International Journal of Genetics 5(2): 48-52, 2015
ISSN 2222-1301

DOI: 10.5829/idosi.ijg.2015.5.2.95240

# In Silico Sequence Analysis of Pks Gene of Ochratoxin A Producing Aspergillus Section Nigri

*[1]B.T. Thomas, [2]M.O. Oni, [4]G.C. Agu, [3]O.M. Lakunle and [5]A. Davies*

[1]Department of Cell Biology and Genetics, University of Lagos, Akoka, Lagos, Nigeria
[2]Department of Biological Sciences, Oduduwa University, Ile-Ife, Nigeria
[3]Department of Medical Laboratory Science, State Hospital Ijebu-Ode, Ogun State, Nigeria
[4]Department of Microbiology, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria
[5]Department of Medical Microbiology and Parasitology, Babcock University, Ilisan-Remo,Ogun State, Nigeria

**Abstract:** This study compared the pks gene sequence of eighteen ochratoxin A producing Aspergillus section Nigri in order to investigate the molecular basis for variation among the studied isolates in the regulation of ochratoxin A production. Comparison of the pks promoter sequences from 18 isolates showed differences in length and homology with the pks gene ranging from 220bp in a strain of the *Aspergillus niger* to 2188bp in *Aspergillus carbonarius*. The alignment of the studied pks gene revealed putative binding sites for homologs of the known fungal transcription factors namely AreA, Aba and PacC while all the studied isolates lack the binding site for BrlA. Consequently, ten of the eighteen studied isolates had at least one HGATAR/YTATCD site. The sequence identical to binding site for the transcription factor involved in pH regulation of gene expression in fungi (Pac C) was found in four of the isolates, three of the four isolates (Isolate 1, 4 and 7) having Pac C binding site corresponding to GCCAAG with isolate 7 also having the binding site CCTGGC together with isolate 6. It can be concluded that AreA and Aba are potential enhancers for ochratoxin A biosynthesis and no single transcriptional factors is enough for the activation of ochratoxin A production.

**Key words:** In Silico · Sequence · Pks Gene · Ochratoxin A · Aspergillus Section Nigri

## INTRODUCTION

Black Aspergilli are notorious ochratoxigenic moulds known for their ubiquitous contamination of several pre and post harvest commodities in which they produce ochratoxin A (OTA) especially in different food matrices in warm countries [1]. This OTA has been reported on foods such as cereals, wine, grapes, cocoa, coffee, spices and dried fruits [2-5] while its nephrotoxicity, hepatotoxicity, genotoxicity, teratogenicity and immunotoxicity to animals and humans as well as its carcinogenicity is well-established [6].

Ochratoxin A is one of the factors involved in causing Balkan endemic nephropathy and tumours of the human urinary tract [7]. The International Agency for Research on Cancer has classified OTA as a possible carcinogen to humans [8]. The contamination of foods with ochratoxigenic fungi is associated with discolouration, production of off odours, losses in nutritional value, deterioration in technological quality as well as contamination with ochratoxins [9, 10].

Annual costs resulting from crop losses and the need to limit food contamination have been estimated to be more than $100million [11]. Of the ochratoxin-producing species, *A.niger* and *A. carbonarius* are the most common species implicated as causal agents of ochratoxin A contamination especially in garri [12].Transcriptional regulation of ochratoxin biosynthesis might be contingent upon environmental signals particular to different niches. Nitrogen source, pH and even antimicrobial agents are known to differentially influence mycotoxin biosynthesis among species, isolates and strains [13, 14]. Variation in the molecular structure of mycotoxin regulatory genes and synthesis among strains has not been described. This report therefore aimed to compare the nucleotide sequences of the pks region of 18 isolates of two species

**Corresponding Author:** Thomas Benjamin Thoha, Department of Cell Biology and Genetics, University of Lagos, Akoka, Lagos, Nigeria.

of ochratoxin A producing fungi within Aspergillus section Nigri to deduce variations in pks region and inferred protein structure, as well as phylogenetic relationships among these species

## MATERIALS AND METHODS

**Fungal Isolates:** Eighteen ochratoxin A producing fungal isolates belonging to Aspergillus section Nigri namely *Aspergillus niger* and *Aspergillus carbonarius* retrieved from gene bank at NCBI were used in this study. These isolates were chosen based on disparity on the production of ochratoxin A .

**Alignments and Phylogenetic Analyses:** DNA sequences were aligned using Clustal W in DNAMAN (Lynnon Biosoft, Vandreuil, Canada). Data sets included aligned DNA sequences from the Pks region. Phylogenetic trees were obtained by parsimony analyses using heuristic search methods with stepwise sequence addition and the tree-bisection–reconnection (TBR) branch-swapping algorithm. Node support was assessed with 10000 bootstrap replicates. Gaps were completely deleted. The partition homogeneity test (PHT) in PAUP* [15, 16] was performed on parsimony informative sites only, with 10000 randomized data sets using heuristic search methods with stepwise sequence addition. A two-tailed Kishino–Hasegawa (KH) test using 10000 RELL bootstrap replicates [17] in PAUP* was employed to further assess the likelihood of the different tree topologies.

## RESULTS

Comparison of pks promoter region sequences from 18 isolates of ochratoxin A producing Aspergillus section Nigri showed differences in length and homology. The pks gene ranged from 220bp in a strain of the *Aspergillus niger* to 2188bp in *Aspergillus carbonarius*. The pks region of three different strains of *Aspergillus niger* and *Aspergillus carbonarius* were found sharing 100% similarities in length and homology. However, these strains of *Aspergillus carbonarius* lack the putative binding site for Are A. The alignment of the pks region from the eighteen isolates revealed the putative binding sites for homologs of the known fungal transcription factors namely Are A Aba and PacC while all the studied isolates lack the binding site for Brl A. Consequently, ten of the eighteen studied isolates had at least one HGATAR/YTATCD site. Isolate 6 however contained two of these sites separated by 30bp. Sequences for Aba binding site (CATTCT) were found ranging between 79-344bp while the CATTCC binding site was found in isolate 4 and 14. Isolates 14 had the CATTCC at 1939bp. The other (isolate 4) however, harbored two of these sequence separated by 479bp. The sequence identical to binding site for the transcription factor involved in pH regulation of gene expression in fungi (Pac C) was found in four of the isolates, three of the four isolates (isolate 1, 4 and 7) having Pac C binding site corresponding to GCCAAG with isolate 7 also having the binding site CCTGGC together with isolate 6.

Table 1: Presence of putatitive binding sites for AreA in the partially sequenced pks gene

| Isolates | pks gene length | GATA1 CTATCT | GATA2 TTATCT | GATA3 TTATCA | GATA4 CTATCA | GATA5 TTATCT |
|---|---|---|---|---|---|---|
| 1 | 873bp | - | - | - | - | 141 |
| 2 | 280bp | - | - | - | - | - |
| 3 | 747bp | 419 | - | - | - | - |
| 4 | 897bp | - | - | - | - | - |
| 5 | 683bp | 471 | - | - | - | - |
| 6 | 747bp | 455/425 | - | - | - | - |
| 7 | 649bp | - | - | - | 275 | - |
| 8 | 220bp | - | - | - | - | - |
| 9 | 822bp | - | - | - | - | - |
| 10 | 951bp | 433 | - | - | - | - |
| 11 | 951bp | 433 | - | - | - | - |
| 12 | 951bp | 433 | - | - | - | - |
| 13 | 725bp | 557 | - | - | - | - |
| 14 | 2188bp | - | - | - | - | - |
| 15 | 726bp | 412 | - | - | - | - |
| 16 | 630bp | - | - | - | - | - |
| 17 | 630bp | - | - | - | - | - |
| 18 | 630bp | - | - | - | - | - |

Table 2: Putatitive binding sites for other fungal transcriptional factors in the partially sequenced pks gene

| Isolates | pks gene length | Aba site CATTCT | A Aba site CATTCC | 2 Brl A site 1 AGAGGGG | Brl A site2 CAAGGGA | PacC site 1 CCTGGC | PacC site 2 GCCAAG |
|---|---|---|---|---|---|---|---|
| 1 | 873bp | - | - | - | - | - | 141 |
| 2 | 280bp | - | - | - | - | - | - |
| 3 | 747bp | - | - | - | - | - | - |
| 4 | 897bp | - | -306/785- | - | - | - | -17 - |
| 5 | 683bp | -79- | - | - | - | - | - |
| 6 | 747bp | - | - | - | - | -694- | - |
| 7 | 649bp | -344- | - | - | - | -175- | -491- |
| 8 | 220bp | - | - | - | - | - | - |
| 9 | 822bp | 328 | - | - | - | - | - |
| 10 | 951bp | 109 | - | - | - | - | - |
| 11 | 951bp | 109 | - | - | - | - | - |
| 12 | 951bp | 109 | - | - | - | - | - |
| 13 | 725bp | - | - | - | - | - | - |
| 14 | 2188bp | - | 1939 | - | - | - | - |
| 15 | 726bp | - | - | - | - | - | - |
| 16 | 630bp | -323- | - | - | - | - | - |
| 17 | 630bp | -323- | - | - | - | - | - |
| 18 | 630bp | -323- | - | - | - | - | |

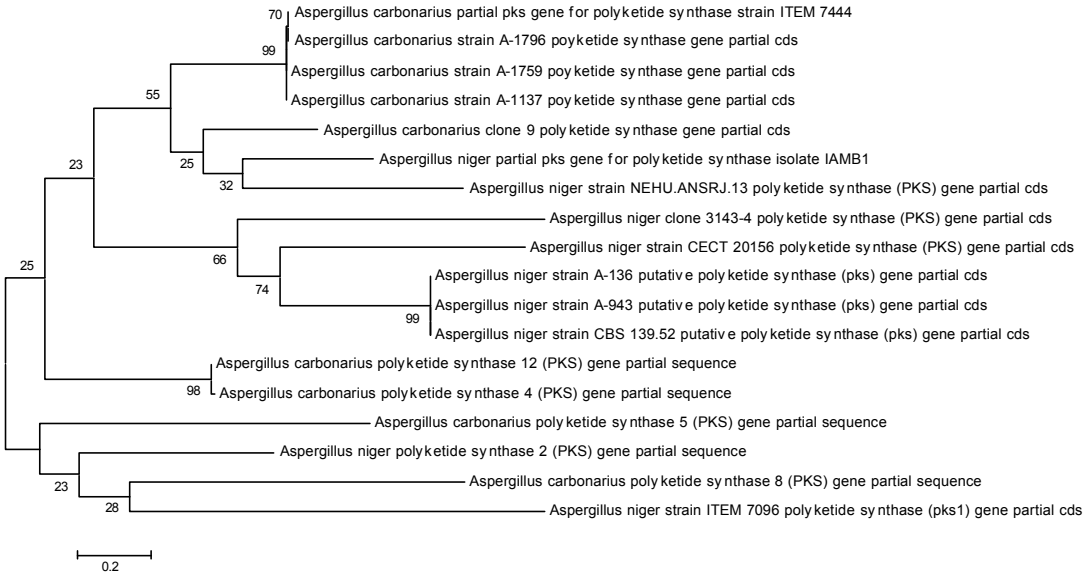


Fig. Phylogenetic relationship between the studied ochratoxigenic fungi

## DISCUSSION AND CONCLUSION

The use of bioinformatic tools for sequence comparison in order to deduce important gene structure and functions have been well documented [18]. In this study, pks gene sequence as well as several fungal transcriptional factors compared revealed variation in homology and length. This observation is not unexpected as such observation has been reported for Aspergillus section Flavi [18]. However, our findings is a strong pointer that these genetic traits may not be necessary among the factors that triggers ochratoxin A production. Sequence differences in the pks region and promoter structure provide a basis for predicting the roles of environmental and developmental cues in differential regulation of ochratoxin production among ochratoxin A producing fungi. The possibility of inhibition or stimulation of ochratoxin A production by different nitrogen sources was assessed with the presence or absence of AreA-binding sites of pks gene. Results obtained revealed a considerable 55.6% of the total isolates harboring these gene and associated high level of OTA. The variability of GATA sites in the pks region and differences among fungi in ochratoxin A production

provides a way to test the role of nitrogen sources on transcriptional regulation of ochratoxin A biosynthesis genes. Transcription factors Aba A and Brl A may mediate expression of genes involved in development-specific processes in fungi [19], but have not yet been directly implicated in regulation of secondary metabolite biosynthesis. Putative BrlA-binding sites was absent in the pks region of all isolates. This observation may not be unconnected to the fact that this important gene is not involved in OTA biosynthesis. The presence or absence of PacC-binding sites in the pks region could partly account for differential sensitivity of ochratoxin A production to pH in different species of Aspergillus [20]. Precedent for interference by PacC in the expression of acid-expressed genes has been reported for the gabA gene in *A. nidulans* [21]. In this study, PacC site was found in only four of the total eighteen isolates representing only 22.2%. This observation could be suggesting that pH may not be that important in the regulation of expression of ochratoxin A gene. The phylogenetic relationship using the maximum likelihood also suggests very little to high similarities among the studied isolates. In conclusion, no single transcriptional factors is enough to activate ochratoxin A biosynthesis, brlA is not involved in OTA production while AreA and Aba could only be regarded to as an enhancers of OTA biosynthesis.

## REFERENCES


1. Kapetanakou, A.E., E.Z. Panagou, M. Gialitaki, E.H. Drosinos and P.N. Skandamis, 2009. Evaluating the combined effect of water activity, pH and temperature on ochratoxin A production by Aspergillus ochraceus and Aspergillus carbonarius on culture medium and Corinth raisins. Food Control, 20: 725-732.
2. JEFCA, 2001. Ochratoxin A. Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives. http://www.inchem.org/documents/jefca/ jecmono /v47je04.htm.
3. O'Brien, E. and D.R. Dietrich, 2005. Ochratoxin A: the continuing enigma. Critical Reviews in Toxicology, 35: 33-60.
4. Ogunledun, A., 2007. Incidence of microbial contaminant and nutrients composition of selected cocoa based beverages in Ibadan, Nigeria. Ph.D thesis submitted to the Department of Microbiology, University of Ibadan, pp: 1-144.
5. Jayeola, C.O. and A. Oluwadun, 2000. Mycoflora and nutritional components of cocoa powder samples in South West Nigeria. Africa Journal of Agric Research, 5: 2694-2698.
6. Castegnaro, M., U. Mohr, A. Pfohl-Leszkowicz, J. Esteve, J. Steinmann, J. Tillmann, T. Michelson and J. Bartsch, 1998. Sex and strain specific induction of renal tumors by ochratoxin A in rats correlated with DNA adduction. International Journal of Cancer, 77: 70-75.
7. Radic, B., R. Fuchs, M. Peraica and A. Lucis, 1997. Ochratoxin A in human sera in an area with endemic nephropathy in Croatia. Toxicology Letters, 91: 105-109.
8. International Agency for Research on Cancer (IARC), 1993. Some Naturally Occurring Substances: Food Items and Constituents. Heterocyclic Aromatic Amines and Mycotoxins, Vol. 56.,World Health Organization, Lyon, France, pp: 599.
9. Basilico, J.C., M.Z. De Basilica, C. Chierizatti and C.G. Vinderola, 2001. Characterization and control of thread mould in cheese. Letters in Applied Microbiology, 32: 419-423.
10. Magnolia, C., C. Hallak, A. Astoreeca, L. Ponsone, S. Chiazchiera and A.M. Dalcero, 2006. Occurrence of ochratoxin A producing fungi in commercial kernels in Argentina. Mycopathologia, 161: 53-58.
11. Robens, J., 2001. The costs of mycotoxin management to the USA: management of aflatoxins in the United States. APSnet Feature http:// www.apsnet.org/online/feature/mycotoxin/top.html, pp: 2-8.
12. Thomas, B.T. and L.A. Ogunkanmi, 2014. Ochratoxin A producing filamentous fungi in garri circulating in Ogun State, Nigeria. Elixir Biosciences, 75: 27788-27794.
13. Cotty, P., 1988. Aflatoxin and sclerotial production by Aspergillus flavus: influence of pH. Phytopathology, 78: 1250-1253.
14. Cotty, P.J. and K.F. Cardwell, 1999. Divergence of West African and North American communities of Aspergillus section Flavi. Appl. Environ. Microbiol., 65: 2264-2266.
15. Farris, J.S., M. Kallersjo, A.G. Kluge and C. Bult, 1994. Testing significance of incongruence. Cladistics, 10: 315-320.
16. Swofford, D.L., 1998. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Sinauer Associates, Sunderland, MA.

17. Kishino, H. and M. Hasegawa, 1989. Evaluation of the maximum liklihood estimate of the evolutionary tree topologies from DNA sequence data and the branching of the Hominoidea. J. Mol. Evol., 29: 170-179.
18. Ehrlich, K.C., J.W. Cary and B.G. Montalbano, 1999. Characterization of the promoter for the gene encoding the aflatoxin biosynthetic pathway regulatory protein AFLR. Biochim. Biophys. Acta, 1444: 412-417.
19. Sewall, T.C., 1994. Cellular effects of misscheduled brlA, abaA and wetA expression in Aspergillus nidulans. Can. J. Microbiol., 40: 1035-1042.
20. Ehrlich, K.C. and P.J. Cotty, 2000. Variability in nitrogen regulation of aflatoxin production by Aspergillus flavus strains. In: 101st General Meeting, Orlando, FL, May 20-24. American Society of Microbiology, pp: 533. (Abst O1).
21. Espeso, E.A. and H.N. Arst Jr, 2000. On the mechanism by which alkaline pH prevents expression of an acid-expressed gene. Mol. Cell. Biol., 20: 3355-3363.