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Pseudozyma flocculosa Y-1: A potent hydrolytic yeast isolate from the biogas digester effluent

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Abstract

Vegetable waste serves as source of nuisance in markets. The current unscientific disposal methods lead to environmental pollution. Biomethanation is a promising biological treatment method for vegetable wastes. Biomethanation process converts organic matter into biogas and manure. The different types of microorganisms are involved in production of biogas. Hydrolysis is the first step in biomethanation and different kinds of aerobic and anaerobic microorganisms are involved in the process. The biomethanation experiment was carried out in 5litre biogas digester under ambient temperature conditions. The yeasts were isolated by using Sabourauds agar medium. The yeast isolates were subjected to determine their ability for the production of hydrolytic enzymes. The yeast isolate with maximum hydrolytic potential was identified by morphological, cultural, biochemical characterization and molecular identification by 24 S rRNA.

Keywords: Biomethanation, yeasts, hydrolytic, cellulolytic, ligninolytic, etc.

Introduction

Vegetable waste is produced everyday in plenty amount in markets. The collection, transportation and disposal of vegetable waste is a very serious issue these days. Current unscientific treatment methods results in air, land and water pollution[1].

Biomethanation process converts organic waste in anaerobic condition to produce biogas and fertilizer. The process can be divided into four major steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis[2]. Hydrolysis step degrades insoluble complex organic matter into its soluble monomers with the help of extracellular enzymes produced by hydrolytic microorganisms.

Biomethanation is accomplished by a consortium of microorganisms working synergistically. Different types of microorganisms mainly bacteria, yeast, molds and actinomycetes are involved in biogas production process. There are several reports on involvement of bacteria in hydrolysis step[3-6]. The involvement of fungi in biomethanation is also reported well[7-10]. There are very few studies on isolation of yeasts from biogas digester. The work was undertaken to study hydrolytic yeasts from digester effluent.

Materials and methods

Collection and preparation of sample: The vegetable wastes collected from the local vegetable market mainly contained Potato, Onion, Cabbage, Cauliflower, Tomato and Brinjal. The paste prepared from equal quantities of these individual wastes was used as a substrate for biomethanation study.

Biomethanation of vegetable waste: Biomethanation study was performed in locally fabricated 5L digesters. The digesters were operated with feeding of the vegetable waste slurry at organic loading rate 0.320g VS/l.d, and substrate pH of 7.0 at mesophilic conditions for 40 days.

Standard Plate Count (SPC) and Isolation of yeasts: The SPC for yeasts from digester effluent was carried out in triplicates using Sabourauds agar plates. The representative yeast isolates obtained were preserved at refrigeration temperature.

Determination of hydrolytic capabilities of yeast isolates: Starch agar, Skim milk agar, Gelatin agar, Tributyrin agar, Carboxy-methyl cellulose agar and Mineral salt medium (MSM) agar containing lignin with methylene blue as indicator dye were used for determining amylolytic, caseinolytic, gelatinolytic, lipolytic, cellulolytic and ligninolytic potential of yeast isolates respectively. The individual cultures were spot inoculated onto respective media plates and room temperature incubation was carried for 2-3 days. Amylolytic activity was detected by exposing the starch agar plates to iodine crystals. Skim milk agar plates and tributyrin agar plates were observed for clear zones around growth. Gelatinolytic activity was detected by using Fraziers reagent. Cellulolytic activity was determined by congo red detection method[11]. The MSM-lignin agar plates were monitored for decolorization of methylene blue dye[12].

The degradation potential of individual culture was determined in terms of hydrolysis capacity (HC)[13].

Identification of yeast isolates: The preliminary identification of potent yeast isolates from digester effluents upto species level was carried out by morphological, cultural and biochemical characterization as per standard literature[14-19]. Further, the confirmation of potent hydrolytic yeast isolates was done by molecular characterization using 24S rRNA analysis.

Results and discussion

Biomethanation of vegetable waste: The range of biogas yield for mixture of six vegetable wastes at ambient temperature conditions was found to be 510-1340mL/d. The average biogas production was 0.633L/g VS.d with the 59% methane.

SPC and Isolation of yeasts: SPC of bacteria from digester effluents was found to be $1.11X10^4$cfu/mL. The two yeast isolates obtained were maintained in refrigeration conditions.

Determination of hydrolytic capabilities of yeast isolate: Two yeasts obtained from digester effluent were tested for determining their hydrolytic potential. The Y-1 isolates was selected as exhibited highest hydrolytic potential as compared to other (Table-1).

Identification of yeast isolates: The morphological, cultural and biochemical characterization of potent yeast isolate revealed the identity as *Pseudozyma flocculosa* Y-1. The colonial morphology of Y-1 is shown in Figure-1. The 24 S rRNA analyses of selected yeast isolate confirmed the identification results (Figure-2 and Table-2).

Discussion: Two yeasts were isolated from digester effluent run on vegetable waste. They were screened for production of hydrolytic enzymes. The identification of the potent hydrolytic yeast isolate was confirmed by molecular methods as *Pseudozyma flocculosa.* It showed production of amylases, proteases, lipases and lingo-cellulases. *Pseudozyma flocculosa* is reported to have biopesticidal activity. This basidiomycetous yeast showed strong antagonistic activity against powdery mildew fungi[20-22]. This antagonistic ability is proved to be due to the release of flocculosin[23,24].

Figure-1: Colonial morphology of Y-1 on Sabourauds agar (RT/3 days).

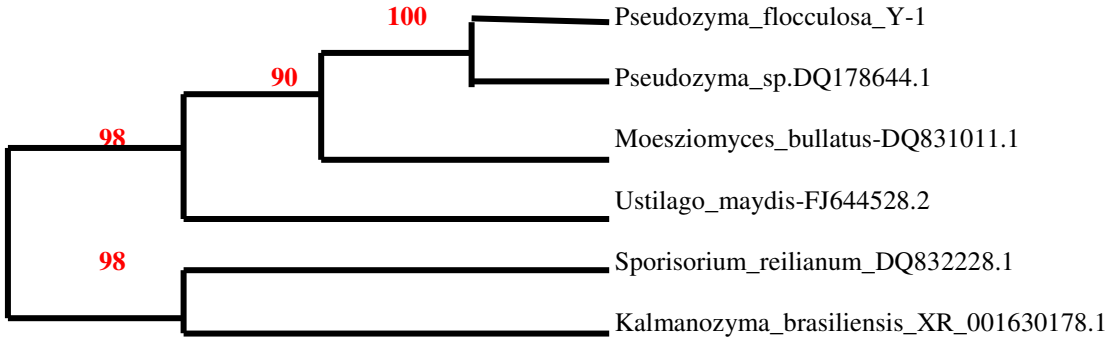

Figure-2: Phylogenetic tree of Y-1 (identified as *Pseudozyma flocculosa* Y-1).

Table-1: Enzymatic characteristics of yeast isolate (RT/48 hrs)

Yeast isolate code	Amylase (HC)	Caseinase (HC)	Gelatinase (HC)	Lipase (HC)	Cellulase (HC)	Ligninase (HC)
Y-1	1.31	1.06	1.25	1.04	1.89	1.01

Table-2: Blast N Report for Y1 (Query name: Y-1_contig_1, Query length: 1360)

Query		Subject					Score			Identities			Strand
Start	End	Description	AC	Length	Start	End	Bit	Raw	E-value	Match	Total	Pct (%)	
1	1349	JCC 207 26S	DQ178644.1	1421	72	1421	2486	1346	0	1349	1350	99	+/+
1	1358	AFTOL-ID 1820 25S	DQ831011.1	1437	40	1397	2475	1340	0	1352	1358	99	+/+
3	1358	AFTOL-ID 1398 25S	DQ094784.1	1399	1	1357	2422	1311	0	1342	1357	99	+/+
1	1358	TUB 019110 28S	FJ644528.2	2118	6	1364	2420	1310	0	1343	1359	99	+/+
1	1358	SRZ 2	FQ311431.1	642761	351770	350411	2399	1299	0	1340	1360	99	+/–
1	1358	AFTOL-ID 490 25S	DQ832228.1	1408	43	1402	2399	1299	0	1340	1360	99	+/+
1	1358	SY 62 28S	XR001099828.1	3660	509	1866	2392	1295	0	1337	1358	98	+/+
1	1358	GHG 001	KF737866.1	5685	2534	3891	2392	1295	0	1338	1359	98	+/+
1	1358	SSC 39	CP010935.1	140422	121665	123022	2386	1292	0	1337	1359	98	+/+
31	1358	AFTOL-ID 864 25S	AY745712.1	1343	1	1328	2381	1289	0	1315	1328	99	+/+

Result: *Pseudozyma flocculosa*

Strand No.	Description
JCC 207 26S	Pseudozyma sp., ribosomal RNA gene, partial sequence
AFTOL-ID 1820 25S	Moesziomyces bullatus, large subunit ribosomal RNA gene, partial sequence
AFTOL-ID 1398 25S	Ustilago tritici isolate, ribosomal RNA gene, partial sequence
TUB 019110 28S	Ustilago maydis voucher, ribosomal RNA gene, partial sequence
SRZ 2	Sporisorium reilianum, chromosome 10 complete DNA sequence
AFTOL-ID 490 25S	Sporisorium reilianum, large subunit ribosomal RNA gene, partial sequence
SY 62 28S	Pseudozyma hubeiensis, ribosomal RNA rRNA
GHG 001	Pseudozyma sp., small subunit ribosomal RNA gene, partial sequence
SSC 39	Sporisorium scitamineum strain, chromosome 23, complete sequence
AFTOL-ID 864 25S	Pseudozyma flocculosa isolate, large subunit ribosomal RNA gene, partial sequence

Conclusion

Pseudozyma flocculosa Y-1, yeast isolated from digester effluent shows good hydrolytic potential. This hydrolytic yeast thus seems to be having important role in hydrolysis step of biomethanation. The source of this yeast could be either vegetable waste or cattle dung slurry or both. This organism is also reported to have strong antagonistic activity against Powdery mildew fungi. Thus, with the further research, this organism can be a promising isolate for biofertilizers and also a eco-friendly biocontrol agent of the plant diseases which finally will help in sustainable development of agriculture.

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