Effect of Exogenous ALA to Increase the Production of Endogenous Porphyrin Staphylococcus aureus Bacteria For Applying Photodynamic Inactivation of Bacteria

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Abstract

Aminolevulinic acid (ALA) is a naturally occurring metabolite in the synthesis pathway of cellular heme production. In bacteria, addition of ALA may induce porphyrin synthesis. ALA induction leads to an increase in the synthesis of uroporphyrin, coproporphyrin and protoporphyrin IX, which are the immediate precursors of heme. In this research, the suspension of Gram positive *Staphylococcus aureus* bacteria ATCC 6538P was added with exogenous ALA (Sigma) in order to increase the production of bacteria endogenous porphyrin, with varying concentration (0, 1.5 mM, 2.5 mM, 3.5 mM, and 5 mM). Bacteria suspension was incubated in the dark at 37°C for 3 h for induction and production of endogenous porphyrin. Each extract, containing the produced endogenous porphyrins, characterized by fluorescence, UV-Visible spectroscopy and HPLC using reverse phase chromatography. Porphyrin production was demonstrated by the fluorescence emission peaks that appeared in the extracts of the ALA treated bacteria. The result showed that the excitation of the *Staphylococcus aureus* endogenous porphyrin at 405 nm yielded a fluorescence peak at 623 nm. The increasing of bacteria porphyrin absorbance comparable with increasing of ALA concentration. A significantly absorbance increased in the incubation of 3.5 mM ALA. The HPLC analyzed showed that the amount of porphyrin produced increased comparable with incubation of ALA concentration. A significantly endogenous porphyrin production increased in the incubation of 3.5 mM ALA. So the incubation of 3.5 mM ALA concentration significantly increased the bacteria endogenous porhyrin production and the absorption of porphyrin for applying the effectiveness photoinactivation of pathogen bacteria.

Keywords: Staphylococcus aureus, exogenous ALA, endogenous porphyrin production and absorption

1 Introduction

Staphylococcus aureus is a Gram-positive spherical and aerobic facultative bacteria which grows with aerobic respiration or fermentation that yields principally lactic acid. These bacteria are linked by lesion to the acne skin condition and causing skin infection [21]. Hospital strains of *Staphylococus aureus* are usually resistant to a variety of different antibiotics, a few strains are resistant to all clinically useful antibiotics [6]. Since bacteria resistance to antibiotics is becoming an increasing problem, this research is being directed towards photodynamic therapy as an alternative methods for killing bacteria [10]. Photoinactivation of various Gram positive bacteria, including *Staphylococcus aureus* has been demonstrated using exogenous photosensitizer [16].

Staphylococcus aureus glow when exposed to Wood's light, broad-band near UV-light (in this study by dyes laser exposure), be due bacteria produce several endogenous porphyrins [15; 7]. The porphyrins make the bacteria light-sensitive. It has also been shown that when illuminated with blue light, porphyrins damage the cells very efficiently [1; 5]. The emission peak of the best