Mini Review

Metabolites of *Botryodiplodia theobromae* for therapeutic agent and food industry

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**Abstract**

This paper reviews production of some metabolites by a soil fungus *Botryodiplodia theobromae*, including enzymes, hormones, exopolysaccharides, and paclitaxel/taxol, for therapeutic use, and for food industry. Paclitaxel, an anti-cancer agent, is produced when the fungus grows as an endophytic fungus. *B. theobromae* produces linear and branched exopolysaccharide β-glucan, called lasiodiplodin, which shows various therapeutic activities: anti-tumor, hypoglycemic, anti-coagulant, anti-cancer, while protecting against DNA damage. Lasiodiplodins also show cytotoxicity against murine leukemia cell line. The fungus is known to produce amylase and glucoamylase to degrade starch into sugar for various process in food industry.

**Introduction**

*Botryodiplodia theobromae* is well known as a high virulent plant pathogen in tropic and subtropic area. This soil-borne fungus infects important commodities such as mango (Khanzada et al., 2004; Twumasi et al., 2014), cocoa pod (Twumasi et al., 2014), yam (Twumasi et al., 2014), banana (Twumasi et al., 2014), citrus (Zhao et al., 2015), kenaf seed (Norhayati et al., 2016), rubber (Anh et al., 2012), cashew (Adeniyi, 2013), and mulberry (Xie et al., 2014), leads to significant economic loss. For example, the fungus infected up 52%, and killed 8% of citrus plant, with around USD 3 million lost in a county in China (Xie et al., 2014). It affects around 280 species of plants, in various stages including postharvest time. Its high virulence resulted in cross infection with surrounding plants (Twumasi et al., 2014).

However, *B. theobromae* is utilised in the process of making gathot, a traditional fermented cassava originated from Java, Indonesia (Purwandari, 2000). Although various fungi grow during fermentation, *B. theobromae* is the dominant species growing inside cassava tuber and gives characteristic of black colour of gathot. *B. theobromae* has several synonyms. Some of them are *Botryodiplodia gossippii*, *Lasiodiplodia theobromae*, *L. tubericola*, *L. nigra*, *Diplodia theobromae*, *D. natalensis*, *D. gossypina*, and *Botryosphaeria rhodina* (www.mycobank.org). A noodle made from gathot showed high antioxidant activity which is lacking in cassava (Purwandari et al., 2014).

Besides its pathogenicity, the fungus was reported to produce valuable natural products. This paper reviews some metabolites namely lasiodiplodin, paclitaxel, and amylase produced by *B. theobromae* which can be beneficial in therapeutic use and food industry.

**Paclitaxel**

Paclitaxel has a brand name taxol, is a molecule capable of inhibiting tumor cell proliferation. The name of taxol is seemingly derived from yew tree (*Taxus brevifolia*) where it was isolated for the first time in the 60’s. According to a traditional folk medicine, the substance of the bark can cure tumor (Zwawiak and Zaprutko, 2014). However, due to small concentration of the substance presents in the bark, the production of substance analogs to taxol from microorganism has been explored. A fungus, *Pestalotiopsis* sp., was successfully employed to produce taxol. The fungus was isolated from several medicinal plants: *Eucalyptus perriniana*, *Marchantia polymorpha*, *Rauwolfia serpentine*, *Nicotiana tabacum*, Glycine max, and *Jatropha curcas* (Zwawiak and Zaprutko, 2014).

Taxol is an important drug for treatment of...
ovarian cancer, breast cancer, non-small cell lung cancer, and AIDS related Kaposi’s sarcoma (Cancer Research UK, 2014). It is usually used in combination with other chemotherapy. There are several methods have been studied to improve the efficacy of taxol. Attaching nano particle of taxol with albumin is one of the methods, since albumin is a carrier of nutrition for tumor cells. As albumin absorb by the tumor cells, taxol attached to it is easily reach the target cells and inhibit its mitosis (Cancer Research UK, 2014). Combination of taxol and albumin is called abraxane or nab-paclitaxel which is designated to reduce side effects of taxol. Other method of improving the drug efficacy is by altering molecular structure. There are several alterations of the molecule to increase its activity against tumordevision, including replacement of phenyl, acetate, or acyloxy groups, and derivatisation of hydroxyl group (Zwawiak and Zaputko, 2014). Method of drug administration also played important role, with low dose and frequent weekly administration gave more effect and less allergic reaction, than high dose with two weeks interval (Pandi et al., 2011; Mulcahy 2013).

Taxol was produced by endophytic B. theobromae. Endophytes are fungi grow in mutualistic way inside medicinal plants without showing any disease symptoms (Schulz et al., 2002). Endophytic fungi produced exoenzymes to colonize the host plant, while utilizing apoplastic fluid of plant for their growth. On the other hand, host plant showed improved growth in the presence of endophytic fungi, presumably due to higher availability of minerals (Schulz et al., 2002). Endophytes demonstrated strong correlation with biological activity of host plants, and there was apparently a balance between fungal virulence and plant defence (Schulz et al., 2002). Due to its interaction with host and niche, endophytic fungi produced higher concentration of secondary metabolites than do pathogen or soil fungi (Schulz et al., 2002). Therefore, endophytic fungi are important in the production of natural products such as paclitaxel.

Botryodiplodia theobromae endophytic to some plants, showed significant production of taxol. The fungus isolated from Morinda citrifolia (Pandi et al., 2011), Bidens pilosa (Abdou et al., 2010), and Salacia oblonga (Roopa et al., 2015), were among those tested for production of taxol. Taxol producing B. theobromae was isolated from various species and different parts of plant, such as leaf of Morinda citrifolia (Pandi et al., 2011), fresh aerial part of Bidens pilosa (Abdou et al., 2010), Taxus baccata (Venkatachalam et al., 2008). Nevertheless, B. theobromae does not seem to be extensively explored for its taxol production, despite it infects broad types of plant species.

The taxol showed inhibition of proliferation of breast cancer cells. Giving the fungal taxolintraperitoneally at 7 mg/kg in 1 mL saline weekly for four weeks period increased some essential antioxidative enzymes in tumor-induced rats (Pandi et al., 2010). Concentration of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in serum or tissue of diseased rats treated with fungal taxol was also higher than those without taxol treatment. Meanwhile, concentration of non-enzymic antioxidants in the form of vitamin C, E, and glutathione (GSH) also increased in breast tissue of cancer-induced rats treated with fungal taxol. There was higher concentration of lipid peroxidase in breast tissue with fungal taxol treatment, while concentration of cyclooxygenase (COX) was lower, and cell proliferation in breast tissue was inhibited. The anti-cancer activity shown by fungal taxol was not different from that shown by commercial taxol at 1mg/kg concentration with the same frequency of treatment.

Several other biochemical parameters for anticancer activity, such as aspartate amino transaminase, alanine amino transaminase, and alkaline phosphatase reduced following B. theobromaeataxol treatment (Pandi et al., 2011). Decrease in expression of matrix metalloproteinase and proliferating cell nuclear antigen (PCNA) also happened in breast cancer bearing animals treated with fungal taxol. Taxol compounds called Botryorhodine A, and Botryorhodine B exhibited inhibition of breast cancer cell proliferation by more than 50%, at concentration as low as 0.1 µg/mL level (Abdou et al., 2010). The metabolites also showed toxicity to HeLa cells at level close to 50%. Whilst, when applied to MCF-7, a human breast cancer cellline, B. theobromaeataxol reduced cell viability up to 80% at concentration of 600 µg/mL (Pandi et al., 2011).

Taxol production by B. theobromae is still low. The quantity of taxol produced by the fungus was around 280 µg/L medium (Venkatachalam et al., 2008; Gond et al., 2014), while the maximum taxol production by endophytic fungi isolated from Taxus brevifolia was over 800 µg/L (Gond et al., 2014). Cladosporium cladosporioides produced 800 µg/L, and Metarhizium anisopliae yielded 846 µg/L (Gond et al., 2014). There were several media used for taxol production by B. theobromae, including M1D medium (Venkatachalam et al., 2008; Pandi et al., 2010; Pandi et al., 2011), malt extract, potato dextrose, casein-flesh-peptone, or corn steep-dextrose-yeast medium (Abdou et al., 2010). Fermentation was carried out
in 21 (Abdou et al., 2010), or 22 days (Pandi et al., 2010) at 23°C (Abdou et al., 2010).

Taxol is also an antifungal agent. Antimicrobial effect of taxol from *B. theobroma* (synonym of *Botrysospheria rhodina*) was shown against some pathogens (Abdou et al., 2010). There were four types of taxol produced by the fungus, called Botryorhodidine A, B, C, and D. Only Botryorhodidine A and B exhibited inhibition of growth of *A. terreus*, *F. oxysporum*, and *Bacillus subtilis*. Botryorhodidine C was the major product, but showed no antifungal effect. Similarly, Botryorhodidine D also did not have antifungal activity. Fermentation method affects antifungal properties, with stationary cultures produced taxol of better anti-fungal effect than that produced by shaken cultures (Abdou et al., 2010).

Lasiodiplodin

Lasiodiplodins, also called lasiodiplodans, are metabolites produced extra cellulary as exopolysaccharides by *B. theobroma*. The study about lasiodiplodins was reportedly started back in 1998 when three lasiodiplodins were isolated from *Lasiodiplodia theobroma*IFO 31059 (Matsuura et al., 1998). They consist of glucose monomer with β-(1→3)-D-linkage or β-(1→6)-D-linkage. Depending on type of isolate, the linear main chain of lasiodiplodin can have (1→3)-linkage at O-6 or (1→6)-linkage (Vasconcelos et al., 2008). The exopolysaccharide had triple helix conformation (Vasconcelos et al., 2008). Production of exocellular polysaccharide owing β-(1→6)-D-glucans by fungi is rare.

Growth medium influenced type of branch of lasiodiplodins produced. Lasiodiplodins produced in glucose medium on a submerged fermentation, yielded up to 2.2 g/L (Oliveira et al., 2015). Glucose was the only building block of the polysaccharide. Most of the lasiodiplodins (67%) was branched (1→3) (1→6)-β-glucans, which were insoluble in water. The linear lasiodiplodins have (1→6)-linkage, with two distinct molecular weight of 1.8 x 10⁶ Da and 7.0 x 10⁷ Da. Whengrown in a fructose containing medium, more branched lasiodiplodins were produced, than those produced in glucose medium (Queiroz et al., 2015).

β-glucan is related to antioxidant activity which was affected by molecular structure and water solubility. Lasiodiplodinof branched structure i.e. with (1→3; 1→6)-β-D-glucan showed high antioxidant effect, up to 80% total antioxidant activity (Giese et al., 2015). Linear lasiodiplodin, on the other hand, showed very limited antioxidant activity (Giese et al., 2015). Some works had been done to improve its antioxidant activity by increasing its water solubility. Carboxymethylation of lasiodiplodin increased its water solubility, and consequently, improved antioxidant activity (Kagimura et al., 2015). There was a positive correlation between molecular size and antioxidant activity, as the result of carboxymethylation of lasiodiplodin (Kagimura et al., 2015). However, there was only slight increase in antioxidant activity as shown by 36.28% ABTS radical removal, while DPPH scavenging activity was also negligible (Kagimura et al., 2015).

Exopolysaccharides of *B. theobroma* showed tumor inhibition activity. The activity is due to high antioxidant level, and was affected by branch type, growth medium, as well as dosage and duration of exposure. Linear (1→6)-β-D-glucan and branched (1→3; 1→6)-β-D-glucan showed inhibition of breast cancer cell proliferation (Quiroz et al., 2015). Branched lasiodiplodan produced from fructose medium had higher degree of branching and higher activity in reducing cell apoptosis and necrosis, while that produced in glucose medium showed lower activity. Direct effect of lasiodiplodan on cell death was demonstrated, while the indirect effect was shown by increasing oxidative stress of tumor cells, resulted in increasing tumorsuppressor and apoptotic genes (Quiroz et al., 2015). The action of anti-proliferation was shown in time and dose dependent manner, with 1500 µg/mL as the most effective concentration. The longer exposure to lasiodiplodin, the more effective inhibition of cell proliferation.

Pro-inflammatory response was also induced by lasiodiplodins especially those highly branched with (1→3)-linkage chain (Oliveira et al., 2015). Lasiodiplodin containing main chain with (1→6)-linkage did not induce anti-inflammatory response such as IL and TNF-α. Molecular weight, degree of branching, and water solubility positively correlated with the response. Moreover, it also prevented DNA damage induced by doxorubicin, and reduced inflammatory response (Mello et al., 2016).

Unlike endophytic fungi which are expected to produce health supporting substance(s), *B. theobroma* is a plant pathogen, thus it was not expected to produce such substances. Therefore, research on biosynthesis of lasiodiplodin by *B. theobroma*seems lacking. However, there was a detail mechanism of EPS production by a plant pathogenic fungus *Aspergillus fumigatus* (Sheppard and Howell, 2016). The EPS was the main component of biofilm produced by the fungus, and categorized as galactosaminogalactan (GAG), a structure different from that of bacterial EPS. It is composed of galactose (Gal) and partially de-acetylated galactose (GalNAc), via several stages...
started from synthesis of amino sugar within the cell, followed by polymerization of the sugars with \(\alpha-1,4\)-linked and subsequently export of the polymer outside cells by transmembrane glycosyltransferase. The glycan polymer was then de-acetylated to form polycationic glycan which assisted attachment to surface of hyphae as well as host and inorganic material such as plastic. GAG also demonstrated prevention of recognition by innate immune receptor of host and resistance to killing by host neutrophil extracellular trap (Sheppard and Howell, 2016). There was no information whether lasiodiplodin was synthesized inside or outside cell. How this infection system of a plant pathogen turns into production of pharmacological substance does not seem to have been studied thoroughly. Nevertheless, EPS with moderate antioxidant effect was produced by a parasitic filamentous fungus *Cordyceps sinensis* (Leung et al., 2009). EPS is not only produced by pathogenic fungi, but also by endophytic species such as *Fusarium oxysporum* isolated from *Dioscorea zingiberensis* (Li et al., 2014).

**Amylase**

Enzymes belong to amylase group have important role in broad range of industries including food processing, fermentation, textile production, pharmacy, brewing, detergent production, bio-ethanol manufacturing, oligosaccharide mixture, and high branched dextrin for powdery food and cakes (Dey et al., 2016).

Broad range of fungi are amylase producers, including plant pathogens, endophytics, and post-harvest spoilage fungi. *Phytophtora* sp. is a plant pathogen produced amylase for carbohydrate degradation of host plant’s cells (Brouwer et al., 2014). These carbohydrate-related enzymes were important for pathogenicity of plant pathogens (Brouwer et al., 2014). Amylase was also produced by spoilage fungi of agricultural commodities such tomato (Brindha et al., 2011) and legumes (Saleem et al., 2014). Moreover, endophytic fungi also capable of excreting amylase in considerable amount (Schulz et al., 2002; Sunitha et al., 2012).

*B. theobromae* produces extracellular amylases (Ogundere 1987; Nwuo and Fajola 1988), as well other enzymes including cellulase,polygalacturonase (Salami and Akintokun, 2008), and pectin methyl esterase (Ogundere, 1987; Salami and Akintokun, 2008). Most microorganisms produces only \(\alpha\) - and \(\beta\)-amylase (Adjuwon, 2011), however *B. theobromae* also produces extracellular glucoamylase (Umezurike, 1971). \(\beta\)-glucosidase was produced by the fungus when grown in cellulbiose medium (Umezurike, 1971). The enzyme hydrolyzes starch by releasing every single molecule of glucose from non-reducing end (Adjuwon, 2011). This type of enzyme is important for ethanol production.

In general, amylase production of *B. theobromae* grown in starch medium was low. Amylase of *B. theobromae* grown in cassava starch residue showed only 3.25 U (mg glucose released/mL.h) (Ray, 2004). The production was increased by addition of inorganic ammonium salt, to reach 4.8 U (Ray, 2004). The fungus grown in cassava medium yielded 280 U/mL glucoamylase at 46 h incubation (Navaratnam et al., 1996). Upon addition of soybean powder, \((NH_4)_2PO_4\), peptone, \(K_2PO_4\), and \(CaCO_3\), glucoamylase production increased to 1950 U/mL (Navaratnam et al., 1996).

*B. theobromae* is capable of producing amylase in raw starch at room temperature and neutral pH, to enable easy control of process. Maximum amylase production by the fungus was at around neutral pH 6 (Ray 2004), or 6-7 (Nwuo and Fajola, 1988), at 25°C (Nwuo and Fajola, 1988) or 30°C (Ray 2004). Enzyme production was positively correlated to starch concentration in the medium (Nwuo and Fajola, 1988), and soluble starch produced less amylase (Salami and Akintokun, 2008). Cassava starch residue was a substrate for amylase production. Complex starch-rich medium such as bread resulted in high amylase activity (Adjuwon, 2011). Contrary, lowest activity of the enzyme was resulted from glucose medium (Adjuwon, 2011). It was highlighted that crude enzyme even showed the highest activity as compared to purified enzyme (Adjuwon, 2011).

**Conclusion**

*B. theobromae* shows great potential to be used in production of anti-microorganism and anti-tumor agent, antioxidant, and amylase. Its production needs to be improved, and its use for therapeutic use for other metabolic disorders such as hyperglycemia and prebiotic is worth exploring.

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**References**


