INVESTIGATION OF GROWTH CONDITIONS ON THE LACCASE ACTIVITY OF *PLEUROTUS OSTREATUS* AND *CERIPORIOPSIS RIVULOSUS* DURING LOW QUALITY FORAGE UPGRADE AS POSSIBLE DRY SEASON FEED

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ABSTRACT

This paper investigated the effects of some growth conditions i.e. forage-liquid ratio and fermentation time on the laccase activity of Pleurotus ostreatus (PO) and Ceriporiopsis rivulosus (CR) when used to upgrade selected low quality forages as possible dry season feed for ruminants. PO and CR were cultivated on Brachiaria decumbens (BD), Andropogon gayanus (AG), Triticum aestivum (TA) and Lolium perenne (LP) alongside untreated forages using two forage-liquid ratios (1:3 and 1:5) for each selected solid state fermentation times (14 and 28 days). The laccase activity of CR increased when the forages were in liquid ratio of 1: 5 at higher fermentation time with the exception of BD in ratio 1:3 at 28th day. The laccase activity of PO was increased when the forages were in liquid ratio of 1:3 at higher fermentation with the exception of TA in liquid ratio of 1:5. Generally increased fermentation time did not have much effect on the laccase production of each of the fungi on LP and TA; had minimal effect on AG; and had much effect on BD. It can therefore be concluded that for a higher laccase production to be recorded, CR should be cultivated on LP and TA in ratio 1:5 for shorter time, AG in ratio 1:5 for longer time, and BD in ratio 1:3 for a longer period. PO should be cultivated on AG and BD in ratio 1:3 for longer time, LP in ratio 1:3 for shorter time, and TA in ratio 1:5 for shorter time.

Keywords: Fungi, fermentation time, forages, laccase, ruminant feed

INTRODUCTION

The pre-treatment of low quality forages (LQF) with aerobic fungi (i.e. selective white rot fungi) have been one of the possible ways of upgrading the nutritive value of LQF found during the dry season. Among these white –rot fungi, PO and CR have increased ligninolytic ability to upgrade agro-industrial by-products and crop residues (Niu *et al*., 2018). However, the conditions of growth needed to be favourable for the release of ligninolytic enzymes especially laccase that are involved in the lignin degradation, a constituent that limits the access of other carbohydrates in feed to animals. Among these growth conditions, substrate-liquid ratio and incubation time were selected as they influences the release of enzymes. Substrate-liquid ratio affects the moisture content and optimal moisture content tends to positively influence fungal growth and laccase production by preventing substrate porosity or agglomeration (Patel *et al*., 2009). On the other hand, the incubation time has been identified to affect the rate and extent at which the fungi are capable of exhibiting their enzymatic functions, an essential or basic pre-requisite for fungal improvement of low quality feed (Arora and Sharma, 2009). This current work was carried out to identify the best substrate-liquid ratio and fermentation time that supports the release of laccase enzyme by each of the selected fungi on the selected low quality forages, as a criteria needed in the upgrade of low quality feed.

MATERIALS AND METHODS

A 2 (forage-liquid ratio) x 2 (fermentation time) factorial arrangement was used to obtain the main and interactive effects of the treatment on the laccase production of the fungi when used to inoculate the forages. Five (5g) of each of the forage samples (i.e. BD, AG, TA straw, and LP) used were weighed into 250ml Erlemneyer flasks, rehydrated with two different amounts (15ml and 25ml) of 1% Potato dextrose broth (CR) and 1% malt extract broth (PO) respectively to set the solid/liquid ratio for each of the fermentation time (14 and 28 days) respectively and then autoclaved at 121^0C for 15mins. Each of the Erlenmeyer flasks was then inoculated with three agar plugs (5mm) obtained from plates cultured on Potato dextrose agar at 20^0C (CR) and Malt extract agar at 30^0C (PO) for 10 days respectively and was replicated thrice for each treatment. This growth conditions have been identified in previous experiment. The flasks were thereafter incubated at either 20^0C (CR) or 30^0C (PO) in a dark environment at 95% humidity. Triplicated control flasks containing the substrates and the media but no fungal mycelium were also made available. After each time, 25ml of 10mM sodium acetic buffer solution (pH 5.0) was added to each selected flask for extraction of enzymes. The flasks were then kept in a shaking incubator (200rpm) for 20mins at 20^0C. The contents of each flask were filtered through a dried what-man filter paper no. 1 (150 mm diameter) and the filtrate was centrifuged at 10,000 rpm at 4^0C for 20 min. The supernatant was collected and laccase was determined by monitoring the oxidation of the diammonium salt of ABTS (2, 2' – azino – bis (3 – ethylbenz – thiazoline – 6 – sulfonic acid) as a substrate (Geng *et al*., 2004).

RESULTS AND DISCUSSION

Figure 1 and 2 shows the main effect of forage-liquid ratio at each inoculation time on laccase production by CR and PO respectively on the selected forages after 14th and 28th days of inoculation respectively. The highest laccase activity produced by *C. rivulosus* was at 1:5 ratio with the exception of BD at 28th day. The highest laccase activity produced by *P. ostreatus* for BD, AG and LP was at 1:3 ratio and that of TA was at 1: 5 ratio. The increase in laccase production with increase in inoculation time for CR was not different ($P > 0.05$) for LP; minimal for AG; and maximal for BD and TA. However, that of PO was not different ($P > 0.05$) for LP and TA but was different for AG and BD. The variations recorded in this study can be attributed to the effect of forages and fungi. Forage characteristics and the capacity of the system plays a major role on the fungal activity (Daniel, 2016). Fungi tends to require different optimal moisture content in their substrate before their ligninolytic activity can be exerted. Furthermore, fungi also vary in the incubation time at which they liberate solubles from substrates in preparation for lignocellulose degradation (Sharma and Arora, 2015)

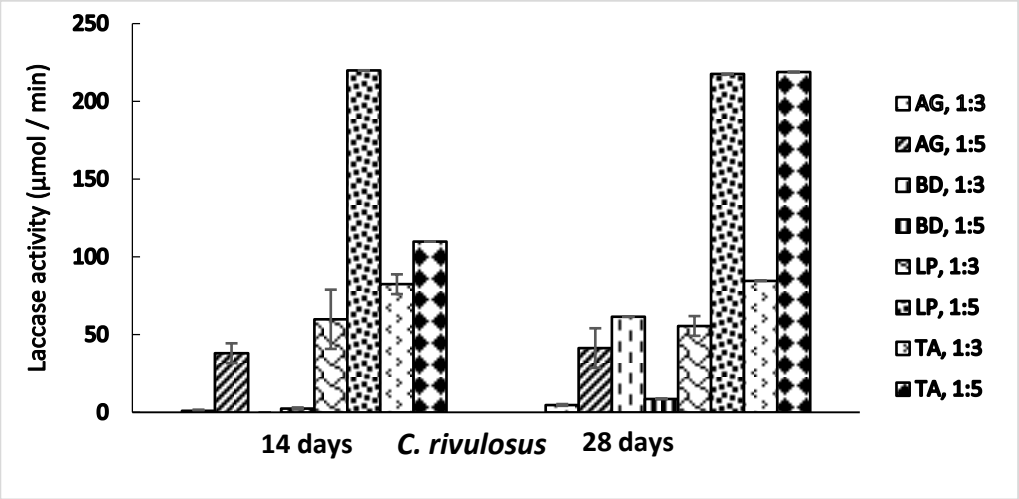

Figure 1.The main effect of substrate-liquid ratio on the laccase activity (µmol / min) of C. rivulosus *after 14th and 28th days of inoculation with the selected forages*

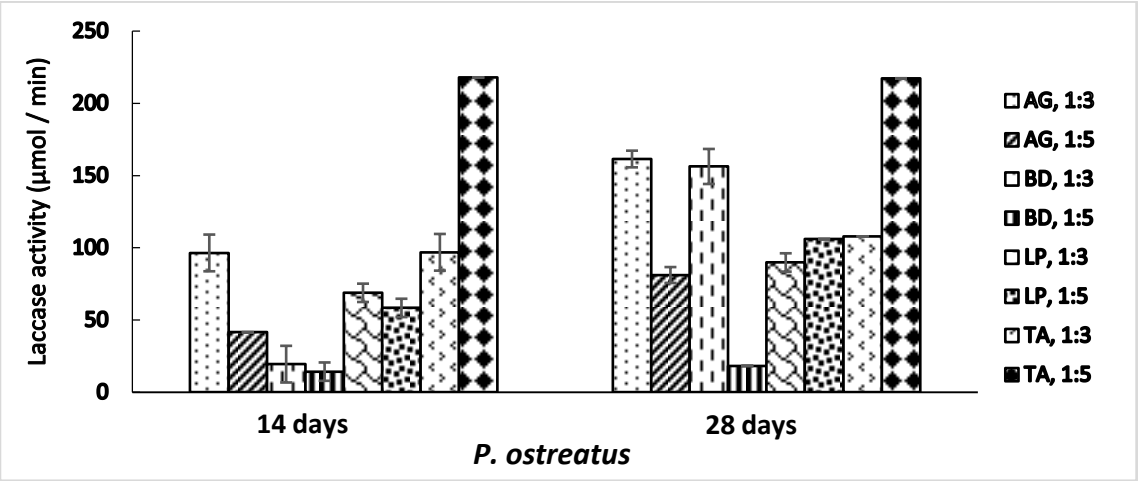

Figure 2.The main effect of forage-liquid ratio on the laccase activity (µmol / min) of P. ostreatus *after 14th and 28th days of inoculation with the selected forages*

Figure 3 shows the laccase activity produced by each of the fungi on the forages at different liquid ratios for 28 days is presented in Figure 3. In general substrate-liquid ratio 1:3 increased the laccase activity in *A. gayanus* and *B. decumbens* than ratio 1:5 and vice versa in *L. perenne* and *T. aestivum*. The forages can be grouped into two groups based on their ability to support laccase activity by the fungi: the low laccase producing group (i.e. BD and A) and the high laccase producing group (i.e. TA and LP). The forages varied in that LP and TA supported a higher production of laccase activity by the fungi by the 14th day of inoculation while for *BD* and AG it was by the 28th day of inoculation. The variations recorded in the forages is an indication that forages used for fungal treatment played a significant role in the liberation of solubles (Bhargav *et al*., 2008). Several factors such as their nutrient composition, degree of crystallinity of structural components especially cellulose, differences in the cell wall composition of C_3 and C_4, forage particle size etc. did have an influence on the ability of the fungi to get the required nutrients needed to initiate their growth and activity (Daniel, 2016).

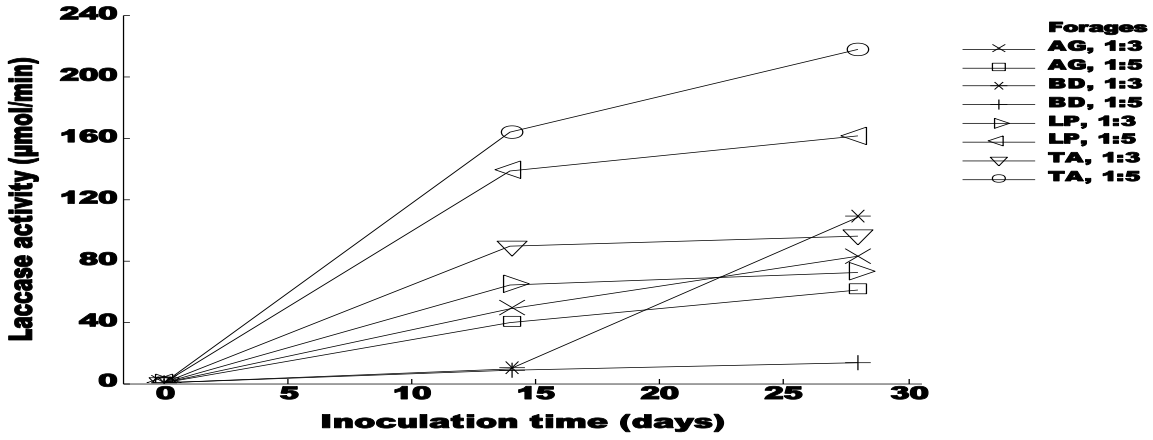

Figure 3 The laccase activity produced by the fungi as influenced by the forages at different sub – liquid ratios for 28 days.

CONCLUSION AND RECOMMENDATION

The fungi varied in their growth conditions requirement as increased laccase production was influenced by substrate-liquid ratio and fermentation time. CR should be cultivated on LP and TA in ratio 1:5 for shorter time, AG in ratio 1:5 for longer time, and BD in ratio 1:3 for a longer period. PO should be cultivated on AG and BD in ratio 1:3 for longer time, LP in ratio 1:3 for shorter time, and TA in ratio 1:5 for shorter time. The shorter fermentation helped in achieving laccase activity by the fungi earlier on LP and TA while longer fermentation time helped in achieving a higher laccase activity by the fungi on BD and AG. Further research in evaluating the effect of these fungi on the chemical composition and degradability potential of the treated forages should be investigated.

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