates

3.1.2. Selection of microbial strains with high cellulose-degradation activity

From the results of isolating 32 strains of microorganisms capable of degrading cellulose on media containing synthetic cellulose substrate, CMC, two strains of bacteria (TG2.1, M4.1) and 06 actinomycete strains (TG8.1, CM4.3, CM 3.1, CM 9.2, PU1.1, PU2.1) were chosen to evaluate the ability to degrade hemp Hurd, natural cellulose substrate (experimented in a triangular flask). The selected microorganisms had the largest cellulosic clearing zone (D- d from 2.5 cm to 3.5 cm).

After shaking the culture for 48 hours, the 2 bacterial strains (TG2.1, CM4.1) and 6 strains of actinomycetes (TG8.1, CM4.3, CM 3.1, CM 9.2, PU1.1, PU2.1), each culture suspension was transferred to a triangular flask containing crushed hemp hurd then incubated for 30 days. The weight reduction of the hemp hurd treated with cellulose-degrading microorganisms compared to mocked treatment was shown in **Figure 2**. The rate of post-composted hemp is calculated by excluding the decomposed parts.

Figure 2. Visualization of hemp hurd weight reduction after 30 days of cellulose-degrading microbial treatment

Table 2. The reduction rate of microbial culture suspension-treated samples after 30 days of incubation

No	Microbial suspension	Weight (g)	Reduction rate (%)
1	TG2.1	50,75	8,62 ± 0,11
2	CM4.1	50,50	12,59 ± 0,33
3	TG8.1	50,30	15,82 ± 0,18
4	CM4.3	50,84	7,30 ± 0,04
5	CM 3.1	50,50	12,65 ± 0,30
6	CM 9.2	40,51	28,41 ± 0,13
7	PU1.1	20,34	62,80 ± 0,20
8	PU2.1	20,60	58,68 ± 0,14
9	Control	60,30	-

The results showed that the addition of microbial culture suspension was effective in degrading significantly the hemp hurd in the flasks. The supplemented with culture suspension of CM 9.2, PU1.1, and PU2.1 strains indicated the highest weight reduction suggesting these strains may be capable of highly degrading cellulose.

3.2. Species identification and biosecurity assessment of selected microbial strains

3.2.1. Determination of several biological characteristics of selected cellulose-degrading microorganisms

To optimize growth conditions and relevant enzymes production of the microbial strains (TG2.1, CM4.1; TG8.1, CM4.3, CM 3.1, CM 9.2, PU1.1, PU2.1) and to promote the cellulose-degradation capability, several morphological, physiological and biochemical characteristics such as oxygen demand, pH, temperature, carbon source utilization, salt tolerance and others were investigated.

Table 3. Colony morphology characteristics of the selected cellulose-degrading microorganisms on Hans and GauzeI medium

No	Strain	Isolating source	Characteristics	Cellulolytic potential (D-d, mm)
1	TG2.1	Hemp production area, Cam Thuy, Thanh Hoa	Size of colonies: 2 - 3 mm, round-shape, a yellowish, serrated edge, wrinkled surface	2,5
2	CM4.1	Topsoil, Ngoc Lac, Thanh Hoa	Size of colonies: 3 - 3,5 mm, round-shape, yellowish, flat surface	2,7
3	TG8.1	Hemp production area, Cam Thuy, Thanh Hoa	Size of colonies: 2 - 3 mm, light pink in the perimeter, dark pink in the center area, substrate mycelium	2,7
4	CM4.3	Hemp production area, Cam Thuy, Thanh Hoa	Size of colonies: 2 - 3 mm, round-shape, white in the perimeter, grey in the center area, substrate mycelium	2,5
5	CM3.1	Hemp production area, Cam Thuy, Thanh Hoa	Size of colonies: 1,5 - 2 mm, round-shape, white, substrate mycelium	2,7
6	CM 9.2	Topsoil, Tho Xuan, Thanh Hoa	Size of colonies: 1,5 - 2 mm, round-shape, white in the perimeter, grey in the center area, substrate mycelium	2,7
7	PU1.1	Compost	Size of colonies: 3 - 4 mm, round-shape, grey in the perimeter, brown in the center area, substrate mycelium	3,0
8	PU2.1	Compost	Size of colonies: 3 - 4 mm, round-shape, grey in the perimeter, brown in the center area, substrate mycelium	3,5

Table 4. Physiological and biochemical characteristics of selected cellulose-degrading microorganisms

Strain notation	Oxygen demand	Gram	Optimal pH	Culture medium	Optimal temperature (^{0}C)	Heat resistance (^{0}C)	Salt tolerance (% NaCl)
TG2.1	+	+	6,5 – 7,5	Hans, King B	28	37	0,05 – 0,6
CM4.1	+	+	6,5 – 7,5	Hans King B	28	37	0,05 – 0,6
TG8.1	+	+	6,5 – 7,5	Gauze I	37	45-55	0,05 – 0,6
CM4.3	+	+	6,5 – 7,5	Gauze I	37	45-55	0,05 – 0,6
CM 3.1	+	+	6,5 – 7,5	Gauze I	37	45-55	0,05 – 0,6
CM 9.2	+	+	6,5 – 7,5	Gauze I	37	45-55	0,05 – 0,6
PU1.1	+	+	6,5 – 7,5	Gauze I	37	45-55	0,05 – 0,6
PU2.1	+	+	6,5 – 7,5	Gauze I	37	45-55	0,05 – 0,6

Gauze I: Gauze medium supplemented with CMC powder

The results showed that the selected actinomycete strains and bacteria are Gram-positive, spore-forming, aerobic, and medium salt tolerant. A salt concentration of 0.05 – 0.6%, pH between 6.5 and 7.5 (optimal pH = 7), and high-temperature resistance (37-45) are optimal growth conditions for these isolated microbial strains (**Table 4**).

To evaluate the ability to use different carbon sources, the selected microorganisms were cultured in suitable media (bacteria – Hans; actinomycetes – GauzeI) in which a variety of carbon sources was examined.

It was shown that glucose, as the primary carbon source, provides the best growth conditions compared to other carbon sources (**Table 5)**.

Table 5. Effects of different carbon sources on the growth of selected microorganisms

Strain	Growing level				Zone of clearance (size: D-d, mm)			
	Glucose	Saccharose	Starch	CMC	Glucose	Saccharose	Starch	CMC
TG2.1	++++	+++	++	++++	2,0	2,0	1,5	2,0
CM4.1	++++	+++	++	++++	1,5	1,2	1,0	1,5
TG8.1	++++	++	++++	+	2,5	2,0	2,6	1,6
CM4.3	++++	+++	++++	++	2,5	2,0	2,5	2,0
CM 3.1	++++	+++	++++	+++	1,7	1,7	1,7	1,5
CM 9.2	++++	+++	++++	+++	2,5	2,0	2,4	1,8
PU1.1	++++	++	++++	++	3,2	2,7	3,2	2,5
PU2.1	++++	+++	++++	+++	3,0	3,0	3,5	2,5

Note: *++++: Strong growth; +++: Fair growth; ++: Moderate growth; +: Weak growth*

Our results suggested that the selected microorganisms can utilize different carbon sources. Furthermore, glucose and CMC (but not starch) is the optimal carbon source for the growth of bacterial strains. Actinomycetes strains could grow well when glucose and starch are the primary carbon source. However, CMC and saccharose provide intermediate and poor growing conditions as compared to glucose and starch.

We next compared the biological characteristics of the selected bacteria and actinomycetes in this study with Bergey and Waksman's taxonomy, S. A [12, 13]. It was found that selected bacterial strains are from the genus *Bacillus*, while actinomycetes belong to the genus *Streptomyces*.

3.2.2 Classification of microbial strains and biosafety assessment of selected microbial strains

Identification of the names of 04 selected microorganisms with high cellulose-degrading activity (cellulosic bacteria

TG2.1 and CM4.1; actinomycetes PU1.1 and PU1.2) was based on sequencing of 16S ribosomal RNA then compared with available gene sequences in EMBL database by FASTA 33 method. Sequencing results were analyzed with Software Clustal W 1.83. The assembled DNA sequences were compared by NCBI BLAST.

Table 6. Identification of selected microbial strains using NCBI BLAST

No	Strain	Microorganism with highest similarity	Query Coverage* (%)	Max Ident** (%)	Risk group***
1	TG2.1	Bacillus subtilis	100	99	1
2	CM4.1	Bacillus subtilis	100	99	1
3	PU1.1	Streptomyces lilaceus	100	99	1
4	PU1.2	Streptomyces misionensis	100	99	1

** Max Ident (percentage of the sequence is used to match with the database)*

*** Querry coverage (a percentage that matches with the database)*

**** The evaluation follows guidelines published by the Scientific Institute of Public Health, Division of Biosafety and Biotechnology, Belgium.*

Sequencing results indicated that two cellulose-degrading bacterial strains TG2.1 and CM4.1 were *Bacillus subtilis*. Additionally, PU1.1 is likely *Streptomyces lilaceus*, and PU1.2 is likely *Streptomyces Misiones*.

Moreover, the obtained biological characteristics compared with the Belgian document (a reference on the level of influence of microorganisms on humans, animals and plants) suggested that the above 04 selected microorganisms belong to the risk group of level 1 (Safety group). However, it is important to note that the selected bacterial strains are classified as safe, the results should be regarded as a reference because of the lack of the assessment of pathogenicity.

4. Conclusion

Thirty-two strains of microorganisms capable of degrading cellulose have been isolated from collected soil samples from various places in Thanh Hoa province. Eight strains of microorganisms with the strongest ability to degrade cellulose have been selected, including 02 bacterial strains (TG2.1, M4.1), 06 actinomycete strains (TG8.1, CM4.3, CM 3.1, CM 9.2, PU1.1, PU2.1); cellulosic clearing zone is ranged from 2.5 cm to 3.5 cm. Preliminary results of classification of the selected microorganisms suggest that the bacterial strains are from the genus Bacillus and actinomycetes belonging to the genus Streptomyces.

Sequencing results of 16S RNA of the two cellulose-degrading bacteria strains imply that TG2.1 and CM4.1 are Bacillus subtilis; PU1.2 is Streptomyces lilaceus, PU1.2 is Streptomyces Misiones is with very high query coverage (99%). The above microorganisms appear to belong to the risk group of level 1 (Safety group), safe for humans, animals, and plants suggesting no harmful impact to the environment.

Therefore, these strains can be used for further experiments in the production of microbial inoculants to potentially treat hemp hurd in Thanh Hoa province as well as other areas. However, other questions remain to encourage investigate such as in-depth testing the biological activity of the selected strains in a larger scale and what mechanism drives these microorganisms to degrade cellulose.

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