International Journal of Biotechnology and Biochemistry
ISSN 0973-2691 Volume 12, Number 1 (2016) pp. 55-61

http://www.ripublication.com

Isolation of Novel Fungal Strains from Marine Environment capable of Cashew Nut Shell Liquid Degradation

Sindhu.M.R* and Potty.V.P

Department of Biotechnology,
The Cashew Export Promotion Council Laboratory and Technical Division,
Cashew Bhavan, Mundakkal, Kollam-691001, Kerala, India
**Corresponding author. sindhumr85@gmail.com*

Abstract

Cashew Nut Shell Liquid or CNSL is the byproduct of the cashew industry which is widely used in paints, enamels, laminating resins, varnishes, rubber compounding resins, epoxy resins, cashew cements, polyurethane based polymers etc. Biodegradation of paints is a serious threat to the marine paint industry. Cashew Nut Shell Liquid is an eco friendly antifouling agent which is widely used in marine antifouling paints that prevents fouling of microorganisms to the hull of marine vessels. The present study was to isolate and identify the fungal strains from the marine environment that degrade phenolic compounds like CNSL which helps in developing new paint products which are resistant to these organisms. By enrichment culture technique, microorganisms that degrade CNSL were obtained. By screening all the microorganisms, it was found that *Trichoderma atroviride*, *Aspergillus terreus and Fusarium sp*. play a major role in the degradation of CNSL. Estimation of degradation of CNSL was done spectrophotometrically at 650 nm by Folins-Ciocalteau method. These fungal strains can be used for bioremediation of CNSL contaminated soils in cashew industries.

Key words: Cashew Nut Shell Liquid, Biodegradation.

INTRODUCTION

Cashew nut shell liquid (CNSL) is a byproduct of cashew industry. It is a mixture of phenolic compounds like anacardic acid, cardanol, cardol and 2-methyl cardol. Of these, anacardic acid and cardanol are monohydric phenols and cardol and 2-methyl cardol are dihydric phenols. CNSL is widely applied in polymer based industries, like

paints, varnishes, friction linings, laminating resins, rubber compounding resins, epoxy resins, wood preservatives etc [1]. Cardanol extracted from CNSL is commonly used in marine antifouling paint which is widely applied to fishing boats, wooden boats, houseboats etc. Researchers developed thermoplastic unsaturated polyester resin from cardol isolated from CNSL and maleic anhydride [2].
Studies show the degradation of CNSL by bacterial species such as *Pseudomonas pseudoalcaligenes, Pseudomonas stutzeri, Enterobacter sakazakii, Enterobacter cloacae,* and *Sphingomonas paucimobilis* [3]. CNSL was found degraded more efficiently by *Pseudomonas* species than that *of Arthrobacter* species [4]. Many studies on biodegradation of phenolic compounds have been reported [5, 6, 7, 8]. Phenolic compound degradation by fungal species is very well studied. Fungi adapt more easily than bacteria and can grow in extreme conditions like nutrient deficiency, low pH and limited water supply [9, 10]. The phenolic compound degradation studies by *Graphium* species [11], *Fusarium* species [12] and *Aspergillus* species [13] were also done. Studies on *Fusarium flocciferum* that degrade some phenolic compounds like gallic, vanillic, protocatechuic, syringic, caffeic and ferulic acids and syringic aldehyde, in agro-industrial effluents were done [14]. Phenol degradation by *Fusarium oxysporum* is inhibited by presence of copper oxide [15]. Studies on CNSL degradation by fungus especially from marine environment is less known. In this study we isolated and identified CNSL degrading fungal species, estimated the extend of degradation of this phenolic compound by Folin-Phenol method [16].

MATERIALS AND METHODS

Sample Collection

Water and soil samples were collected in sterile bottles and bags from different areas of the major fishing Harbor Neendakara, and also from Astamudi lake and Thankasseri port, Kollam, Kerala. The collected samples were brought to laboratory for further studies.

Enrichment and Isolation of CNSL Degrading Fungus

1g of soil sample and 1mL of water sample were suspended in 100 mL of Mineral salt media containing KH_2PO_4 (0.5 g/L), K_2HPO_4 (1.5g/L), NaCl (0.5 g/L), $MgSO_4.7H_2O$ (0.5 g/L), NH_4NO_3 (1 g/L), $FeSO_47H_2O$ (0.01 g/L), $CaCl_2.H_2O$ (0.01g/L) and NH_4SO_4 (0.5 g/L). CNSL was used as a sole source of carbon and incubated at 32°C in 250 mL flask in rotary shaking incubator at 120 rpm for a week. 10mL of the enriched media was transferred asceptically to sterile mineral media containing CNSL as the carbon source and incubated for a week at 32°C.The enriched medium was then streaked to mineral media agar containing CNSL as the sole source of carbon [17].

Identification of CNSL Degrading Fungus

The fungal species obtained as a result of enrichment isolation technique were identified by colony morphology, slide culture technique, lactophenol cotton blue staining and microscopic observation. The species was confirmed by National Fungal Culture Collection of India (NFCCI, Pune).

SCREENING OF DOMINANT PHENOLIC COMPOUND DEGRADING FUNGUS

Inoculum preparation

The fungal strains obtained by enrichment isolation method were inoculated into sterile Mineral Media broth containing catechol 10 mg/L as sole source of carbon and incubated 3-4 days at 32°C in shaking incubator at 120 rpm. The mycelial mat formed after 4 days of incubation was used as inoculums for further studies.

Estimation of phenolic compound Degradation

The fungal mycelial mat were inoculated into sterile mineral media broth containing catechol 10 mg/L as sole source of carbon and incubated 72 hours at 32°C in shaker at 120rpm. The sterile mineral media broth containing 10mg/L catechol as carbon source without any inoculum was kept as control. At regular intervals, the estimation of phenolic concentration was done by using Folin's Ciocalteau method. The decrease in concentration of the phenolic compound was determined by using catechol as the standard at 650 nm by using UV-VIS –NIR Spectrophotometer (SHIMADZU UV-3600) [16]. The percentage degradation of catechol by fungus was calculated by the following equation [18].

$$\text{Percentage of Degradation} = \frac{(Co - Cf)}{Co} \times 100$$

Estimation of CNSL Degradation

Fungal strains were inoculated into sterile Mineral Media broth containing CNSL 500 mg/L as sole source of carbon and incubated 3-4 days at 32°C in shaker at 120rpm. The mycelial mat formed after 4 days of incubation was used as inoculums and transferred to sterile mineral media containing 500mg/L CNSL and incubated at 32°C in shaker at 120 rpm. The decrease in concentration of the phenolic compound was determined by Folins Ciocalteau method at 1hour interval at 650 nm by using UV-VIS –NIR Spectrophotometer (SHIMADZU-UV-3600).

ESTIMATION OF BIOMASS

Biomass was estimated by Dry cell weight method. The fungal mycelium was inoculated to Mineral Salt Medium containing catechol as the sole source of carbon ($MSMC_1$) and Mineral Salt Medium containing CNSL as the sole source of carbon ($MSMC_2$). At regular intervals, the inoculated medium was filtered through Whatmann no.1 filter paper. The filter paper was dried in oven at 70^0C overnight and cooled and again weighed. The difference between pre weighed filter paper and final weight gives the biomass concentration.

RESULTS AND DISCUSSION

By enrichment culture method, various fungal strains capable of utilizing phenolic compound were obtained in MSM CNSL medium (Fig.1). They were screened out and three of the fungal strains showed maximum phenolic compound degrading

capacity. It was identified by slide culture technique and lactophenol cotton blue staining. The fungal strains identified were confirmed as *Trichoderma atroviride, Aspergillus terreus and Fusarium sp* by National Fungal Culture Collection of India (NFCCI, Pune). The identified funguses were maintained in Potato Dextrose Agar slants for further studies.

After 24 hours of inoculation, the *Trichoderma atroviride, Aspergillus terreus and Fusarium sp.* showed a decrease in concentration of phenolic compound, ie catechol to 3.86 ± 0.10, 3.49 ± 0.16 and 3.64 ±0.20 µg/mL respectively. After 48 hours it was reduced to 2.08 ±0.07, 1.94 ± 0.18 and 2.03 ± 0.23 µg/mL respectively and after 72 hours it was reduced to 0.64 ±0.10, 0.40 ± 0.11 and 0.55 ±0.22 µg/mL respectively (Fig.2). After 4 hours of incubation, the 92.2%, 95.8% and 95% of phenolic compound in CNSL has been reduced by *Trichoderma atroviride, Aspergillus terreus and Fusarium sp* respectively (Fig.3). These results were supported by Santos and Linardi (2004) [19]. They studied the phenol degradation by *Graphium* sp. and *Fusarium* sp., and found that 75% degradation of (10 mM) phenol occurs in 168 hours. Abedin and Barakat (2013) [20] studied Phenol degradation and p-cresol degradation by *Fusarium oxysporum RA* and the highest rate of degradation of phenol and p-cresol were found 86 % and 78% within 168 h, respectively. The Phenol degradation by *Fusarium oxysporum RA* showed the degradation at an optimum pH 8. When pH falls below 8, the degradation rate was found lower, and at pH 4, phenol degrading activity of *F. oxysporum RA* was found to be inhibited. Phenolic compound degradation by *Aspergillus terreus* [21] confirmed the ability of *Aspergillus terreus* to degrade phenol and phenolic compounds. Estimation of biomass concentration was done by Dry cell weight method. The maximum degradation was obtained after 24 hours of inoculation and the dry cell weight of *Trichoderma atroviride*, *Aspergillus terreus* and *Fusarium sp* were found maximum within 24 to 48 hours (Table 1, 2).

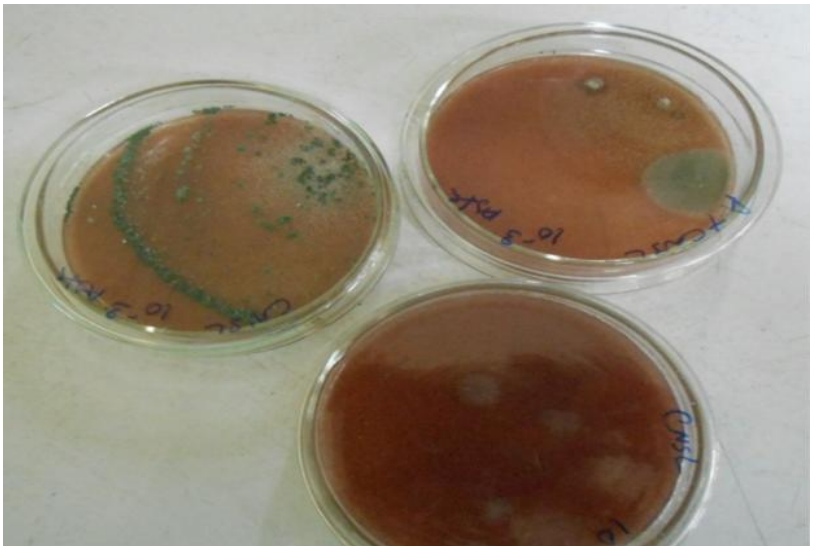

Figure 1: Fungal colonies in mineral salt medium containing Cashew Nut Shell Liquid

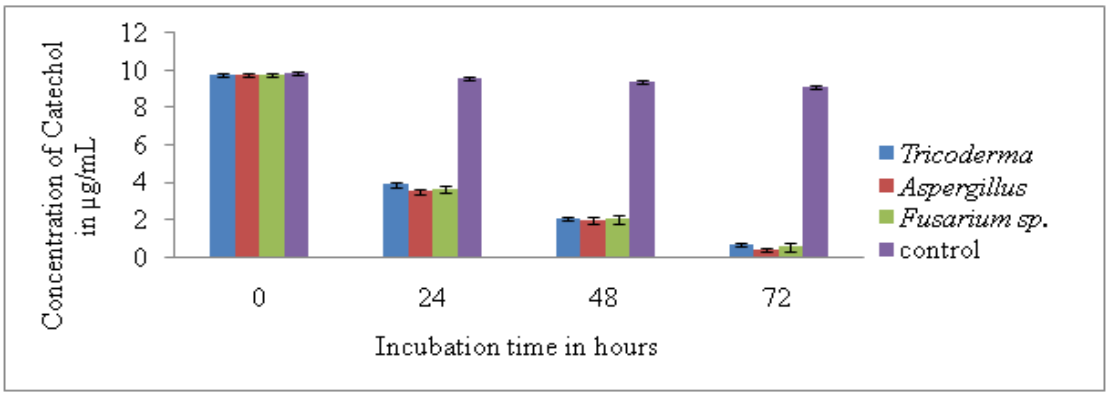

Figure 2. Catechol degradation by *Trichoderma atroviride, Aspergillus terreus and Fusarium sp.* Values expressed as means ±SD (n=3)

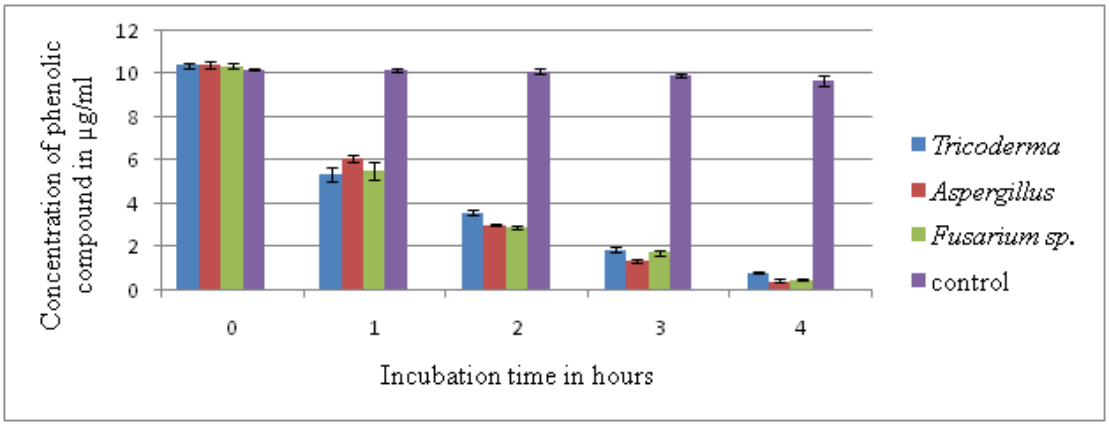

Figure 3. Degradation of CNSL by *Trichoderma atroviride, Aspergillus terreus and Fusarium sp.* Values expressed as means ± SD (n=3)

Table 1: Estimation of biomass in mg/10 mL of $MSMC_1$medium.

Fungus	After 0 hrs	After 24hrs	After 48 hrs	After 72 hrs
Trichoderma atroviride	5.33 ± 0.57	22.00 ± 2.64	19.00 ± 2.64	13.66 ± 2.08
Aspergillus terreus	5.66 ± 1.50	35.00 ± 5.00	34.00 ± 2.00	22.33 ± 3.21
Fusarium sp	7.00 ±1.00	23.66 ± 5.50	16.66 ± 5.77	14.00 ± 2.00

Values expressed as means ± SD (n=3)

Table 2: Estimation of biomass in mg/10 mL of $MSMC_2$ medium.

Fungus	After 24 hrs	After 48 hrs	After 72 hrs
Trichoderma atroviride	42.96 ± 2.89	49.33 ± 0.83	43.76 ± 1.07
Aspergillus terreus	42.66 ± 0.90	47.63 ± 1.25	41.5 ± 0.95
Fusarium sp	41.33 ± 1.52	42.06 ± 1.66	37.33 ± 2.08

Values expressed as means ±SD (n=3)

Conclusion

From the study, we can conclude that *Trichoderma atroviride, Aspergillus terreus and Fusarium sp* are potent phenolic compound degraders and will be helpful in developing new paint products that resists these organisms. These strains can also be used for bioremediation purposes in CNSL contaminated sites in cashew industries.

Acknowledgement

The authors are thankful to The Department of Biotechnology, The Cashew Export Promotion Council of India, for providing the facility and also to Kerala University for providing University JRF.

References

[1] Kathir Viswalingam., and Emerson Solomon, F., 2013, "A process of selective extraction of cardanol from cashew nut shell liquid (CNSL) and its useful applications", International Journal of Scientific and Engineering Research., 4 (3).

[2] Patrick Muturi Mwangi., and Bildad Kaggia Mbugua., 2013, "Novel unsaturated polyester (UPE) resin based on cashew nut shell liquid (CNSL)", Int.J.Cur.Tr.Res., 2(1), pp. 285-292.

[3] Sabna Prabha, S., Sisu Pramod, S., and Potty, V. P., 2011, "Isolation, identification and methanogenesis of CNSL degrading bacteria and immobilized bioremediation techniques of CNSL and its contaminated water from cashew industry," Middle East Journal of Scientific Research., 10(3), pp. 327-331.

[4] Rajeshwari, T., Padmapriya, B., Teesha, K., and Kavithakumari, P., 2011, "Degradation of cashew nut shell liquid by *Pseudomonas* sp. Isolated from soil", International Journal of Microbiological Research., 2(2), pp. 172-175.

[5] Bhavna V Mohite., Ruby E Jalgaonwala., Shradha Pawar and Ankush Morankar., 2010, "Isolation and characterization of phenol degrading bacteria from oil contaminated soil", Innovative Romanian Food Biotechnology., 7, pp 61-65

[6] ArunKumar, A., and Anitha, A., 2014, "Biodegradation of phenol and emulsification properties of native microorganisms from coal carbonization plant", International Journal of Applied Biology and Pharmaceutical Technology., 5 (4), pp. 249-256.

[7] Chakraborty, S., Bhattacharya, T., Patel, T.N., and Tiwari, K.K., 2010, "Biodegradation of phenol by native microorganisms isolated from coke processing waste water", Journal of Environmental Biology., 31, pp. 293-296.

[8] Zia Ur Rahman Awan., Abdul Haleem Shah., and Mohammad Amjad., 2013, "Microbial degradation of phenol by locally isolated soil bacteria. Global Advanced Research Journal of Microbiology," 2(4), pp. 72-79.

[9] Atagana, H. I., 2004, "Bioremediation of creosote-contaminated soil in South Africa by land farming", J. Appl. Microbiol., 96, pp. 510-520.

[10] Stoilova, I., Krastanov, A., Bui, H., 2008, "Biodegradation of mixed phenolic compounds by a microbial association of *Aspergillus awamori* and *Thermoascus aurantiacus*", EJEAFCHE., 7, pp. 2625-2633.

[11] Santos, V. L., Heilbuth, N. M., Braga, T., Monteiro, A. S., and Linardi,V. R., 2003, "Phenol degradation by a *Graphium* sp.FIB4 isolated from industrial effluents," J Basic Microbiol., 43, pp. 238-48.

[12] Anselmo, A. M., and Novais, J. M., 1992, "Degradation of phenol by immobilized mycelium of *Fusarium floccieferum* in continuous culture", Water Sci Technol., 25, pp. 161-8.

[13] Jones, K. H., Trudgill, P. W., and Hopper, D. J., 1995, "Evidence of two pathways for the metabolism of phenol by *Aspergillus fumigates*", Arch Microbiol., 163, pp. 176-81.

[14] Elsa Mendonca., Aida Martins., and Ana Maria Anselmo., 2004, "Biodegradation of natural phenolic compounds as single and mixed substrates by *Fusarium flocciferum*", Electronic Journal of Biotechnology., 7 (1).

[15] Park, J. Y., Hong, J.W., and Gadd, G. M., 2009, "Phenol degradation by *Fusarium oxysporum* GJ4 is affected by toxic catalytic polymerization mediated by copper oxide", Chemosphere., 75(6), pp. 765-71.

[16] Sadasivam, S., and Manickam, A., 1996, Biochemical methods, New Age International (p) Limited publishers, Second edition pp. 193-194.

[17] Nagamani, A., Soligala, R., and Lowry, M., 2009, "Isolation and characterization of phenol degrading *Xanthobacter flavus*", African Journal of Biotechnology., 8 (20), pp. 5449-5453.

[18] Shweta., and K. Dhandayuthapani., 2013, "Influence of media supplements on phenol biodegradation by *Pseudomonas aeruginosa* SPD 10," Int. J. Curr. Microbiol. App.Sci., 2 (6), pp.64-69.

[19] Santos, V. L., and Linardi, V. R., 2004, "Biodegradation of phenol by a filamentous fungus isolated from industrial effluents-identification and degradation potential," Process. Biochem., 39, pp. 1001-1006.

[20] Rania M.A Abedin., and Khouloud M. I Barakat., 2013, "Evaluation of medium components using Plackett-Burman design for phenol degradation by marine degrading *Fusarium oxysporum",* African Journal of Microbiology Research., 7(7), pp.541-550.

[21] Borja, R., Alba, J., Garrido, S. E., Martinez, L., Garcia, M. P., Incerti, C., and Ramos-Cormenzana, A., 1995, "Comparative study of anaerobic digestion of olive mill waste water (OMW) and OMW previously fermented with *Aspergillus Terreus",* Bioprocess Engeneering., 13(6), pp. 317-322.