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Studies on effect of various agro-industrial waste on milk-clotting activity extracted from *Aspergillus Tamarii*

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Abstract: The impact of five agro-industrial wastes and casein on the protease activity of Aspergillus tamarii was studied using solid-state fermentation. Protease is one of the most indispensable industrial enzymes worldwide. Therefore, screening substrates and organisms for better production can add to a country's economy. In this study, six substrates were screened as media to produce protease from A. tamari by using 5.0g of each substrate and 1 mL spore suspension (106 spores/mL) of A. tamari. Crude enzyme was purified by ammonium sulphate precipitation, and milk clotting and protease activity analysis were conducted for the enzymes extracted from each substrate. Results revealed that supernatant enzyme from banana peel powder had the highest milk-clotting activity of 351.2 SU/mL within 382.7 seconds. While rice bran supernatant enzyme had the lowest MCA. The pellet enzymes extracted from the fermentation of the substrate with A. tamarii showed low milk-clotting activity, but still, the pellet enzyme from Bpp had a high MCA of 387.3 SU/mL at 310 seconds. However, protease activity was high with the supernatant of millet bran (0.40±0.01). A. tamarii had shown a high milk-clotting activity when treated with Banana Peel Powder and high protease activity on treatment with millet bran. Further studies on the production of protease from A. tamarii with the combination of the two substrates are necessary to determine their implication when combined.
Keywords: agro-industrial waste; Aspergillus tamarii; industrial enzymes

1. Introduction:

Aspartic proteases (acidic proteases) are endopeptidases that have been isolated from bacteria, fungi, plants, and animals (Chen et al., 2009). They are used as a coagulating agent to produce cheese (Yegin et al., 2011). Proteases from different animals, plants, and microbes have been used as dairy coagulants (Chazarra et al., 2007). Increasing population and food processing are creating huge deposits of agro-industrial wastes. Such agro-industrial wastes, particularly agricultural and forest residues, could be better renewable resources as they harbor a lot of alternative feed stocks such as starch, protein, xylan, lignin, pectin, and cellulose, which several microbes can utilize, to produce vast arrays of enzymes (with the use of fermentation processes, especially SSF). In this study, various substrates that use low-cost agro-industrial wastes, which included wheat bran (WB), rice bran (RB), millet bran (MB), soya bean husk (SBH), and banana peel powder (BPP), along with casein, were screened against different protease fungi using SSF.

2. Materials And Methods

Six types of media (casein, wheat bran, millet bran, rice bran, banana peel powder, and soya bean meal) were screened as media to produce protease from the isolated fungi using the Solid State Fermentation (SSF) method. Crude enzyme was extracted after the fermentation and assayed for the acid protease activity. For the SSF, 5.0g of each substrate was taken in a 250mL Erlenmeyer flask separately, each was moistened with salt solutions; composition as follows: sodium nitrate 0.2 mL, potassium dihydrogen phosphate 0.1 mL, magnesium sulphate 0.05 mL, potassium dihydrogen phosphate 0.1 mL, magnesium sulphate 0.05 mL,

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potassium chloride 0.05 mL, ferrous sulphate concentration, and zinc sulphate concentration at pH of 7.0 were used to achieve the desired content. The mixture was sterilized at 121 0C at 15 min, cooled, and inoculated with 1 mL of fungal spore suspension (106 spores/mL) and incubated at 30 0C for 72 hrs (Shivakumar, 2012). The same procedure was used for the remaining substrates.

2.1. Enzyme Extraction (EE)

The enzyme was extracted after 72 hours of fermentation, and the rest of the procedure was adopted from Shivakumar (2012).

2.2. Purification of Extracted Enzymes

The crude enzyme extracted from the samples was purified according to the method of Ramachandran and Arutselvi (2013).

2.3. Assay of enzyme activity (Milk clotting activity) of Supernatant and Pellets

The milk-clotting activity (MCA) of the enzyme extracted was measured by the method described by (Otani et al., 1991), and the milk-clotting activity was calculated by using the formula of Kawai and Mukai (1970).

2.4. Protease Activity (PA) of Enzyme Supernatant and Pellets

The protease activity of each protease was measured using the method of Kunitz (1947), which was further adopted by Khademi et al. (2013). A standard curve was generated by plotting the change in absorbance in the standard on the Y axis versus the amount (in micromoles) for each of the standard concentrations on the X axis, and the formula below was used to calculate values of both the standard and test samples, and the result was expressed in units per milliliters (Cupp-Enyard, 2008).

2.5. Statistical analysis

Obtained values were mean ± SD of three replicates. Mean values with asterisk were significantly ($p<0.05$) different from one another (Two-way ANOVA followed by Bonferroni's Multiple Comparison Test).

3. Results

Enzyme supernatant extracted from BPP treated with A. tamarii proved to have high enzyme Activity (PA) 351.2 SU/mL (Fig. 1) within 382.7 secs (Fig. 2), followed by WB (207.1 SU/mL), CS (202.8 SU/mL), and MB (181.2 SU/mL) at 580, 593.3, and 666.7 seconds, respectively. The RB supernatant enzyme had the lowest activity (Fig. 1). The results also revealed no significant difference ($P>0.05$) between MCA, CS, and WB. Equally for the pellet enzymes, there was a significant difference for MCA and MCT values of the substrate treated with A. tamarii (Figs. 1 and 2). Protease activity values of supernatant obtained from treatment of the substrate with A. tamarii (Figure 3) showed that there was no significant difference between the values of PA obtained for MB (0.409±0.021) and those for BPP (0.359±0.096). Protease activity of the pellet enzymes treated with A. tamarii was generally low as compared to the various treatments for the supernatant enzymes. Treatment with A. tamarii showed no significant difference between PA obtained for WB, BPP, and CS, which had 0.027±0.005 U/mL, 0.0276±0.006, and 0.024±0.003U/mL, respectively. However, pellet enzymes from SBH showed a remarkable PA (0.146±0.020 U/mL) on treatment with A. tamarii.

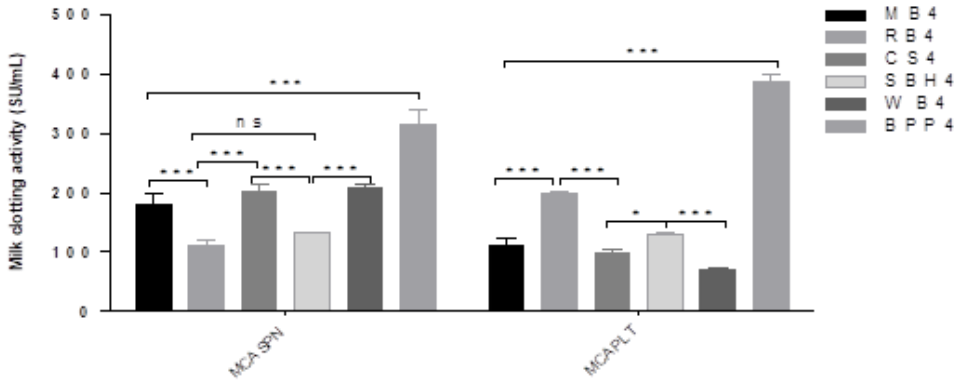

Figure 1: Effects of Various Substrates on Milk Clotting Activity of Supernatant and Pellet Enzyme Produced by A. tamarii.

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Values are mean ± SD of three replicates. Mean values with asterisk are significantly ($p<0.05$) different from one another. NS: Non-Significant ($p>0.05$); * significant ($p<0.05$); ** significant ($p<0.001$); *** significant ($p<0.0001$).

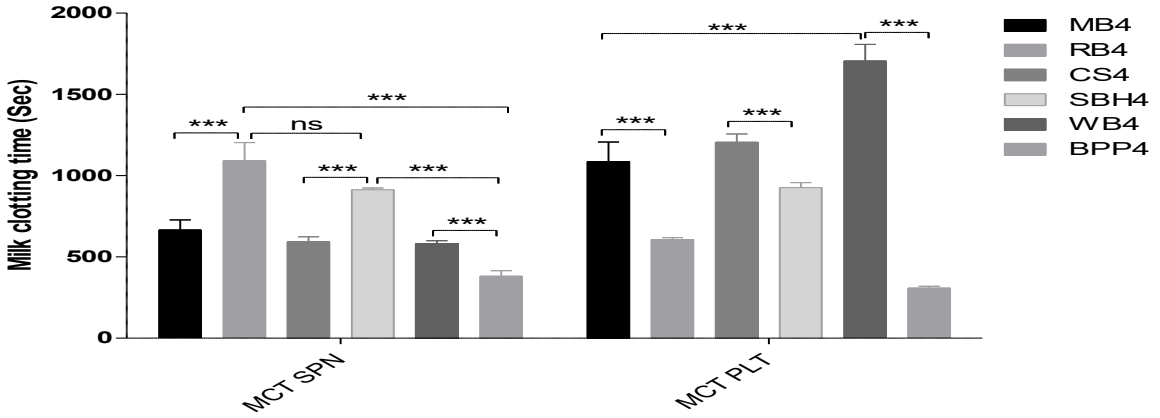

Figure 2: Effects of Various Substrates on Milk Clotting Time of Supernatant and Pellet Enzyme Produced by A. tamarii. Values are mean ± SD of three replicates. Mean values with asterisk are significantly ($p<0.05$) different from one another.

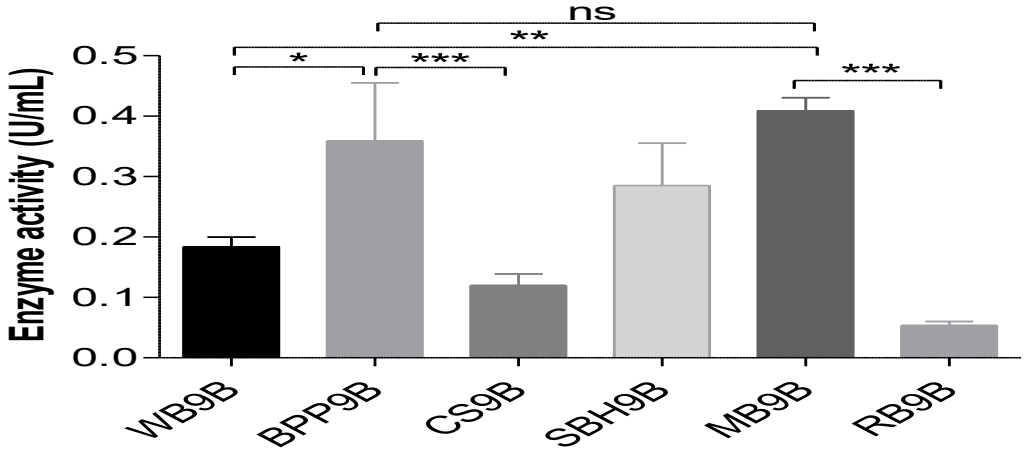

Figure 3: Effects of Various Substrates on Protease Activity of Supernatant Enzyme Produced by A. tamarii Values are mean ± SD of three replicates. Mean values with asterisk are significantly ($p<0.05$) different to one another.

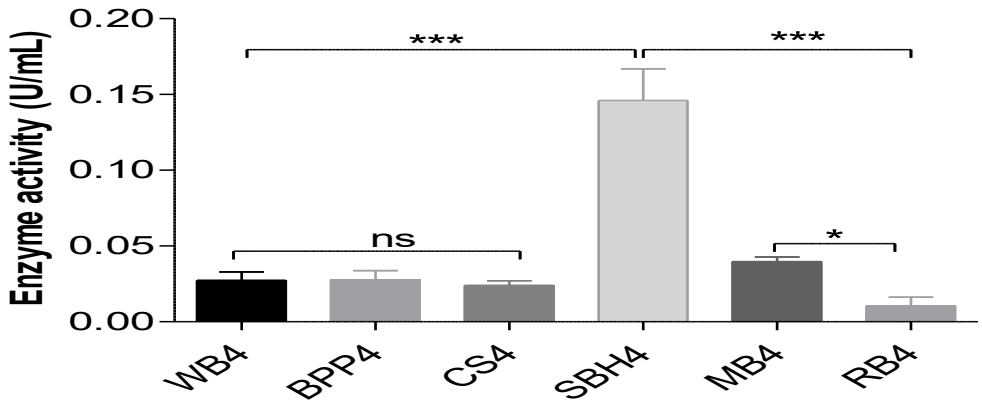

Figure 4: Effects of Various Substrates on Protease Activity of Pellet Enzyme Produced by A. tamarii

Values are mean ± SD of three replicates. Mean values with asterisk are significantly ($p<0.05$) different from one another.

4. Discussion

The MCA and MCT of the supernatant enzymes from the six substrates, BPP treated with A. tamarii supernatant, proved to have the best with the highest MCAs of 351.14SU/mL with the shortest clotting times of 382.7 seconds. The shortest clotting time of A. tamarii is related to the findings of Benlounissi et al. (2012), who reported that *A. tamarii* and *A. niger* fermented industrial waste of cheese to clot milk within 5 minutes. Sambo et al. (2021), also reported high clotting activity from *A. tamarii*. Sethi et al. (2016) identified banana peel suitable for proteases biosynthesis with an activity of 6500±1116.2 µg/L.

Wheat bran also proved to be a good medium for protease enzyme cultivation using fungal species, as the result obtained across the supernatant enzyme treatment for A. tamarii yielded results close to and above 100SU/mL. This agrees with the findings of Silva et al. (2014), whose results on maximum rennin production by Mucor mehei in SSF on wheat bran with HCL addition at 0.4N and 0.3N yielded 157.0 and 264.0SU, respectively. The results are also in agreement with those previously reported by Chazarra et al. (2007), and Vishwanatha et al. (2010). The highest enzyme production of 271.7±3.6SU/mL from M. circineloides was on wheat bran, and 60.5U/mL of MCA from Thermomucorindicae-sedaticae N31. Wheat as substrate and nearly 40.000U/g bran of milk clotting activity from A. oryzae on wheat bran.

Casein also had high SU units in both supernatant and pellet. Silva et al. (2014) also found that casein supplementation on wheat bran during SSF yielded more SUs of rennin. Therefore, casein can be a good supplement for rennin production.

5. Conclusion

Production of enzymes by bioprocesses is a good alternative to add value to agro-industrial waste, because five out of six substrates used are abundant and cheap carbon sources found in our localities, with MB, A. tamarii thriving well and producing PA of 0.409±0.021U/mL. BPP (an underutilized waste) had supported the growth of the fungal strain used in this study. There is a need for optimizing the use of BPP to yield high enzyme volume for milk-clotting activity since it has been revealed to be effective.

CRediT authorship contribution statement

Sambo Sadiya, Experiment and analyze data, Salau Ibrahim, Data analysis, writing original draft, Adamu Shahida Ahmed, editing, proofreading.

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Declaration of competing interest

The authors declare no conflict of interest.

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