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ISOLATION AND CHARACTERIZATION OF CELLULOSE DEGRADING BACTERIA, BACILLUS FLEXUS FROM GOBAR GAS DIGESTER

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ABSTRACT

The major objective of the research is to isolate the potential bacteria from gobar gas digester and analyze the cellulose degrading ability of bacteria for degrading the category III biomedical waste. The standard techniques such as morphological, microscopic, biochemical characterization and preliminary cellulose screening were carried to assess the morphological and biochemical characteristics of selected bacteria. The result showed *Bacillus flexus* (I2) is the potential cellulose degrading bacteria availed for the degradation of category III biomedical waste.

KEYWORDS: ***Biomedical waste, Isolation, B.flexus, Cellulose Degrading Bacteria, Characterization.***

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INTRODUCTION

Hospital wastes refers to all wastes, biological or non biological from hospitals, that is discarded and not intended for further use and these include: pathological and cellulosic waste, hazardous chemicals, radioactive wastes, stock cultures, blood and blood products, animal carcasses, pharmaceutical wastes, pressurized containers, batteries, plastics, low level radioactive wastes, disposable needles, syringes, scalpels and other sharp items.[1] These waste are categorized as biomedical waste, these biomedical waste based on their toxicity are classified into several categories. Category: I (human anatomical waste), Category: II (needle and sharp waste), Category: III (cellulosic waste), Category: IV (discarded medicines, Radionuclide waste).[2] There is deleterious effect produced from these biomedical wastes. The source from which frequent effect of health issues occur is majorly Category III waste.[3] WHO (1999) reported that, about 85% of health hazard to the health workers, public and air hospital waste is non-hazardous, 10% infective, among the 10% of infective waste 3% is the category III waste.[4] The impact of biomedical waste is immense, it causes major pollution problems in the environment.[5]The pathogens present in the cellulosic waste can easily contaminate air.[6] Other biomedical waste such as cytotoxic drugs, radionuclide waste can cause serious issues majorly carcinogenic effect.[7] Environment is at a great risk based on the percentage of biomedical waste excluded from hospitals and labs.[8] Serious pollution problem are faced through the release of toxic agents from biomedical waste.[9] Several measures were taken to tackle this problem. Improper recycling and waste management systems in developing countries are majorly responsible for plastic pollution. Incineration is the major and easier techniques applied to decrease the percentage of biomedical waste.[10] There are several demerits for the incineration technique, the prime demerit is by burning the biomedical waste, the amount of biomedical waste will be drastically reduced creatin air pollution and soil pollution.[11] Among the various techniques available, innovative and economical method are recycling of medical waste.[12] Several studies were carried out to isolate cellulose degrading organisms from anaerobic digesters.[13] The major cellulose degrading bacteria isolated from the digester were *Pseudomonas, Cellulomonas* sp*, Bacillus* sp*, Micrococcus* sp*,* and *Cellovibrio* sp.[14]The cellulose system consist of either secreted or cell associated enzymes belonging to different classes categorized based on their mode of action and structural properties.[15] The efficiency of cellulose degradation for these bacterial species has a significant role to play in conversion of unusable cellulosic waste to usable form.[16] The aim of the present study is to isolate a potential bacteria from the gobar gas digester for degrading the category III biomedical waste.

MATERIALS AND METHODS

Sample Collection

Gobar gas digester samples were collected from Palakkad (10.7867°N, 76.6548°E), Kerala.

Isolation and Characterization of Bacteria

The bacterial colonies were isolated and enumerated.[2] Bacteria were identified and classified based on their physical, microscopic and biochemical characteristics. Various biochemical tests such as Indole, Methyl Red, Voges Prausker,Citrate Test IMViC), Triple sugar Iron(TSI), Carbohydrate fermentation, Catalase, Urease, Starch hydrolysis test were performed.[17]

Degradation of cellulose screening

The ten isolates were inoculated by spot inoculation and incubated at 24-48 hrs. 1% of Congo red solution was added to the spot inoculated plates and then excess stains were removed by 1M NaCl and the zone of clearance were observed.[18]

Molecular characterization

Among the ten isolates based on the screening, isolate 2 (I2) isolate was found to be having the potential for cellulose degradation. Bacterial DNA isolation and 16SrRNA sequencing were carried.[19]

STATISTICAL ANALYSIS

The results obtained from the present investigation were statistically analyzed, SPSS 16.0 and the result data were expressed as mean ± S.D.

RESULT AND DISCUSSION

Isolation of bactreia from gobar gas digester

One gram of gobar gas digester sample was taken serially diluted and followed by spread plating in 10^{-4} dilution; ten unique colonies were selected and morphologically identified. These results are in agreement with the isolation of bacteria from gobar gas digester.[20] Further works are in agreement with identification of cellulose degradation.[21] After the morphological identification, the isolates were labeled as I1, I2, I3, I4, I5, I6, I7, I8, I9 and I10.These ten isolates were then taken for further study (Table 1and 2).

Preliminary identification of bacteria

Three gram positive bacterial isolates and seven gram negative bacterial isolates were identified using series tests such as IMViC, TSI, Carbohydrate fermentation, Catalase, Urease, Starch hydrolysis. The microscopic and biochemical characters of isolated (ten) bacterial species are presented in table 3.The microscopic and biochemical characteristics reveals the properties of the bacteria isolated.

Degradation of cellulose screening

Among the ten isolates, I2 (Isolate 2) has produced large zone of clearance by efficiency to degrade the cellulose which was assessed. Figure 1 refers the cellulose degradation efficiency by ten isolates which were isolated from gobar gas digester. The former studies reveals that the bacteria isolated from the gobar gas digester where majorly having cellulose degrading ability (Mandels *et al.,*1969).

Molecular characterization

DNA isolation and 16SrRNA sequencing of the I2 isolate were performed. After the molecular identification, the I2 (Isolate 2) was found as *B.flexus.* Phylogenetic analysis was done by CLUSTAL - W. (Figure 2).

Table 1

Enumeration of microorganisms

Serial dilution	Colonies obtained (one quadrant)	Colonies obtained(four quadrant) (CFU)
10^{-2}	101 ± 5.0	404 ± 0.3
10^{-3}	96 ± 7.3	384 ± 1.0
10^{-4}	**77** ± 5.2	**308** ± 0.8
10^{-5}	66 ± 1.3	264 ± 1.0
10^{-6}	52 ± 1.0	208 ± 1.2
10^{-7}	15 ± 1.4	60 ± 1.0

From the serially diluted tubes 10^{-4} was showing unique colonies which was further used for the study

Table 2

Morphological characterizations

Sl no:	Name	Size	Shape	Color	Margin	Surface	Elevation	Transparency	Viscosity
1	I1	Large	Circular	Creamy	Entire	Glossy	Flat	Opaque	Moist
2	I2	Large	Irregular	White	Entire	Finely granular	Flat	Tranlucent	Dry
3	I3	Large	Circular	White	Entire	Glossy	Flat in growing	Opaque	Moist
4	I4	Small	Circular	White	Entire	Glossy	Flat	Opaque	Moist
5	I5	Small	Circular	White	Entire	Glossy	Flat	Opaque	Mucoidal
6	I6	Large	Irregular	White	Diffuse	Smooth	Flat in growing	Opaque	Moist
7	I7	Small	Irregular	Creamy	Diffuse	Glossy	Flat	Opaque	Moist
8	I8	Medium	Irregular	Yellow	Diffuse	Smooth	Flat	Opaque	Moist
9	I9	Large	Irregular	Creamy	Diffuse	Glossy	Flat in growing	Opaque	Dry
10	I10	Small	Circular	Yellow	Entire	Glossy	Flat	Transparent	Dry

The colonies obtained from 10^{-4} tubes were morphologically characterized
Key: I –Isolate

Table 3

Microscopic and biochemical examination

Sl no:	Bacterial isolates	Microscopic features	Indole	Methyl red	VP	Citrate utilization	Catalase	Urease	Starch hydrolysis	TSI	Glucose	Sucrose	Lactose
1	I1	Gram negative	-ve	-ve	-ve	-ve	+ve	-ve	-ve	AS	-VE	-VE	-VE
2	I2	Gram positive	-ve	+ve	+ve	-ve	+ve	-ve	+ve	AS	-VE	-VE	G & NA
3	I3	Gram negative	-ve	-ve	-ve	-ve	+ve	-ve	-ve	AS	-VE	-VE	G & NA
4	I4	Gram negative	-ve	-ve	-ve	-ve	+ve	-ve	-ve	AKS&AKB	-VE	-VE	G & NA
5	I5	Gram negative	-ve	-ve	-ve	-ve	+ve	-ve	-ve	AS	-VE	-VE	NA &NG
6	I6	Gram negative	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-VE	G & NA	-VE	NG &NA
7	I7	Gram negative	-ve	+ve	+ve	-ve	+ve	-ve	-ve	AS	-VE	-VE	NG &NA
8	I8	Gram positive	+ve	+ve	+ve	+ve	+ve	-ve	+ve	AKS & AB	AG	AG	NG &NA
9	I9	Gram positive	-ve	-ve	+ve	+ve	+ve	-ve	+ve	AS & AB	G &NA	AG	NG &NA
10	I10	Gram negative	+ve	+ve	-ve	-ve	+ve	-ve	-ve	AKS & AB	AG	AG	AG

KEY :- ***AS –ACID SLANT***
-VE – NO CHANG
AG – ACID & GAS
AB –ACID BUTT
AKS –AKLANINE SLANT
G & NA – GAS & NO ACID
AKB –ALKALINE BUTT
NG & NA –NO ACID & NO GAS,
A & NG – ACID & NO GAS
TSI – TRIPLE SUGAR IRON

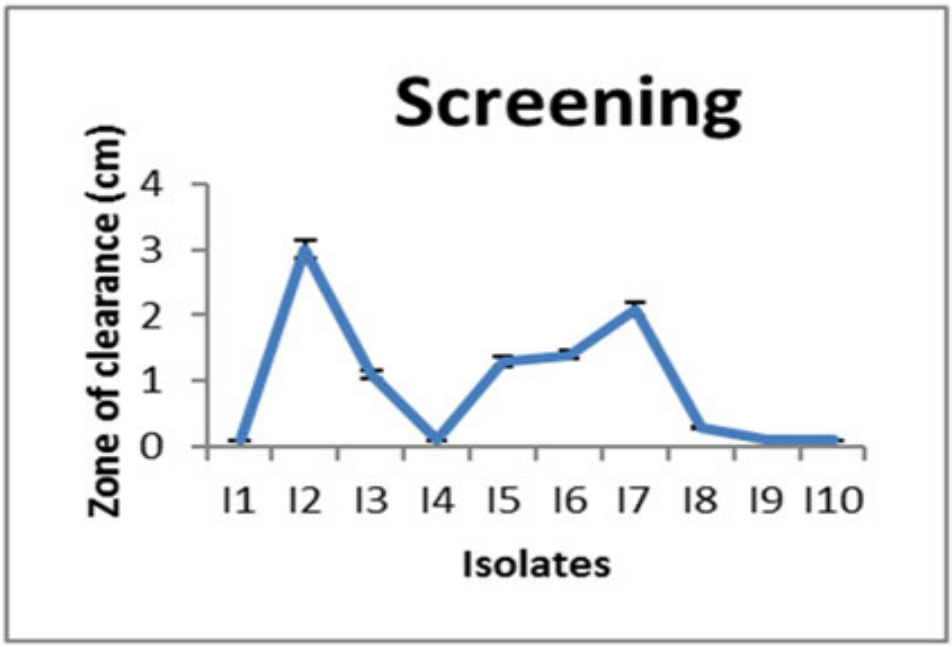

Figure 1
Celllulase screening test

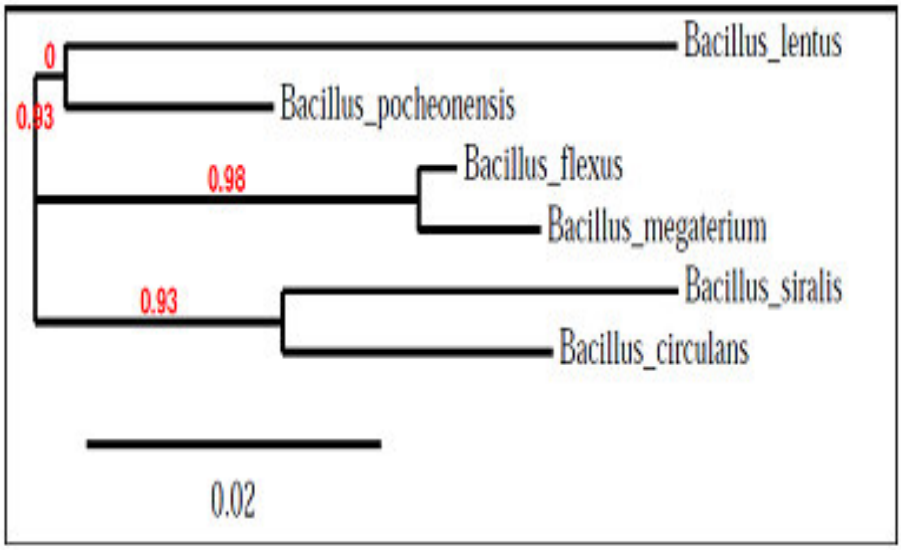

Figure 2
Phylogenetic characterizations

CONCLUSION

The bacterial species were isolated from the gobar gas digester, after pure culturing, it was labeled as I1, I2, I3, I4, I5, I6, I7, I8, I9 and I10. The efficiency of bacterial species to degrade cellulose was analyzed through the screening. Among the ten isolates, I2 (Isolate 2) produced major zone of clearance, this organisms has the potential ability to degrade the cellulosic compounds. The potential isolate I2 was identified as *Bacillus flexus*. The potential isolate may have the ability to degrade the biomedical waste (Category III).

CONFLICT OF INTEREST

Conflict of interest declared none.

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