UNIVERSITI PUTRA MALAYSIA

ISOLATION, IDENTIFICATION AND CHARACTERISATION OF SULPHUR-OXIDISING BACTERIA ISOLATED FROM HOT SPRING FOR REDUCTION OF HYDROGEN SULPHIDE IN CHICKEN MANURE

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By

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The rapid development of poultry industry has led to the production of large amounts of manure which contributed to odour pollution such as hydrogen sulphide (H_2S). Hydrogen sulphide is widely known as the most undesirable gas component and therefore, H_2S removal from the environment is necessary. Generally, chemical and physical methods have been used for the removal of H_2S, however, this method is costly and often results in secondary pollution. Recently, the biological method has drawn so much attention due to its efficiency, low-cost method, and environmentally friendly. It has been widely known that sulphur oxidising bacteria (SOB) can be used to remove contaminating H_2S due to its ability to oxidise the reduced sulphur compounds. Thus, this study was conducted to isolate, characterise and identify a potential SOB from hot spring in Malaysia in reducing the H_2S from chicken manure. Three potential SOB has been isolated in this work which namely as isolate AH18, AH25, and AH28. Isolate AH18 was identified as *Pseudomonas* sp. meanwhile isolate AH25 and AH28 was identified as *Achromobacter* sp. based on 16S rRNA phylogenetic analysis. The optimum pH for growth of all the isolates occurred at pH 8.0. Moreover, the optimum temperature for isolate AH18, AH25 and AH28 occurred at 45 °C, 30 °C and 30-45 °C respectively. The three isolates were classified as facultative chemolithotroph with the capability of growth at thiosulphate concentration as high as 100 mM. The pure culture and the mixed culture of the isolates were immobilised on perlite and alginate for cell immobilisation to test their H_2S removal performance in chicken manure. The laboratory-scale experiments revealed that the most active isolate was AH18 with a reduction rate of 67.3% and 74.7% when carried on perlite and alginate respectively. Meanwhile, the reduction rate for isolate AH25 was 59% and 54.2% when carried on perlite and alginate respectively, and for isolate AH28 was 63.2% and 60.8% when carried on perlite and alginate respectively. However, the removal performance of H_2S was enhanced in mixed culture with 69.6% and 81.9% of reduction rate carried on perlite and alginate respectively. Additionally, based on the results obtained, the reduction rate of H_2S in chicken manure was observed higher when the potential SOB and the mixed culture were carried on alginate than on perlite. In conclusion, three potential SOB isolates were successfully isolated from hot spring in Malaysia with their ability in reducing the H_2S from chicken manure in the form of pure culture and

mixed culture. Moreover, based on the results obtained, these potential SOB isolates could be a potent candidate for biological deodorisation due to their pH, temperature adaptability, metabolic flexibility and H_2S removal efficiency in chicken manure. In addition, to achieve the higher H_2S removal ability, the mixed culture carried on alginate could be the best alternative for H_2S deodorisation application.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia Sebagai memenuhi keperluan untuk Ijazah Master Sains

PEMENCILAN, PENGENALPASTIAN DAN PENCIRIAN BAKTERIA PENGOKSIDASI SULFUR DARI KOLAM MATA AIR PANAS UNTUK PENGURANGAN HIDROGEN SULFIDA DALAM TAHI AYAM

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Perkembangan pesat industri ternakan ayam telah membawa kepada pengeluaran sejumlah besar tahi ayam yang menyumbang kepada pencemaran bau seperti hidrogen sulfida (H_2S). Hidrogen sulphide secara meluasnya dikenali sebagai komponen gas yang paling tidak diingini dan oleh itu, penyingkiran H_2S di persekitaran adalah perlu. Secara umumnya, kaedah fizikal dan kimia telah digunakan untuk penyingkiran H_2S, walau bagaimanapun, kos kaedah ini adalah tinggi dan sering menyebabkan pencemaran sekunder. Baru-baru ini, kaedah biologi telah menarik begitu banyak perhatian kerana kecekapannya, kos yang rendah, dan mesra alam sekitar. Ia telah diketahui bahawa bakteria pengoksidasi sulfur (SOB) boleh digunakan untuk menyingkirkan H_2S kerana kemampuannya untuk mengoksidakan sebatian sulphur. Oleh itu, kajian ini dijalankan untuk memencilkan, mencirikan dan mengenal pasti SOB berpotensi dari kolam mata air panas di Malaysia untuk tujuan mengurangkan H_2S daripada tahi ayam. Tiga SOB berpotensi telah dipencilkan dalam kerja ini iaitu dinamakan sebagai AH18, AH25, dan AH28. Pencilan AH18 telah dikenal tpasti sebagai *Pseudomonas* sp. sementara itu pencilan AH25 dan AH28 telah dikenal pasti sebagai *Achromobacter* sp. berdasarkan analisis filogenetik 16S rRNA. Optimum pH untuk pertumbuhan pada semua pencilan berlaku pada pH 8.0. Tambahan lagi, suhu optimum untuk pencilan AH18, AH25 dan AH28 masing-masing berlaku pada 45 ° C, 30 ° C dan 30-45 ° C. Tiga pencilan ini diklasifikasikan sebagai chemolithotroph fakultatif dengan kemampuan pertumbuhan pada kepekatan tiosulfat setinggi 100 mM. Kultur tulen dan campuran kultur tulen telah disekat-gerakan dalam perlite dan alginate untuk tujuan penyekat-gerakan sel dalam menguji prestasi penyingkiran H_2S dari tahi ayam. Eksperimen berskala makmal telah mendedahkan bahawa pencilan yang paling aktif adalah AH18 dengan kadar pengurangan H_2S sebanyak 67.3% dan 74.7% apabila masing-masing disekat-gerakan dalam perlite dan alginate. Sementara itu, kadar pengurangan H_2S untuk pencilan AH25 adalah 59% dan 54.2% apabila masing-masing disekat-gerakan dalam perlite dan alginate, dan untuk pencilan AH28 adalah 63.2% dan 60.8% apabila masing-masing disekat-gerakan oleh perlite dan alginate. Walau bagaimanapun, prestasi penyingkiran H_2S telah dipertingkatkan dalam campuran kultur tulen dengan kadar pengurangan sebanyak 69.6% dan 81.9% apabila masing-masing disekat-gerakan dalam perlite dan alginate. Secara umumnya, berdasarkan keputusan

yang diperolehi, kadar pengurangan H_2S dalam tahi ayam diperhatikan lebih tinggi apabila SOB berpotensi dan campuran kultur tulen disekat-gerakan dalam alginate berbanding dengan perlite. Kesimpulannya, tiga pencilan SOB telah berjaya diasingkan daripada kolam mata air panas di Malaysia dengan keupayaan mereka mengurangkan H_2S daripada tahi ayam dalam bentuk kultur tulen dan campuran kultur tulen. Selain itu, berdasarkan keputusan yang diperolehi, pencilan-pencilan SOB ini boleh menjadi calon yang sesuai untuk penyahbauan secara biologi kerana kesesuaian pH, suhu, metabolik yang fleksibil dan kecekapan mereka menyingkirkan H_2S dalam tahi ayam. Di samping itu, untuk mencapai keupayaan penyingkiran H_2S yang lebih tinggi, campuran kultur tulen yang dibawa dalam alginate boleh menjadi alternatif terbaik untuk aplikasi penyahbauan H_2S.

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I certify that a Thesis Examination Committee has met on 20 April 2017 to conduct the final examination of Hidayat Mohd.Yusof on his thesis entitled "Isolation, Identification and Characterisation of Sulphur-Oxidising Bacteria Isolated from Hot Spring for Reduction of Hydrogen Sulphide in Chicken Manure" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student is awarded the Master of Science degree.

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LIST OF ABBREVIATIONS

%	Percent
mg/mL	Milligram per liter
kg	Kilogram
g	Gram
mL	Milliliter
µL	Microlitter
nm	Nanometer
MW	Molecular weight
min	Minutes
EDTA	Ethylenediamine tetraacetic acid
M	Molar
°C	Degree Celcius
PCR	Polymerase Chain Reaction
dNTP	Deoxyribonucleotide triphosphate
DNA	Deoxyribonucleic acid
rpm	Revolution per minute
ppm	Part per million
bp	Base pair
kb	Kilobases
w/v	Weight per volume
v/v	Volume per volume
spp.	Species
SE	Standard Error
H_2S	Hydrogen sulphide
H_2O_2	Hydrogen peroxide
NaOH	Sodium hydroxide
HCl	Hydrochloric acid
$MgCl_2$	Magnesium chloride
$CaCl_2$	Calcium chloride
K_2HPO_4	Dipotassium hydrogen phosphate
KH_2PO_4	Potassium dihydrogen phosphate
NaCl	Sodium chloride
$MgSO_4$	Magnesium sulphate
NH_4Cl	Ammonium chloride
$MgCl_2.6H_20$	Magnesium Chloride Hexahydrate
$CaCl_2.2H_2O$	Calcium Chloride Dihydrate
$Na_2S_2O_3 \cdot 5H_2O$	Sodium thiosulphate
K_2SO_4	Potassium sulphate
P	Probability value
SOB	Sulphur-Oxidising Bacteria
SRB	Sulphur-Reducing Bacteria
TSM	Thiosulphate mineral medium
OD	Optical density
CFU	Colony forming unit
<	Less than
>	More than

CHAPTER 1

INTRODUCTION

One of Malaysia's National Agro-food Policy, 2011-2020 (Ministry of Agriculture and Agro-based Industry Malaysia) goals is to ensure an adequate domestic supply of egg and poultry. The poultry sector has become an integral part of the livestock industry in Malaysia. According to Federation of Livestock Farmer's Association of Malaysia, there are currently over 3000 broiler farms in Peninsular Malaysia producing about 491 million of birds. Besides the supplementation of protein (meat and eggs) to the people, this industry also helps the country by employment, income generation and supplying nutrient source for maintaining proper health. Although poultry industry plays a key role in strengthening our socio-economic, it might be a great threat to our environment. Tonnes of poultry manure produces daily could become hazardous to the environment and causing detrimental to the health and safety of both humans and animals.

Poultry farm emits a large number of odorous gasses such as ammonia, carbon monoxide, carbon dioxide, methane, hydrogen sulphide, dimethylamine, mercaptans, and phenolic compounds (Burgess *et al.,* 2001) which derived from the manure. However, of all of the manure gasses, hydrogen sulphide (H_2S) is known as the most toxic and dangerous gasses. In the sulphur cycle, nature balances the inorganic sulphur oxidations through the biological reduction of sulphate to sulphide (Kleinjan, 2005) and the reduction process is a synonym to the H_2S production or sulfidogenesis by sulphate-reducing bacteria (Kelly and Wood, 2006). On the other hand, H_2S is also produced during the anaerobic decomposition of manure. It can be identified by its characteristics smells of rotten eggs causing community problems by creating an unpleasant condition of working and living ambient. This situation has created never-ending conflicts between the poultry farm and the surrounding residential area. Apart from the offensive odours, it is highly undesirable in the environment because of acute neurotoxicity towards human and animals and also corrosive to metallic infrastructure. Therefore, removal or reduction of H_2S, especially in the chicken manure, is necessary.

The removal of H_2S can be done through physicochemical methods including allowing it to oxidise in the air with the presence of several catalysts such as potassium permanganate ($KMnO_4$), ferric ion (Fe^{3+}) and active coal. Apart from that, the detrimental activities of sulphur reducing bacteria (SRB) can be controlled by the effective use of oxidising biocides such as chlorine (Cl_2), hydrogen peroxide (H_2O_2) and sodium hypochlorite (NaClO) (Oprime *et al.,* 2001) which could prevent SRB from producing H_2S in the surface facilities. However, processes based on such agents are expensive due to the high cost involved in the facility installation, as well as the operational cost due to higher energy demand and toxic chemical usage that have a greater tendency to generate secondary pollution. (Dehghanzadeh *et al.,* 2011).

On the other hand, the biological treatment of reduced sulphur compounds such as sulphide and H_2S by using microorganisms has drawn much attention. Biological treatment works on the principle which microorganisms such as bacteria act as a

catalyst for the conversion of volatile pollutants into a less harmful form. The bacteria which involved in the aid of degradation reduced sulphur compound are known as sulphur-oxidising bacteria (SOB). The elimination of H_2S by SOB is due to the fact that this compound can serve as an energy source and/or a carbon source for bacterial metabolism and thus, the harmful gasses can be removed. Additionally, biological treatment is believed to be cost-effective with higher removal efficiency and environmentally friendly (Mohapatra *et al.,* 2007). The application of SOB to remove harmful H_2S and others reduced sulphur compounds may potentially utilise for the industrial processes and wastewater treatment. Many studies focused on mitigation of sulphides from effluent streams, landfills, wastewater facilities and also oil-field brine. A few studies also have been done to reduce the offensive odours from farm animal faeces using bacteria isolated from the animal faeces.

Sulphur oxidising bacteria can be found in a variety of environments including soil, water, and geothermal area. Hot spring is believed to have a high amount of saturated sulphur and various reduced sulphur compounds in the water, in which can be used as electron donors for microbial growth (Nakagawa and Fukui, 2003). Additionally, microorganisms that live in hot spring have minimal requirements for nutrients and their metabolic activities only depend on the biogeochemical cycle such as sulphur and other mineral contents (Skirnisdottir *et al.,* 2000). To our knowledge, the study using sulphur oxidising bacteria isolated from hot spring in order to reduce the level of H_2S in poultry manure is not available publicly.

Studies on odour pollution especially H_2S gas have become a serious concern especially among the developed country such as EU, and Australia. It involved various aspects including the impact of pollution on health, control and mitigation and odour management (Casey *et al.,* 2006). In Malaysia, the studies on odour pollution are still new and at an infancy stage which only involved few studies focusing on issues and challenges of odour pollution (Othman *et al.,* 2008). Additionally, ammonia emitted from livestock farming has been more thoroughly studied than H_2S.
Therefore, the general objective of this research was to isolate, characterise, identify and evaluate the performances of potential SOB isolated from the hot springs in Malaysia in reducing the H_2S gas from chicken manure. Based on this main goal, the specific objectives of this study were:

i. to isolate, characterise and identify the potential SOB from hot springs in Malaysia.
ii. to evaluate the performance of isolated potential SOB carried on perlite and alginate for reduction of H_2S gas in chicken manure.

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LIST OF PUBLICATIONS

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Hidayat, M. Y., Saud, M. H., and Samsudin, **A. A.** (2017). Isolation and characterisation of sulphur oxidizing bacteria isolated from hot spring in Malaysia for biological deodorisation of hydrogen sulphide in chicken manure. *Media Peternakan* 40(3): 178-187.

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Hidayat, M. Y., Saud, M. H., and Samsudin, A. A**.** (2018). Reduction of hydrogen sulphide in chicken manure by cells immobilisation of sulphur oxidising bacteria isolated from hot spring. *Microbiology and Biotechnology Letters.*