Enzyme assisted citronella oil extraction using coloniser of cymbopogan roots

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ABSTRACT: Conventional method for citronella oil extraction often low in yield (0.25%-3%). In this study, cellulase producing strain isolated from citronella root was applied to enhance oil extraction. The root contains 38.21% cellulose, 30.49% hemicellulose, 21.12% lignin and 4.97% extractives. It is likely that the oil is protected by these compounds. Hence, approach taken is to disrupt the lignin and digest the cellulose by using microbial cellulases. Screening of cellulase producing microbes resulted in 4 potential candidates. Cellulase growth and induction phase of the selected 4 strains were evaluated. Laboratory scale oil extraction of pre-treated root showed a 2.5 fold increase in oil yield. Finding suggested that UniMAPF7 capable to enhance citronella oil extraction and can also be applied in various cellulase related fields.

Keywords: *Enzyme Assisted Extraction; Essential oill; Serai Wangi*

1. INTRODUCTION

Current trend of modern living prefers the use of essential oil infusion for physical and emotional health[1]. Citronella oil is experiencing high demand due to the current shift. However, conventional extraction method often low in yield[2]. Without pre-treatment, the oil was trapped within the lignocellulosic structure. Suggestive of finding in rosemary, cloves, and garlic, enzyme pre-treatment can be applied to induce collapse of root structure to enhance release of oil bodies into the extraction environment[1]. As the oil itself possess antimicrobial activity, isolation through objective approaches will ensure tolerance towards oil biocide activity. Cellulase production is a two-stage process, thus characterizing the growth and induction stage is important to determine time for enzyme harvesting[3]. The current study intended to investigate cellulase production from fungus isolated from the root of *Cymbopogan winterianus* and evaluate its effectiveness in enzymatic enhancement of citronella oil extraction.

2. METHODOLOGY

Lignocellulosic content of Cymbopogon roots was determined following method by [4]. Samples isolated from surface sterilized roots were serially plated on either PDA-CMC or NA-CMC to encourage the growth of cellulase producing fungus and bacteria respectively. Colonies with clearing zone upon flooding with Gram Iodine were selected for Cellulolytic Index (CI) analysis. Of these, only four colonies with highest CI were chosen for evaluation of cellulase production. The growth and induction phase of these four colonies were investigated and FPase activity over time was measured. Colony with the highest FPase activity was selected for extraction. The root of C. *winterianus* was then pre-treated with crude cellulase. Extraction yield of pre-treated roots was then compared to control (without crude enzyme)

3. RESULTS AND DISCUSSIONS

Table 1: Lignocellulosic compositions of dried *C. winterianus* roots

Components	Composition
Cellulose	38.2±1.65
Hemicellulose	30.5±0.93
Lignin	21.1±1.86
Extractives	4.9±0.22

In Table 1, cellulose build the largest structural support in *C. winterianus* roots. Therefore, the use cellulase to disrupt the lignocellulosic structure to enhance release of oil bodies is promising. The major component of the root decides the type of enzyme used as shown in extraction of oleoresin in turmeric. Oleoresin yield improved significantly upon pre-treatment with xylanase and cellulase due to the very high content of xylans and cellulose in the turmeric rhizomes [5].

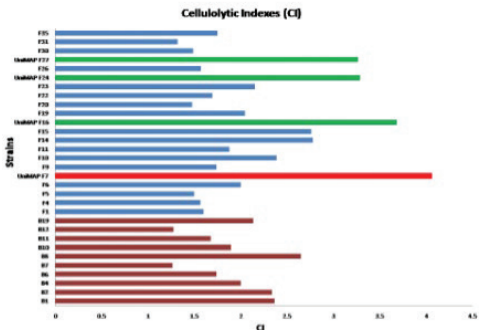

Figure 1: Cellulolytic Index (CI) of 31 isolates grown on PDA-CMC and NA-CMC flooded with Gram Iodine.

Figure 1 showed CI for 31 isolates ranging from 1.3 to 4.1. UniMAPF7, UniMAPF16, UniMAPF24 and UniMAPF27 were the best four isolates with CI of 4.05, 3.68, 3.28 and 3.26, respectively. Nonetheless, CI only screen for presence of cellulases without reflecting its activity. A quantitative cellulase assay is hence needed.

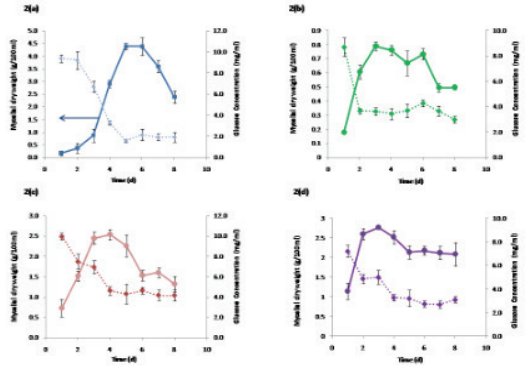

Figure 2: Mycelial dry weight (g/100ml) and glucose consumption profiles (2a) UniMAPF7 (2b) UniMAPF16 (2c) UniMAPF24 (2d) UniMAPF27

Figure 2 showed a biphasic growth in every strains due to availability of glucose which is critical to initiate cell growth. Presence of glucose however, suppressed cellulase activity in certain strain while enhancement was also observed in others. Suggestive of these contradicting findings is the uniqueness of cellulases across species thus justifying the needs to investigate the level of cellulase activity in every isolate.

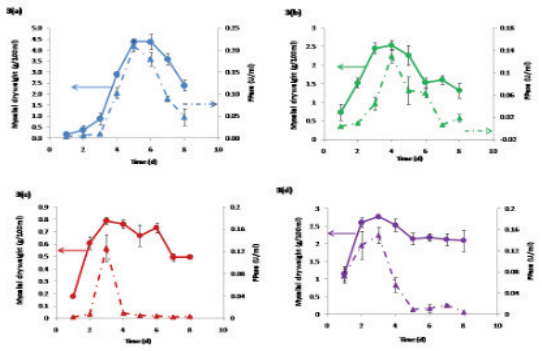

Figure 3: Mycelial dry weight (g/100ml) and FPase activity over time (3a) UniMAPF7 (3b) UniMAPF16 (3c) UniMAPF24 (3d) UniMAPF27

Figure 3 showed increased in cellulase activity concomitant to the accumulated biomass. Activity was maximum towards the end of exponential phase. In agreement with past research, cellulase is secondary metabolites produced upon stress such as depletion of glucose which is toward the end of exponential phase. Hence, the optimal harvesting time for every strain was day 5, 4, 3 and 3 for UniMAPF7, F16, F24 and F27, respectively. Figure 4 showed improvement in citronella oil extraction by all four strains using crude enzyme optimally harvested at the aforementioned days. UniMAPF7 yielded an outstanding 2.5-fold increased, similar to extraction of lycopene in tomatoes which showed a 2-fold enhancement through enzymatic pre-treatment[6].

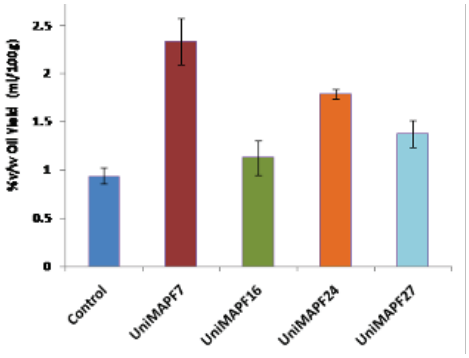

Figure 4: Enzymatic extraction of citronella oil using UniMAPF7, UniMAPF16, UniMAPF24 and UniMAPF27.

4. CONCLUSION

Crude microbial cellulase showed promising efficacy to enhance citronella oil extraction. Knowing the enzyme harvesting time is critical to ensure successful application. Genomic identification of each strain and optimization of cellulase production can be attempted in the future to further enhance the enzyme activity.

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