Production of α-arbutin from Hydroquinone by *Bacillus subtilis* TISTR 1248 and *Xanthomonas campestris* TISTR 2065

Aurasorn Saraphanchotiwitthaya[1,2*], and Pattana Sripalakit[2,3]

[1]*Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok 65000, Thailand*
[2]*Pharmaceutical Biotechnology Research Unit, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok 65000, Thailand*
[3]*Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok 65000, Thailand*

**Corresponding author. E-mail: aurasorns@nu.ac.th*
https://doi.org/10.12982/CMUJNS.2020.0060

Received: *November 13, 2019*
Revised: *March 3, 2020*
Accepted: *April 17, 2020*

ABSTRACT

α-Arbutin has been used in the cosmetic industry as a skin-whitening agent and in pharmaceutical applications. It can be synthesised by a chemical reaction and a microbial enzyme. This investigation aims to study the production of α-arbutin by microbial fermentation, using whole cells of Bacillus subtilis TISTR 1248 or Xanthomonas campestris TISTR 2065 as a biocatalyst for the transglucosylation reaction between hydroquinone and disaccharide (sucrose and maltose). The amount of α-arbutin in the biotransformation medium under different concentrations of hydroquinone, disaccharides and ascorbic acid was analysed by a high-performance liquid chromatography (HPLC) technique. The results showed that X. campestris TISTR 2065 was more effective than B. subtilis TISTR 1248 on α-arbutin production. The production yield obtained from using sucrose or maltose as a donor was not statistically different. The addition of ascorbic acid resulted in a slight increase in α-arbutin production, spending less time to reach the maximum yield. The maximum production of α-arbutin of approximately 366.33 ± 21.13 mg/l (day 12) was achieved from the biotransformation medium containing 5%w/v hydroquinone and 3%w/v sucrose together with 0.2 mM ascorbic acid by X. campestris TISTR 2065. The elucidation of the α-arbutin structure and the biological activity of the biotransformation mixture containing α-arbutin needs further investigation.

Keywords: α-Arbutin, Hydroquinone, *Bacillus subtilis* TISTR 1248, *Xanthomonas campestris* TISTR 2065

INTRODUCTION

α- and β-arbutin are glycosylated hydroquinones that have been used in phytotherapy and the cosmetic industry (Kwiecień et al., 2013). Arbutin has been used for the treatment of urinary tract infection, kidney stones and diuresis (Seo et al., 2012a). The *in vivo* antitussive activity of arbutin from plants was reported (Strapková et al., 1991). Moreover, the anti-inflammatory effects of arbutin in lipopolysaccharide-stimulated BV2 microglial cells has been shown (Lee et al., 2012). Its antifungal and antioxidant activities have also been proven (Azadbakht et al., 2004). In cosmetics, both the compounds exhibited inhibitory activity on tyrosinase, an enzyme involved in melanin synthesis (Akiu et al., 1988); this enzyme catalyses the hydroxylation of tyrosine to 3-(3, 4-dihydroxyphenyl)-alanine (DOPA) and the oxidation of DOPA to dopaquinone (Sugimoto et al., 2005). Therefore, α- and β-arbutin has been wildly used as skin whitening agents (Akiu et al., 1988; Seo et al., 2012b); however, the whitening effect of α-arbutin was more than ten times higher than β-arbutin (Sugimoto et al., 2003). The half maximum inhibitory concentration (IC_{50}) of α-arbutin on human tyrosinase was 2.0 mM which was more potent than natural arbutin at a concentration higher than 30 mM (Sugimoto et al., 2003; Sugimoto et al., 2007). Recently, the demand for α-arbutin has increased significantly in many parts of the world (Liu et al., 2013). β-arbutin can be extracted from various plant species including bearberry, wheat and pear (Liu et al., 2013), it can be produced via the biotransformation of hydroquinone in *in vitro* cultures of numerous plant species (Seo et al., 2012a). α-Arbutin is mainly produced via microbial biotransformation by using whole cells and carbohydrate-active enzymes (Liu et al., 2013). From previous studies, *Bacillus subtilis* X-23 was reported as an effective strain among 600 microbial strains screened from soil for α-arbutin production by glycosylation of hydroquinone and possessed α-amylase activity. Using hydroquinone as a glycosyl acceptor and maltopentaose as the donor reached an α-arbutin production yield of 32.4% (Nishimura et al., 1994). When using *Xanthomonas campestris* WU-9701 cells as biocatalysts and maltose as a glucosyl donor for α-arbutin production, a maximum molar conversion yield of 93% was obtained (Kurosu et al., 2002). Mutant *Xanthomonas maltophilia* BT-112 was screened using various mutation techniques. Under optimised conditions, the maximum α-arbutin yield of 30.6 g/l (93.6%) was obtained from 120 mM hydroquinone (Liu et al., 2013).

In this study, we aimed to investigate the efficiency of two culture collections, *Bacillus subtilis* TISTR 1248 and *Xanthomonas campestris* TISTR