

Dissolved oxygen on xylanase production by *Trichoderma reesei* using *Reutealis trisperma* press cake as an additional substrate

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Abstract. Dissolved oxygen (DO) level in media plays a big role in the succession of the fermentation process by a microorganism. In this project, *Trichoderma reesei* was cultivated in two types of media, media A and B, both in 250 mL shake flask and 1.5 L fermenter at varying DO level. Media A and B contained the same carbon source and mineral salts, the difference between both media was the presence of press cake in Media B as urea and ammonium sulfate replacement, whereas the nitrogen sources in media A consists of urea and ammonium sulfate. The batch fermentation process was conducted in a 7.8 C/N ratio with an initial pH of 6. The purified enzyme was shown to convert xylan to xylose. Media B that contained *Reutealis trisperma* press cake gives a higher xylanase activity than media A without press cake, for both shake flask and fermenter cultivations. A xylanase activity of 101.07 ± 1.65 U/mL and 39.30 ± 0.64 U/mL was produced in a shake flask under 140 rpm for 96 hours of fermentation with ratio of 0.6 media B/flask volume and 0.6 media A/flask volume, respectively. A xylanase activity of 205.90 ± 0.84 U/mL and 88.87 ± 1.45 U/mL was produced in 72 hours at 0.8 L/min of aeration rate in a 1.5 L fermenter with media A and media B, respectively. Thus, this study identified the *Reutealis trisperma* cake can be used as a beneficial additional substrate and nitrogen sources. The increase in DO level, which indicated by increasing aeration rate, can generate a higher xylanase activity for both media in shake flask and fermenter.

Keywords: dissolved oxygen, press cake, purification, *Reutealis trisperma*, xylanase

INTRODUCTION

Xylanase, is an extracellular enzyme with molecular weight approximately 29.8-35 KDa, that catalyzes the hydrolysis of xylan to xylooligosaccharide and xylose (Kamble & Anandrao, 2012; Polizeli *et al.*, 2005; Sanghi *et al.*, 2010). Xylanase has been widely applied in several industries, i.e., food and beverage, pulp and paper, textile, biofuel and animal feed, which increases the xylanase demand (Motta *et al.*, 2015; Rahmani *et al.*, 2020). The most broadly xylanase application is in pre-bleaching of pulp especially kraft pulp to reduce the use of bleaching chemical. Xylanase synthesis is carried out in several

optimum conditions of pH and temperatures depending on the source of microorganisms (Beg *et al.*, 2001). Among microorganism sources, filamentous fungi gave a higher level of xylanase production than those produced by yeast and bacteria (Polizeli *et al.*, 2005). *Trichoderma reesei*, a microbial, is a mesophilic and filamentous fungus that could produce enzymes capable of degrading polysaccharides cell walls (Coffman *et al.*, 2014). There are a wide range of carbon sources available for *T. reesei* growth relates to enzyme production (Druzhinina *et al.*, 2006), which showed the

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production level is different among various conditions.

Recently, large amount of agricultural residue was produced each year. Most agricultural industrial wastes are removed by open burning, causing severe air pollution (Hou *et al.*, 2019). Hence, there has been increasing interest in converting agricultural waste into various valuable raw materials, such as biofuels, chemicals, and it also has been used as substrates for various microbial growth.

This study introduced the use of *Reutealis trisperma* press cake which is an agro-industrial residue resulting from the mechanical pressing in biodiesel production, as additional nitrogen, and carbon sources in xylanase fermentation. This study also investigated the effect of dissolved oxygen in the fermentation. *Reutealis trisperma* press cake residues contain (weight percent) 34.03% of protein, 18.56% of total sugar, 15.58% of lipid, 6.32% of moisture and 25.51% of others (Riadi *et al.*, 2019). The solid waste of press cake still contains a high percentage of protein; hence we use it to increase the nitrogen content in our media formulation.

Nutrient medium and culture conditions have a significant impact on xylanase production. Physical and chemical factors known to affect xylanase production are temperature, pH, incubation time, carbon and nitrogen sources, concentrations, and agitation speed (Dhaver *et al.*, 2022). It has been reported that aeration determines the oxygenation of the cultures and bulk mixing of fermentation broth. However, there is limited report on the effect of aeration in xylanase production. An inadequate supply of oxygen is one of the major problems in industrial and lab-scale production of microbial metabolites, as solubility of oxygen in aqueous media is limited.

During its growth in xylan, certain microorganism produces specific xylanase with little or no cellulase. However, when microorganisms are grown in culture medium containing cellulose, cellulase are produced together with xylanase (Bueno & Brienza, 2020). Hence, some operation parameters must be investigated to give maximum xylanase production from fungi which has been known as major source of xylanase (Garcia-Ochoa & Gomez, 2009). Those parameters are agitation, aeration, temperature, and dissolved oxygen concentration.

Some studies on the effect of DO level in aldolase and serratiopeptidase production have been carried out (Nagai *et al.*, 1971; Pansuriya & Singhal, 2011). Since *T. reesei* is an aerobic fungus, the presence of dissolved oxygen will affect the production of xylanase.

T. reesei was cultivated in a media both in shake flask and in a batch fermenter. The shake flask was filled with the various volume ratio of media to flask to get different DO level, while the fermenter batch mode was carried out under different aeration rates to determine their effect on xylanase production. The C/N ratio and initial pH was used based on our previous study (Riadi *et al.*, 2019). Two types of media, defined and complex media, were used in this experiment. The defined media, labelled as media A, contained Avicel cellulose as the carbon source, ammonium sulfate, urea, and proteose peptone as the nitrogen source. Meanwhile, the complex media, labelled as media B, has an addition of *Reutealis trisperma* press cake as carbon and nitrogen source.

For further application, purification and characterization of xylanase generated from microorganism fermentation is important. There have been reported in some studies of purification and characterization of xylanase from many species of *Trichoderma* (Chen *et al.*, 2009; da Silva *et al.*, 2015; Törrönen *et al.*, 1992). The xylanase produced by *Trichoderma reesei* was then purified to confirm that xylanase presents in crude extract, hence the total activity of xylanase should be higher after final purification. The C/N ratio of 7.8 and initial pH of 6.0 were used as optimum parameters which were referred from previous study to produce xylanase by *Trichoderma reesei* which used *Reutealis trisperma* press cake as an additional substrate (Riadi *et al.*, 2019). The results showed the same parameters as reported by prior experiments (Bailey *et al.*, 1993; Coffman *et al.*, 2014; Xiong *et al.*, 2004).

MATERIALS AND METHODS

Press cake preparation

The press cake was obtained from a company named Energi Baru Sentosa (EBS) located in Gresik, Indonesia. It was ground to 40 mesh and dried at 50°C for 24 hours to remove the

remaining oil and water. The press cake powder was then stored in a vacuumed container. The media was made by mixing ingredients of media A and media B respectively. The pH was subsequently adjusted to 6 and the media was then autoclaved (Lejeune & Baron, 1995).

Media and culture preparation

Both media A and media B contained 1 g/L proteose peptone, 2 g/L KH_2PO_4 , 0.4 g/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.3 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2 g/L tween 80, 5 mg/L $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1212 mg/L $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 1.4 mg/L $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 2 mg/L $\text{CoCl}_2 \cdot 7\text{H}_2\text{O}$, and 10.55 g/L Avicel cellulose. Additional N-sources for media A are 1.4 g/L $(\text{NH}_4)_2\text{SO}_4$, and 0.3 g/L urea. In media B, $(\text{NH}_4)_2\text{SO}_4$ and urea were substituted by 8.7 g/L (to achieve C/N ratio of 7.8) *Rentealis trisperma* press cake as alternative N-sources. All chemicals were purchased from Merck, Germany. The pH of both media was adjusted to 6.0 (Xiong *et al.*, 2004).

T. reesei was obtained from a culture collection source at Gadjah Mada, Indonesia and was stored on PDA (Potato Dextrose Agar) at 4°C and subcultured regularly. Approximately 0.016 g of *T. reesei* from PDA slants were cultivated in 20 mL media A and media B, and were incubated for 3 days at 28°C. A 10 % inoculum was transferred from each media into 250 mL shake flasks with several working volumes and into 1.5 L fermenter with 800 mL of working volume.

Batch fermentation in 250 mL flask

A 250 mL shake flask was used to study the effect of DO by varying working volume (125, 150, and 175 mL) for both media. The ratio of working volume to flask volume were 0.5, 0.6, and 0.7, respectively. The incubation was carried out under 140 rpm in a rotary shaker (GFL 3017, Burgwedel, Germany) at 28°C (Irfan *et al.*, 2016) for 5 days. Sample was taken (5 mL) every 24 hours and the pH were measured. It was then centrifuged with 10,000 rpm angular velocity for 10 minutes. The aliquot was kept at temperature -20°C for enzyme activity assay.

Batch fermentation in 1.5 L fermenter

For 1.5 L batch fermenter, the DO was varied by varying the supply oxygen rate for 0.6 and 0.8 L/minute on media A, and 0.2, 0.6, and 0.8 L/minute for media B. The additional parameter of 0.2 L/minute for fermentation using media B is to assure the limited DO will significantly affect to the fermentation. The parameter of aeration rate for media B was added (0.2 L/min) since the difference of xylanase production for both aeration rates in media A is small. The initial pH was 6.0 and monitored by pH meter (Mettler Toledo). The fermentation was carried out for 5 days at 150 rpm agitation and the dissolved oxygen level was monitored using DO meter (Aplicon). Each sample was taken every 24 hours and centrifuged for 10 minutes with 10,000 rpm angular velocity. The aliquot was kept at temperature -20°C for enzyme activity assay.

Enzyme purification

An Amicon® Ultra Centrifugal Filter with 3,000 Da molecular weight cut off (MWCO) was used to concentrate the crude extract obtained from the experiment. A 10 mL crude extract was loaded into the filtration tube and centrifuged at 4000 rpm for an hour. The remaining solution above the filter (approximately 20% of the initial extract volume) which is called as the concentrated extract was used for further step.

A 2 mL of the concentrated extract was loaded into a (1×10 cm) Ion Exchange Chromatography (IEC) resin of Bio-Rex™ 70, which was previously equilibrated with 0.01 M citrate buffer (pH 5.3). The protein was then eluted using linear gradient of NaCl solution (0.05-0.5 M NaCl in 0.01 M citrate buffer (pH 5.3). The eluted fractions were assayed for protein concentration using Bradford colorimetric dye-binding assay (Bradford, 1976) with bovine serum albumin (BSA) as a standard. The purification process was expressed as yield and purification factor which were calculated as shown by equations [1] and [2]. The fraction with the highest yield and purification factor was selected for further step.

$$\text{Yield (\%)} = \frac{\text{total activity at certain purification step}}{\text{total activity of crude extract}} \times 100 \%$$

[1]

$$\text{Purification factor (fold)} = \frac{\text{specific activity at certain purification step}}{\text{specific activity of crude extract}} \quad [2]$$

A 12.5% SDS-PAGE gel was used to estimate the molecular mass of the purified enzyme. An approximately 0.15-1.5 µg of the purified protein was loaded into each lane along with pre-stained protein ladders of 11-245 kDa (GangNam-Stain, Intron). The electrophoresis was run at 20 mA for 50 mins. The gel was stained using Coomassie Brilliant Blue R250 staining reagent.

Xylanase activity

Xylanase activity was analyzed using a modified method of Bailey *et al.* (1993). A solution of 2 g of beechwood xylan (Sigma Aldrich, St. Louis, MO) in 200 mL of 0.05 M sodium citrate buffer (pH 5.3)

was used as a substrate. To start the reaction, a mixture of 100 µL of enzyme solution and 900 µL of substrate solution was put into 25 mL test tube consecutively, and incubated on water bath at 50°C for 5 minutes along with the blank. The reaction was terminated by the addition of 3 mL of DNS reagent, after which were boiled for 10 mins for color development. The total volume of each mixture was adjusted to 25 mL by the addition of distilled water. The concentration of xylose released was measured using UV-Vis Spectrophotometer (Hewlett Packard 8453) at 540 nm, and used to measure the xylanase activity according to the following equation [3].

$$\begin{aligned} \text{Xylanase} \left(\frac{\text{U}}{\text{mL}} \right) &= \frac{\text{xylose released (mg)}}{5 \text{ mins} \times 0.1 \text{ mL enzyme sample}} \times \frac{1 \text{ mmol}}{150.13 \text{ mg}} \times \frac{1000 \text{ } \mu\text{mol}}{1 \text{ mmol}} \\ &= 13.32 \times \text{xylose released (mg)} \end{aligned} \quad [3]$$

RESULTS AND DISCUSSION

Effect of dissolved oxygen on xylanase production in flask system

We observed the fermentation ratio of media volume to flask volume of 0.5; 0.6 and 0.7 to observe the effect of DO level on the xylanase production in flask fermentation. We found the lower the ratio, the higher the DO level is. Table 1 shows the xylanase production in the three flask systems, both in media A and B. In the flask system with media A, both 0.5 and 0.6 ratios give the highest xylanase activity of 29.20 and 39.30 U/mL within 72 hours of cultivation, whereas 0.7 ratio gives the lowest xylanase activity of 21.83 U/mL within 120 hours. Compared to the system with media A, the system of media B is able to produce the highest xylanase activity within 96 hours at 96.05, 101.07, and 65.49 U/mL, for media's /flask's volumes ratio of 0.5, 0.6, and 0.7, respectively.

The best xylanase production was at ratio of 0.6 in media B. After 96 hours (data not shown), xylanase production decreased, which may be associated with oxygen deprivation in the flasks. The initial DO level in media B affected the xylanase production. Though the ratio of 0.5

provided higher DO level, the amounts of media may not be enough to support the growth of *Trichoderma reesei*, which could result in smaller xylanase production. The lowest xylanase production was in the system with the ratio of 0.7 both in media A and B, as DO level might not enough to support the growth. There is a difference in production time for highest xylanase yield between media A and media B. The difference could be caused by additional protein and sugar contents in media B since the presence of press cake, which contains protein gives amino acids as nitrogen source leading to higher xylanase production as depicted in Table 1.

Cultivation in batch fermenter system

Effect of aeration on xylanase production

Trichoderma reesei is an aerobic microorganism for which high DO is preferable for metabolite production. The role of the oxygen is to accept H⁺ ion from NADH oxidation to produce water and energy (ATP). A low dissolved oxygen level could lead to a decrease in energy available for the growth of *T. reesei*, resulting in the decrease production of enzyme. Lack of energy would lower the consumption of substrate and the production of xylanase. On the other hand, an excess of oxygen content may result in hydrolysis

of *avicel cellulose* which lower the microbial's growth and the xylanase production (Lejeune & Baron, 1995).

The utilization of dissolved oxygen varies with the aeration rate for 0.6 and 0.8 L/min both in media A and media B (Figure 1 and Figure 2). We used additional aeration rate of 0.2 L/min in the media B since the DO profile and xylanase production in media A are not much different. Rapid utilization of the DO was observed within 48 hours of fermentation both in media A and B. The pattern of DO increase after 72 hours of fermentation with the aeration rates at 0.6 and 0.8 L/min. It means that the oxygen uptake rate (OUR) is lower compare to the oxygen transfer rate (OTR). The highest xylanase production was at 72 hours and then decreased as the utilization of DO is reduced after 72 hours (Table 1 and Table 2), which implied that the metabolite activity is reduced as the xylanase activity decrease. The decrease in xylanase activity could be due to other protease-mediated degradation (Li *et al.*, 2017). The protease can degrade protein to amino acids, hence can degrade xylanase and reduce the activity. In the case of 0.2 L/min the DO concentration was less than 5 % after 48 h fermentation, suggesting the oxygen depleted fermentation which is associated with low production of xylanase. In this case, the highest

xylanase produced was at 48 hours and decrease afterwards. According to Xiong *et al.* (2004), the DO for xylanase fermentation must be kept above 40% saturation. In our study, % DO above 40 % occurred for highest xylanase production in both media A and media B for 72 hours at 0.8 L/min aeration rate (Figure 1 and Figure 2).

The highest xylanase production (205.9 U/mL) was found in the batch system media B with 0.8 L/min aeration rate, 72 hours fermentation time. The concentrated xylanase activity is lower than xylanase activity reported from fermentation of L-arabinose-rich hydrolysates by *T. reesei*, which is 510 U/mL (Xiong *et al.*, 2005) and higher than xylanase activity produced from rice straw by *T. reesei* (123 U/mL) (Colina *et al.*, 2003).

***Reutealis trisperma* induces xylanase production**

The time profiles of xylanase activities produced by *T. reesei* are shown in Figure 3 and Figure 4. The concentrated xylanase activity started to decrease after 72 hours of cultivation, hence 72 hours was chosen as a cut off time for consistent comparison of xylanase production. The summary of enzyme activity in media B cultivation at the highest time production is presented in Table 3.

Table 1. Production of xylanase by *Trichoderma reesei* on different media in shake flasks at initial pH of 6.0.

	Ratio of media volume to flask volume	Concentrated xylanase activity (U/mL)	Fermentation time for highest xylanase activity (h)	Final pH
Media A	0.5	29.20±0.24	72	4.40
	0.6	39.30±0.64	72	5.18
	0.7	21.80±0.53	120	4.19
Media B	0.5	96.05±0.78	96	5.84
	0.6	101.07±1.65	96	5.12
	0.7	65.50±1.07	96	5.56

Table 2. Xylanase activity in media A with 150 rpm at different aeration rate.

Aeration rate (L/min)	Concentrated xylanase activity (U/mL)	Fermentation time for highest xylanase activity (h)
0.6	78.53 ±0.64	72
0.8	88.87±1.45	72

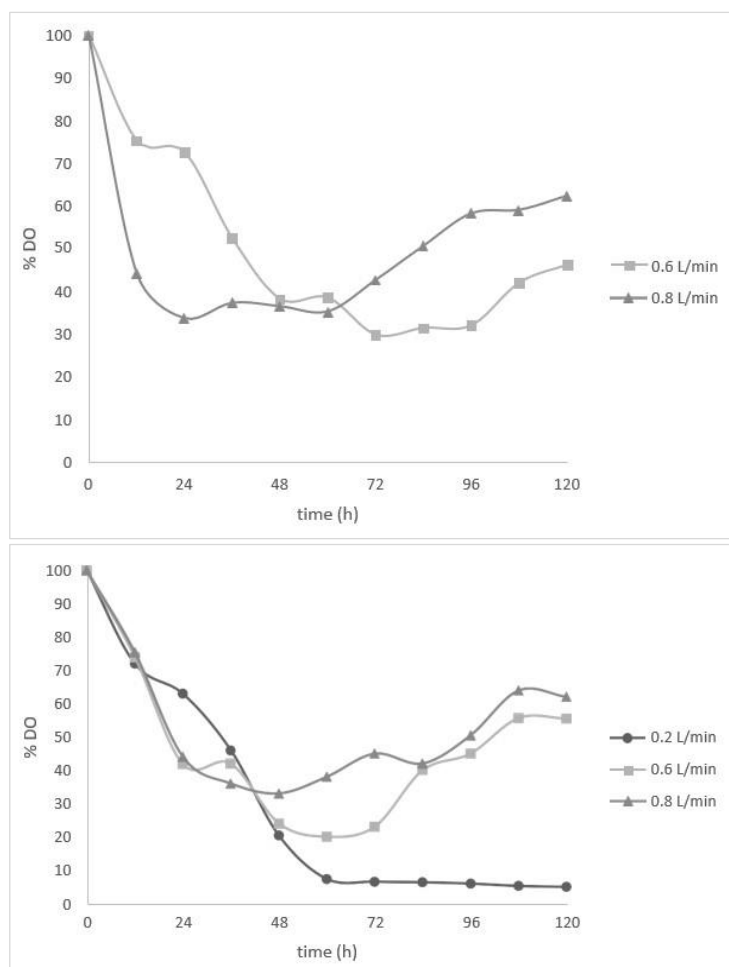


Figure 1. Percentage of DO during fermentative production of xylanase by *Trichoderma reesei* in batch culture media A at various aeration time.

Figure 2. Percentage of DO during fermentative production of xylanase by *Trichoderma reesei* in batch culture media B at various aeration rate.

T. reesei produced more xylanase in media B compare to media A, which is more than two folds in the system with 0.8 L/min aeration rate. It implied the presence of *Rentealis trisperma* shows the substrate induced xylanase production. The importance of *Rentealis trisperma* as an inducer of xylanase needs further investigation. In cultivation on cellulose substrates (media A) at pH 6.0, xylanase production is superior compare to cellulase, but cellulase will be dominant at pH 4.0 (Bailey *et al.*, 1993). Since we focused on xylanase production, the starting pH was set at 6.0.

The purified xylanase has approximately 32 kDa size which is similar to the previously reported xylanase (Xiong *et al.*, 2004). The detail will be explained in the next section.

Enzyme purification

Table 4 summarizes the results of each purification step. The supernatant of the culture was concentrated and purified by ultrafiltration and ion exchange chromatography. Ultrafiltration with 3000 Da MWCO was used to separate and concentrate xylanase from other biomolecules smaller than 3000 Da resulting in the reduction of sample volume, slightly increasing of purification factor and higher intensity of every band on SDS-PAGE analysis (Figure 5). The concentrated extract was then purified further using cationic IEC resin at pH 5.3, to separate xylanase which has a PI of 5.5.

Table 3. Xylanase activity in media B with 150 rpm at different aeration rate.

Aeration rate (L/min)	Concentrated xylanase activity (U/mL)	Fermentation time for highest xylanase activity (h)
0.2	32.20±0.26	48
0.6	189.30±2.32	72
0.8	205.90±0.84	72

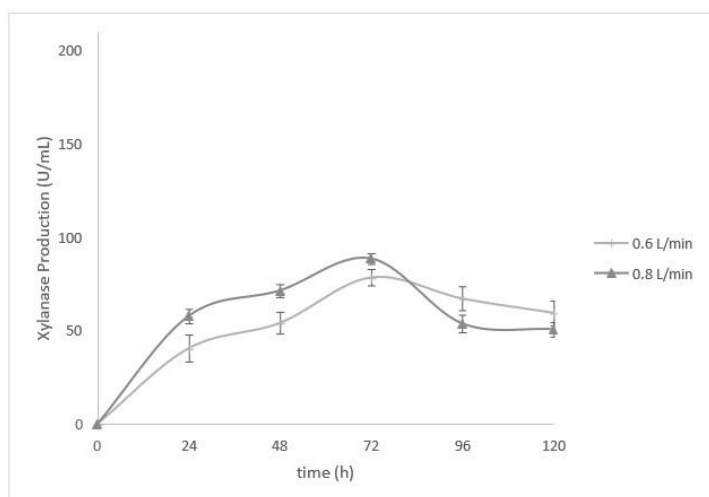


Figure 3. The time course of xylanase production by *Trichoderma reesei* in media A at various aeration rate. The highest xylanase activity was obtained at 72 hours fermentation.

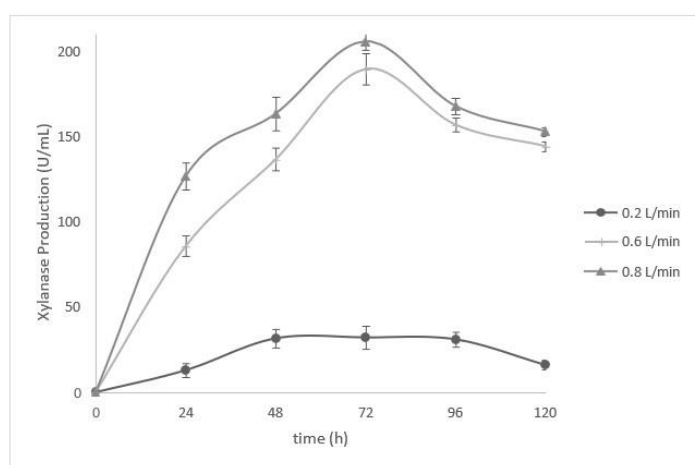


Figure 4. The time course of xylanase production by *Trichoderma reesei* in media B at various aeration rate. The highest xylanase activity was obtained at 72 hours fermentation.

Table 4. Purification of xylanase from *Trichoderma reesei*.

Step	Volume (mL)	Activity (U/mL)	Total Activity (U)	Total Protein (mg)	Specific Activity (U/mg)	Yield (%)	Purification Factor (fold)
Crude enzyme	120	60.173	7220.760	91.248	79.133	100	1
Ultrafiltration	24	106.457	2554.968	30.348	84.189	35.384	1.064
IEC	1	4.787	4.787	0.009	518.700	7.184	6.555

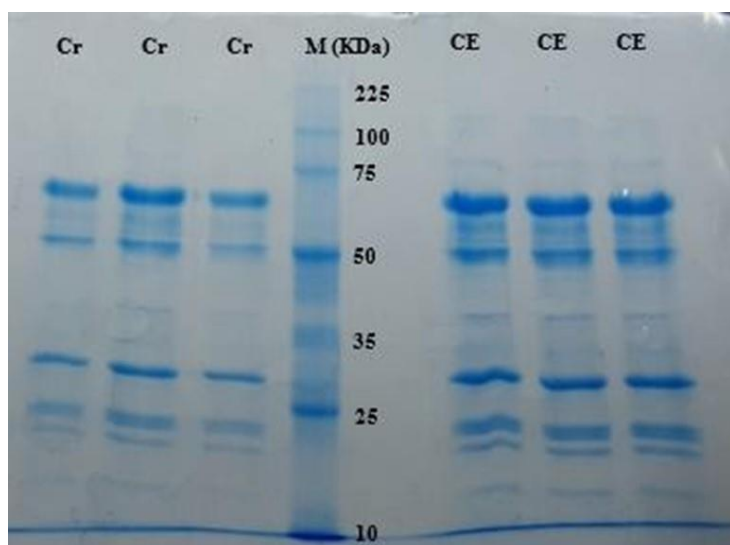


Figure 5. SDS-PAGE analysis of crude extract (Cr) and concentrated extract (CE) produced by *Trichoderma reesei*. M: protein molecular weight marker.

The yield was obtained by dividing the total enzyme activity after purification with the initial total activity. The purification factor is the ratio of enzyme specific activity after purification to initial its specific activity.

The fraction number 10, 20, and 30 of IEC gave three peaks which have both high yield and purification factor (Figure 6). The absence of band from fraction 10 might be due to very low protein concentration. The fraction 10, 20, and 30 were then analyzed using SDS-PAGE which gave two bands (Figure 7); ~32 kDa from fraction 30 and ~65 kDa from fraction 20. The highest yield and purity were shown by fraction 30, indicates the purification process was successful. The

fraction 30 was confirmed further by the xylanase activity assay, which showed that it can convert xylan to xylose. These findings are in line with the previous report that stated *Trichoderma reesei* PC-3-7 has three forms of xylanase with a size of 32 kDa (Xu *et al.*, 1998). Previously, *Trichoderma reesei* was also reported as having two forms of xylanases with a size of 19 and 21 kDa, respectively (Törrönen *et al.*, 1992). The ~32 kDa band could be predicted as xylanase, meanwhile the ~65 kDa seems belong to be the size cellobiohydrolase I (CBH I), which is known as one of the major enzymes produced by *Trichoderma reesei* (Abuja *et al.*, 1988; Chen & Hayn.,1993).

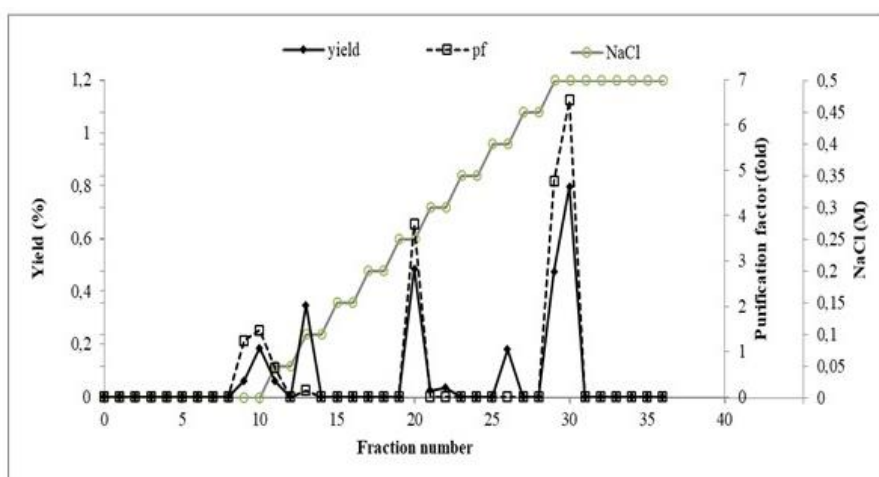


Figure 6. Ion Exchange Chromatography (IEC) chromatogram of purified xylanase from *Trichoderma reesei*.

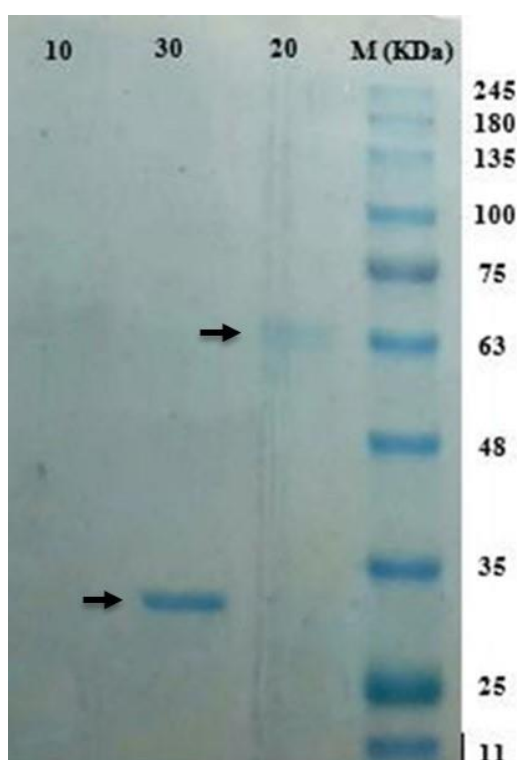


Figure 7. SDS-PAGE analysis of IEC fractions. M: protein molecular weight marker. Fraction 30 give a band with a size of 32 kDa, meanwhile fraction 20 give a band with size 65 kDa.

CONCLUSION

The results of this study showed that dissolved oxygen (DO) level affected the xylanase production by *Trichoderma reesei* using *Reutealis trisperma* press cake as an additional substrate. Xylanase production in media that contained *Reutealis trisperma* press cake gives a higher xylanase activity than that in media A without press cake. In flask system, the highest xylanase activity was obtained at media volume to/flask volume ratio of 0.6 in media B, i.e. 101.07 ± 1.65 U/mL, with 96 hours fermentation time. In batch system using media B, the highest xylanase activity was obtained with 0.8 L/min aeration rate, i.e. 205.90 ± 0.84 U/mL, within 72 hours fermentation time. The purified xylanase had ~32 kDa and ~65 kDa molecular weight. Further study should observe oxygen mass transfer to understand volumetric mass transfer constraints in relation to the availability of oxygen at different aeration rate.

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CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

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Michelle Teo Yee Mun is currently a lecturer in the Faculty of Applied Sciences, UCSI University. Previously, she obtained her BSc and MSc from the University of the West of England in Bristol, United Kingdom, followed by a PhD from UCSI University. Her research interests are molecular oncology and cancer immunology, with specialization in immunotherapies such as immunotoxins and antibody engineering. Michelle has been actively participating in various international and national conferences and symposiums and received several awards, including the Best Abstract Award during the Hong Kong Croucher Summer Course in 2018.



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Khatijah has over 32 years' experience investigating the molecular virology of Newcastle disease virus. Her current research interest is in the development of NDV as a therapeutic oncoviral vaccine through the use of reverse genetics. She is also involved in the molecular biology of other organisms, in particular on the use of nanobiotechnology, surface display technology and phage therapy. She is currently in the Vice-President of World Academy of Sciences (TWAS); and sits in the Councils for the Academy of Sciences Malaysia, the Islamic World Academy of Sciences, TWAS, and the Council of Science Advisers (CSA) of the International Centre for Genetic Engineering and Biotechnology (ICGEB).

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Professor Dr Lau Yee Ling is at present the Head of the Department of Parasitology, Faculty of Medicine at University of Malaya (UM). She started her academic career as a lecturer at Monash University Sunway Campus while waiting for her PhD viva in 2008. During her time as a lecturer in Monash University, she was awarded two Monash University Research Grants in which enabled her to continue her research in the field of molecular parasitology. She then returned to her alma mater, University of Malaya, as a Senior Lecturer in 2009. She was granted tenure in 2010 and promoted to Associate Professor in 2013, and Professor in 2019. Professor Lau has been awarded University of Malaya Excellent Service Award three times in 2011, 2013 and 2015. She was awarded MSPTM Nadchadtram Medal in 2014. She has also been awarded a few times for her innovation in research including the Grand Prize in National Exclusive Innovation Challenge Award 2018.



Suet Lin Chia, PhD



Suet Lin obtained his PhD in the field of Medical Biotechnology from Universiti Putra Malaysia in year 2012 under the supervision of Prof. Dr. Khatijah Yusoff. He underwent a two-year post-doctoral training in the Seymour's lab in the University of Oxford. His field of interest is to develop a potent oncolytic Newcastle disease virus for the treatment of cancer. Currently, his research involves genetic modification of NDV genome to reduce the virus pathogenicity towards birds. In addition, he is also working on cloning immunostimulatory genes into the virus backbone to enhance the oncolytic properties of the virus.

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Molecular characterization and phylogenetic analysis of kaurene synthase protein in *Stevia rebaudiana* MS007

Nur Fathiah Rosilan, Muhammad Amirul Husni Samsulrizal, Nor Adilah A. Rani, Nurul Hidayah Samsulrizal, Zarina Zainuddin, Tamil Chelvan Meenakshi Sundram

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Stevia rebaudiana is a plant under the Asteraceae family and has been reported as a healthier alternative to sugar. Steviol glycosides (SGs) is the group of secondary metabolites responsible for the sweet taste. (1) Among nine SGs synthesised by *S. rebaudiana*, stevioside and rebaudioside A are the sweetest. The biosynthetic pathway of SGs partly involves conversion of geranylgeranyl diphosphate (GGDP) into steviol, catalysed by *ent*-kaurene synthase (KS), *ent*-copalyl diphosphate synthase (CPPS), and kaurene oxidase (KO). This study focuses on *in silico* molecular characterization and phylogenetic analysis of KS from Malaysia's *S. rebaudiana* MS007 variety (*Stevia* MS007). The transcriptomic dataset of *S. rebaudiana* accession MS007 was used in initial experiment toward analysing the KS. Through the blastx homology search using transcriptomic dataset query Cluster-31069.42907, the *Stevia rebaudiana* kaurene synthase (*SrKS*) sequence was identified with the highest similarity percentage identity (99.62%). The protein domain prediction using InterPro yields IPR005630 (terpene synthase metal-binding domain) at positions 490 to 755 and IPR001906 (terpene synthase-N-terminal-domain) at positions 258 to 477. Multiple sequence alignment was conducted using MUSCLE and MEGA-X as phylogenetic tree analysis tool for constructing the phylogenetic analysis tree. Based on the bootstrap value from the phylogenetic analysis, Cluster-31069.42907 represents relationships between the ancestors. Since both *Helianthus annuus* and *S. rebaudiana* are Asteraceae species, the bootstrap value for both species was 100%. In conclusion, this research contributes to a better understanding of *Stevia* MS007 KS via *in silico* analysis.

Production and functional characteristics of exopolysaccharide by *Lactobacillus plantarum* co-cultivation with *Saccharomyces cerevisiae*

'Aina Nabilah Faizah Ahmad Bustamam, Nur Sazwani Daud, Zaheda Mohamad Azam, Mohamad Azzuan Rosli, Solleh Ramli, Noorazwani Zainol, Muhammad Helmi Nadri, Hong Yeng Leong, Nor Zalina Othman

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Exopolysaccharides (EPS) are well-known biopolymers secreted by several lactic acid bacteria with combination of various strains. The aim of this study is to increase EPS production by co-culturing *Lactobacillus plantarum* ATCC 8014 with *Saccharomyces cerevisiae* ICA-Y01 and study the changes in the functional characteristics of the EPS from both cultivations. In this study, the production and functional characteristics of EPS from co-cultivation culture of *L. plantarum* ATCC 8014 with *S. cerevisiae* ICA-Y01 were evaluated. The co-cultivation of *L. plantarum* ATCC 8014 with *S. cerevisiae* ICA-Y01 was markedly increased EPS production up to 55.84% with 6.8 g/l yield after 20 hours cultivation. The pH of the co-cultivation culture was remained constantly at 5.2 until the end of cultivation. Furthermore, co-cultivation under pH 6 in the 16L bioreactor showed a higher growth rate of 0.214 h⁻¹ and EPS production increased up to 104.44% when compared with single cultivation of *L. plantarum* ATCC 8014. This result clearly indicates the importance of growing the cells in the controlled pH condition when cultivated with *S. cerevisiae* ICA-Y01 to enhance EPS production. The functional characteristics of EPS secreted from both cultivation strategies were also evaluated. FT-IR spectroscopy confirmed EPS presence from both cultivations, indicating functional group of the polysaccharide with D-glucose units bound by α -(1→6). The EPS production from single cultivation showed a higher DPPH radical scavenging activity (88.21%) and IC₅₀ (19.57%) as compared to EPS produced from co-cultivation with DPPH scavenging exhibited 32.45% with no IC₅₀ value detected. Furthermore, solubility and water uptake of EPS from single cultivation are higher in comparison to co-cultivation. In conclusion, higher efficiency in the bioactivity of EPS from the single cultivation of *L. plantarum* ATCC 8014 was confirmed even though the EPS yield is low as compared to EPS synthesis through inter-kingdom cultivation.

Characterisation of capsid polypeptide P1 and capsid protein VP1 of the Malaysia foot and mouth disease virus (FMDV) serotype O and A isolates

Farah Najwa Abd-Halin, Zunita Zakaria, Saila Ismail, Sarah Othman

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Foot and mouth disease virus (FMDV) is the cause of foot and mouth disease (FMD) outbreaks in livestock worldwide, which affects domestic and international trade, resulting in significant economic losses and social consequences. For efficient monitoring and prevention of FMD outbreaks, the need for improved strategies to control FMDV and achieve FMD-free status with various control measures including vaccination can be established. In vaccinology, major advances and discoveries in vaccination variations including DNA and protein subunit vaccines proved to be more economical and sustainable. To develop a safe vaccine for animals, possible antigenic genes or antigens need to be identified and characterised. The FMDV is a single-stranded RNA virus consisting of a capsid precursor polypeptide, P1, which encodes for four structural proteins (VP4-1), leading to antigenic variation and VP1 potentially carrying the key epitope for vaccine development. This study aims to identify and characterise the capsid polypeptide, P1 and capsid protein, VP1 of the Malaysian FMDV serotype O and serotype A isolates. The nucleotide and protein sequences were identified based on the FMD outbreaks in Malaysia and the antigenicity of the P1 and VP1 was predicted by Kolaskar and Tongaonkar's semi-empirical method. Subsequently, the P1 and VP1 genes were inserted into pET-28a, respectively, and used for protein expression analysis. The P1 and VP1 were predicted to be antigenic via *in silico* analysis and successfully expressed and characterised through *in vitro* analysis. Hence, this study can be exploited as a tool to design a new novel vaccine for vaccine development against FMD in bovines.

Primer characterization of *in-house* real time PCR (RT-PCR) for BCL2 gene using saliva sample

Indra Wahyu Nufroha, Adri Nora, Henny Saraswati

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In organisms, cells perform apoptosis to remove damaged or mutated cells from the body. The Bcl-2 family protein encoded by the BCL2 gene plays a role in regulating apoptosis. Abnormalities in the function and expression level of the Bcl-2 protein are associated with several cancers. Saliva is a body fluid that can be used for biomedical research because it contains essential biomarkers of the body and genetic material derived from free cells in the oral cavity. This study aims to get primer characterization for the RT-PCR method for the BCL2 gene. We used RNA samples isolated from saliva to optimize the primers' annealing temperature, concentration, and combination pairs. Previous studies produced three primer candidates, i.e., primers A, B, and C, used in this research. The optimization results showed that primer C was the best primer to be used in the real-time PCR of this study. The optimal annealing temperature used was 60.3°C with a primer concentration of 400 nM. This study also shows the potential of saliva as a material for biomedical studies on the BCL2 gene. The results of the primer characterization resulting from this research are the first step in establishing the in-house RT-PCR method. The validation research will use a larger sample to validate this method.

Simple approach for expression and rapid purification of *Taq* DNA polymerase in three *Escherichia coli* strains

Xue Chi Teng, See Yin Ang, Marimuthu Citartan, Thean Hock Tang, Siti Aminah Ahmed

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Background and objectives: One of the most commonly employed bacterial hosts for the synthesis of recombinant proteins is *Escherichia coli* (*E. coli*). Choosing an ideal host for the production of the protein of interest is an important step in the large-scale production process. Due to its thermostable characteristic, *Taq* Pol I, which was first isolated from the bacterium *Thermus aquaticus* (*Taq*), is now a typical enzyme found in many laboratories. This study aimed to identify the ideal host for large-scale production of *Taq* Pol I and purify the enzyme under a simple and rapid purification method. **Methods:** *Taq* Pol I gene in pSE420 plasmid was overexpressed in *E. coli* strains DH5α, TOP10 and BL21(DE3) pLysS. The enzyme was purified using Pluthero's method and dialyzed using Amicon® Ultra-4 Centrifugal Filter. **Results:** The host strain *E. coli* TOP10 produced the highest amounts of *Taq* Pol I, followed by DH5α and BL21(DE3) pLysS. An estimated 4.5 U/μL of *Taq* Pol I was produced from a 200 mL culture of TOP10 host. **Conclusion:** This study provides data on the capacity of the *E. coli* strains used for *Taq* Pol I protein production. This information can be used to accelerate future targeted strain selection for the production of a specific protein of interest.

Validation of endophytic bacteria colonisation in tissue culture-derived oil palm plantlets via green fluorescent visualization

(/)Salwa Abdullah Sirajuddin, Intan Nur Ainni Mohamed Azni, Nur Diyana Roslan, Shamala Sundram

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This study aimed to validate the colonisation capability of endophytic bacteria (EB) isolates, *Bacillus cereus* EB2 and *Pseudomonas aeruginosa* EB35, that previously exhibited their potentials as biological control agents (BCAs) against the *Ganoderma* spp., a pathogen for *Ganoderma* disease in oil palm. Here, we demonstrated a rapid method to determine the colonisation capacity of the selected EB using oil palm tissue culture plantlets and a green fluorescent protein (GFP) visual marker. Wounded plantlet roots were inoculated with GFP-tagged *B. cereus* EB2 and *P. aeruginosa* EB35 while the plantlets without EB inoculation served as controls. The GFP signals appeared as bright green spots or lines in the inoculated GFP-tagged EB cells in root and leaf plantlet tissues, respectively, under the confocal laser scanning microscopy (CLSM) 5 days post-inoculation. In contrast, there was no intense GFP spots in neither the control root nor leaf tissues. The cracks in the roots by wounding facilitated the entry of the GFP-tagged EB cells into root tissues, allowing for endophytically colonisation of the root and above-ground tissues. Subsequent result of polymerase chain reaction (PCR)-GFP analysis further displayed the endophytic nature and early chronological colonisation of the tested EB. This is a preliminary report on root colonisation by a Gram-positive endophyte, *B. cereus* EB2 and leaf tissues colonisation by both EB isolates as internal colonisers, demonstrating their potential as BCAs to protect oil palm against *Ganoderma* spp. for a sustainable disease management.

Influence of IL-28B serum level and gene polymorphism in a sample of Iraqi patients with ankylosing spondylitis

Hadeel Waleed Abdulmalek, Reema Mohammed Abed, Laith Ahmad Yaaqoob, Maha Fakhry Altaee, Zaid Kadim Kamona

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Ankylosing spondylitis (AS) represents one kind of advanced arthritis formed via inflammatory stimuli long-term in the spine's joints. Interleukin (IL)-29 (interferon- λ 1 (IFN- λ 1)), interleukin (IL)-28A (interferon- λ 2 (IFN- λ 2)) and interleukin (IL)-28B (interferon- λ 3 (IFN- λ 3)) are three interferon lambda (IFN- λ s) molecules that have recently been identified as new members of the IFN family. IL-28B expression in ankylosing spondylitis (AS) is not well understood. 150 male healthy controls (HC) and 160 males with AS as patients group participated in this study. Serum level and gene polymorphism were assessed using an enzyme-linked immunosorbent assay and Sanger sequencing for IL-28B, respectively. The results showed significantly lower serum IL-28B concentrations in the AS groups in comparison to the HC groups (both p values equal to 0.003). There was a large difference in IL-28B genotype and allele frequency between the two individuals. IL-28B heterozygote genotype CT of rs12979860 SNP exhibits a substantial correlation with AS (P = 0.008). While the genotypes of rs12980275 SNP were not shown any significant correlation with AS. The findings suggest that serum concentration of IL-28B is a potential diagnostic biomarker in patients with AS, and that the heterozygote CT of rs12979860 SNP serves as a potential risk factor for the onset of AS in the Iraqi population.

The effect of HIV protease gene mutations to protease inhibitor drugs resistance in Papua patients: In silico analysis

Mirna Widiyanti, Yustinus Maladan, Setyo Adiningsih

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The use of phenotypic assay requires laborious work to culture HIV isolates to observe the phenotypic change of the virus in the presence of antiretroviral drugs. The genotypic approach may rely on the secondary data of documented mutations that are responsible for phenotypic alterations to antiretroviral-resistant HIV. HIV genomes were extracted from patients' plasma, which was subsequently subjected to RT-PCR and Sanger sequencing. The obtained HIV genome sequencing data were analyzed for mutation detection. Three-dimensional (3D) structures of mutant HIV protease were constructed using FoldX software. The binding affinity of the mutant HIV protease with protease inhibitor drugs (Saquinavir, Ritonavir, Nelfinavir, Indinavir, and Lopinavir) was analyzed using AutoDock Vina. There were 90 patients involved in this study. The patients attended the Voluntary Counseling Test (VCT) of Mitra Masyarakat Hospital in Mimika, Papua, Indonesia. Among recruited subjects, the HIV genomes corresponding to the protease-encoded gene of 30 patients were successfully sequenced. There was only one patient (RSMM_70) infected with HIV harboring minor mutations (L10V, I15V, M36I, and R41K) in the protease-encoded gene that was not a new finding mutation. The 3D structure showed that the hydrophobicity and stability of mutant HIV protease were different from the wild genotype. Docking analysis showed decreasing binding affinity of the mutant HIV protease to the protease inhibitor drugs, which may lead to the alteration of inhibitory effectiveness. *In silico* docking, the analysis may provide an alternative approach to predict the effect of minor mutations in the HIV protease gene on the effectiveness of protease inhibitor drugs.

Invasive non-typhoidal *Salmonella* in adult patients: A three-year review in a Malaysian General Hospital

Karshini Jeya Pirathaba, Maria Kahar Bador Abdul Kahar, Nurzam Suhaila Che Hussin

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Invasive non-typhoidal *Salmonella* (iNTS) are recognized as an important cause of infection among immunocompromised patients with high morbidity and mortality. This study focused on the epidemiology, risk factors, antibiotic susceptibility, and clinical outcome of iNTS in Hospital Kuala Lumpur over 3 years period (2017-2019). **Methods:** A retrospective study was conducted on 43 adult patient who had NTS isolated from a sterile sample. The patient's microbiology worksheets and medical notes were reviewed and analyzed. **Results:** The overall incidence rate for iNTS was 2.4 per 10,000 admissions. The patients median age was 57 years, with majority of them being above ≥ 55 years old (n=24, 55.8%). The most prevalent diseases are hypertension (28%), human immunodeficiency virus (20.9%), diabetes mellitus (18.6%) and heart disease (6.3%). Primary bacteraemia (95.3%) was the most frequent cause of iNTS, followed by tubo-ovarian abscess (2.3%) and urinary tract infections (2.3%). The overall mortality rate was 30%. The mortality rate was greater in patient presenting with shock on admission (p=0.04). Most of the invasive diseases were caused by *Salmonella enterica* serovar Enteritidis (95.3%). Overall, most isolates were susceptible to ceftriaxone (97.7%). Five isolates were intermediately resistant to ciprofloxacin (11.6%). Only one isolate (2.3%) was multidrug resistant. **Conclusions:** The comorbidities of iNTS varies even within the same country. Mortality rates are higher in those with primary bacteraemia and shock during admission. By understanding the epidemiology of iNTS in Malaysia, it will provide valuable information about the vulnerable population at risk of iNTS and improve the treatment and management of this patients.

Role of type IV pilin biosynthesis genes in biofilm formation of *Aeromonas hydrophila*

Nancy Garg, Geetika Sharma, Daad Saffarini, Shivani Sharda, Rachana Sahney, Sheetal Shirodkar

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Aeromonads resides in aquatic environments and infect humans and fish among other animals. This opportunistic pathogen is predicted to have several pili and fimbriae genes which may promote biofilm formation and attachment affecting the infection process. The present study compares biofilm formation and subsequent infection on MDCK cell lines using wildtype *Aeromonas hydrophila* and putative type IV pilin biosynthesis gene mutant generated by standard protocol. The results indicate the involvement of putative pilus biosynthesis operon AHA0686-AHA0696 in biofilm formation of *Aeromonas hydrophila* and infection of MDCK cells. In silico analysis of the operon predicts to contain putative type IV pili and pilin biosynthetic genes. Detailed analysis of these genes is required to evaluate the applicability of these mutant strains as potential vaccine candidates.

Volume 31(2); 2023

***Escherichia coli* phages isolated from broiler chickens showed ideal characteristics in gut modulation**

Mohd Asrore Mohd Shaufi, Chin Chin Sieo

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Phage has gained interest as an alternative antibiotic growth promoter (AGPs) in poultry production. Most phage studies only focus on phages that target pathogens. In this study, we isolated and characterised phages that target non-pathogens from chicken intestines. This study aimed to isolate and characterise phages that target non-pathogenic *Escherichia coli* for gut modulation study in broiler chickens. Based on a morphological study, the C1 phage belonged to the *Podoviridae* family, whereas C2, C3, and C4 phages belonged to the *Siphoviridae* family. The C1, C2, C3, and C4 phages appeared to be unique based on restriction fragment length polymorphisms (RFLPs), amplification of phage signature genes, and protein profiling (SDS-PAGE). The C1 phage had an ideal multiplicity of infection (MOI) of 0.001, followed by 0.1 for the C2, C3, and C4 phages. C1 had the highest adsorption rate of 99.7% in 1 min, followed by C2 (98%), C3 (98.7%), and C4 (98.2%), all of which were within 2 min. C1 also exhibited the largest burst size (72 PFU/infected cell) and the shortest latent period (5 min). The latent period of the C2, C3, and C4 phages was longer, lasting 10 minutes, and their burst sizes were 70, 77, and 46 PFU/infected cells, respectively. All phages had optimum lytic activity at pH 7 and 37°C. Each phage was unique and possessed favourable lytic characteristics, making all of them suitable for gut modulation study in chickens.

Structural complexity and physical mechanism of self-assembled lipid as nanocarriers: A review



(/ Nazhan Ilias, Rocky Vester Richmond, Gayathri Thevi Selvarajah, Intan Diana Mat Azmi, Mokrish Ajat

APJMBB 31(2): 26-35

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Lipids such as glyceryl monooleate, phosphatidylcholine, and monoglyceride (CITREM) possess an amphipathic property that allows them to self-assemble into a complex internal structure when interacting with an aqueous solution. Since amphiphilic molecules possess hydrophilic heads and lipophilic tails, hydrophobic effects cause the spontaneous activity of the molecular rearrangement. The self-organization of the molecules often results in the phases of lipid polymorphism, for example microemulsion, inverse bicontinuous cubic (Q2), discontinuous hexagonal (H2), and micellar cubic (I2) Fd3m. Interestingly, these lamellar and non-lamellar phases have been applied in the development of nanocarriers for drug delivery due to their ability to provide a sustained drug release system, better drug bioavailability, and improved overall treatment. However, the attention that they are receiving from their application is not comparable to our understanding of the mechanisms involved in their synthesis. Elucidation of the spontaneous process helps in predicting and tuning the internal structure of an amphiphilic molecule to suit its application. Therefore, this review discusses the formation of lipid polymorphism from the thermodynamic point of view, critical packing parameter, and modified stalk theory.

Long non-coding RNA in glioblastoma invasion: Angiogenesis and mesenchymal transition via PI3K and Wnt signalling

Dexter Hoi Long Leung

APJMBB 31(2): 36-52

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Glioblastoma (GBM) is the deadliest type of primary brain tumours with a high mortality rate, attributing to high post-surgical tumour recurrence. This unfavourable prognosis is due to the highly invasive phenotype observed in GBM cells, especially within the mesenchymal (MES) subtype of GBM. In recent years, the involvement of non-coding RNA (ncRNA) such as long non-coding RNA (lncRNA) and microRNA (miRNA) have been extensively deliberated in cancers and GBM. They were reported to be involved in the regulation of multiple biological pathways and cellular processes, which leads to increased cell invasion observed in tumours. This review focuses on two cellular processes; angiogenesis and MES transition, which can stimulate the invasive nature of GBM cells. Additionally, the extracellular matrix (ECM) and the hypoxic environment of GBM microenvironment which are central factors regulating both cellular processes will be discussed. Both cellular processes affiliated with cell invasion are downstream of signalling pathways such as PI3K/Akt or Wnt/ β -catenin signalling, which will also be elaborated. Finally, recent studies characterising novel lncRNAs in the regulation of cell-invasion in GBM, specifically via the biological processes and signalling pathways discussed previously will be compiled and reviewed.

Bacteria as promising biofactory for pigment production: A prospective insights into production strategies and industrial applications

Priya Sundararajan, Shanmuga Priya Ramasamy

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The pigments are the coloured substances obtained from various organic and inorganic sources. Due to the negative impact of chemically synthesized dyes and pigments, there is a significant demand for (//)microbially derived natural colourants. Microorganisms secrete pigments as their secondary metabolites. Microbial pigments are found as an alternative to synthetic pigments as they are produced significantly in higher quantities through biotechnological processes. Microbial pigments replaced by artificial colourants are easily decomposable and do not cause hazardous effects on the ecosystem. Different microbes like bacteria, fungi and actinomycetes could be exploited in pigment production. Despite varied groups of microorganisms acting as a source of pigment, bacteria are predominant source for pigment production because of their genetic simplicity. Moreover, bacterial pigments are recognized for their biological activities which accomplish their usage as colourants and therapeutics in various industries. Regarding bacterial pigment production, fermentation strategies are essential to overcome the market demand, which is applied in the pharmaceutical, chemical and food industries. The importance of bacterial pigments over synthetic pigments in various industrial applications and their production strategies is well narrated in this review.

The biosensor application in cancer detections: A review

Norhaniza Emrizal, Zaini Haryati Mohd Zain, Khor Goot Heah

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Cancer has a higher possibility to be cured if the cancer can be detected earlier. However, many malignancies are currently diagnosed only after they have spread throughout the body due to late diagnosis. Current devices have limitations to detect early cancer cells as the huge variations in the signal and limiting repeatability and sensitivity. Thus, more specific, and sensitive device of biosensor is required urgently for early cancer cells detection. The design and advancement of biosensor technology has become a focal point in recent years due to a broad variety of biosensor applications of cancer detections. These devices convert the genomic materials into an electric signal that can be identified by recognizing a specific biological analyte. In addition, the recent application of biosensors together with nanomaterials has constituted an excellent strategy in cancer monitoring and detection. This review recaps the latest literature search insights the biosensors development and application on their biological recognitions. Finally, the up-to-date approaches applied in biosensors using the nanomaterials and micro-technologies as advancement in detecting various cancers are highlighted in this review paper.

Utilizing waste mango and avocado seeds for highly effective dye removal with activated carbon



(/)Rawa Ghassan Yousuf, Huda Adil Sabbar, Zainab Yaqoub Atiyah

APJMBB 31(2): 71-79

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Activated carbon (AC) is a highly important adsorbent material, as it is a solid form of pure carbon that boasts a porous structure and a large surface area, making it effective for capturing pollutants. Thanks to its exceptional features, AC is widely used for purifying water that is contaminated with odors and removing dyes in a cost-effective manner. A variety of carbonic materials have been employed to prepare AC, and this study aimed to evaluate the suitability of utilizing waste mango and avocado seeds for this purpose, followed by testing their efficacy in removing dye from aqueous solutions. The results indicate that using waste mango and avocado as AC is technically feasible, achieving dye removal percentages of 98% and 93%, respectively. Equilibrium isotherms were explained using Langmuir and Freundlich adsorption models, with the former proving to be the best fit for the experimental data ($R^2=0.99$). Additionally, adsorption kinetics were analyzed and found to be well represented by the pseudo-2nd kinetic model.

The first DNA barcode of medically important cockroaches in Bangladesh

Faria Farhana Rain, Abu Faiz Md. Aslam

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Cockroaches can spread various pathogenic agents which are accountable for food adulteration and the dispersing of foodborne pathogens. Pest management depends on proper identification. (1) Nowadays, the COI gene of mitochondrial DNA has been anticipated as a recent systematic method functional in taxonomy and evolutionary study on species identification. The present research work is an initiative to identify the medically important cockroaches based on mitochondrial COI gene sequences. Eight (8) cockroach species (*Periplaneta americana*, *Periplaneta brunnea*, *Periplaneta australasiae*, *Blatella germanica*, *Hebardina concinna*, *Pycnoscleus surinamensis*, *Blaberidae* sp. and *Balta notulata*) were identified. Among them, four cockroach species (*Balta notulata*, *Blaberidae* sp., *Hebardina Concinna* and *Pycnoscelus surinamensis*) were the new record from Bangladesh. AT base content in DNA fragments of cockroaches was higher than GC base content. The highest AT content was 70% and the lowest GC content was 30%. The interspecific genetic divergence range of medically important cockroach species was 0.01-0.25. According to mutational steps, *Pycnoscelus surinamensis* was mostly diverged from its common ancestor by 88 mutational steps. Phylogenetic analysis revealed that species belonging to the same family were in the same major clade. This research is the first molecular approach to identify the medically important cockroach species based on MT-COI gene sequences in Bangladesh.

Phytochemical, anti-microbial activity, and anti-proliferation tests against human cancer-origin cell lines using water and ethanolic extracts of *Momordica cochinchinensis* (Gac fruit)

Priscilla Jayanthi Thavamany, Ming Thong Ong, Sreeramanan Subramaniam, Vikneswaran Murugaiyah

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Momordica cochinchinensis (Gac fruit) is a perennial tropical fruit which nutritional benefits have drawn significant attention in Southeast Asian countries but are not completely explored in this region. In addition to aril (/) extracts, pulp and seed extracts were the focus of this study in terms of their phytochemical composition, antioxidant, antimicrobial, antiproliferative, and wound healing properties. The extracts obtained were aril water extract (AW), pulp water extract (PW) and seed extracts (SW), and its ethanolic counterpart, namely aril extract (AE), pulp extract (PE) and seed extract (SE). Both water and ethanolic extracts of the aril, pulp and seed contain alkaloids, flavonoids, saponins, volatile oil and reducing sugars. However, glycosides were only present in water extracts (AW, PW, SW), meanwhile tannins were detected only in SW. The PW exhibited an increased level of total phenolic content (TPC); 0.0215 ± 0.00060 mg GAE/g whereas, total flavonoid content (TFC) was quantitated at 0.083 ± 0.022 mg QE/g FW (TFC), respectively. Apart from that, the PW extract also exhibited potent antibacterial activity, with MIC values between 5 and 20 mg/ml and MBC values between 10 and 20 mg/ml against *E. coli*, *P. aeruginosa*, *S. flexneri*, and *B. cereus*. Cancer-origin cell lines MCF7, HepG2, A549, HCT116 and HT29 have been discovered to be most susceptible to AW and PW at 72 hours (h) post-treatment. The concentrations ranged between 1 µg/ml and 10 µg/ml of PE and SW extracts showed positive effects in the wound healing experiment.

Dissolved oxygen on xylanase production by *Trichoderma reesei* using *Reutalis trisperma* press cake as an additional substrate

Lieke Riadi, Yuana Elly Agustin, Ruth Chrisnasari, Tjie Kok, Meyta Sanoe

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Dissolved oxygen (DO) level in media plays a big role in the succession of the fermentation process by a microorganism. In this project, *Trichoderma reesei* was cultivated in two types of media, media A and B, both in 250 mL shake flask and 1.5 L fermenter at varying DO level. Media A and B contained the same carbon source and mineral salts, the difference between both media was the presence of press cake in Media B as urea and ammonium sulfate replacement, whereas the nitrogen sources in media A consists of urea and ammonium sulfate. The batch fermentation process was conducted in a 7.8 C/N ratio with an initial pH of 6. The purified enzyme was shown to convert xylan to xylose. Media B that contained *Reutealis trisperma* press cake gives a higher xylanase activity than media A without press cake, for both shake flask and fermenter cultivations. A xylanase activity of 101.07 ± 1.65 U/mL and 39.30 ± 0.64 U/mL was produced in a shake flask under 140 rpm for 96 hours of fermentation with ratio of 0.6 media B/flask volume and 0.6 media A/flask volume, respectively. A xylanase activity of 205.90 ± 0.84 U/mL and 88.87 ± 1.45 U/mL was produced in 72 hours at 0.8 L/min of aeration rate in a 1.5 L fermenter with media A and media B, respectively. Thus, this study identified the *Reutealis trisperma* cake can be used as a beneficial additional substrate and nitrogen sources. The increase in DO level, which indicated by increasing aeration rate, can generate a higher xylanase activity for both media in shake flask and fermenter.

Homeopathic interventions against *Salmonella typhi*: A narrative review

Chitram Umashankar, Bipinraj Nirichan Kunchirman, Chetan H. Shinde

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This review discusses the importance of the antimicrobial action of homoeopathic medicine in the general management of typhoid. The main aim is to provide individualized homoeopathic treatment with symptomatic improvement, minimizing complications, and promoting early recovery. Justifying homoeopathy as preventive and curative in epidemic diseases. To review the effectiveness of homoeopathic medicine against *Salmonella typhi* by agar well diffusion and MIC value methods. Google scholar and PubMed databases were searched for this study to analyse the effects of homoeopathic medicine against *Salmonella typhi*. After scrutiny, shortlisted studies were reviewed for the study. The Google scholar search yielded 41 studies, a bibliography from 13 different sources books, and the Selection of 6 clinical rubrics from Radar 10-Synthesis Repertories after analysis of abstracts of 60 studies, 27 were shortlisted including five *in-vitro* and two *in-vivo* studies. Most of the studies showed the significance of minimum inhibitory concentration (MIC) for homoeopathic medicine exhibited antibacterial potential against the related miniature organisms. The studies regarding antimicrobial action and other mechanisms of activity were heterogeneous. Homoeopathic remedies will allow the vital force immediately to deal with any exposure agents viz. various antigenic variants of salmonella. It appears to actuate resistant framework by initiating both T and B cells by the arrangement of antibodies. Further expansion of experimental studies is needed to know the exact action mechanism of homoeopathic medicines against *Salmonella typhi*.

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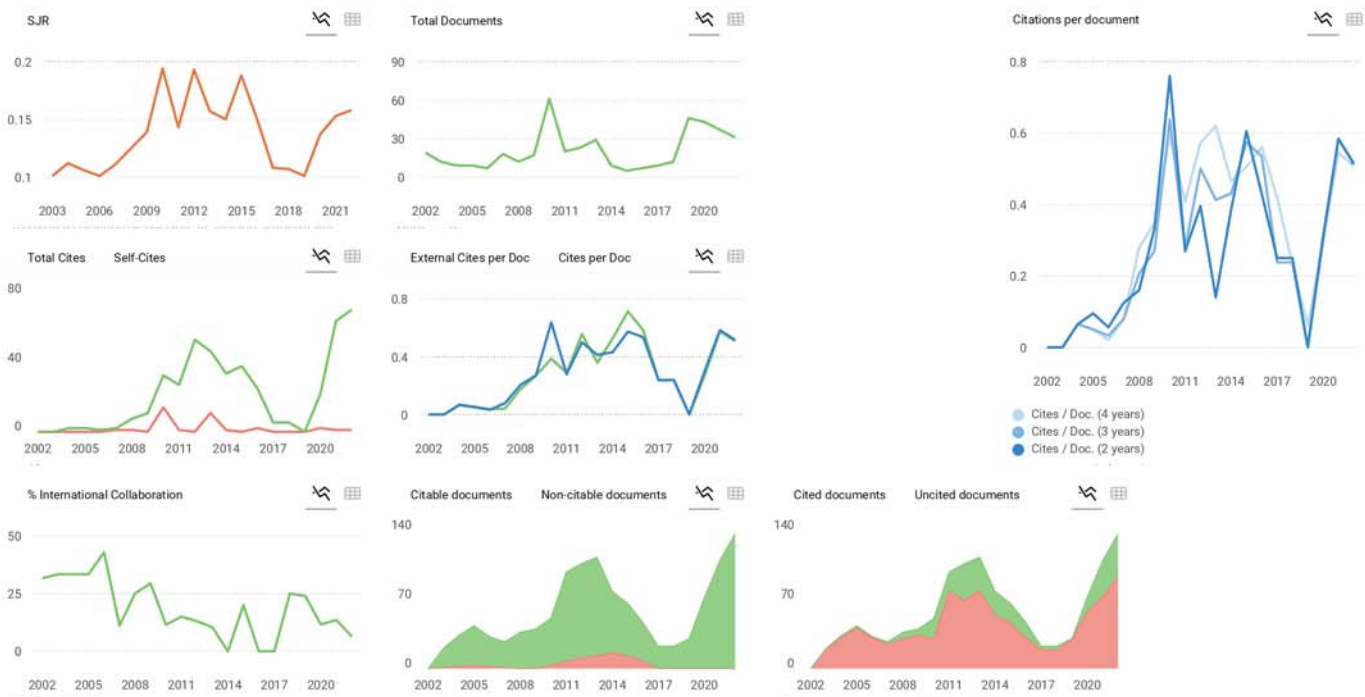
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