Bioresource Technology 142 (2013) 436–444

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Molecular fingerprinting of bacterial communities in enriched azo dye (Reactive Violet 5R) decolorising native acclimatised bacterial consortia

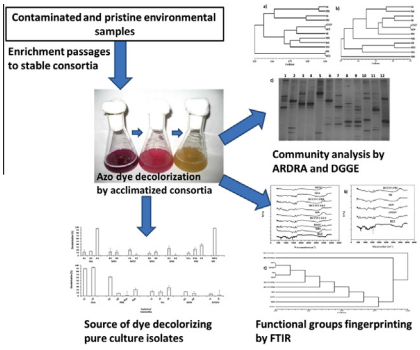
Jagat Rathod, G. Archana *

Department of Microbiology and Biotechnology Centre, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara 390 002, Gujarat, India

HIGHLIGHTS

- Both pristine and contaminated niches lead to development of acclimatised consortia.
- Total 12 efficient RV5R decolorising acclimatised consortia were enriched in lab.
- Community profiling was done using ARDRA and DGGE analysis.
- Consortia were comprised of decolorising and non-decolorising members.
- Acclimatised consortium Gly was found to degrade RV5R.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 12 April 2013
Received in revised form 11 May 2013
Accepted 15 May 2013
Available online 22 May 2013

Keywords:
Azo dye decolorisation
Acclimatised bacterial consortia
Amplified ribosomal DNA restriction digestion analysis (ARDRA)
Denaturing gradient gel electrophoresis (DGGE)
Fourier transformed infrared spectroscopy (FTIR)

ABSTRACT

Reactive Violet 5R (RV5R) decolorising acclimatised bacterial consortia were enriched from industrial effluent contaminated and pristine samples from Gujarat, India on several different media. Twelve acclimatised consortia were selected for the study which were able to decolorise 100 mg/L RV5R in 30 h under shaking or static conditions. Eubacterial diversity was studied by 16S rRNA gene based culture-independent methods, using HaeIII and Hinf1 enzymes for ARDRA and V3 region based DGGE analysis, forming total 6 clusters in both analysis. Decolorised end products of all the consortia were analysed by FTIR showing cleavage of the azo bond and group modifications. GC–MS data of dye decolorised end products of Gly consortium obtained from hydrocarbon contaminated soil demonstrated benzene ring cleavage activity. Present study suggests that enrichment of acclimatised consortia under different conditions can result in diverse microbial communities that differentially degrade RV5R and can provide rich source of dye decolorising strains.

1. Introduction

Azo dyes are the largest and versatile group of dyes having wide range of applications in the textile, paper, food, leather, cosmetics and pharmaceutical industries. They are xenobiotic and toxic and found to have high recalcitrance and stability in environment (Puvaneswari et al., 2006) as a result create aesthetic and environment problems. Conventional treatment of coloured effluents by physical or chemical procedures suffers from formation of hazardous by-products, high cost and intensive energy requirements (Taylor et al., 2013). Several microorganisms including algae, yeast, filamentous fungi and bacteria are reported to bring about dye decolorisation (Saratale et al., 2011). Biological decolorisation and degradation of azo dyes has gained importance as a method of treatment, as these methods are inexpensive, eco-friendly and can be applied to wide range of dyes (Stolz, 2001). The azo bond

* Corresponding author. Tel.: +91 265 2794396; fax: +91 265 2792508.
E-mail addresses: archanagayatri@yahoo.com, garchana@india.com (G. Archana).

0960-8524/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2013.05.057

(–N=N–), being the important chromophore group, can be reductively cleaved resulting in aromatic amines which are colourless in nature. However, the presence of different aromatic rings with methyl, methoxy, nitro, and sulfo groups make dyes more resistant to microbial degradation and their residues accumulate in biota (Saratale et al., 2011). Although decolorisation does not lead to complete degradation or detoxification, it is a rate-limiting bottleneck of the biodegradation pathway. Bacterial dye decolorisation is facilitated by enzymatic reactions or by nonspecific reduction by different reduced metabolites (H_2S) or redox mediators of intra or extracellular nature (Singh et al., 2007). Decolorisation of azo dyes can occur under anaerobic, anoxic or aerobic conditions by different groups of microorganisms depending on the enzyme system and microbial metabolites (Chen, 2006; Chengalroyen and Dabbs, 2013; Solís et al., 2012).

Pure cultures of several bacteria such as *Pseudomonas*, *Acetobacter*, *Bacillus*, *Sphingomonas*, *Xanthomonas*, *Aeromonas*, *Klebsiella* etc. are reported as azo dye decolorisers (Solís et al., 2012). Recent trend has been to use co-cultures comprising of a mixture of well-characterised pure cultures (Saratale et al., 2011; Solís et al., 2012). Mixed cultures have an advantage over pure cultures, since the mixed population attains additional co-metabolic potentials and possesses higher degree of biodegradation and mineralisation due to synergistic metabolic activities of microbial community (Senan and Abraham, 2004). Jain et al. (2012) have shown that acclimatised mixed culture consortium SB4 decolorised RV5R dye efficiently in 18 h under static conditions which was better than the individual pure bacterial strains from the same consortium. In mixed cultures, the individual strains may attack the dye molecule at different positions or may utilise metabolites produced by the co-existing strains for further mineralisation or have broad spectrum for dye decolorisation due to variation in the enzymatic machinery in the individual members (Chengalroyen and Dabbs, 2013).

Mixed cultures that are artificially formulated by combining individual pure cultures have certain disadvantages such as the requirement of thorough optimisation of the appropriate mixture as well as unstable nature of the artificially mixed populations whose proportions may vary with time. As against artificially mixed cultures, acclimatised consortia, encompassing enriched microbial community derived by repeated transfers of native microbial community from an appropriate sample in dye containing media, have several advantages. The members of the acclimatised consortia may have functional or dormant populations, both of which could be vital for the slow adaptation of the community to deliver stably the desired process (Dafale et al., 2008). Acclimatised consortia offer the advantages of mixed cultures but have additional features such as natural adaptation of the community members with each other (Mikesková et al., 2012). Adaptation of a microbial community in the presence of recalcitrant and toxic dyes improves the rate of decolorisation process by acclimatised consortia (Beydilli and Pavlostathis, 2005). Community succession in bacterial adaptation results in stable consortium development (Desai et al., 2009). Additionally efficient acclimatised consortia can be a potential source of individual efficient strains (Moosvi et al., 2005).

The development of acclimatised consortia could be expected to depend on the original microbial community present in the environmental sample and the enrichment medium and conditions. However, no systematic reports exist on how these factors affect consortia development and dye degradation patterns in such differently developed enriched consortia. The enrichment culture technique combined with advanced molecular methods for community fingerprinting could be utilised to compare the populations from different consortia. With this in view, the objective of the present study was to enrich dye decolorising consortia from various pristine and contaminated samples to develop acclimatised indigenous populations effectively decolorising azo dye. The acclimatised consortia were further studied for their bacterial diversity by 16S rRNA gene based molecular fingerprinting techniques such as amplified ribosomal DNA restriction analysis (ARDRA) and denaturing gradient gel electrophoresis (DGGE) analysis. Decolorised end-products were analysed by FTIR and GC–MS analysis. Reactive Violet 5R (RV5R), a mono azo dye having complex sulphonated moieties on benzene and naphthalene rings was used as the model dye for the current study. Our results show significant bacterial diversity in differently developed consortia correlating with differences in the degradation patterns and indicate the complexity of ecological microbial interactions.

2. Methods

2.1. Sampling

Various samples of soil, sand, mud, water and effluents from pristine and contaminated areas from different geographic regions of Gujarat, India were collected in sterile containers. Marine samples were from coastal areas of western India located near Bhavnagar, Gujarat and were considered as pristine samples. Hydrocarbon contaminated soil sample was taken from area surrounding petroleum refinery, Vadodara, Gujarat; aeration tank sample was from a common effluent treatment plant, Green environment and effluent sample from Meghmani dye industry were from Vatva, Gujarat; petroleum industry effluent sample was from petroleum refinery, Vadodara, Gujarat and sample from canal for disposal of treated industrial effluent at Amalakhadi, Gujarat.

2.2. Development of enrichment cultures of Reactive Violet 5R decolorising bacteria

The ready-made Bushnell Haas Medium (BHM) [Composition (g/L): $MgSO_4$, 0.2; K_2HPO_4, 1.0; $CaCl_2$, 0.02; $FeCl_3$, 0.05; NH_4NO_3, 1.0] and media additives used in this study were from HiMedia Laboratories, India. RV5R was obtained from Meghmani Dyes And Intermediates Ltd., GIDC Vatva, Ahmedabad, India. Enrichment was started in within 12 h of sampling. Samples were suspended or diluted in phosphate buffered saline (PBS; 8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4, 0.25 g KH_2PO_4 for 1000 mL, pH 7.4) at 10% w/v or 10% v/v, mixed thoroughly and the resultant suspension was used as inoculum (5% v/v) into the different media and incubated under static or shaking (80 rpm) conditions at 30 °C. Considering the sample location, following different media were used for acclimatising the dye decolorising consortia: medium A: BHM with glucose (0.5% w/v) and yeast extract (0.5% w/v); medium B: BHM with glycerol (0.5% v/v) and yeast extract (0.5% w/v); medium C: peptone 2% w/v, K_2HPO_4 0.15% w/v, $MgSO_4$ 0.15% w/v, glycerol 1% (v/v) and medium D: tryptone 1.5% w/v, Soya peptone 0.5% w/v, NaCl 0.5% w/v. All the media were amended with filter sterilised RV5R solution to give a final concentration of 100 mg/L. Visual colour change of the medium from violet to colourless was used as an indication of dye decolorisation. After decolorisation was obtained, the enrichment culture was re-inoculated (5% v/v) into fresh medium and in this manner several successive passages were given. Out of an initial 23 enrichment setups, 12 consortia able to decolorise 100 mg/L RV5R in 30 h up to 15 successive transfers were selected for further studies.

2.3. Quantification of Reactive Violet 5R decolorisation

Aliquots (1 mL) were withdrawn from the bacterial suspensions and centrifuged at 14,000*g* for 10 min to separate the bacterial cell