

Comparative assessment of mycelial biomass and exo-polysaccharide production of wild type and mutant strains of *Schizophyllum commune* grown in submerged liquid medium

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Abstract: Wild *Schizophyllum commune* and mutant strains of the same organism generated by exposure of the wild type fungus to ultraviolet rays at various time intervals of 30minutes, 60minutes and 90minutes respectively were assessed for fungus biomass and exopolysaccharide production (EPS). Growth conditions such as temperature, pH, and carbon sources were optimized. Results showed that temperature of 28°C was optimal for both mycelia biomass yield [3.18, 4.42, 3.58, 3.13 (g/100mls)] and EPS production [330, 460, 400, 290 (mg/100mls)] for both the wild type and the three mutant strains respectively with SCM 1 having the highest values of 4.42 for mycelial biomass and EPS of 4.60. The optimal pH for both mycelia yield and EPS production was 5.8 for both the wild type and the mutant strains with SCM 2 having the highest mycelial yield of 3.40 and EPS of 4.1 as compared to other strains. Glucose was the carbon source that most supported both the mycelia biomass yield and EPS production for both wild type and the mutant strains [3.18, 3.02, 3.20, 3.13 (g/100mls)] and [160,400,450,350(mg/100mls)] respectively out of the five carbon sources tested with SCM 2 having the highest mycelial yield of 3.20g/100mls and EPS of 4.5mg/mls. These results showed that significant improvement in mycelial yield and EPS production by *S.commune* wild type and mutant strains could be enhanced through submerged cultivation under appropriate optimized conditions.

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1. Introduction

Mushrooms are the reproductive structures (fruiting body or sporocarp) of certain fungi (Jonathan,2002; Pilz *et al.*, 2003). Prasad and Wesely (2008) have defined mushrooms as macrofungi with distinctive fruiting bodies that are large enough to be seen by the naked eye and to be picked by hand. It is estimated that there are approximately 1.5 million species of mushrooms in the world of which approximately 70,000 species are described (Prasad and Wesely, 2008).

Edible mushrooms are nutritionally endowed fungi (mostly ascomycetes and basidiomycetes) that grow naturally on the trunks, leaves and roots of trees as well as decaying woody materials (Iwalokun *et al.*, 2007). Some mushrooms including edible mushrooms possess a new class of compounds with nutritional and medicinal features extractable from either the mycelium or the fruiting bodies of mushrooms referred to as "mushroom nutraceuticals" (Quereshi *et al.*, 2010; Chang and Buswell, 1996). Medicinal mushrooms accumulate a wide variety of bioactive compounds including terpenoids, steroids, phenols, nucleotides and their derivatives glycoproteins and

polysaccharides that display a broad range of biological activities (Borchers *et al.*,1999; Teissedre and Landrault, 2000). These different bioactive compounds have been extracted from the fruiting body, mycelia and culture medium of various medicinal mushrooms such as *Lentinula edodes*, *Ganoderma lucidum*, *Schizophyllum commune*, *Trametes versicolor*, *Inonotus obliquus* and *Flammulina velutipes* (Wasser and Weis, 1999 a).

Schizophyllum commune Fries, is a higher fungus which belongs to family *Schizophyllaceae*, Order *Aphylllophorales*, phylum *Basidiomycota* of the kingdom fungi (Reyes *et al.*, 2003). It is known to produce exopolysaccharides called schizophyllan. The family *Schizophyllaceae* contains only one genus; *Schizophyllum* and there is a single common worldwide species, although there are a few less common species of *Schizophyllum*. The genus name means "split gill," and thus it is called the split gill fungus. *S. commune* is one of the common gill-bearing bracket fungi of world-wide distribution (Zoberi, 1972).

S. commune mutants are the resulting strains after the wild type has been exposed to ultra-violet

radiation for various time intervals. This in turn would cause a mutation that affects all the cell formation of the organism. The higher the exposure to the ultra-violet light, the more mutated the organism would be thus bringing about a major variation between the original organism and the mutated organism. In biology and especially genetics, a mutant is an individual, organism, or new genetic character arising or resulting from an instance of mutation, which is a base pair sequence change within the DNA of a gene or chromosome of an organism resulting in the creation of a new character or trait not found in the wild type (Prescott *et al.*, 2008).

Many types of polysaccharides could be produced by submerged cultures of higher fungi including mushrooms. The polysaccharides have been studied and used for pharmaceutical purposes due to their diverse biological activities (Song *et al.*, 1998; Kim *et al.*, 2005). These include antitumor and immunomodulating activities (Tokunaka *et al.*, 2000).

Exopolysaccharides (EPS) are high molecular weight polymers of monosaccharides (>20) and are secreted by a microorganism into the surrounding environment. They have found multi-various applications in various food and pharmaceutical industries (Suresh and Mody, 2009).

This study aimed at optimizing growth conditions for mycelia biomass and EPS productions from *S. commune* wild type and mutants.

2. Materials and methods

2.1. Collection of microbial sample

Schizophyllum commune was collected from dead wood of *mangifera indica* at Ogbomoso, Oyo State, Nigeria and identified by its characteristics using the descriptions of Zoberi (1972) and Alexopolous *et al.*, (1996).

2.2. Sample preparation and establishment of mycelial cultures

Tissue culture was carried out on fresh carpophores of *S. commune* using the method of Jonathan *et al* (2009). The mycelial thus generated were cultured on plates of potatoes dextrose agar (PDA).

2.3. Production of *Schizophyllum commune* mutants.

Various mutants of *Schizophyllum commune* were prepared as follows; Fresh plates of *Schizophyllum commune* were allowed to sporulate. The spores were removed with sterile distilled water and re-inoculated on three different freshly prepared plates. The plates were exposed to UV light at 260nm at various time intervals to induce mutation (Peak *et al.*, 1984 with modification). The first plate was exposed to UV for

30mins and labelled as *Schizophyllum commune* mutant 1 (SCM 1).

The second plate was exposed to UV for 60mins and labelled as *Schizophyllum commune* mutant 2 (SCM 2). The third plate was exposed to UV for 90mins and labelled as *Schizophyllum commune* mutant 3 (SCM 3). Fresh plate of the organism before exposing to UV radiation was also prepared and labelled as *Schizophyllum commune* wild type (SCW).

Four different strains that eventually resulted were SCW, SCM1, SCM2 and SCM3.

2.4. Culture preparation for biomass and EPS

The basal medium used consisted of 100mls of *Hibiscus sabdariffa* solution added with 6g Glucose, 1.6g Malt extracts, 2g Peptone, 1.2g Yeast extracts, 0.8g KH₂PO₄, 0.4g MgSO₄.7H₂O, 0.4g Urea and P^H adjusted to 5.8. (Yap and Ng 2001 with modification) *S. commune* wild type and mutants (i.e SCW, SCM1, SCM2 and SCM3) were initially sub-cultured on PDA plates and then 6mm of the vigorous growing agar plate culture (5-day old) was removed using sterile cork borer. The sterilized basal medium was inoculated with this mycelial disc of *S. commune* wild type and mutants. The fermentation experiments were set-up under aerobic condition with the use of aeration pumps to ensure continuous aeration and agitation. The fermentors were fixed to the hose connected to two aeration pumps to supply oxygen and to stimulate fermentation rate. Two fuel filters were fixed to the end of the aeration pumps so as to filter air coming to the media. The set-up was allowed to undergo batch fermentation at 28°C for a period of 6 days with constant supply of electricity powering the pumps (Jonathan *et al.*, 2009 with modifications).

2.5. Optimization of growth conditions for biomass and EPS

This was done by varying the different growth conditions that affect fungus biomass and EPS to determine the best conditions for the production.

2.5.1 Influence of Temperature

The organisms were cultivated in the chemically defined medium as described above. One hundred millilitres of the chemically defined medium were dispensed into 250mls mayonnaise bottles and sterilized, then inoculated with 6mm agar disc plug of the fungus and incubated at 24°C, 28°C, 32°C and 36°C for 6 days. On the 6th day of fermentation, the experiment was terminated. The products formed were carefully sieved using pre-sterilized muslin cloth. The residues (Biomass) wet weight from the various fermentors were recorded. The biomass dry weight was also recorded after drying the wet biomass in free

air. The filtrates (containing the EPS) were then collected (Adejoye and Fasidi, 2009 with modification). The liquid fermented products were centrifuged at 4,000rpm for 20mins using electrical centrifuge. After centrifugation, the residues were discarded and the filtrates that contained the EPS were transferred into separating funnels. Equal volume of acetone was used to extract the EPS in the separating funnel. This was achieved by thoroughly shaking the acetone and the filtrate and thereafter allowed to settle. Two different layers were formed; the upper layer which contained the acetone and the lower layer containing the EPS. The EPS were carefully removed by decantation and was quantified (Yap and Ng,2001 with modifications). The mutant that produced the highest EPS was noted alongside the wild type.

2.5.2. Influence of pH

The organisms were cultivated in the chemically defined medium as described above. One hundred millilitres of the chemically defined medium were dispensed into 250mls mayonnaise bottles and pH adjusted by using 0.1N HCl and 0.1N NaOH to 4.0, 5.8, 7.0 and 8.5 and sterilized, then inoculated with 6mm agar disc plug of the fungus and incubated at 28°C, for 6days. On the 6th day of fermentation, the experiment was terminated. The products formed were carefully sieved using pre-sterilized muslin cloth. The residues (Biomass) wet weight from the various fermentors were recorded. The biomass dry weight was also recorded after drying the wet biomass in free air. The filtrates (containing the EPS) were then collected (Adejoye and Fasidi,2009 with modification). The liquid fermented products were centrifuged at 4,000rpm for 20mins using electrical centrifuge. After centrifugation, the residues were discarded and the filtrates that contained the EPS were transferred into separating funnels. Equal volume of acetone was used to extract the EPS in the separating funnel. This was achieved by thoroughly shaking the acetone and the filtrate and thereafter allowed to settle. Two different layers were formed; the upper layer which contained the acetone and the lower layer containing the EPS. The EPS were carefully removed by decantation and was quantified (Yap and Ng,2001 with modifications). The mutant that produced the highest EPS was noted alongside the wild type.

2.5.3. Influence of Carbon sources

The organisms were cultivated in the chemically defined medium containing the nitrogen sources as described above. The carbon sources tested were glucose, fructose, lactose and maltose. One hundred millilitres of the chemically defined medium were

dispensed into 250mls mayonnaise bottles and sterilized, then inoculated with 6mm agar disc plug of the fungus and incubated at 28°C, for 6days. On the 6th day of fermentation, the experiment was terminated. The products formed were carefully sieved using pre-sterilized muslin cloth. The residues (Biomass) wet weight from the various fermentors were recorded. The biomass dry weight was also recorded after drying the wet biomass in free air. The filtrates (containing the EPS) were then collected (Adejoye and Fasidi,2009 with modification). The liquid fermented products were centrifuged at 4,000rpm for 20mins using electrical centrifuge. After centrifugation, the residues were discarded and the filtrates that contained the EPS were transferred into separating funnels. Equal volume of acetone was used to extract the EPS in the separating funnel. This was achieved by thoroughly shaking the acetone and the filtrate and thereafter allowed to settle. Two different layers were formed; the upper layer which contained the acetone and the lower layer containing the EPS. The EPS were carefully removed by decantation and was quantified (Yap and Ng,2001 with modifications). The mutant that produced the highest EPS was noted alongside the wild type.

3. Results and Discussion

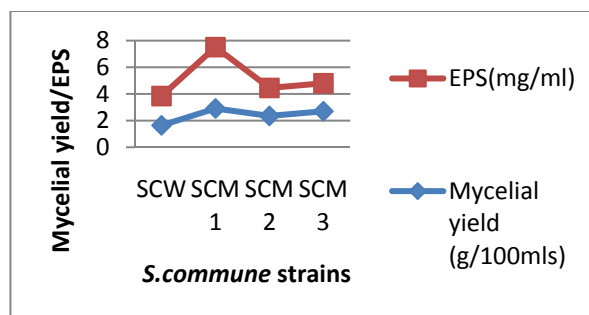


Fig. 1a: Comparison of biomass yield and EPS production from wild type and mutant strains of *S. commune* grown in submerged liquid medium at incubation temperature of 24°C
EPS= Exopolysacharides, SCW=*Schizophyllum commune* wild type, SCM1= *Schizophyllum commune* mutant one, SCM 2=*Schizophyllum commune* mutant two, SCM 3= *Schizophyllum commune* mutant three.

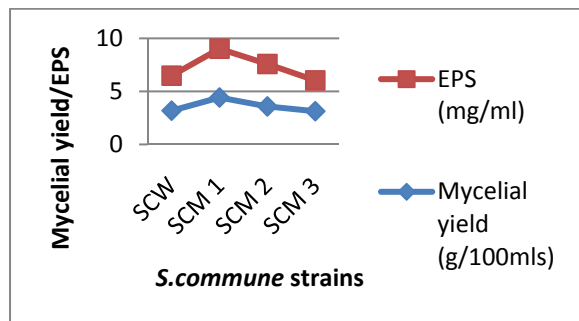


Fig. 1b: Comparison of biomass yield and EPS production from wild type and mutant strains of *S. commune* grown in submerged liquid medium at incubation temperature of 28°C
EPS= Exopolysacharides, SCW=*Schizophyllum commune* wild type, SCM1= *Schizophyllum commune* mutant one, SCM 2=*Schizophyllum commune* mutant two, SCM 3= *Schizophyllum commune* mutant three.

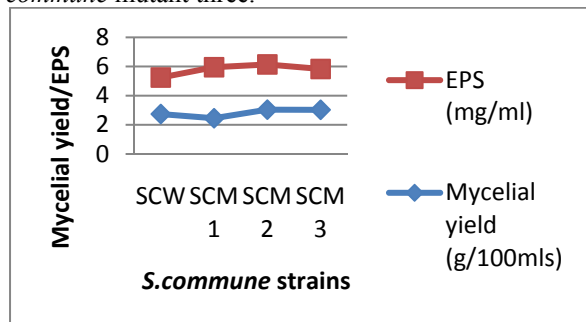


Fig. 1c: Comparison of biomass yield and EPS production from wild type and mutant strains of *S. commune* grown in submerged liquid medium at incubation temperature of 32°C
EPS= Exopolysacharides, SCW=*Schizophyllum commune* wild type, SCM1= *Schizophyllum commune* mutant one, SCM 2=*Schizophyllum commune* mutant two, SCM 3= *Schizophyllum commune* mutant three.

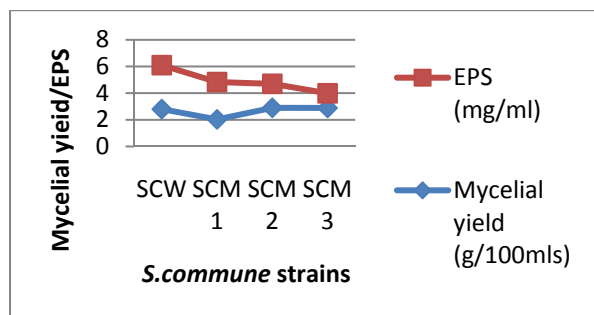


Fig. 1d: Comparison of biomass yield and EPS production from wild type and mutant strains of *S. commune* grown in submerged liquid medium at incubation temperature of 36°C

EPS= Exopolysacharides, SCW=*Schizophyllum commune* wild type, SCM1= *Schizophyllum commune* mutant one, SCM 2=*Schizophyllum commune* mutant two, SCM 3= *Schizophyllum commune* mutant three.

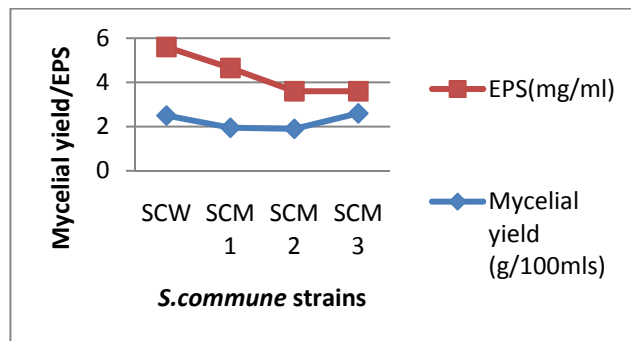


Fig. 1e: Comparison of biomass yield and EPS production from wild type and mutant strains of *S. commune* grown in submerged liquid medium at incubation temperature of 40°C
EPS= Exopolysacharides, SCW=*Schizophyllum commune* wild type, SCM1= *Schizophyllum commune* mutant one, SCM 2=*Schizophyllum commune* mutant two, SCM 3= *Schizophyllum commune* mutant three.

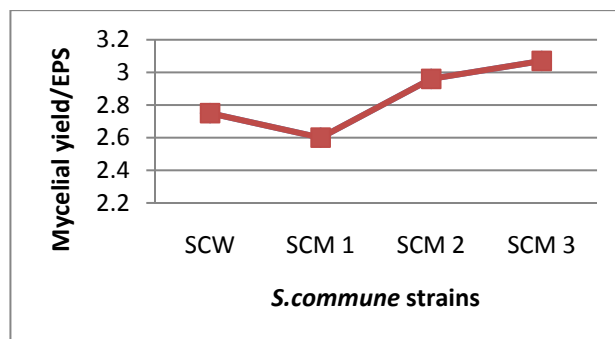


Fig. 2a: Comparison of biomass yield and EPS production from wild type and mutant strains of *S. commune* grown in submerged liquid medium at initial pH of 4.0
EPS= Exopolysacharides, SCW=*Schizophyllum commune* wild type, SCM1= *Schizophyllum commune* mutant one, SCM 2=*Schizophyllum commune* mutant two, SCM 3= *Schizophyllum commune* mutant three.

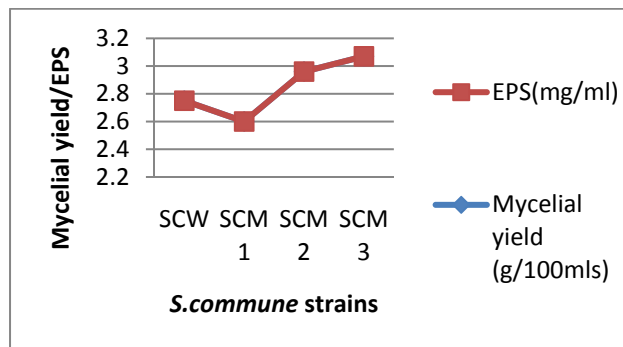


Fig. 2b: Comparison of biomass yield and EPS production from wild type and mutant strains of *S. commune* grown in submerged liquid medium at initial pH of 5.8

EPS= Exopolysacharides, SCW=*Schizophyllum commune* wild type, SCM1= *Schizophyllum commune* mutant one, SCM 2=*Schizophyllum commune* mutant two, SCM 3= *Schizophyllum commune* mutant three.

EPS= Exopolysacharides, SCW=*Schizophyllum commune* wild type, SCM1= *Schizophyllum commune* mutant one, SCM 2=*Schizophyllum commune* mutant two, SCM 3= *Schizophyllum commune* mutant three.

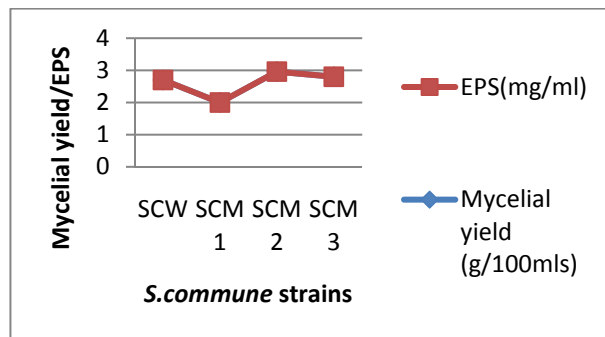


Fig. 2e: Comparison of biomass yield and EPS production from wild type and mutant strains of *S. commune* grown in submerged liquid medium at initial pH of 9.5

EPS= Exopolysacharides, SCW=*Schizophyllum commune* wild type, SCM1= *Schizophyllum commune* mutant one, SCM 2=*Schizophyllum commune* mutant two, SCM 3= *Schizophyllum commune* mutant three.

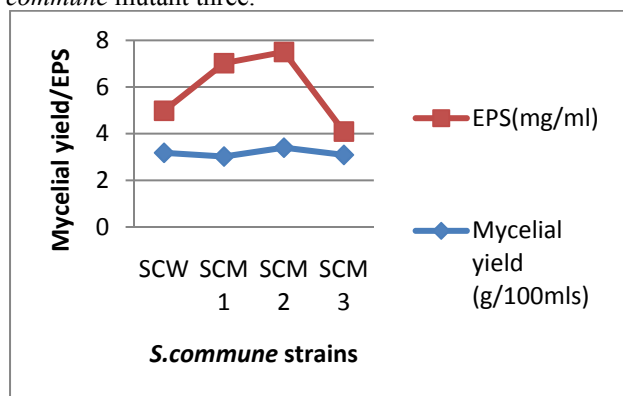


Fig. 2c: Comparison of biomass yield and EPS production from wild type and mutant strains of *S. commune* grown in submerged liquid medium at initial pH of 7.0

EPS= Exopolysacharides, SCW=*Schizophyllum commune* wild type, SCM1= *Schizophyllum commune* mutant one, SCM 2=*Schizophyllum commune* mutant two, SCM 3= *Schizophyllum commune* mutant three.

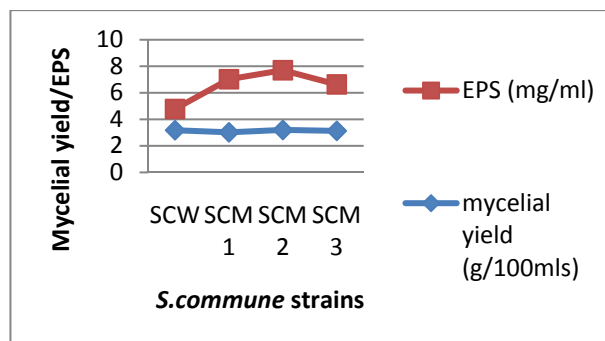


Fig. 3a: Comparison of biomass yield and EPS production from wild type and mutant strains of *S. commune* grown in submerged liquid medium using glucose as carbon source

EPS= Exopolysacharides, SCW=*Schizophyllum commune* wild type, SCM1= *Schizophyllum commune* mutant one, SCM 2=*Schizophyllum commune* mutant two, SCM 3= *Schizophyllum commune* mutant three.

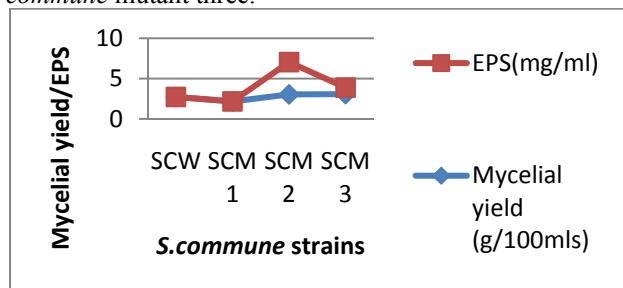


Fig. 2d: Comparison of biomass yield and EPS production from wild type and mutant strains of *S. commune* grown in submerged liquid medium at initial pH of 8.5

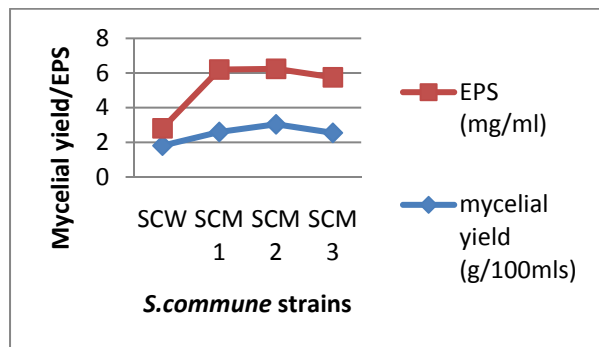


Fig. 3b: Comparison of biomass yield and EPS production from wild type and mutant strains of *S.commune* grown in submerged liquid medium using fructose as carbon source
EPS= Exopolysacharides, SCW=*Schizophyllum commune* wild type, SCM1= *Schizophyllum commune* mutant one , SCM 2=*Schizophyllum commune* mutant two, SCM 3= *Schizophyllum commune* mutant three.

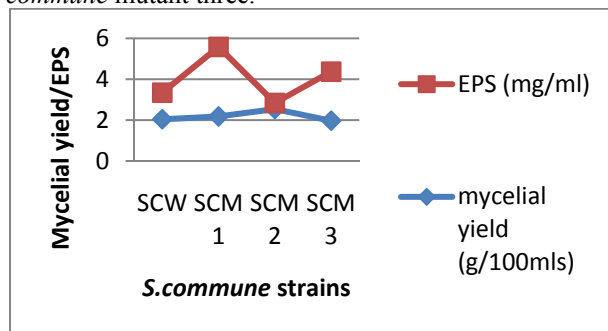


Fig. 3c: Comparison of biomass yield and EPS production from wild type and mutant strains of *S.commune* grown in submerged liquid medium using maltose as carbon source
EPS= Exopolysacharides, SCW=*Schizophyllum commune* wild type, SCM1= *Schizophyllum commune* mutant one , SCM 2=*Schizophyllum commune* mutant two, SCM 3= *Schizophyllum commune* mutant three.

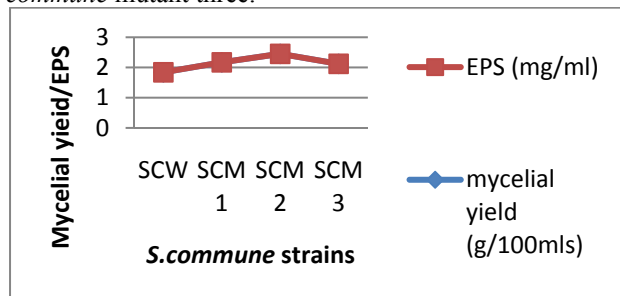


Fig. 3d: Comparison of biomass yield and EPS production from wild type and mutant strains of *S.commune* grown in submerged liquid medium using lactose as carbon source
EPS= Exopolysacharides, SCW=*Schizophyllum commune* wild type, SCM1= *Schizophyllum*

commune mutant one , SCM 2=*Schizophyllum commune* mutant two, SCM 3= *Schizophyllum commune* mutant three.

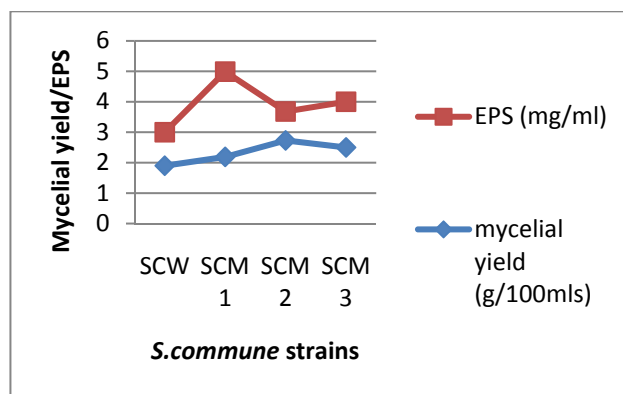


Fig. 3e: Comparison of biomass yield and EPS production from wild type and mutant strains of *S.commune* grown in submerged liquid medium using sucrose as carbon source
EPS= Exopolysacharides, SCW=*Schizophyllum commune* wild type, SCM1= *Schizophyllum commune* mutant one , SCM 2=*Schizophyllum commune* mutant two, SCM 3= *Schizophyllum commune* mutant three

The results showed that the higher the exposure of the organism to UV light, the higher the mycelia biomass as well as EPS production. This notable observation further reaffirms the previous reports of Rau (1999) on the behaviour of *S.commune* and the positive correlation between mycelial growth and polysaccharide production. Wang and Lu (2005) also stated that mycelial growth from submerged fermentation of fungal mycelia is known to be affected by the fermentation conditions and the types of genes encoded in the fungus.

Temperature had a significant effect on mycelia yield and EPS-production by *S.commune* wild type and its mutant strains. To determine the optimal temperature for mycelial growth and EPS-production, these organisms were cultivated at various temperatures of 24⁰C, 28⁰C, 32⁰C , 36⁰C and 40⁰C. The optimum temperature for growth was found to be 28⁰C for both the wild type and the mutant strains (Fig.1a-e). At incubation temperatures of 24⁰C and 28⁰C, *S.commune* mutant one i.e the strain obtained by exposing the wild type organism to UV rays for 30 minutes produced the highest mycelial mats and exopolysacharides (Fig.1a and 1b). At incubation temperature of 32⁰C, *S.commune* mutant strain of 60minutes produced the highest mycelial mat and exopolysacharides, however at 36⁰C and 40⁰C ,*S.commune* wild type performed better than all the mutant strains in mycelial and EPS productions.

These observations agree favourably with the findings of chi *et al.*, (1996) and that of Jonathan and Fasidi (2003) who reported optimum temperature for the growth of *Phellinus linteus* and *Psathyrella atroumbonata* as 25^oC to 30^oC. These reports however contrast that of Yang and Liau, 1998 who proved that temperature range of between 30^oC and 33^oC was found to be suitable for EPS production by *Ganoderma lucidum*.

The results in fig 2 a-e showed that the optimal pH for both mycelia yield and EPS-production was 5.8 with *S.commune* mutant exposed for 60minute having the highest mycelial yield and EPS of 3.40g/100mls and 4.10mg/ml respectively. The mycelia yield and EPS production declined at pHs lower or higher than 5.8. At pHs 4.0 and 9.5, no EPS was produced. This result showed that initial pH had a profound effect on the studied parameters by *S.commune* wild type and mutant strains. This result correlates with the report of Nour *et al.*, 2004 that other EPS synthesis of fungi such as *Pleurotus pulmonarius* had optimal pH of 5.5. The pH of medium is often a neglected environmental factor but it has a remarkable influence on morphology of fungi mycelia which further affect biomass accumulation and metabolite formation (Wang and Neil, 1995; Shu and Lung,2004; Gbolagade *et al.*; 2006b; Jonathan *et al.*; 2009)

Since carbohydrates are a major component of the cytoskeleton and an important nutritional requirement for the growth and development of higher fungi (Xiao *et al*; 2006), investigation was done to determine the best carbon source on mycelial yield and EPS-production by *S.commune* wild type and mutant strains. It was observed that among the 5 carbon sources used, glucose supported the highest mycelia yield and EPS production with *S.commune* mutant exposed for 60minutes to UV rays i.e *S.commune* mutant two having the overall best mycelial yield and EPS of 3.20g/100mls and 4.5mg/mls respectively. (Fig.3a-e).

S.commune has the ability to utilise large numbers of sugars all of which have varied degree of stimulatory effect on biomass and EPS production as shown in fig 3a-e. Different carbon sources had different effects of catabolic repression on the cellular secondary metabolism (Adebayo-Tayo *et al.*, 2011). Such phenomenon was also demonstrated in submerged cultivation of different kinds of mushrooms (Jonathan and Fasidi, 2001; Hwang *et al.*, 2003; Kim *et al.*, 2003). Alofe (1995) and Kadiri (1990) reported that glucose and fructose were the most readily utilised carbohydrate source for the growth of *Pleurotus tuber-regium* and *P.squarosulus* respectively. Coincidentally the result of this research work confirmed such reports.

4.0. Conclusion

This work has shown that optimum mycelia and EPS production by *S.commune* wild type and mutant strains could be attained through submerged cultivation of the fungus at 28^oC, pH 5.8, glucose and fructose as most utilised carbon source. It is interesting to note that the parameters tested exhibited the same order in both the wild type and the mutant strains though the mutant strains slightly performed better than the wild types in terms of mycelial yield and EPS production in submerged medium.

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