International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS | Volume 9 | Special Issue 2- ICESMT| 2019 | 152-158
International Conference on "Empowering Society with Microbial Technology"
Held at Tuljaram Chaturchand College of Arts, Science and Commerce, Baramati - 413 102,
Dist. Pune, Maharashtra, India, 7-9 February 2019
| Research Article | Biological Sciences |Open Access |MCI Approved|
|UGC Approved Journal|

Isolation of *Thiobacillus* Species from Distillery Spentwash and Its Sulfide Oxidation Activity

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Received: 30 Jan 2019 / Accepted: 20 Feb 2019 / Published online: 01 Apr 2019
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Abstract
In the present study a total of 14 microorganisms were isolated from distillery spentwash samples using standard methods of isolation and enrichment. Three of them had the ability to oxidize sulfur in liquid culture and able to grow autotrophically using elemental sulfur. These microbial cultures were able to produce sulfates from sulfides within pH range of 3.6 to 8.0. Based on the morphological as well as physiological studies and on comparison with the reference strain of *Thiobacillus novellus* (NCIM 2858), all the isolates were confirmed to belong to the genus *Thiobacillus*. The effect of initial sulfide concentration on the activity of isolated *Thiobacillus* species was studied. Experiments on shake flask level (1L scale) were conducted using three selected isolates based on maximum pH reduction (SOM 05, SOM 06 and SOM 12) against reference strain with initial sulfide concentration of 200 mg/l. Out of three isolates, SOM 05 oxidized 200 mg/l of sulfide within 120 hours. SOM 06 and SOM 11 required 168 hours for oxidation of sulfide. Utilization of sulfide and formation of sulphate was estimated periodically. Presence of sulfides in raw spentwash results in H_2S formation (0.1-2.0 %, v/v) in biogas which is not desirable. The results from the study indicate that it is possible to isolate *Thiobacillus* species from distillery spentwash for further use in sulfide oxidation in sulfide rich effluents.

Keywords
Sulfide oxidizing, sulphate, spentwash, *Thiobacillus*

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INTRODUCTION:
Sulfur compounds are among the chief pollutants in the environment since they cause unfavorable impacts in the ecosystem. It is widely known that the discharge of hydrogen sulfide (H_2S) and reduced sulfur compounds from anthropogenic sources produces corrosion to metallic materials, terrible odour and under particular conditions, they are toxic to human health [1]. To reduce the harm caused by sulfur contaminants, several physicochemical processes are being employed which in most cases are costly and are not ecofriendly [2]. Biological techniques have appeared as probable alternatives to solve this problem since there are microorganisms which are

Indian Phytopathology (2019) 72:51–61
https://doi.org/10.1007/s42360-018-0075-2

RESEARCH ARTICLE

Detection of resistance to demethylation inhibitor fungicides in *Erysiphe necator* from tropical India by biological and molecular assays

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Received: 16 May 2018 / Revised: 3 October 2018 / Accepted: 13 October 2018 / Published online: 22 October 2018

Abstract
Fungicides of demethylation inhibitor (DMI) group are used worldwide for the management of *Erysiphe necator* but are associated with medium to high risk of development of resistance in the pathogen. Till date there was no report on the presence of DMI resistance in *E. necator* isolates from the major grape growing regions in tropical India, though there were instances of DMI fungicides providing less than accepted levels of powdery mildew control. In this study, 54 *E. necator* isolates were collected during 2015–2017 from vineyards located in different geographical regions of India. The isolates were tested for their sensitivity to the commonly used DMI fungicide, myclobutanil, using leaf disc bioassay. Four isolates were sensitive (MIC < 1.0 µg/ml), nine were moderately resistant (MIC 1.0 to < 10 µg/ml) and 41 were resistant (MIC > 10 µg/ml) to the fungicide myclobutanil. The resistance factor (RF) ranged from 1.5 to 295. In PCR amplification of a specific allele, the product specific for A495T mutation was produced only in the 43 isolates with RF > 4. The *CYP51* gene sequence analysis confirmed A495T mutation leading to Y136F change associated with high levels of resistance to DMI fungicides. Cross resistance studies among the DMI fungicides showed that 11 out of 13 myclobutanil resistant isolates were also resistant to difenoconazole and tetraconazole. Three myclobutanil sensitive isolates were also sensitive to difenoconazole and tetraconazole. Detection of resistance in *E. necator* isolates from the major grape growing region of tropical India stresses on the need for developing resistance management strategies.

Keywords *CYP51* gene · Fungicide resistance · Maharashtra · Powdery mildew

Introduction

Grapevines are mainly grown in the tropical regions of India, especially in Maharashtra and adjoining regions of Karnataka. In these regions, powdery mildew is a serious disease as it occurs throughout the year, except during the hot summer months (Sawant et al. 2015). In these regions the grapevines do not undergo dormancy and are pruned twice in a year, although the crop is harvested only during October to March which is designated as the fruiting season. Powdery mildew decreases the vine productivity and also affects the fruit quality resulting in lower marketable yield and poor shelf-life of table grapes (Ashtekar et al. 2017).

Various fungicides, and few low risk chemicals and biocontrol agents are available for disease management. However, fungicides are preferred during high risk periods. Until 2016, only four different FRAC groups were available for management of powdery mildew in India. These were the demethylation inhibitors (DMIs) and quinone outside inhibitors (QoIs), dinitrophenylcrotonates and inorganic.

The Fungicide Resistance Action Committee (FRAC) categorizes the fungicides into different groups based on their mode of action. The DMI and the QoI fungicides have a single-site mode of action which is associated with medium to high risk of development of resistance in pathogen populations. The other two fungicides, sulfur and dinocap, are non-systemic fungicides and are classified as low risk fungicides.

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[illegible] Vol. 92 No. 6 pp. 467–471 © Allerton Press, Inc., 2018

Assessment of the Utility of TRAP and EST-SSRs Markers for Genetic Diversity Analysis of Sugarcane Genotypes[1]

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Received December 19, 2017

Abstract—The TRAP and EST-SSRs technique were utilized for assessing the genetic diversity of 55 sugarcane genotypes (28 wildtypes and 27 cultivars). The total number of polymorphic bands amplified by TRAP primers ranged from 7 to 11 with an average of 9 amplified by SuSy + Arb2, SAI + Arb1, PPDK + Arb3 and PPDK + Arb2. The polymorphism was found to be high (≥50%), ranging from 78 to 100% with an average of 87% for all the markers. Polymorphic Information content (PIC) value ranged from 0.11 (SuSy+Arb2) to 0.44 (SuSy + Arb3) primers with an average of 0.27. Also, the highest resolving power (Rp) was found 6.9 in (SAI + Arb1) between nine primers. A total 15 sets of EST-SSRs primers were used for PCR amplification. 179 amplified fragments is produced which 174 were polymorphic. The total numbers of polymorphic alleles amplified by the various EST-SSRs markers were ranged from 5 (ESSR07 and ESSR10) to 22 (ESSR09), with an average of 13.5 alleles. The polymorphism was found to be high (≥50%), ranging from 83.33 to 100% with an average of 97.2% for all the markers. Polymorphic Information content (PIC) value ranged from 0.29 (ESSR15) to 0.83 (ESSR04) primers with an average of 0.56. Also, the highest resolving power (Rp) was found in 8.55 ESSR05 between 15 primers. For the TRAP nine combination primers was used for the work. A total 85 amplified fragments were produced which 74 (85%) were polymorphic. In cooperation of both the markers, dendrogram was constructed using UPGMA method from the present study. Hence, the TRAP and EST-SSRs techniques jointly helped to identify the genetic diversity of sugarcane clones/varieties which could be used in breeding program for sugarcane improvement.

Keywords: sugarcane, genetic diversity, molecular assisted selection, PIC, TRAP, EST-SSRs

DOI: 10.3103/S0095452718060026

INTRODUCTION

Sugarcane (*Saccharum* spp. hybrids) is the main bioenergy crop as well as important agricultural commodity, provides more than 70% of the global sugar and 30% ethanol production in tropical and subtropical countries [1–3]. In tropical climate, the cultivation of sugarcane is more successful in terms of cane yield and sugar recovery throughout the year [4]. Sugarcane is a C4 grass belongs to the genus *Saccharum* of the family Poaceae in the tribe Andropogoneae composed of hybrids derived from *S. officinarum* and *S. spontaneum* [5]. It is a genetically complex crop showing unstable and irregular chromosomal behavior [1]. The noble sugarcane varieties are developed from interspecific hybridization of *S. officinarum* L. and having high sugar content with less disease tolerance, and *S. spontaneum* which provides stress, disease tolerance, as well as high fiber content for biomass. The taxonomy and genetic constitution of sugar cane are complicated due to the complex inter-specific aneupolyploid genome ($2n = 80–120$). Some of the major wild species of sugarcane in the repository of *S. officinarum*, *S. sponteneum*, *S. barberi*, *S. robustum*, and *Erianthus arduniaceus* were used for the inter-specific and inter-generic crossing [6].

Genetic profiling by using DNA-based molecular marker technique is an important tool to study genetic diversity. Several molecular marker systems have been developed and used in genetic studies and taxonomic complexity of sugarcane [2, 7]. The genetic variability within sugarcane germplasm estimates the usage of the different molecular markers, including, RFLP (Restriction Fragments Length Polymorphism) [8], AFLP (Amplified Fragment Length Polymorphism) [9], RAPD (Random amplified polymorphic DNA) [10], SSR (Simple Sequence Repeat) or microsatellite [11], ISSR (Inter Simple Sequence Repeat) [12, 13], STMS (Sequence Tagged Microsatellite Site) [14], TRAP (Target Region Amplified Polymorphism) [15], 5S rRNA intergenic spacer sequences [16], has been used in explaining genetic diversity among different successions of sugarcane. Among all the molecular markers

[1] The article is published in the original

DOI : 10.5958/0974-0112.2018.00073.7

Indian J. Hort. 75(3): September 2018: 434-439

Detection of G143A mutation in *Erysiphe necator* and its implications for powdery mildew management in grapes

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ABSTRACT

Quinone outside inhibitor (QoI) fungicides are used worldwide for the management of *Erysiphe necator* but with associated problem of resistance development in the pathogen. Twenty nine *E. necator* isolates were collected during 2015-2016 from different geographical regions of India. In leaf disc bioassay using azoxystrobin, the EC_{50} of four isolates from research farms was <1 µg/ml, while 25 isolates from commercial vineyards had EC_{50} more than 115 µg/ml. The 256 fold resistance factor indicated G143A mutation. All the resistant isolates produced a 100 bp PCR product with G143A mutant allele specific primer which was not produced by the four sensitive isolates. A primer pair was designed for partial amplification of cytochrome b gene (*Cyt b*) and used for amplification of the gene from two resistant and two sensitive isolates. Alignment of amino acid sequences showed that the QoI resistant isolates harboured a G143A mutation, which was absent in the sensitive isolates. The two haplotypes of *Cyt b* gene from a resistant isolate, SAA2, and a sensitive isolate, HP1, have been deposited in GenBank under accession numbers KY418049 and KY418048, respectively. This is the first report of presence of QoI resistant isolates of *E. necator* from India. Studies point out the need for developing resistance management strategies by interspersing bio-control agents with judicious use of fungicides.

Keywords: *Vitis vinifera*, leafdisc bioassay, *Cytochrome b* gene, quinone outside inhibitor, fungicide resistance

INTRODUCTION

Grapevine powdery mildew caused by the obligate pathogen, *Erysiphe necator* (Schwein.) Burrill (earlier *Uncinula necator*), is one of the most widespread diseases of grapevines in India and can be seen for most part of the year on green tender parts (Chadha and Shikhamany, 4; Sawant *et al.*, 19). It decreases vine productivity and diminishes fruit quality impacting marketable yield and shelf-life of the produce (Ashtekar *et al.*, 1). Powdery mildew is controlled mainly by fungicides, such as demethylation inhibitors, sulphur and strobilurins. The strobilurin fungicide azoxystrobin belongs to the quinone outside inhibitors [QoI] group and has preventive and curative activity against several fungal pathogens.

The QoI fungicides disrupt electron transport during cellular respiration at the ubiquinol oxidation centre (Qo site) of the cytochrome bc1 enzyme complex (complex III). This ultimately results in depletion of adenosine triphosphate (ATP) and disrupts spore germination due to lack of energy (Grasso *et al.*, 13). Being single site inhibitors they are included in the high risk group for development of resistance against them in more than 30 phytopathogeneic species including grapevine powdery mildew. *E. necator* is also classified as a pathogen with medium and high risk of development of resistance to fungicides by FRAC and EPPO respectively (FRAC, 10).

Within a few years of the introduction of QoI fungicides in disease management, field resistance to these fungicides was reported in cucurbit and cereal powdery mildew. Resistance in *E. necator* to QoI fungicides occurred in New York and Pennsylvania, Hungary, Austria, France, Virginia (Baudoin *et al.*, 2; Dufour *et al.*, 6; Fontaine *et al.*, 7; Miles *et al.*, 17; Fraaije *et al.*, 8; FRAC, 10). Three point mutations in *Cyt b* gene were reported as mechanism of resistance against QoI fungicides. Substitution of phenylalanine to leucine at position 129 (F129L) and substitution of glycine to arginine at position 137 (G137R) are associated with the low level of resistance usually controlled by the recommended field levels of QoIs, while substitution of glycine with alanine at position 143 (G143A) was reported for higher level of resistance (Ishii *et al.*, 15). These point mutations in positions 127-147 (cd loop) of the amino acid sequence results in peptide sequence changes that prevents the binding of fungicide.

Resistance factor (RF= effective dose to control 50% of resistant strain/ effective dose to control 50% of sensitive strain) caused by G143A mutation is generally greater than 100 and shows complete resistance. RF associated with mutation F129L and

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GSC Biological and Pharmaceutical Sciences, 2018, 05(02): 017-025

Available online at GSC Online Press Directory

GSC Biological and Pharmaceutical Sciences

e-ISSN: 2581-3250, CODEN (USA): GBPSC2

Journal homepage: https://www.gsconlinepress.com/journals/gscbps

(RESEARCH ARTICLE)

Assessment of genetic diversity among different sugarcane genotypes using internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA)

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Publication history: Received on 29 September 2018; revised on 09 October 2018; accepted on 15 October 2018

Article DOI: https://doi.org/10.30574/gscbps.2018.5.2.0108

Abstract

Internal transcribed spacer or ITS region of nuclear ribosomal DNA (rDNA) has been used to evaluate genetic assortment and phylogenetic relationship in nine sugarcane genotypes including *Saccharum* species and another related genus as *Erianthus*, Narenga and hybrid. DNA was extracted from selected genotypes and ITS (ITS-1 and ITS-2) regions were amplified using specific primers. The sequence lengths ITS-1 showed 205- 207 bp, while ITS2 was ranged from 211- 218 bp. However, G+C content (%) 65.2% - 67% in ITS-1 and in ITS-2 68.4% - 99.7%. The sequence lengths of fragment and GC content of ITS-1 and ITS-2 regions showed variable. To evaluate the phylogenetic association of both the region of ITS (ITS-1 and ITS-2) neighbor-joining (NJ) method was employed. The cluster A of ITS-1 and cluster B for ITS-2 and cluster C combined between ITS1+ ITS2 sequences gave two distinct groups A and B. The group A represented the ITS1 sequences which showed two subgroups I and II. The A-I subgroup consisted of wild species of sugarcane; *Erianthus*, *Narenga* and *S. robustum*, whereas the A-II subgroup consisted of the *Saccharum* species and hybrid. The ITS2 sequences in the group B showed better correlation amongst each other. The sequences ITS-1 & ITS-2 combined and compared with some selected sequences from NCBI database using NJ method. The results have confirmed that ITS region can be used for evaluating the genetic assortment in *Saccharum* and its closely related genes.

Keywords: Genetic diversity; ITS; rDNA; PCR; Sugarcane

1. Introduction

Sugarcane (*Saccharum* spp. hybrids) is secure second rank an imperative crop and acting as significant role in agricultural and industrial economy [1], provides more than 70% sugar and more than 30% ethanol production in tropical and subtropical countries [2-5]. Currently, sugarcane is cultivated in 20.42 million ha producing 1,333.2 million tonnes with an average cane productivity of 65.20 tonnes/ha [6]. Sugarcane belongs to family *Poaceae* tribe Andropogoneae.

Present sugarcane cultivars are highly polyploidy in nature and often aneuploid, (2n=100-130) [7-8]. The *Saccharum* genus having six spp. viz., the noble cane *S. officinarum* L. two wild spp. Including *S. spontaneum* L. and *S. robustum* Brandes et Jeswiet ex Grassl, and three sub spp., *S. sinense* Roxb., *S. barberi* Jeswiet., and *S. edule* Hassk. And four other sugarcane genus including *Erianthus* Michx., *Narenga* Burkiee., *Miscanthus* Anderss., and *Sclerostachya*. For the evolutionary relationships among species in *Saccharum* complex, it is still unclear although researchers have attempted to disclose the phylogenetic relationships of *Saccharum* complex using molecular markers, plastid intergenic spacers, and ITS of nrDNA [9-10].

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ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 10, Issue, 05, pp.68947-68951, May, 2018

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

MOLECULAR CHARACTERIZATION OF SUGARCANE GENOTYPES FOR THEIR SALINITY AND SUSCEPTIBILITY USING TRAP MARKERS

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ARTICLE INFO

Article History:
Received 18th February, 2018
Received in revised form
08th March, 2018
Accepted 30th April, 2018
Published online 31st May, 2018

Key words:
ESTs, Molecular markers,
Polymorphism, Salinity,
Sugarcane, TRAP

ABSTRACT

Sugarcane is a cash crop in India and salinity affects its productivity and crop growth. Identification of molecular markers linked to salinity tolerance traits has provided plant breeders a new tool for selecting cultivars with improved salt-tolerance and hence, molecular characterization of eighteen sugarcane genotypes of salinity tolerant and susceptible was carried out using thirty combinations of 5 TRAP markers related salinity tolerant ESTs and 3 arbitrary primer combinations. Out of 124 alleles, 81 (65%) were found polymorphic and The PIC values were ranged from 0.10 to 0.41 with an average of 0.30 furthermore Rp value varied from 0.33 to 4.33 with an average of 1.48. Also, the range of polymorphism was found about 25% to 100%. The genetic similarity coefficients ranged from 0.53-0.91 with an average of 0.72 which revealed the existence of limited genetic variation among 18 sugarcane genotypes.

Abbreviations:
ESTs: Expressed sequence tag
MA: Monomorphic Alleles
P: Percentage Polymorphism
PA: Polymorphic Alleles
PCR: Polymerase chain reaction
PIC: Polymorphic Information Content
PIC: Polymorphism Information Content
RAPD: Random Amplified Polymorphic DNA
Rp: resolving power
TA: Total Alleles
TM: Temperatures of primers
TRAP: Tartrate-resistant acid phosphatase

Citation: Forough Jomhe Farsangi, Avinash S. Thorat and Rachayya M. Devarumath. 2018. "Molecular characterization of sugarcane genotypes for their salinity and susceptibility using trap markers", *International Journal of Current Research*, 10, (05), 68947-68951

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is a member of the Poaceae family and imperative cash crop. Sugar production contributes around 70–80% globally and 100% in India (Thorat, 2017 and Breusegem, 2001). Sugarcane is a tropical plant and grows well under tropical condition all over the world. Nearly 120 countries produce sugar from sugarcane, 57 countries produce sugar from sugar beet and 12 countries produce it from both these crops. India is the second biggest sugarcane producer next to Brazil and contributes around 15% production. However, in India, it is cultivated under a wide range of agro-climatic conditions. Abiotic stress is the most harmful factor concerning the growth and productivity of crops worldwide (Breusegem, 2001 and Guo, 2007). Excess amount of salt in the soil harmfully affects plant growth and development. Nearly 20% of the world's cultivated area and nearly half of the world's irrigated land are affected by salinity (Zhu, 2001). About 12 million hectare of land has been affected by saline and alkaline conditions in India (Yadav, 1984). Salinity in agricultural fields, a major environmental stress, is a severe constraint on crop growth and productivity in many regions, and the situation has become a global concern.

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Biotechnological Communication

BBRC
Bioscience Biotechnology Research Communications

Plant regeneration from direct and indirect organogenesis and assessment of genetic fidelity in *Saccharum officinarum* using DNA-based markers

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ABSTRACT

Sugarcane has acquired significant importance in the world economy because of the sugar and ethanol production. Therefore, rapid multiplication of outstanding sugarcane variety is necessary in developing countries. The aim of this investigation was to compare regeneration efficiency of various explants and genetic fidelity of regenerated plantlets certified by using DNA-based molecular markers, that is, random amplified length polymorphism (RAPD) and inter simple sequence repeats (ISSR). The sugarcane plantlets were obtained through direct (axillary buds, apical meristem, and leaf whorl disk) and indirect (callus culture) shoot organogenesis from the variety Co 86032. Among all the explants, highest shoot forming ability was observed in axillary buds showed 97.66±0.66% shoot formation and the highest number of shoots per explants (4.33±0.24) and a total number of regenerated shoots (173.00±8.11) were observed in the leaf whorl disk. Morphological variation was not observed among the regenerated plants from various explants and therefore, genomic DNA was isolated from fresh leaves and genetic fidelity assessment was carried out using RAPD and ISSR. Both the markers produced 1068 and 2271 bands, respectively, including all the tested plants, indicates that plants derived from direct organogenesis did not show any polymorphism. However, a genetic variation has been observed in the plants derived from callus and showed 4.54% polymorphism during analysis. The results suggested that plants regenerated from direct organogenesis are of more true-to-type, whereas genetic variation occurs during indirect organogenesis. Combination of RAPD along with ISSR can be used for detection of genetic variation in the early stage in sugarcane micropropagation. High performance of regeneration and low risk of genetic variation ascertains the efficiency of this operation.

KEY WORDS: FIDELITY, ISSR, MICROPROPAGATION, REGENERATION, RAPD, SUGARCANE

ARTICLE INFORMATION:

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Received 19th Jan. 2018
Accepted after revision 20th March, 2018
BBRC Print ISSN: 0974-6455
Online ISSN: 2321-4007 CODEN: USA BBRCBA
Thomson Reuters ISI ESC / Clarivate Analytics USA and Crossref Indexed Journal
NAAS Journal Score 2017: 4.38 SJIF 2017: 4.196

Online Contents Available at: http://www.bbrc.in/
DOI: 10.21786/bbrc/11.1/9

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