International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 14 Issue 12 , December - 2025

Screening Through Bioinformatic Studies of Secondary Metabolites from the Rare Alkaline-Halophilic *Stachybotrys Microspora* Compared to Related Fungi

Salma Abdeljalil
Molecular Biotechnology of Eukaryotes Laboratory, Centre of Biotechnology of Sfax- University of Sfax, Tunisia
Centre de Biotechnologie de Sfax, Route Sidi Mansour, BP «1177» 3018,
Université de Sfax – Tunisia.

***Abstract* - Screening for microbial secondary metabolites (SMs) has attracted the attention of the scientific community since 1940s. In fact, since the discovery of penicillin, intensive researches have been conducted worldwide in order to detect and identify novel microbial secondary metabolites.**

However, the development and advances of omics-based techniques such as metabolomics and genomics have revealed the potential to discover novel SMs encoded in the DNA of microorganisms. However, we have not yet tested their production in the laboratory to determine the biosynthesis gene clusters (BGCs) actually associated with the biosynthesis of these secondary metabolites.In fact, Fungi produce a number of structural classes of secondary metabolites including polyketides (PKs), non-ribosomal peptides (NRPs), hybrid PK-NRPs, indole alkaloids, and terpenes. In almost all cases the genes responsible for the production of these classes of secondary metabolites are organized as a gene cluster. We report in this paper a large scale analysis of secondary metabolites produced by the rare fungus *Stachybotrys microspora* using different bioinfomatic tools.

Keywords: Stachybotrys microspora; metabolism; Secondary metabolites; Prism 4; OmicsBox platform; anti-SMASH database.

I. INTRODUCTION

Numerous facets of society are impacted by fungi, which have incalculable effects on ecosystems and are crucial to ecology, agriculture, health, and biotechnology. Because of their typical haplobion life cycle, which promotes the phenotypic of mutations, and the fact that the majority of cells can differentiate throughout the organism, they are utilized as appropriate organisms for fundamental research. Numerous fungi are also beneficial for molecular, genetic, and microbiological methods and are simple to cultivate. As a result, fungi were among the earliest model organisms used in the study of cells, developmental biology, biochemistry, and genetics.

We are working on a rare, locally isolated fungal strain belonging to the *Stachybotrys microspora*. *Stachybotrys* is related to the genus *Memnoniella*, most *Stachybotrys* species inhabit cellulose-rich substances [1]. This genus is widely distributed and contains about 50 species [2]. Our previous biochemical studies on *S. microspora* shows the production of various cell wall degrading enzymes. Interestingly, our strain is characterized by growing in a cellulosic-based medium over a wide pH range of 4-9. This is rarely reported in most known fungal species. In addition to its ability to grow at alkaline pH, *S. microspora* produces neutral and alkaline endoglucanases [3] and secretes several β-glucosidases [4-9] whereas fungi generally have one or two beta-. Produces glucosidase.

To take advantage of the potential of this strain, we have also conducted a molecular study of the cellulolytic genes to investigate the molecular expression profile of the corresponding gene. We succeeded in isolating three β-glucosidase genes from the glycoside hydrolases 1 and 3 families, confirming the abundance of *S. microspora* in the production of β-glucosidase, and showed different expression patterns [7,8].

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 14 Issue 12 , December - 2025

In addition to secreting cellulase, the fungus produces xylanase, protease, chitinase, β-glucanase and pectinase, some of which share the same regulatory mechanism to induce or suppress their respective activities [10].

Sequencing of the whole genome using next-generation sequencing can identify sequences that are potentially associated with toxin production and other yet unknown secondary metabolites.
In fact, the most infamous species, *S. chartarum* (also known as *S. atra*) and S.*chlorohalonata* are known as "black mold" or "toxic black mold" and are frequently associated with poor indoor air quality that arises after fungal growth on water damaged building materials. *S. chartarum* produces several mycotoxins, highly toxic macrocyclic trichothecenes and related trichoverroids as well as immunosuppresants and endothelin receptor antagonists [11].
Recently, a comparative genome sequencing study has revealed chemotype-specific gene clusters in the toxigenic black mold *Stachybotrys*, coding for satratoxins and other less-toxic atronomes [12].
Triprenyl phenols (SMTPs), a novel family of small, advantageous compounds that improve plasminogen activation and fibrin-binding, are secreted by *Stachybotrys microspora* and are non-toxic. Little is known regarding their action in vivo with regard to plasminogen activation and blood clot clearance, despite the fact that their effects on fibrinolysis have been described in vitro [13].
Furthermore, at recommended dosages, enzymes are categorized as non-toxic. Ben Hmad et al. assessed the cytotoxicity effect of an enzyme cocktail produced by Stachybotrys microspora on human endothelial cells (HUVEC) in light of the growing consumer safety concerns related to enzymes in the food sector.
Remarkably, the results showed that cell viability and replication remained between 120 and 150 percent across enzyme cocktail concentrations ranging from 1 to 10 percent. This suggests that the enzyme cocktail from *Stachybotrys microspora* does not exhibit cytotoxicity but rather promotes the growth of human endothelial cells [14].

II. MATERIALS AND METHODS

A. Screening for secondary metabolites clusters using antiSMASH database

Microbial secondary metabolites are a potent source of antibiotics and other pharmaceuticals. Genome mining of their biosynthetic gene clusters has become a key method to accelerate their identification and characterization. In 2011, antiSMASH was developed as a web-based analysis platform that automates this process available at http://antismash.secondarymetabolites.org/.

In fact, many fungal secondary metabolites are made by products of genes that are found adjacent to one another in a single contiguous locus [15]. These genetic loci are known as secondary metabolite biosynthesis (SMB) clusters. SMB clusters were identified with the anti-SMASH cluster prediction software.

B. Screening of secondary metabolites screening using OmicsBox platform

Recently, we published the first draft of the genome sequence of S. microspora consisting of 3,715 contigs with a genome size of 35,343,854 bp and a GC content of 53.31 percent.Two bioinformatic tools, Omicsbox and the fungicompanion web server, were used for functional annotation [9].

We report in this paper the screening of secondary metabolites using eggnog map annotation via Omicsbox platform.

C. Screening of secondary metabolites screening using Prisma 4

All kinds of antibiotics now used in clinical settings can have their chemical structures predicted using the full PRISM 4 platform. The development of machine-learning techniques to forecast the probable biological activity of encoded molecules is made possible by the precision of chemical structure prediction. Using PRISM 4, we map the production of secondary metabolites in a population of more than 10,000 bacterial genomes from metagenomic and cultivated isolates, uncovering thousands of antibiotics that are encoded. The interactive web application PRISM 4 can be found for free from http://prism.adapsyn.com.

D. Multiple alignment and phylogenetic analysis

Amino acid sequence alignment was performed using the ClustalW-BioEdit program.

The phylogenetic tree was performed with Cobalt Multiple Protein Alignment Tool of the NCBI Web BLAST service using the neighbour-joining method. COBALT is a multiple sequence alignment tool that finds a collection of pairwise constraints derived from conserved domain database, protein motif database, and sequence similarity, using RPS-BLAST, BLASTP, and PHI-BLAST. Pairwise constraints are then incorporated into a progressive multiple alignment.

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 14 Issue 12 , December - 2025

III. RESULTS AND DISCUSSION:

A. Screening of secondary metabolites using the online database anti- SMASH

The screening of secondary metabolites using the online database anti-SMASH gave a total number of 45 compounds classified as12 of T1PKS (Type I PKS (Polyketide synthase),8 of NRPS (Non-ribosomal peptide synthetase cluster),indole, 7 of terpene,NRPS-like, 3 of T3PKS (fig.1).

B. Screening of secondary metabolites screening using OmicsBox platform

Secondary metabolites were also studied using Omicsbox platform under eggnog map annotation.

Some strains from the fungal genus *Stachybotrys* strains comprise two mutually-exclusive toxin chemotypes, one producing satratoxins, which are a subclass of trichothecenes, and the other producing the less-toxic atranones [12].

The whole annotation of *Stachybotrys microspora* toxins has indicated the presence of 6 Satratoxin biosynthesis SC1 cluster protein 4 with two of them were incomplele, Satratoxin biosynthesis SC1 cluster transcription factor SAT9, Satratoxin biosynthesis SC3 cluster transcription factor SAT20, putative HC-toxin efflux carrier TOXA, killer toxin subunits alpha/beta, host-specific AK-toxin Akt2, toxin efflux pump (AflT), structural toxin protein RtxA, killer toxin resistance protein, zeta toxin family protein, Ras-like C3 botulinum toxin substrate 1, gliotoxin biosynthesis but no atranones.

Compared to other *Stachybotrys* species which the largest named the core atranone cluster, which encodes 14 proteins that may suffice to produce all observed atranone compounds via reactions that include an unusual Baeyer-Villiger oxidation. Satratoxins are suggested to be made by products of multiple gene clusters that encode 21 proteins in all, including polyketide synthases, acetyltransferases, and other enzymes expected to modify the trichothecene skeleton. One such satratoxin chemotype-specific cluster is adjacent to the core trichothecene cluster, which has diverged from those of other trichothecene producers to contain a unique polyketide synthase [12].

The eggnog annotation has shown 458 secondary metabolites using OmicsBoxEggNOG Annotation (Table 1) and a report of all eggonog annotation is illustrated as supplementary file (supplementary file 1).

Regarding the biosynthesis of aflatoxin, we must state that among the known mycotoxins, aflatoxins—a class of furanocoumarins generated from polyketides—are the most harmful and carcinogenic substances. However, only four main aflatoxins—B1, B2, G1, and G2 (AFB1, AFG1, AFB2, and AFG2)—contaminate agricultural commodities and may be harmful to animals out of the at least 16 structurally related aflatoxins that have been identified [16].

Interestingly, the annotation of *S.microspora* genome revealed the presence of aflatoxin reductases : aflatoxin B1 aldehyde reductase member 2, 3 and 4 , aflatoxin biosynthesis ketoreductase nor-1, aflatoxin biosynthesis regulatory protein, probable aflatoxin efflux pump AFLT.

According to [17] , these enzymes serve as detoxifying agents against aflatoxins, which are secondary metabolites mostly generated by *Aspergillus flavus* and *Aspergillus parasiticus*. There are currently more than 20 known aflatoxin derivatives. The International Agency for Research on Cancer has designated aflatoxins B1 (AFB1) as a Group I carcinogen, making it the most hazardous of them [18,19]. Many crops, including rice, peanuts, wheat, and maize, naturally become contaminated with AFB1 [20]. Consuming tainted food or feed can result in serious financial losses as well as acute and chronic illnesses in both people and farm animals [21].

C. Screening of secondary metabolites using Prism 4

Due to the limitation of the input file size which was up to 20 Mb we were obliged to divide the whole genomic Fasta file in two distinct files.The first one gives an annotation of about 30 cluster genes mostly are polyketide and non ribosomal peptide wich are sometimes in the direct or oppositedirection in the clusters (Fig.2). RIPP (The ribosomally synthesized and post-translationally modified peptide) and trichodiene-derived terpene are also predicted (Fig.3)

The second file predict only 4 clusters of polyketides (data not shown).

Terpenes and terpenoids have well-known pharmaceutical properties, including anti-cancer, anti-viral, anti-bacterial and anti-inflammatory activity [22].

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 14 Issue 12 , December - 2025

D. Multiple alignment and phylogenetic analysis of aflatoxin B1 aldehyde reductase

To more investigate the aflatoxin B1 aldehyde reductase member 2, 3 and 4 produced by our strain we performed a multiple alignment of the corresponding sequences and we found some region identities (Fig.4).

Moreover, to understand the relationship with other fungi regarding the production of this class of enzymes, we analyzed the sequences blasted (the top 30 sequences obtained after blast protein) by phylogenetic analysis.

At first, we have to say that all the enzymes share 100 % identity with the fungus *Stachybotrys chlorohalonata.*

All the generated phylogenetic trees (Supplementary file 2, supplementary file 3 and supplementary file 4) showed that they are more closely related to *Trichoderma* species.

Multiple alignment and phylogenetic analysis of satratoxin clusters

To more investigate the Satratoxins production by our strain we performed multiple alignment of the corresponding sequences. We should note that they do not share any significant homology between each other (data not shown).

Moreover, to understand the relationship with other fungi regarding the production of this class of toxins, we analyzed the sequences blasted (the top 30 sequences obtained after blast protein) by phylogenetic analysis for one Satratoxin biosynthesis SC1 cluster protein 4.

At first, we have to say that all toxins share 100 % identity with the fungus *Stachybotrys chlorohalonata.*

The analysis of the generated phylogenetic tree (Supplementary file 5) showed that they are more closely related to *Verticillium* and *Colletotrichum* species (sexual stage: Glomerella), which is a genus of fungi that establishes a relationship with plants as endophytes or phytopathogens. Many of the species in this genus are plant pathogens, but some species may have a mutualistic relationship with hosts [23].

These results could suggest that *S. microspora* can act also as a phytopathogen for some plants.

IV. CONCLUSIONS

Since the 1940s, the scientific community has been interested in screening for microbial secondary metabolites (SMs). Actually, since the discovery of penicillin, much research has been carried out all over the world to find and identify new secondary metabolites produced by microorganisms. As a result, employing conventional trials has greatly reduced the finding of novel SMs. Consequently, one of the top priorities was to find new methods for finding novel SMs. The possibility to find novel SMs that were encoded in the microorganisms' DNA but were either not expressed in the lab media or might be created in undetectable amounts has been made clear by the invention and advancement of omics-based approaches like metabolomics and genomics.

Moreover, the identification of some gene in the gene clusters (and not all the cluster) doesn’t mean necessary that we will detect the corresponding products in the media after

In actuality, a large number of microorganisms possess genes that could potentially code for particular tasks but do not operate during the organism's typical lifetime. These genes have been dubbed "cryptic genes," and a mutational event is typically necessary to activate them. They vary from pseudogenes, which are created when a functional gene is duplicated but stay inactive due to the accumulation of many mutations.

Moreover, the identification of our isolated strain as *Stachybotrys microspora* has shown that it is safe for humans but the presence of these toxin genes could suggest that it is only pathogen for plants and insects as it is the most case for the majority of fungal strains. Therefore and again, further laboratory experiments and research must be carried out to confirm or refute all these hypotheses.

The analysis of secondary metabolites through bioinformatics studies have shown the richness of our studied strain regarding secondary metabolites using the tool the EggNOG Annotation (the number of Secondary metabolites biosynthesis, transport and catabolism (Q) is around 458 and also the prediction of interesting detoxification enzymes like aflatoxin B1 aldehyde reductases.

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 14 Issue 12 , December - 2025

ACKNOWLEDGMENTS

This work received financial support from the Ministry of Higher Education and Scientific Research, Tunisia, granted to the "Laboratory of Molecular Biotechnology of Eukaryotes", Biotechnology Center of Sfax, Tunisia.

Author contributions

SA and AG were responsible for the study design. SA and AG supervised the draft of the manuscript. All of the authors approved the final version of the manuscript submitted for publication.

Data Availability

The data obtained or analyzed in the present study have been incorporated in this manuscript.

Declarations

Conflict of interests

The authors declare that they have no conflicts of interest.

Ethical statement

No ethical approval is required for this current study.

REFERENCES

[1] R.A. Haugland, S.J. Vesper, S.M. Harmon. “Phylogenetic relationships of *Memnoniella* and *Stachybotrys* species and evaluation of morphological features for *Memnoniella* species identification”, Mycologia, 2011, vol. 93 (1), pp. 54–65.

[2] P.M. Kirk, P.F. Cannon, D.W. Minter, J.A. Stalpers. “Dictionary of the Fungi” (10th ed.) ,2008, Wallingford: CABI. p. 659. ISBN 9780851998268.

[3] I. Ben Hmad, S. Abdeljalil, W. Saibi, A. Gargouri. “Medium initial pH and carbon source stimulate differential alkalin cellulase time course production in *Stachybotrys microspora”*, Applied Biochemistry and Biotechnology, 2014, vol. 172(5),pp.2640-9.

[4] B. Amouri and A. Gargouri. “Characterization of a novel β-glucosidase from a *Stachybotrys* strain”, Biochemical Engineering Journal, 2006, vol. 32, pp. 191–197.

[5] W. Saibi, B. Amouri and A. Gargouri. “Purification and biochemical characterization of a transglucosilating β-glucosidase of *Stachybotrys* strain”, Applied Microbiology and Biotechnology , 2007, vol. 77, pp. 293–300.

[6] W. Saibi, S. Abdeljalil and A. Gargouri. “Carbon source directs the differential expression of β-glucosidases in *Stachybotrys microspora”* , World Journal of Microbiology and Biotechnology, 2011, vol. 27, pp. 1765–1774.

[7] S. Abdeljalil, H. Trigui-Lahiani, H. Lazzez and A. Gargouri. “Cloning, molecular characterization, and mRNA expression of the thermostable family 3 β-Glucosidase from the rare fungus *Stachybotrys microspora”*, Molecular Biotechnology, 2012, vol. 54, pp. 842-852.

[8] S. Abdeljalil, I. Borgi, S. Carvalho, L. Jmal-Hammami and A. Gargouri. “Molecular and bioinformatic data reveal two differentially-expressed intracellular GH1 β-glucosidases from the rare alkalophilic fungus *Stachybotrys microspore”*, Gene ,2019, vol. 703, pp. 134-144.

[9] S. Abdeljalil *et al.* “Large-scale analysis of the genome of the rare alkaline-halophilic *Stachybotrys microspora* reveals 46 cellulase genes”, FEBS Open Bio, 2023, vol.13(4), pp. 670-683.

[10] S .Abdeljalil *et al.* “Investigations on hydrolytic activities from *Stachybotrys microspora* and their use as an alternative in yeast DNA extraction” Applied Biochemistry and Biotechnology,2014, vol. 72(3), pp. 1599-611.

[11] B. Kendrick. “The Fifth Kingdom, 3rd edn”, 2000, Newburyport, MA, Focus Publishing.

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 14 Issue 12 , December - 2025

[12] J. Semeiks, D. Borek, Z. Otwinowski and N.V. Grishin. "Comparative genome sequencing reveals chemotypespecific gene clusters in the toxigenic black mold *Stachybotrys"*, BMC Genomics, 2014, vol.15(1), pp. 590.

[13] W. Hu, R. Narasaki, N. Nishimura and K. Hasumi. "SMTP *Stachybotrys microspora* triprenyl phenol enhances clot clearance in a pulmonary embolism model in rats" Thrombosis Journal, 2012, vol. 9;10 (1):2.

[14] I. Ben Hmad, A. Mokni Ghribi, M. Bouassida, W. Ayadi, S. Besbes, S. Ellouz Chaabouni and A. Gargouri. "Combined effects of α-amylase, xylanase, and cellulase coproduced by *Stachybotrys microspora* on dough properties and bread quality as a bread improver" International Journal of Biological Macromolecules, 2024, vol. 277, Part 3, 134391.

[15] J. Yu *et al.* "Clustered pathway genes in aflatoxin biosynthesis", Applied and Environmental Microbiology Journal, 2004, vol. 70(3), pp.1253–1262.

[16] S. Kumagai, N. Nakano and K. Aibar. "Interactions of aflatoxin B1 and bloodcomponents of various species in vitro: Interconversion of aflatoxin B1 and aflatoxicol in the blood", Toxicology and Applied Pharmacology,1983, Vol.67, pp. 292-301.
[17] Y. Guan, J. Chen, E. Nepovimova, M. Long, W. Wu and K. Kuca. "Aflatoxin Detoxification Using Microorganisms and Enzymes", Toxins, 2021, (Basel)13(1):46.

[18] J. He *et al. "*Chemical and biological transformations for detoxification of trichothecene mycotoxins in human and animal food chains: a review" , Trends in Food Science and Technology, 2010, vol.21,pp.67-76.

[19] Y. Zhu *et al.* "Strategies and Methodologies for Developing Microbial Detoxification Systems to Mitigate Mycotoxins", Toxins, 2017, (Basel)9(4): 130.

[20] D.V. Neeff *et al.* "In vitro and in vivo efficacy of a hydrated sodium calcium aluminosilicate to bind and reduce aflatoxin residues in tissues of broiler chicks fed aflatoxin B1", Poultry Science, 2013, vol.92, pp.131-137.

[21] C. Verheecke, T. Liboz and F. Mathieu. "Microbial degradation of aflatoxin B1: Current status and future advances", International Journal of Food Microbiology, 2016, vol.237, pp.1-9.

[22] E. J. Downer. "Anti-inflammatory Potential of Terpenes Present in *Cannabis sativa* L", ACS Chemical Neuroscience, 2020, vol.11(5),pp. 659-662.

[23] L. Cai *et al.* "A polyphasic approach for studying *Colletotrichum"*, Fungal Divers, 2009, vol. 39, pp.183–204.

FIGURES LEGENDS

Figure 1. Prediction of secondary metabolites using anti-SMASH database. Colors show different clusters with percentages of similarity.

Figure 2. An example of the output generated by Prism 4 annotated tool for secondary metabolites.

Figure 3. Triterpenoid Biosynthesis prediction pathway using OmicsBox platform.

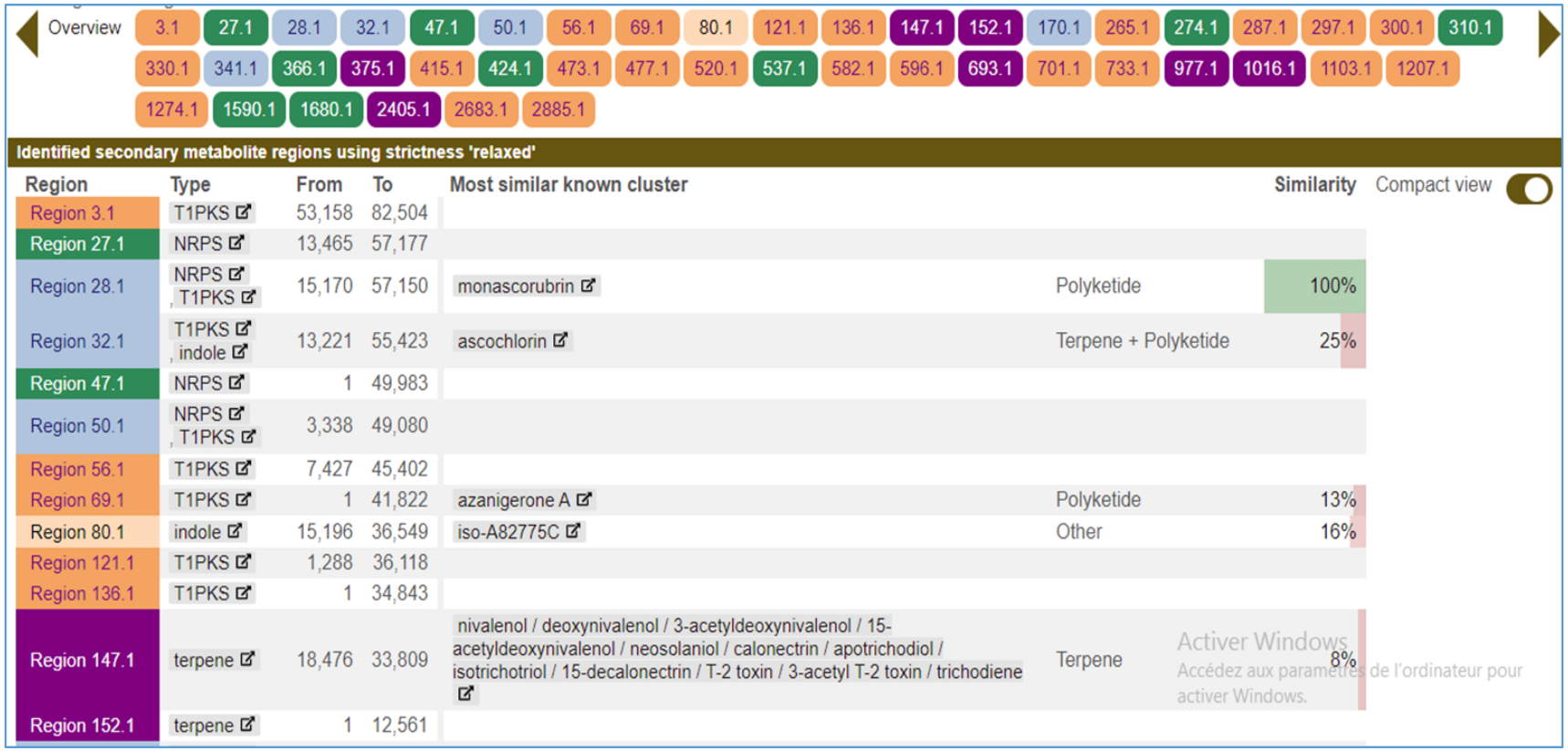
Figure 4. Multiple alignment of aflatoxin B1 aldehyde reductase member 2, 3 and 4

Supplementary figure 1.a report of all eggonog annotation using OmicsBox platform.

Supplementary figure 2, 3, 4 and 5: phylogenetic trees with COBALT:Multiple Protein Alignment Tool for aflatoxin B1 aldehyde reductase member 2, 3, 4 and Satratoxin biosynthesis SC1 cluster protein 4.

Published by :
https://www.ijert.org/
An International Peer-Reviewed Journal

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 14 Issue 12 , December - 2025



Identified secondary metabolite regions using strictness 'relaxed'

Compact view

Region	Type	From	To	Most similar known cluster		Similarity
Region 3.1	T1PKS	53,158	82,504			
Region 27.1	NRPS	13,465	57,177			
Region 28.1	NRPS, T1PKS	15,170	57,150	monascorubrin	Polyketide	100%
Region 32.1	T1PKS, indole	13,221	55,423	ascochlorin	Terpene + Polyketide	25%
Region 47.1	NRPS	1	49,983			
Region 50.1	NRPS, T1PKS	3,338	49,080			
Region 56.1	T1PKS	7,427	45,402			
Region 69.1	T1PKS	1	41,822	azanigerone A	Polyketide	13%
Region 80.1	indole	15,196	36,549	iso-A82775C	Other	16%
Region 121.1	T1PKS	1,288	36,118			
Region 136.1	T1PKS	1	34,843			
Region 147.1	terpene	18,476	33,809	nivalenol / deoxynivalenol / 3-acetyldeoxynivalenol / 15-acetyldeoxynivalenol / neosolaniol / calonectrin / apotrichodiol / isotrichotriol / 15-decalonectrin / T-2 toxin / 3-acetyl T-2 toxin / trichodiene	Terpene	8%
Region 152.1	terpene	1	12,561			

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 14 Issue 12 , December - 2025

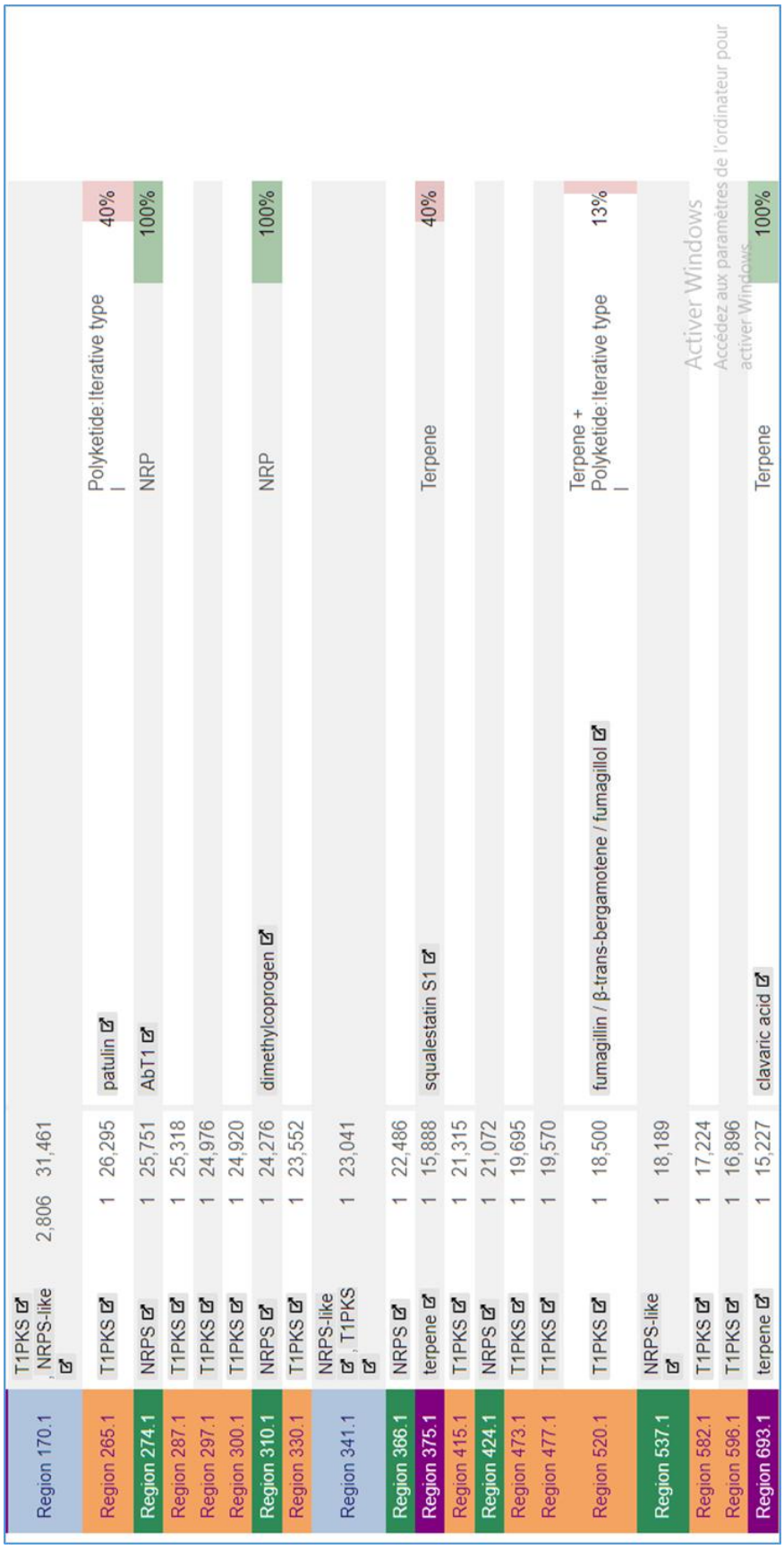


Figure 1

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 14 Issue 12 , December - 2025

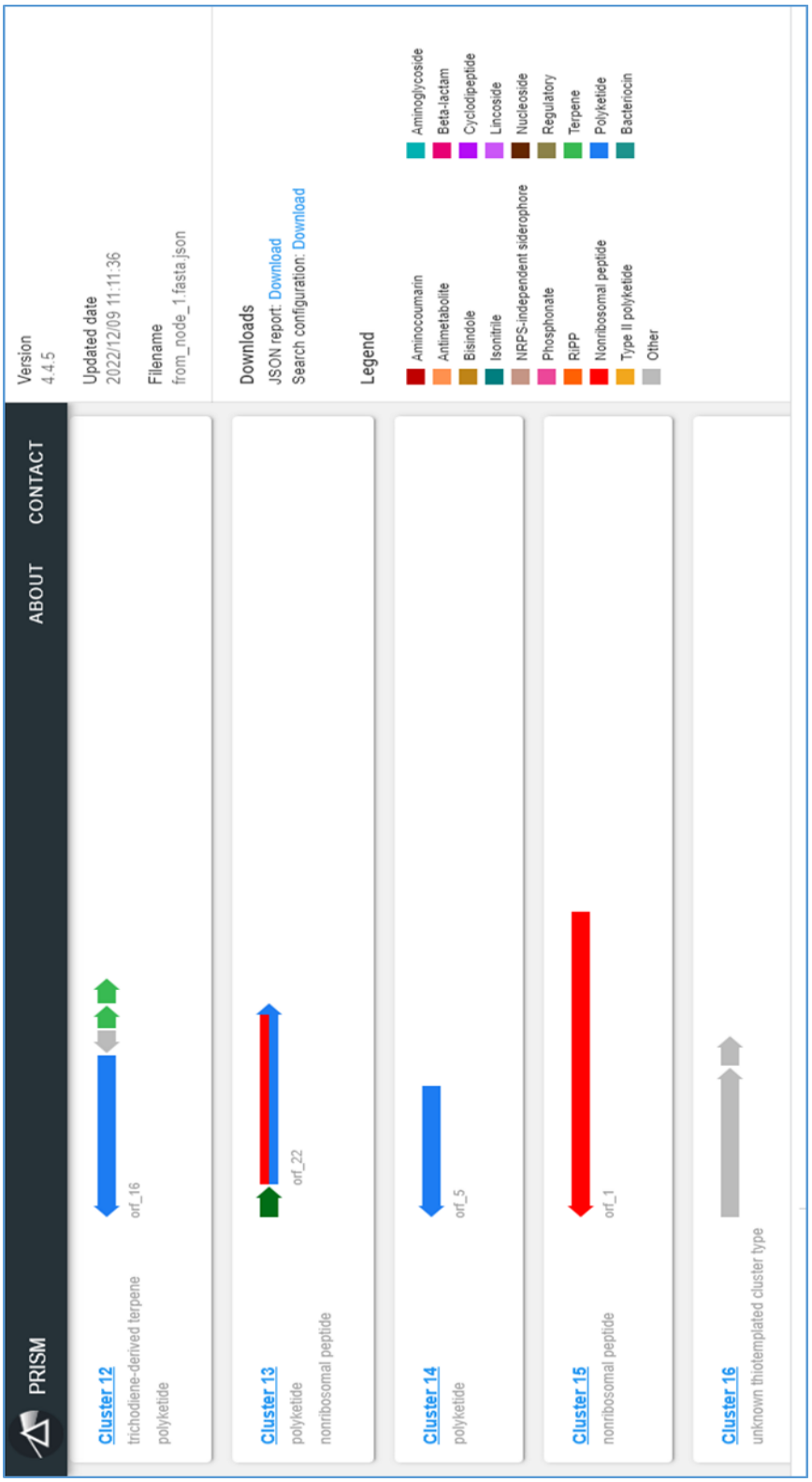


Figure 2

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 14 Issue 12 , December - 2025

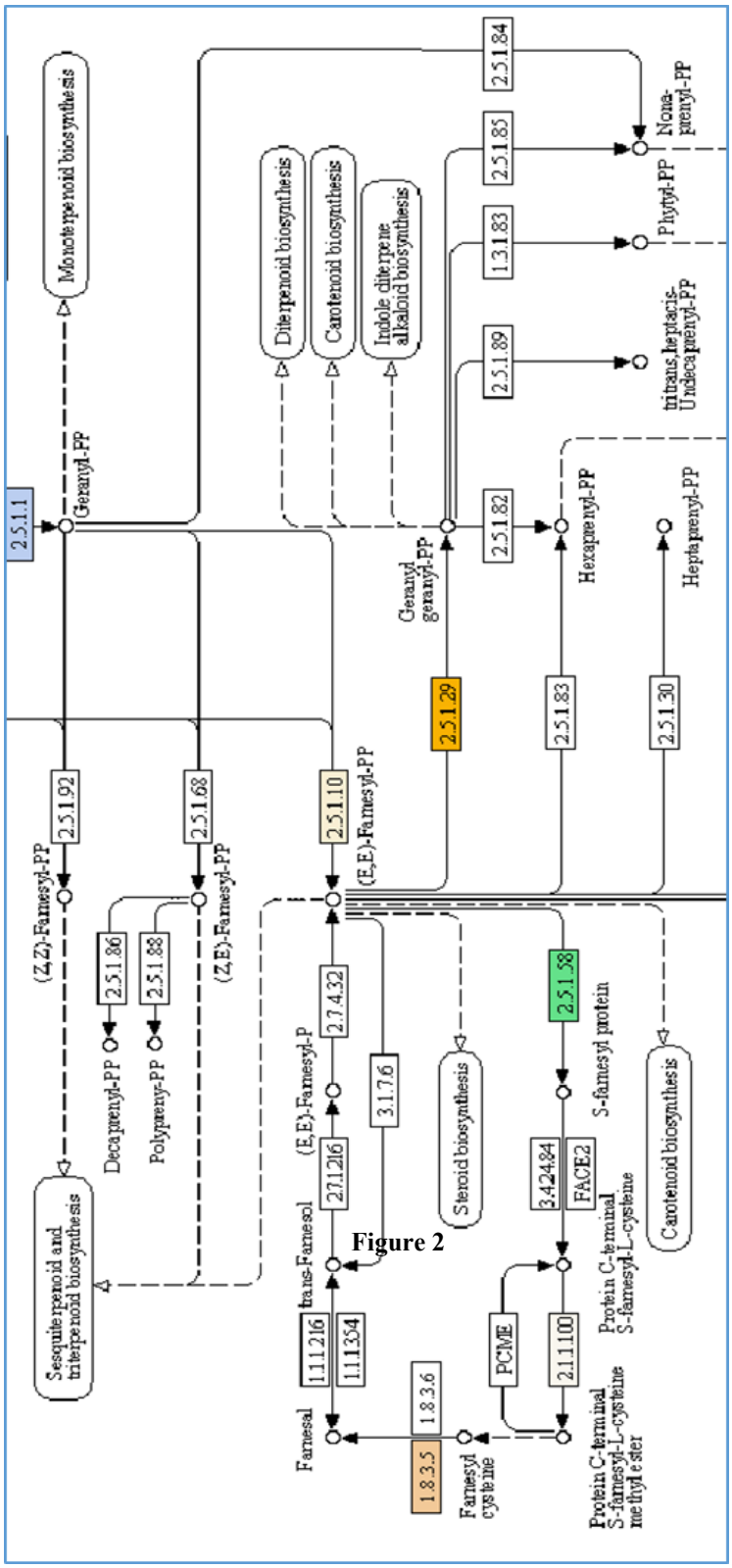
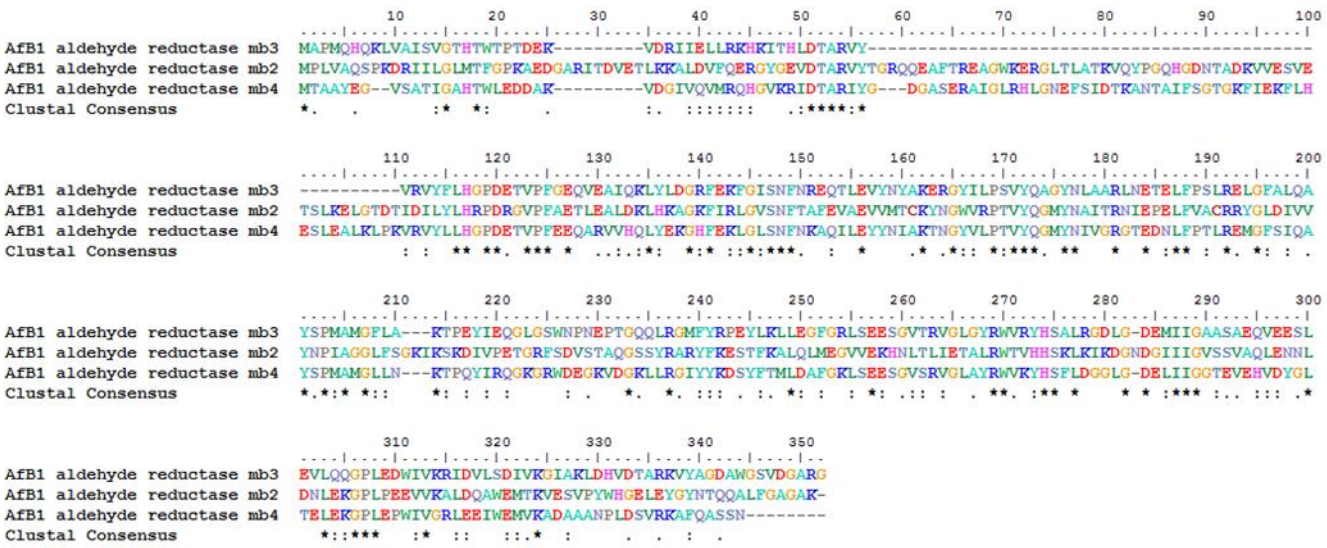


Figure 2

Figure 3

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 14 Issue 12 , December - 2025

Figure 4



International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 14 Issue 12 , December - 2025

Supplementary figure 1

EggNOG Annotation Report

General Information

Total amount of input sequences:	11098
Average length:	490.0
Number of GO annotated sequences:	3730
Number of GO annotations:	32384
Average GOs per sequence:	8.68

COG Categories Distribution

Information Storage and Processing:	
Replication, recombination and repair (L):	390
Translation, ribosomal structure and biogenesis (J):	368
Transcription (K):	331
RNA processing and modification (A):	307
Chromatin structure and dynamics (B):	93
Total:	**1489 / 16.42%**
Cellular Processses and Signaling:	
Posttranslational modification, protein turnover, chaperones (O):	569
Intracellular trafficking, secretion, and vesicular transport (U):	376
Signal transduction mechanisms (T):	286
Cytoskeleton (Z):	106
Cell wall/membrane/envelope biogenesis (M):	102
Cell cycle control, cell division, chromosome partitioning (D):	100
Defense mechanisms (V):	45
Extracellular structures (W):	4
Nuclear structure (Y):	3
Cell motility (N):	3
Total:	**1594 / 17.58%**
Metabolism:	
Carbohydrate transport and metabolism (G):	628
Secondary metabolites biosynthesis, transport and catabolism (Q):	458
Amino acid transport and metabolism (E):	372
Energy production and conversion (C):	346
Lipid transport and metabolism (I):	278
Inorganic ion transport and metabolism (P):	227
Coenzyme transport and metabolism (H):	174
Nucleotide transport and metabolism (F):	94
Total:	**2577 / 28.42%**
Poorly Characterized:	
Function unknown (S):	2914
General function prediction only (R):	0
Total:	**2914 / 32.13%**

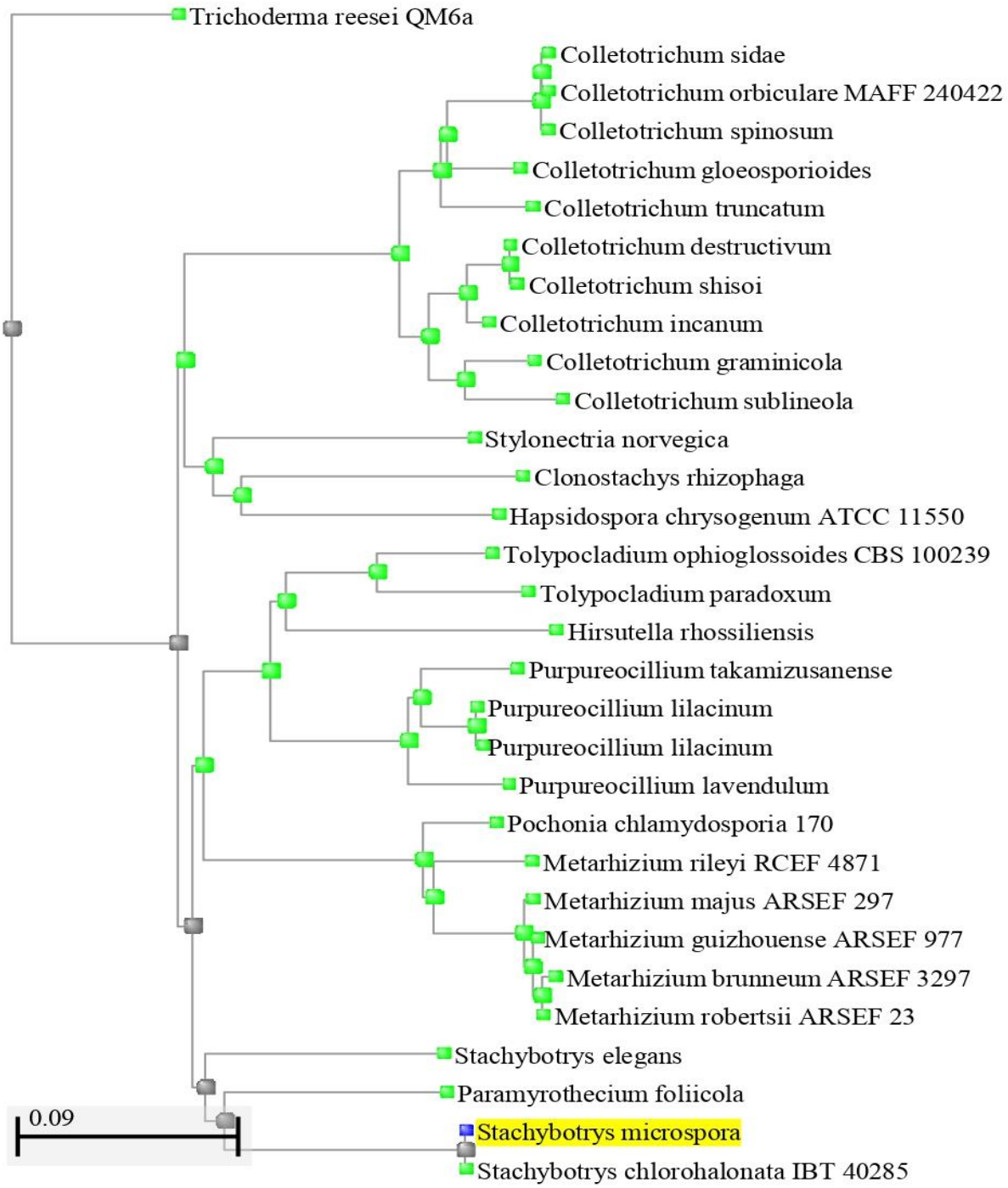
International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 14 Issue 12 , December - 2025

Top 10 Orthologous Groups Distribution

root:	10754 / 13.59%
Eukaryota:	10731 / 13.57%
Opisthokonta:	10473 / 13.24%
Fungi:	10454 / 13.22%
Ascomycota:	10402 / 13.15%
Sordariomycetes:	9564 / 12.09%
Hypocreales:	7496 / 9.48%
Nectriaceae:	3606 / 4.56%
Hypocreaceae:	1515 / 1.92%
Clavicipitaceae:	1421 / 1.8%
Glomerellales:	997 / 1.26%

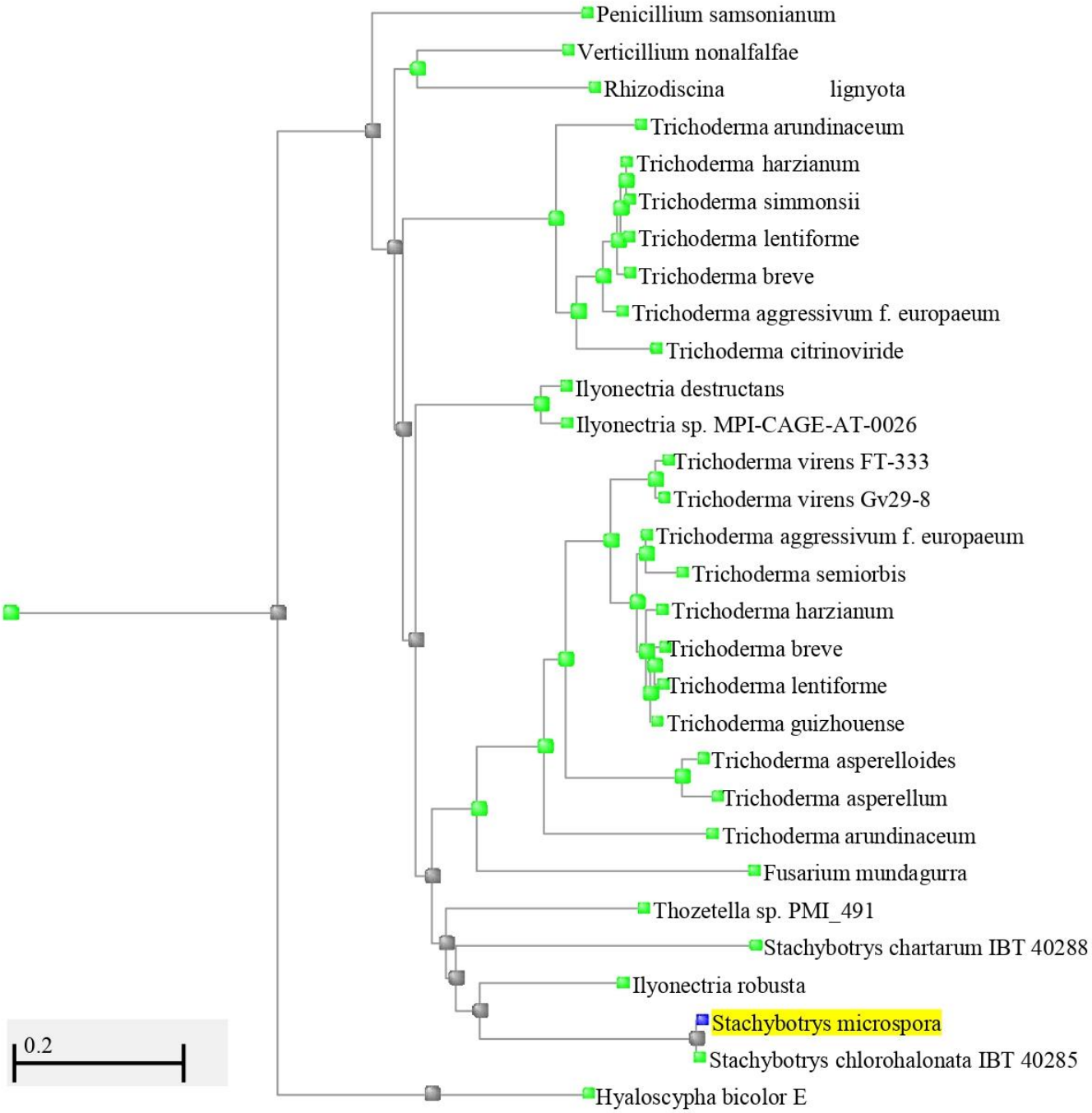
International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 14 Issue 12 , December - 2025

Supplementary figure 2



International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 14 Issue 12 , December - 2025

Supplementary figure 3

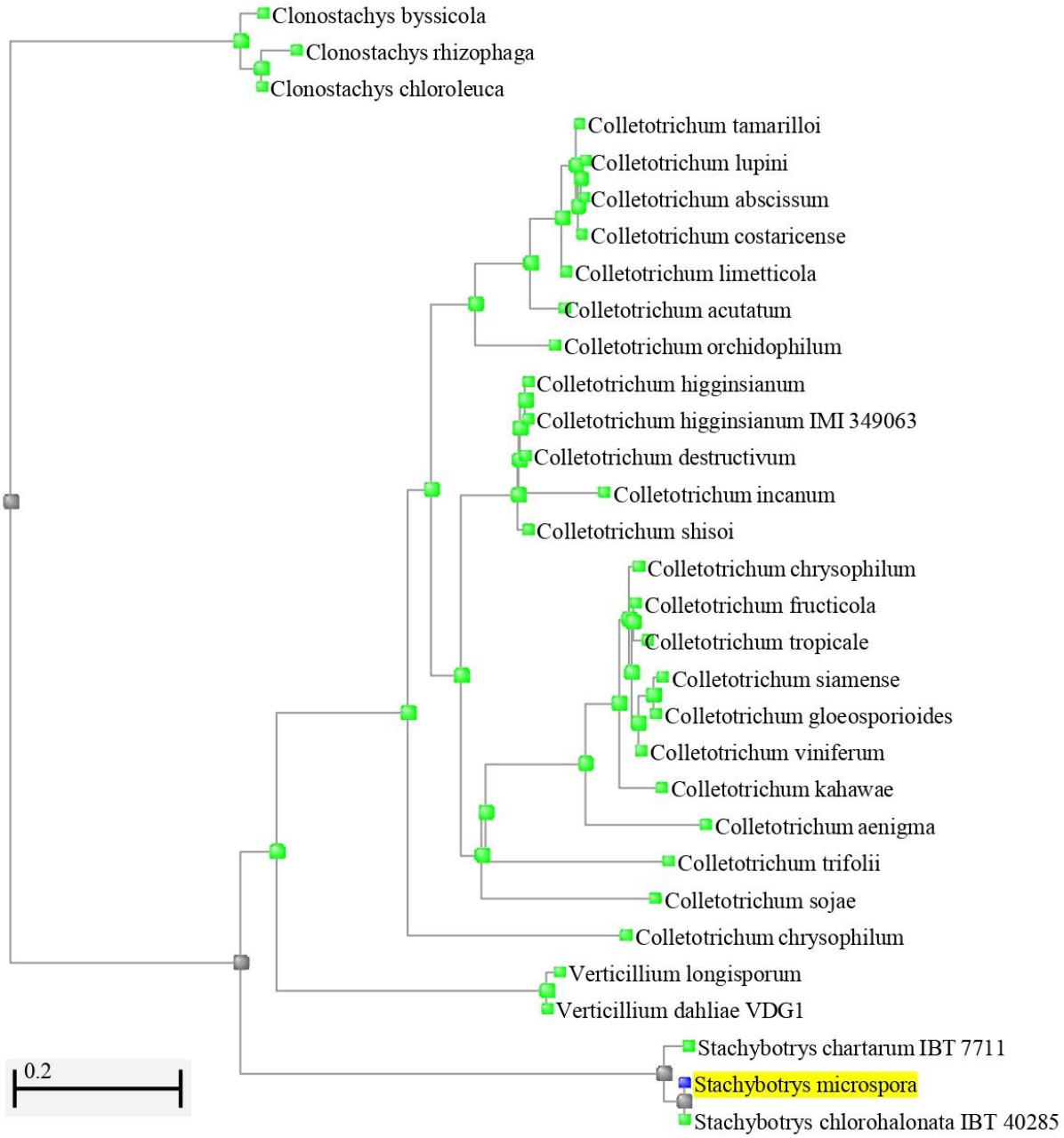


International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 14 Issue 12 , December - 2025

Supplementary figure 4

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 14 Issue 12 , December - 2025

Supplementary figure 5



International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 14 Issue 12 , December - 2025

Table 1. Eggnog pathways results

Pathways	Sequences	Enzymes
Purine metabolism	463	47
Thiamine metabolism	433	12
Drug metabolism-other enzymes	79	13
Starch and sucrose metabolism	31	20
Folate biosynthesis	30	18
Cysteine and methionine metabolism	29	29
Pentose and glucuronate interconversions	29	14
Glycero phospholipid metabolism	27	21
Glycine, serine and threonine metabolism	26	24
Aminoacyl-Trnabiosynthesis	25	22
Glycolysis/gluconeogenesis	25	21
Pyruvate metabolism	24	24
Fructose and mannose metabolism	24	20