**Co-biomass degradation of fluoranthene by marine-derived fungi; *Aspergillus aculeatus* and *Mucor irregularis*: comprehensive process optimization, enzyme induction and metabolic analyses**

Paul Olusegun Bankolea,b\*, Victor Taghoghor Omonib, Sikandar Imamsab Mullac,d, Seun Owolabi Adebajoe, Adedotun Adeyinka Adekunlef

aDepartment of Pure and Applied Botany, College of Biosciences, Federal University of Agriculture P.M.B. 2240 Abeokuta, Ogun State, Nigeria

bLancaster Environment Centre, Lancaster University, Lancaster LA1 4YQ, United Kingdom

cDepartment of Biochemistry, School of Applied Sciences, REVA University, Bangalore 560064, India

dDivision of Biotechnology, Jeonbuk National University, Iksan 54596, Republic of Korea

eDepartment of Microbiology, College of Biosciences, Federal University of Agriculture P.M.B. 2240 Abeokuta, Ogun State, Nigeria

fDepartment of Botany, Faculty of Science, University of Lagos, Lagos State, Nigeria

**Corresponding author:** bankolepo@funaab.edu.ng

*Molecular identification and phylogenetic relationship between AA and MI*

Internal Transcribed Spacer (ITS) 1 and 4 sequences were used in the characterization of the marine fungi; *A. aculeatus* and *M. irregularis*. The Basic Local Alignment Search Tool (BLAST) analysis of the nucleotide sequences revealed that the molecular data on *A. aculeatus* had homologous identities with *A.* *aculeatus* (MH368116) by 99.47%, *A.* *aculeatus* (KJ958359) by 99.61%, *A.* *aculeatus* (KJ439163) by 96.55%, *A.* *aculeatus* strain 4-4 (MF196878) by 95.00%, and *A.* *aculeatus* (AJ279995) by 92.78% (Fig. SM1). Similarly, the BLAST query revealed that *M. irregularis* sequence data had homologous similarities with *M.* *irregularis* strain LZ02 (MN533715) by 93.67%, *M.* *irregularis* strain LZ04 (MN533717) by 91.77%, *M.* *irregularis* (MH102381) by 90.87%, *M.* *irregularis* strain Bpf-19 (MH397495) by 97.21% and *M.* *irregularis* strain E22F (KY425732) by 90.54%.

**Table SM1**. GC-MS data of obtained fluoranthene metabolites after degradation by *Aspergillus* *aculeatus*, *Mucor* *irregularis* and AA+MI co-culture

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Peak | R*t*(min) | m/z | Mol. Weight(g moL-1)  | Description |  Mass spectrum |
| aI | 15.794  | 225 | 224.21 | 2-formylacenaphthylene-1-carboxylic acid |  |
| aII | 12.511  | 182 | 182.22 | 9*H*-fluoren-9-ol |  |
| aIII | 11.324 | 171 | 170.21 | 1,2-dihydroacenaphthylen-1-ol |  |
| aIV, bI | 9.122 | 155 | 154.21 | 1,2-dihydroacenaphthylene |  |
| bII | 8.679 | 186 |  186.32 | 1,2-dihydroacenaphthylene-1,2-diol |  | 11.34 |  |
| bIII | 7.999 | 181 | 180.55 | 9*H*-fluoren-9-one |  |  |  |
| cI | 7.924 | 237 | 238.28 | 1,2- dihydroxyfluoranthene |  |  |  |
| cII | 7.672 | 256 | 254.23 | 9*H*-fluorene-1,9-dicarboxylic acid |  |  |  |
| cIII | 7.101 | 211 | 210.14 | benzene-1,2,4-tricarboxylic acid |  |  |  |
| cIV | 6.907 | 166 | 166.13 | benzene-1,3-dicarboxylic acid |  |  |  |
| cV | 4.966 | 121 | 122.12 | benzoic acid |  |  |  |

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 *Aspergillus* *aculeatus* (KJ439163)

 *Aspergillus* *aculeatus* (KJ958359)

 *Aspergillus* *aculeatus* (MH368116)

 ***Aspergillus* *aculeatus* strain bpo2 (MT492456)**

 *Aspergillus* *aculeatus* (AJ279995)

 *Aspergillus* *aculeatus* strain 4-4 (MF196878)

 *Aspergillus* *aculeatus* strain KU20018.78 (MT487837)

 *Aspergillus* *japonicus* (KF493862)

 *Aspergillus* sp. strain 79-B7 (MT594360)

 *Aspergillus* *aculeatus* (KF493861)

 *Mucor* *irregularis* strain E22F (KY425732)

 *Rhizomucor* sp. UFMGCB 3507 (JQ418437)

 *Mucor* *irregularis* strain Bpf-19 (MH397495)

 *Mucor* sp. (MN968723)

 *Rhizomucor* sp. JJP-2009a (FJ210508)

 *Rhizomucor* sp. JJP-2009a (FJ210515)

 *Mucor* *irregularis* (MH102381)

 *Mucor* sp. (MN853752)

 *Mucor* *irregularis* strain LZ04 (MN533717)

 *Mucor* *irregularis* strain LZ02 (MN533715)

 *Aspergillus* *aculeatus* strain AN5 (KY859793)

 ***Mucor* *irregularis* bpo1 (MK373020)**

 *Aspergillus* sp. (MK332589)

 *Aspergillus* *aculeatus* strain A1.9 (EU833205)

 *Aspergillus* *aculeatus* strain MR10 (KY320594)

*Aspergillus* *aculeatus* (EU326206)

 *Aspergillus* *japonicus* (KX621981)

 *Mucor* *circinelloides* strain SD-8 (KY807767)

 *Rhizomucor* sp. strain SH-16 (MN521812)

 *Mucor* *circinelloides* strain LZ27 (MN533740)

99

98

98

90

98

98

90

96

94

96

91

97

94

95

90

96

99

100

96

91

90

93

90

90

87

99

0.5

**Fig. SM1.** Evolutionary relationships of the marine-derived fungi, *A. aculeatus* bpo 2 and *M. irregularis*bpo 1. The tree was constructed by bootstrap analysis and inferred through the Neighbor-Joining tree method using MEGA X Software.

|  |  |  |
| --- | --- | --- |
| **a**

|  |
| --- |
| **c**  |

 | **b** |

 **Fig. SM2**. Pareto chart showing the influence of (a) 11 standard variables (b) 6 standard variables (c) 4 standard variables (Plackett-Burman Designs) in fluoranthene degradation by AA+MI co-culture (p≤0.05)



**Fig. SM3.** Validation of the FFD using growth-linked experiment of the optimized culture conditions

|  |  |
| --- | --- |
|  |  |
|  |  |

**Fig. SM4.** HPLC Chromatogram of fluoranthene (a) untreated (b) after degradation by AA (c) after degradation by M1 (d) after degradation by AA+MI