Most of the positive tannase-producing fungi were isolated from banana peels with 13 fungal isolates, followed by cocoa residue and rice by-products with 11 fungal isolates and 6 fungal isolates, respectively. The least number of positive tannase producers was found from soybean, desiccated coconut, tomato, and onion, with only 1 fungal isolate obtained. The addition of molasses in the initial stage before the natural fermentation process promotes the growth of fungi. Tannase producer fungi can be found in high tannin-rich agri-industrial by-products such as Jamun (*Syzgium cumini*), amaltash (*Cassia fistula*), tamarind (*Tamarindus indica*), mulberry (*Morus macroura*), keeker (*Acacia nilotica*), pomegranate (*Punica granatum*) and mango (*Magnifera indica*) [22]. Fungal isolates were also obtained from different environmental sources such as various tea dump sites, agri-industrial waste sites, and site nearby tannery industries [5].

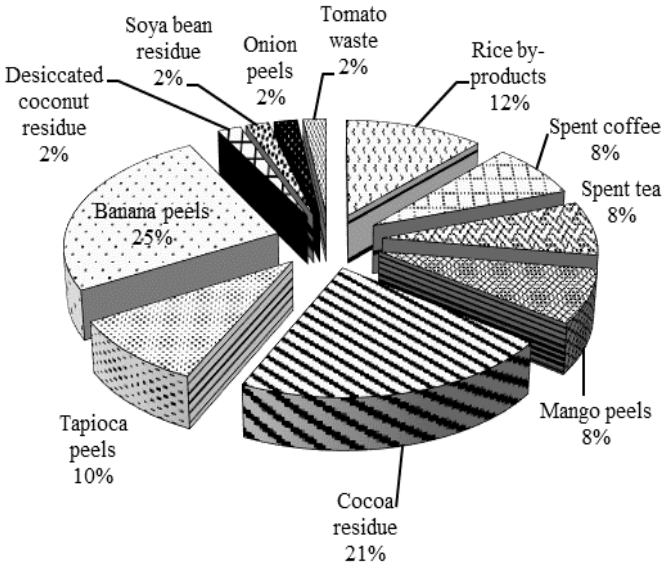

Fig. 2 Percentage of positive tannase-producing fungi isolated from various agri-industrial by-products.

From 56 isolates tested, only 13 isolates which are JI, O1, M1, F0018, I1, I2, H5, G1, E2, A2, B2, C2, and C4 were selected as potential tannase producer due to their large diameter of hydrolytic zone ranging from 51 mm to 61 mm after incubation at 30˚C for 72 hours and these fungal strains were subjected to submerged fermentation for secondary screening.

B. Secondary Screening under Submerged Fermentation

The results showed that fungal strain J1 isolated from banana peels showed the highest tannase activity with (6.86 ± 0.04) U/ml at 72 hours followed by fungal strain I1 and G1 with tannase activity of (6.13 ± 0.08) U/ml and (4.39 ± 0.02) U/ml respectively (Figure 3). Isolate H5 showed the least tannase activity. In general, the highest tannase activity for all fungal strains was observed after 72 hours. The low tannase activity before 48 hours because less mycelium is produced in the early stage; thus, less extracellular tannase is synthesized to break down the medium's tannin. J1 isolate was chosen as the best tannase-producing fungus due to its highest tannase activity.

The tannase activities show a high correlation with r= 0.9204 between the hydrolytic zone diameter and tannase activity in all 13 fungal isolates. These results are in line with previous studies [10], [23], [24]. Their studies showed a correlation between tannase activity and the diameter of hydrolytic zone produced by isolates on plate assay.

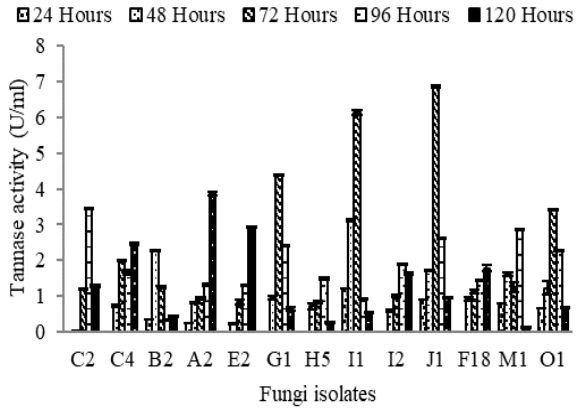

Fig. 3 Tannase activity of selected fungal strains under SmF

C. Identification of Fungal Strain

Fungal strain J1 showed dark green conidia on PDA plate, while black conidia were observed on TAA plate. Besides, white mycelium was observed at the end of the colony on both PDA and TAA, and there was no pigmentation on the reverse side of both plates. A hydrolytic zone can be seen clearly on TAA plate, as shown in Figure 4. Under the light microscope, it was observed that J1 isolate has septate hyphae, biserrate phialides radiate around the conidiophore, long and globose conidiophore, and round-shaped conidia, as shown in Figure 5.

For molecular identification, purified DNA was amplified using polymerase chain reaction (PCR). Two types of primers were used in this study: ITS 1 (Forward Primer) and ITS 4 (Reverse Primer). The band produced was sent to First BASE Laboratories Sdn Bhd for the DNA sequencing. The result from the sequencing analysis was analyzed by using the software; NCBI BLAST Website. It was found that PN1 isolate is *Aspergillus niger*.

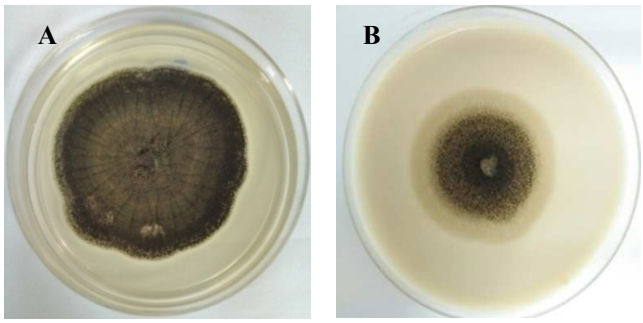

Fig. 4 Growth of J1 Isolate (A) on PDA (B) on TAA

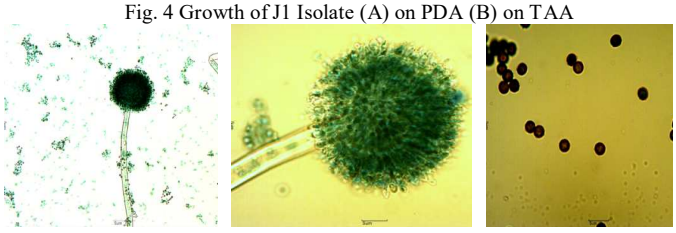

Fig. 5 Light microscopy of J1 isolate

IV. Conclusion

This report showed that tannase-producing fungal strain could be isolated from local agri-industrial by-products such as banana peels, rice bran, brewer's rice, red onion, cocoa pod and husk, mango peels, and desiccated coconut residue. Besides, fungal strain J1 from banana peels was identified as the most potential tannase-producing fungi due to the largest hydrolytic zone diameter of (60.7 ± 0.6) mm and the highest tannase activity (6.86 ± 0.04) U/ml. The study reveals the low-cost agri-industrial by-products' potential as the best source to isolate potential tannase producer fungi.

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