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Research Article

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Molecular Identification of Azo Dye Degrading Fungi Isolated from Azo Dye Contaminated Soil of Local Dyeing Facility in Bida, Niger State

Abubakar Hassana[1*], Balogu Tochukwu Vincent[2], Isah Muhammad Nasiru[1], Ndatsu Yakubu[1], Mayaki Fatima Gogo[1] and Usman Hamza Boko[1]

[1]Department of Biochemistry, [2]Department of Microbiology,
Ibrahim Badamasi Babangida University, Lapai, Nigeria

*Corresponding Author E-mail: ahassana@ibbu.edu.ng

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ABSTRACT

Azo dyes are synthetic organic colorants containing one or more azo bonds (–N=N). These dyes constitute the major chemical in textile industrial waste water and are released into the ecosystem. Biotransformation of these azo compounds produces intermediates like aromatic amines which are very carcinogenic and mutagenic. Several physicochemical methods used in the treatment of these azo compounds are usually ineffective, expensive, and not always applicable. This study aimed at isolating and identifying azo dye degrading fungi from azo dye contaminated soil taken from a local dyeing facility in Bida local government area, Niger State, Nigeria. Isolation was done on mineral salt medium containing 1% congo red azo dye as carbon and energy source. The isolates were characterized based on microscopic and culture morphology and identified with molecular characterization. Degradation potential of the isolates were determined in mineral salt broth with 1% congo red dye as carbon source using UV spectrophotometer at wave length of 590 nm. The isolate with the highest degradation rate was further identified using ITS Gene Seguence. Two fungi were isolated; MSN1 and SN3. MSN1 was identified as Aspergillus fumigatus while SN3 was Penicellium chrysogenum.Aspergillus fumigatus attained the highest degradative activity of 0.93 Optical Density on the sixth day and Penicellium chrysogenum highest degradative activity was 0.70 Optical Density on the ninth day. ITS Gene Seguencing of Aspergillus fumigatus confirmed that this fungi belongs to this species with 99% similarity. This specie is known to have great dye decolorizing ability, hence its application in dye bioremediation is highly recommended.

Keywords: *Azo dye, congo red, degradation, Aspergillus fumigatus.*

INTRODUCTION

Azo dyes constitute the major group of artificial aromatic dyes which are made up of one or more azo bonds (N=N) and sulfonic groups (-SO3-). These dyes usually have between one to three azo bonds linked to naphthyl, phenyl rings typically replaced with certain functional groups such as methyl, hydroxyl, chloro, nitro, sulphonate and triazine amine (Sudha et al., 2014, Bell et al., 2000).

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Over three thousand azo dyes exist which are extensively applied in textile, cosmetics, paper, leather and food coloring industries (Elbanna et al., 2010). In the textile industries, almost eighty percent of azo dyes are utilized, of which about ten to fifteen percent was estimated to be lost during the dyeing process and discharged into the ecosystem (Asad et al., 2007).

The textile industries in various Nigerian towns release raw dye effluents as wastewater into public drainage system which ultimately drains into rivers and consequently changes the color, pH, and rises the chemical and biochemical oxygen demand of the river (Olayinka & Alo, 2004, Ajayi & Osibanjo, 1980, Olukanni et al, 2006). Several azo dyes and their intermediates like aromatic amines are very carcinogenic and mutagenic. Poisonous compounds of azodyes easily blend with water bodies, penetrate into aquatic organisms, eventually get to man via food chain and results in some health problems (Elbanna et al., 2010, Sudha et al., 2014).

Several physicochemical methods such as coagulation, flocculation, adsorption, ozonisation, oxidation, etc have been reported to be used in the treatment of textile wastes to attain decolourization (de Campos Ventura-Camargo et al., 2013, Robinson et al., 2001). However, these techniques are usually ineffective, expensive, and not always applicable. Moreover, at times large quantities of toxic waste is produced that is not readily disposable (Asad et al., 2007, Verma et al., 2003). To substitute these techniques, bioremediation is used to completely degrade azo dyes (Verma et al., 2003). Biological techniques results in full degradation of organic contaminants at cheap rate, hence, generally regarded as environmental friendly techniques (Pandey et al., 2007).

Some species of bacteria, fungi, yeast, algae and plants have been reported to have the ability of degrading azo dyes (de Campos Ventura-Camargo & Marin-Morales, 2013). In bacterial degradation of Azo dyes, the azo bond is cleaved under anaerobic condition to produce aromatic amines, a more toxic intermediate, which is then oxidized under aerobic condition by specific enzymes to produce easily degradable metabolite for TCA cycle (McMullan et al., 2001, Misal et al., 2011). *Alcaligenes spp, lactobacillus spp klebsiella spp bacillus subtillus, Rhizobium spp* etc. are the various species of aerobic and anarobic bacteria widely reported as azodyes degraders (Sudha et al., 2014). In addition, various fungi such as *Aspergillus niger, Aspergillus flavus, Penicillium geastrivous* etc. have been documented regarding azo dyes degradation (Ajayi & Osibanjo, 1980, Asad et al., 2007).

Congo red is among the common and most used azo dyes with its molecular structure containing two azo bonds (-N=N-) chromo phore. This dye is structurally stable, therefore very toxic and not readily biodegradable. Hence, it is essential to isolate and identify various microorganisms that can biode grade this azo dye and completely remove it from our environment in an environmental friendly way. In this study, two fungi were isolated from azo dye contaminated soil taken from a local dyeing area in Bida local government, Niger State. These fungi shows good degrade ative ability on 1% congo red azo dyes and can be used to effectively bio remediate azo dye contaminated soil.

MATERIALS AND METHODS

Growth Media Preparation

The growth media was prepared by adding 1% filter sterilized congo red azo dye to autoclaved mineral salt medium (MSM). The MSM contained K_2HPO_4 (0.9g/500mL); NH_4CL (2g/500mL); MgSO4.7H20 (0.1g/500mL); NaCl (0.05g/500mL); $Na_2S04.7H_20$ (0.005g/500mL); agar agar (10g/500mL).

Isolation and Characterization Of Azo Dye Degrading Fungi

0.1ml of 10^{-3} diluted azo dye contaminated soil sample from local textile dyeing facility in Bida, Niger state was spread on the mineral salt medium containing 1% congo red azo dye as carbon source and energy source. The medium was incubated at 37˚C for 5-15 days.

The fungi growth observed were sub cultured and characterized.

Screening For Azo Dye Degradative Activity

The degrading activities of the fungi were measured using method of Meenakshi sundaram and Bharathi raja with slight modification. Each fungi was inoculated seperately in Mineral salt broth (MSB) supplemented with 1% congo red azo dye and incubated at 22˚C -25˚C for 15 days. The fungi degradative activity was obtained by taking the Optical Density (O.D) of the supernatant at 590nm using a UV spectrophotometer from day1-15days at regular intervals of 3 days against mineral salt broth as blank.

ITS Gene Seguencing and Phylogenetic study

The fungi with the highest decolorizing activity was further identified molecularly using fungal ITS gene sequencing. The fungi DNA was extracted from fungi culture using Bioneer corp Genomic DNA Extraction Kit. Polymerase chain reaction (PCR) was performed to copy the desired DNA regions of the fungi using fungal general primers, ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3')[5]. The thermo cycler was programmed at an initial denaturation (94^{o}C 3min), 35 cycles of denaturation (94^{o}C for 40secs), annealing (54^{o}C for 40secs) extension (72^{o}C for 40secs), final extension (72^{o}C for 10min). The amplicon purification and sequencing was performed by DNA Laboratory Kaduna. From the NCBI blast result, Phylogenetic tree was constructed using neighbor joining method in MEGA 6.

RESULTS

Isolation and Identification of Azo Dye Degrading Fungi

Between 10-15 days of incubation, two fungi (MSN1 and SN3) with the ability of using 1% congo red azo dyes as carbon source were isolated from the azo dye contaminated soil collected from local textile dyeing area in Bida, Niger state. MSN1 was blue green colony, which grew fast and cover the disk in few days (figure 1) and SN3 obverse side was white cream color with White-margin, and pale yellow reverse side (figure 2). The two fungi isolate were first identified based on morphological, microscopic observation on sabourauds dextrose agar and fungus book (Atlas). MSN1 was identified as *Aspergillus fumigatus* while SN3 was *Penicellium chrysogenum.*

Fig. 1: MSN1

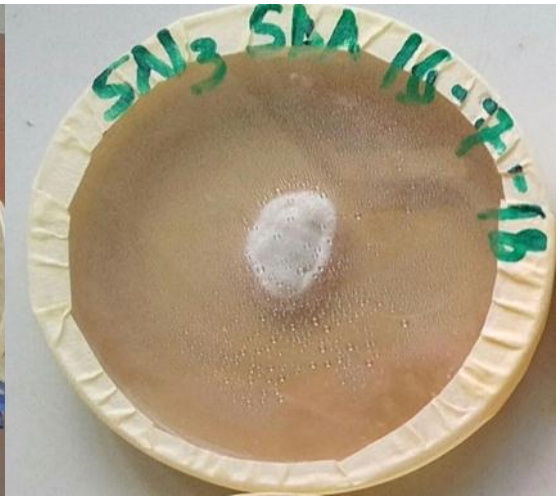

Fig. 2: SN3

Azo Dye Degradative Activity

Figure 3 shows degradative activity of the two fungi isolates on azo dye. The fungi degradative activity on the azo dye was obtained from the O.D values of 1% congo red mineral salt broth turbidity at three days regular interval. Fungi MSN1 (*Aspergillus fumigates*) attained the highest degradative

activity of 93% on the sixth day then fungi SN3 (*Penicellium chrysogenum*) which highest degradative activity was 70% on the ninth day.

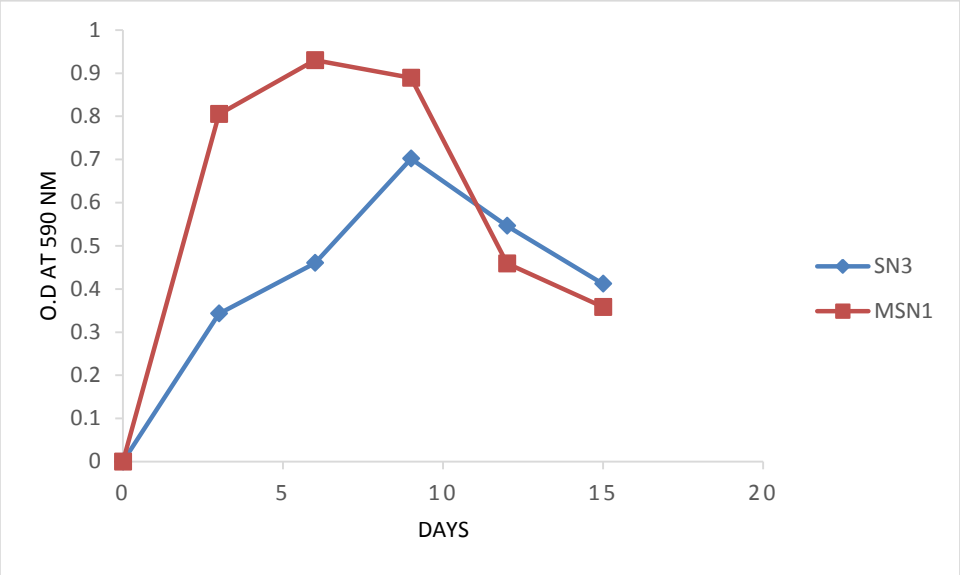

Fig. 3: Degradation activity of the isolated fungi

ITS Gene Seguence and phylogenetic Analysis

The ITS gene of MSN1 was amplified through Polymerase Chain Reaction (figure 4). The subsequent sequencing result showed a sequence of about 600 base pair. From the NCBI blast result, MSN1 shows 99% similarity with *Aspergillus fumigatus* species (table 1).

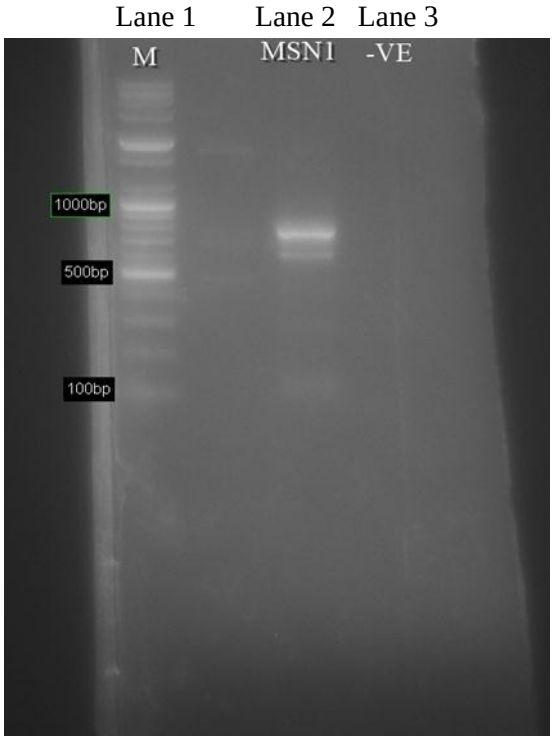

Fig. 4: PCR amplification of ITS rDNA gene on an agarose gel. Lane 1, DNA ladder, Lane 2, MSN1 amplified ITS rDNA gene, Lane 3, control without ITS primers

Table 1: The NCBI database BLASTn result

Accession number	Description	Max Identity
LC317446.1	*Aspergillus fumigatus strain: IFM 54229*	99%
MG659675.1	*Aspergillus fumigatus strain ND81*	99%
LC317447.1	*Aspergillus fumigatus strain: IFM 62665*	99%
LC317445.1	*Aspergillus fumigatus strain: IFM 4942*	99%
MG518471.1	*Aspergillus fumigatus strain ay*	99%

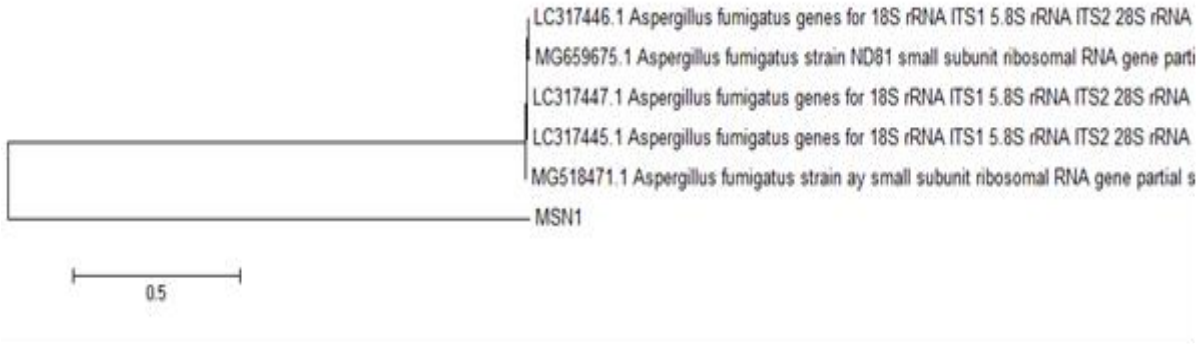

Fig. 5: Phylogenetic tree of Fungi MSN 1 constructed based on ITS gene analysis showing evolutionary relationship of MSN 1 with other related taxa

DISCUSSION

In this study two fungi (MSN1 and SN3) were isolated from the azo dye contaminated soil collected from local textile dyeing area in Bida, Niger state which were able to utilize 1% congo red azo dyes as carbon and energy source. MSN1 and SN3 were first identified using fungus book as *Aspergillus fumigatus* and *Penicellium chryso genum* respectively. These organisms were able to utilize 1% congo red azo dyes as carbon and energy source. The O.D reading of degrading activity of the fungi on the Azo dye (figure 3) shows that *Aspergillus fumigates* and *Penicellium chrysogenum* have great ability to degrade Azo dye contaminated soil. *Aspergillus fumigatus* having highest decolorizing activity was further confirme dusing ITS rRNA gene sequence.

The Internal Transcribed Spacer (ITS) regions are highly preserved in most species having intraspecific resemblances greater than 99% but shows variation between species which make it suitable for application in classification (Prewitt et al., 2008). ITS1 (fungal specific primer) and ITS4 (fungal general primer) are the first PCR primer sets usually employed to copy the fungal ITS regions (White et al., 1990). The ITS rRNA analysis using ITS1 and ITS4 primers confirmed the organism to be 99% identical to *Aspergillus fumigatus* species (table 1). Figure 5 supports this result.

Aspergillus fumigatus isolated in this study showed maximum decolorization activity of 93 % at the end of the 6th day which is in line with (Kalyani et al., 2017) who recently showed the decolorisation of congo red by *Aspergillus fumigatus* with maximum dye decolorization of 90% at the end of 5th day. *Aspergillus fumigatus* have also been found to effectively decolorise other dyes such as Malchite green, Trypan Blue and Viscose Orange-A (Kalyani et al., 2017, Madhuri et al., 2014, Saranraj et al., 2010). (Saranraj et al., 2010) recorded this fungi as the best decolourizer of Viscose Orange-A with 88.70% decolorization. *Aspergillus fumigatus* has also been discovered to degrade certain pollutants such as petroleum, phenol and pesticides (Hara & Uchiyama, 2013, Gerginova et al., 2013. Oliveira et al, 2015). This organism can thrive a large number of environmental circumstances due to the production of huge amount of airborne spores (Abad et al., 2010). The degradation potential of this organism can be attributed to this characteristic.

CONCLUSION

In this study, two fungi that were able to utilize 1% congo red azo dyes as carbon and energy source were isolated. The fungi with the highest degradation rate was identified using ITS gene as *Aspergillus fumigatus.* This organism is recognized to have great dye decolorizing ability, hence its application in dye bioremediation is highly recommended.

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