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細菌血紅素工技

吳誌明
中國文化大學化工系
台北市 111 士林區華岡路 55 號

摘　要

蛋白質帶有血質（heme）且能與氧分子行可逆結合者，稱之爲血紅素。血紅素廣泛地分佈於細菌、原生生物、真菌、植物與動物中。第一個被發現的細菌性血紅素爲 *Vitreoscilla* hemoglobin（VHb），當好氧性細菌 *Vitreoscilla* 處於極低的溶氧環境下時，它本身會合成出 VHb，幫助自己適應氧氣不足的環境而能存活，並且能夠繼續地生長。一般而言，在進行微生物高密度細胞發酵培養時，經常會發生這種氧氣不足的情形。因此，基於 VHb 這種特性，研究人員利用基因工程技術，將 VHb 於各種宿主細胞中表現，其結果通常會加快細胞生長速率，增加細胞密度，提高蛋白質與代謝物產量，加快氧化代謝能力，提高生物降解能力等。這些 VHb 的效應與應用，我們稱之爲細菌血紅素工技（VHb technology）。本文將就 VHb 的特性、目前的應用與作用機制等作介紹。

關鍵詞：細菌血紅素

VHb Technology

JYH-MING WU
Department of Chemical Engineering, Chinese Culture University
55 Hwa Kang Rd., Taipei, Taiwan

ABSTRACT

Hemoglobin, defined as heme proteins that bind oxygen reversibly, is widely distributed in nature, being found in all groups of organisms including prokaryotes, protozoa, fungi, plants and animals. The first bacterial hemoglobin to be discovered was *Vitreoscilla* hemoglobin (VHb). When the obligate aerobic, gram-negative bacterium *Vitreoscilla* is in an oxygen-limited environment, it synthesizes elevated quantities of VHb to adapt itself to the hypoxic condition and grow continuously. Oxygen limitation is a typical problem in large-scale high-density cultivations of cells. On the basis of the characteristics of VHb, researchers therefore use genetic-engineering technology to express VHb in various host cells. Examples of the beneficial effects of VHb technology include increasing the specific growth rate, final cell density, production of proteins and metabolites, oxidative metabolism, and bioremediation of toxic compounds. In this article, the characterization, current applications, and mechanisms of VHb will be introduced.

Key Words*: *Vitreoscilla hemoglobin (VHb), VHb technology

一、前言

不同來源的血紅素，包括微生物、植物或動物等，胺基酸的序列呈現著相關性，如圖 1 所示，它們具有一定的相似程度，而從演化的觀點來看，高等生物的血紅素包括我們人類血紅素在內，都是從細菌血紅素演化而來。在哺乳動物中，肌血紅素與血紅素的功能是儲存與傳送氧氣，但其它生物血紅素的功能與調控機制仍然不清楚。血紅素能與氧結合，是一種氧氣感應器，由於演化的結果，造成各種生物血紅素的氧氣感應與調控機制呈現多樣性，如表 1 所示，在不同的生物中有著完全不一樣的性質，有些血紅素是在環境氧氣不足時才會表現出來，有些則是在環境氧氣濃度太高時才表現 [20]。

二、細菌血紅素及其特性

蛋白質帶有血質（heme）而且能與氧行可逆性結合者，稱爲血紅素。在高等生物中，肌血紅素與血紅素具有儲存與傳送氧氣的功能，而血紅素一般認爲只存在真核生物中，直到 1986 年 Wakabayashi 等人才發現在原核生物 *Vitreoscilla* 細菌中也有血紅素的存在 [54]，而此後陸陸續續地也在各種微生物中找到了血紅素與黃素血紅素（flavohemoglobin），這代表著在微生物的世界中類血紅素蛋白質（hemoglobin-like protein）普遍存在著 [1, 23]。*Vitreoscilla* hemoglobin 早期被稱爲可溶性細胞色素 *o*（soluble cytochrome *o*），因爲它除了具有可與氧行可逆結合的特性外，而且其基因序列與 leghemoglobin 的基因序列相似 [54]，因此被判定成爲第一個細菌血紅素。血紅素廣泛地存在於細菌、原生生物、真菌、高等植物與動物中，血紅素是由一條多胜肽鏈的球蛋白與血質所組成，其中血質是由 Fe^{++} 與吡咯紫質 IX（protoporphyrin IX）所構成的一個平面複合物，如圖 2 所示。

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                  A helix---------->B helix---------->C helix >          D helix>E helix---   ------------>
      1             15 16            30 31           45 46            60 61            75 76            90
                                                        P      F                        'H'
humhbb -------VHLTPEEK SAVTALWGKVN--VD EVGGEALGRLLVVYP WTQRFFESFGDLSTP DAVMGNPK----VKA HGKKVLGAFSDGLAH 77
humhbg -------GHFTEEDK ATITSLWFKVN--VE DAGGETLGRLLVVYP WTNRFFDSFGNLSSA SAIMGNPK----VLA HGKKVLTSLGDAIKH 77
humhba --------VLSPADK TNVKAAWGKVGAHAG EYGAEALERMFLSFP TTKTYFPHF------ DLSHGSAQ----VKG HGKKVADALTNAVAH 72
humhbz --------SLTKTER TIIVSMWAKISTQAD TIGTETLERLFLSHP QTKTYFPHF------ DLHPGSAQ----LRA HGSKVVAAVGDAVKS 72
soyhbn --TTTLERGFSEEQE ALVVKSWNVMKKNSG ELGLKFFLKIFEIAP SAQKLFSFL------ RDSTVPLEQNPKLKP HAVSVFVMTCDSAVQ 82
parhbn MSSSEVNKVFTEEQE ALVVKAWAVMKKNSA ELGLQFFLKIFEIAP SAKNLFSYL------ KDSPVPLEQNPKLKP HATTVFVMTCESAVQ 84
soylbc -------GAFTEKQE ALVSSSFEAFKANIP QYSVVFYNSILEKAP AAKDLFSFL------ ANGVDPTN--PKLTG HAEKLFALVRDSAGQ 75
pealbI --------GFTDKQE ALVNSSSE-FKQNLP GYSILFYTIVLEKAP AAKGLFSFL------ KDTAGVEDS-PKLQA HAEQVFGLVRDSAAQ 74
ytflHb --------MLAEKTR SIIKATVPVLEQQGT VITRTFYKNMLTEHT ELLNIFNRT------ NQKVGAQP----N-A LATTVLAAAKNIDDL 71
bacfhb --------MLDNKTI EIIKSTVPVLQQHGE TITGRFYDRMFQDHP ELLNIFNQT------ NQKKKTQR----T-A LANAVIAAAANIDQL 71
vitrhb --------MLDQQTI NIIKATVPVLKEHGV TITTTFYKNLFAKHP EVRPLFDMG------ RQESLEQP----K-A LAMTVLAAAQNIENL 71
alcfhb --------MLTQKTK DIVKATAPVLAEHGY DIIKCFYQRMFEAHP ELKNVFNMA------ HQEQGQQQ----Q-A LARAVYAYAENIEDP 71
ascehb -----SANKTRELCM KSLEHAKVDTSNEAR QDGIDLYKHMFENYP PLRKYFKNR-----E EYTAEDVQNDPFFAK QGQKILLACHVLCAT 80
ptnohb --AIASASKTRELCM KSLEHAKVGTSKEAK QDGIDLYKHMFEHYP AMKKYFKHR-----E NYTPADVQKDPFFIK QGQNILLACHVLCAT 83
caehbl -----NRQEISDLCV KSLEGRMVGTEAQNI ENGNAFYRYFFTNFP DLRVYFKGA-----E KYTADDVKKSERFDK QGQRILLACHLLANV 80
tetrhb ----------MNKPQ TIYEKLGG---ENAM KAAVPLFYKKVLADE RVKHFFKNT------ --DMDHQT------K QQTDFLTMLLGGPNH 63
chl637 ----------RKCPS SLFAKLGG---REAV EAAVDKFYNKIVADP TVSTYFSNT------ --DMKVQR------S KQFAFLAYALGGASE 63
nosthb -------------MS TLYDNIGG---QPAI EQVVDELHKRIATDS LLAPIFAGT------ --DMAKQR------N HLVAFLGQIFEGPKQ 60
humcyc --------------M GDVEKGKKIFIMKCS QCHTVEKGGKHKTGP NLHGLFGRK------ ---TGQAP------- -GYSYTAANKNKGII 59
            F helix- ------>     G helix------------ >       H helix ------------->
      91           105 106         120 121         135 136         150 151         165 166      176
                         H                                          'AW'
humhbb LDNLKGTFA------ TLSELHCDKLHVDPE -NFRLLGNVLVCVLA HHFGKE--FTPPVQA AYQKVVAGVANALAH KYH-------- 146
humhbg LDDLKGTFA------ QLSELHCDKLHVDPE -NFKLLGNVLVTVLA IHFGKE--FTPEVQA SWQKMVTGVASALSS RYH-------- 146
humhba VDDMPNALS------ ALSDLHAHKLRVDPV -NFKLLSHCLLVTLA AHLPAE--FTPAVHA SLDKFLASVSTVLTS KYR-------- 141
humhbz IDDIGGALS------ KLSELHAYILRVDPV -NFKLLSHCLLVTLA ARFPAD--FTAEAHA AWDKFLSVVSSVLTE KYR-------- 141
soyhbn LRKAGKVTVRESNLK KLGATHFRTGVANE- -HFEVTKFALLETIK EAVPEM--WSPAMKN AWGEAYDQLVDAIKS EMKPPSS---- 160
parhbn LRKAGKVTVKESDLK RIGAIHFKTGVVNE- -HFEVTRFALLETIK EAVPEM--WSPEMKN AWGVAYDQLVAAIKF EMKPSST---- 162
soylbc LKTNGTVVA----DA ALVSIHAQKAVTDP- -QFVVVKEALLKTIK EAVGGN--WSDELSS AWEVAYDELAAAIKK A---------- 143
pealbI LRTKGEVVLG---NA TLGAIHVQKGVTNP- -HFVVVKEALLQTIK KASGNN--WSEELNT AWEVAYDGLATAIKK AMKTA------ 147
ytflHb SVLMDHVKQ------ -IGHKHRALQIKPE- -HYPIVGEYLLKAIK EVLGDA--ATPEIIN AWGEAYQAIADIFIT VEKK------- 139
bacfhb GNIIPVVKQ------ -IGHKHRSIGIKPE- -HYPIVGKYLLIAIK DVLGDA--ATPDIMQ AWEKAYGVIADAFIG IEKDM------ 140
vitrhb PAILPAVKK------ -IAVKHCQAGVAAA- -HYPIVGQELLGAIK EVLGDA--ATDDILD AWGKAYGVIADVFIQ VEADLYAQAVE 146
alcfhb NSLMAVLKN------ -IANKHASLGVKPE- -QYPIVGEHLLAAIK EVLGNA--ATDDIIS AWAQAYGNLADVLMG MESEL------ 140
ascehb YDDRETFNAYTR--- ELLDRHARDHVHMP- ---PEVWTDFWKLFE EYLGKKTTLDEPTKQ AWHEIGREFAKEINK HGRHA------ 153
ptnohb YDDRETFDAYVG--- ELMARHERDHVKIP- ---NDVWNHFWEHFI EFLGSKTTLDEPTKH AWQEIGKEFSHEISH HGRHS------ 156
caehbl YTNEEVFKGYVR--- ETINRHRIYKMDPA- -----LWMAFFTVFT GYLESVGCLNDQQKA AWMALGKEFNAESQT HLKNS------ 151
tetrhb YKGKNMTEA------ -----HKGMNLQNL- -HFDAIIENLAATLK ELG-----VTDAVIN EAAKVIEHTRKDMLG K---------- 121
chl637 WKGKDMRTA------ -----HKDLVPHLSD VHFQAVARHLSDTLT ELGVPP---ED-ITD AMAVVASTRTEVLNM PQQ-------- 126
nosthb YGGRPMDKT------ -----HAGLNLQQP- -HFDAIAKHLGEAMA VRGVS----AEDTKA ALDRVTNMKGAILNK ---------- 118
humcyc WGEDTLMEY------ -----LENP------ ------KKYIPGTKM IFVGIK---KK---E ERADLIAYLKKATNE ---------- 105
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圖 1. 比較從細菌、原生生物、植物、無脊椎動物、哺乳動物等來源的血紅素胺基酸序列 [20]

表 1. 不同來源血紅素的功能與調控呈現多樣性 [20]

Class	Exemplary genus	Hemoglobin	Regulation	Function (demonatrated and proposed)
Vertebrate	*Homo*	HbA	Hypoxia-induced increase in production of erythropoietin, which stimulates proliferation and differentiation of erythroid precursors, the progeny of which express Hb at a high level	Oxygen transport between tissues
Plant	*Glycine*	Lb	Nodulin-specific increase in transcription	May sequester oxygen away from nitrogenase May transport oxygen to electron transport chain in nodule
Plant	*Glycine*	Nonsymbiotic Hb	Induced by hypoxia?	Intracellular oxygen movement
Alga	*Chlamydomonas*	LI637 Hb	Light-inducible expression in chloroplast	Oxygen bound to LI637 Hb can be reduced. It may serve to accept electrons, sequester oxygen or deliver oxygen inside the organelle
Fungi	*Saccharomyces*	YHB (a flavo-hemoglobin)	Induced by high levels of oxygen or reactive oxygen species, or by blocking electron transport Repressed by hypoxia Induction is mediated by the transcription factors HAP1 and HAP2/3/4	Can transfer electrons from NADPH to heme iron May serve to protect from oxidative stress
Bacteria	*Alcaligenes*	FHP (a flavo-hemoglobin)	Induced anaerobically Promoter contains a potential binding site for NarL and FNR	Proposed electron transfer Possible role in anaerobic metabolism, perhaps gas metabolism during denitrification
Bacteria	*Vitreoscilla*	Hb	Induced by hypoxia Promoter contains binding sites for FNR	Can serve as terminal electron acceptor during respiration May scavenge oxygen

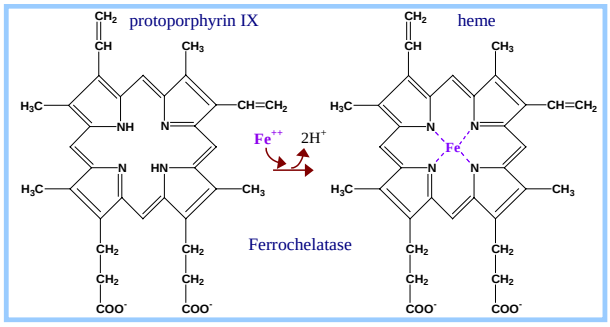

圖 2. Fe^{++} 加入吡咯紫質 IX 而產生血質

微生物血紅素依其蛋白質結構可分爲三類，第一類是只含有一個血質部份（domain），如 *Vitreoscilla* sp. 與 *Nostoc commune* 之血紅素 [48] 。第二類是黃素血紅素，除了血質部份外還有另一黃素（flavin）部份存在，它會與 FAD 結合而具有 NAD(P)H 氧化酶活性，如 *Escherichia coli*、*Erwinia chrysanthemi* 、 *Alcaligenes eutrophus* 、 *Vibrio parahaemolyticus*、*Bacillus subtilis* 之血紅素 [40]。第三類是截短（truncated）血紅素，它通常比一般血紅素短少了 20~40 個胺基酸，其鐵離子具有六個配位基而對氧呈現高親和力 [63]。

然而在這些微生物血紅素中，以 *Vitreoscilla* hemoglobin（*vgb*/VHb）最被廣泛地研究。*Vitreoscilla* sp. 是一株極度好氧、具菌絲性的革蘭氏陰性菌，但卻喜歡生長在氧氣不足（hypoxic）的環境中，例如不流動的池塘與腐爛的蔬菜、植物上。它有一套特殊機制去適應氧氣不足的環境，進而存活與生長，也就是面臨到極低的溶氧環境時，*Vitreoscilla* 細胞會合成出 VHb。

在培養 *Vitreoscilla* 時，當培養液的溶氧濃度降低到微氧程度時，細胞內 VHb 的濃度便會增加兩個級數，達到每克濕菌重約 50 nmol [3]，這種 VHb 大量的表現應是由一個氧氣敏感性啓動子所調控，後來經由報導基因（reporter gene）*CAT* （ chloramphenicol acetyltransferase ） 、 *xyl*E （catechol-2,3-dioxygenase）與 *vgb* 啓動子融合後之轉錄實驗，證實了此一推論 [14, 30] 。此外，FNR（fumarate nitrate reductase regulator）和 CRP（cyclic AMP receptor protein）也會參與 *vgb* 啓動子的調控 [34, 52]。當 *Vitreoscilla* 所處之生長環境從 20% 的溶氧飽和度降到 2-5% 時，*vgb* 啓動子便被高度啓動，呈現最高的誘導表現量，這種在低溶氧啓動了 *vgb* 啓動子表現的情形，在重組 *E. coli* 中亦是如此 [14, 30]。於微氧環境下，細胞內表現出大量的 VHb 被認爲是能

從環境中吸附氧分子，並且幫助傳遞氧氣到細胞膜上之呼吸鏈，讓此極度好氧菌能夠存活。

Vitreoscilla globin gene（*vgb*, 441 bp）[28] 已被分離且定序，在 *Vitreoscilla* 染色體上只有一個樣模數（copy），經基因轉錄與轉譯而產生蛋白質 VHb。VHb 蛋白質有兩個相同的次單元體所組成，每個次單元 15,775 kDa [54]，其結構與動、植物血紅素相似，而其三級結構也被解開。VHb 與一氧化碳的結合是屬於高協和性（high cooperativity），而希爾係數（Hill coefficient）$n_H \approx 2$，然而，VHb 與氧結合之希爾係數卻尚未得知。VHb 含有血質 IX，可與氧可逆地結合，對氧的親和力與 *E. coli* 的細胞色素 *d* 相當 [44]。VHb 與一氧化碳的結合會讓血質還原，吸收圖譜在 Soret 區域，這與細胞色素 *o* 相似，波峰出現在 419nm，而波谷在 437nm [57]，如圖 3 所示。

三、VHb 之應用

基因重組改良後之質體經轉殖作用進入宿主細胞後，此一重組細胞菌株通常會呈現出細胞生長不佳的問題，這生長不佳的原因主要是來自於質體複製與目標蛋白質表現時所需要額外的能量負擔，而使得重組細胞生長地較不好，因此，基因重組細胞菌株對氧氣的需求較高 [6, 33]。此外，在進行好氧性細胞培養時，如何維持培養液中的溶氧濃度在細胞生長與蛋白質表現需求之上，是一個重要的問題，而這個問題在進行高密度細胞培養時更爲重要 [35]。另外，有研究人員利用代謝工程方法嘗試去增進細胞的生長與蛋白質的生產，但最終也面臨到高密度細胞培養時所帶來氧氣供

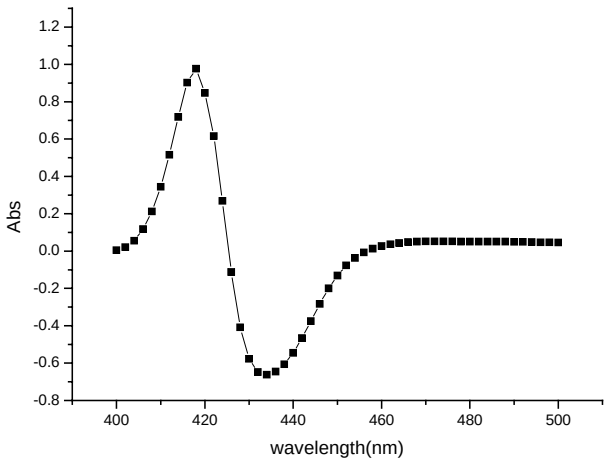

圖 3. VHb 與一氧化碳結合之吸收圖譜

應不足的問題。而到目前爲止，解決溶氧問題的方法大多集中在發酵槽的設計與改良來增加氧氣傳遞速率，或添加化合物來增加培養基的溶氧能力，如全氟碳（perfluorocarbon）[12] 與十六烷（*N*-hexadecane）[21] 等。然而在 1988 年，Khosla 與 Bailey 首次利用 “逆向代謝工程策略”（inverse metabolic engineering methodology: the genetic transfer of useful phenotypes to heterologous organisms）[29] 來解決細胞在生化工程量產上，會因爲氧氣不足所產生不利於細胞生長的現象，亦即在 *E. coli* 細胞中表現外來之 VHb，讓細胞在微氧環境下仍能達到高細胞密度，而且提高了重組蛋白質的產量 [24]。

利用基因工程技術讓 VHb 在各種宿主細胞中表現，通常會提高細胞的生長密度、加快氧化性代謝的能力、增加目標蛋白質的產率、提升生物降解的能力等，VHb 在細胞體內（*in vivo*）的效應與實例，包括（1）使重組 *E. coli* 與 *Pseudomonads* 細胞濃度增加 [14, 29, 31, 37]；（2）使重組 *E. coli* 中的澱粉醣化酶（α-amylase）表現量提高 [32] 以及在 *Acremonium chrysogenum* 中增加抗生素頭孢菌素 C（cephalosporin C）的產率 [13]；（3）加快有毒廢物的降解，例如利用 *Pseudomonads* 分解苯甲酸（benzoic acid）[38] 和 *Burkholderia* 分解二硝基甲苯（2,4-dinitrotoluene）[46] 等。表 2 列出 VHb 在各種宿主細胞中表現時所造成的效應。

VHb 的存在能增加微生物對有毒芳香族化合物（aromatic compound）的降解，因爲這些化合物通常需要在芳香環結構（aromatic ring）中加入氧後才可被細胞所代謝。因此 VHb 的存在，一方面可提供氧給細胞使用，增加 ATP 濃度與促進細胞生長，進而合成出較多降解路徑上之酵素來加快降解。另一方面，VHb 也可直接地供應氧給這些酵素加速降解反應的進行。

四、VHb 作用機制

到目前爲止，對於 VHb 的生合成、功能特性與基因調控有兩種模式被提出來解釋，第一種稱爲促進擴散假設（facilitated diffusion hypothesis）[62]，乃 VHb 能增加氧氣通量到一個或兩個最終氧化酶（cytochromes *bo* 與 *bd*）。在帶有 VHb 的重組 *E. coli* 細胞中，VHb 能增加氧的供應至電子轉移鏈上的最終氧化酶，使其呼吸活性與 ATP 的生產高於不帶有 VHb 的 *E. coli* 細胞，進而增進細胞生長與蛋白質的生產 [25]。在微氧環境時，細胞表現出 VHb 能幫助其生

表 2. VHb 在各種宿主細胞中表現所造成的效應

Host organisms	Effects	Refs
Bacteria		
Bacillus subtilis	Increased total protein secretion, neutral protease activity and α-amylase activity	24
Burkholderia cepacai	Improved cell growth and cell viable number, enhanced 2,4-dinitrotoluene degradation	42, 46
	Increased 2,4-dinitrotoluene dioxygenase activity	36
Escherichia coli	Enhanced growth rates and cell densities	29
	Enhanced total cell protein and cloned proteins	29, 31, 52
	Increased chloramphenicol acetyltransferase activity	31
	Increased catechol-2,3-dioxygenase activity	14
	Enhanced α-amylase production	32
	Increased β-galactosidase activity	31
	Increased Pi-ATP metabolic flux	25
	Increased cytochrome *d* promoter activity	52
	Enhanced level and activity of terminal oxidases, and improved the efficiency of microaerobic respiration and growth, and increased ATP production	51, 53
	Enhanced ribosome and tRNA levels of translation components	43
	Enhanced 2,4-dinitrotoluene dioxygenase activity	17
	Increased green fluorescent protein expression and solubility	27
	Enhanced detoxification of organophosphates	26
	Enhanced cell growth and D-amino acid oxidase activity. Reduced the toxic effect of D-amino acid oxidase on cell growth	9
Gordonia amarae	Increased cell mass, enhanced rehalose lipid and biosurfactant production	15
Pseudomonas aeruginosa	Enhanced cell growth	11, 19
	Enhanced bioremediation of benzoic acid	11
	Increased cell viable number	37
Rhizobium etli	Increased in respiratory activity, chemical energy content, and expression of the nitrogen-fixation gene *nifHc* of free-living cell under oxygen-limited conditions	50
Saccharopolyspora erythraea	Enhanced erythromycin production	41
Serratia marcescens	Enhanced colony size	58
	Increased acetoin and 2,3-butanediol production	59
Streptomyces coelicolor	Increased actinorhodin production	39
Streptomyces lividans	Increased growth rates and final cell densities	39
Xanthomonas maltophilia	Bioremediation of benzoic acid	38
	Increased cell viable number	37
Yeast		
Pichia pastoris	Enhanced β-galactosidase production and cell growth under microaerobic conditions	10, 64
Saccharomyces cerevisiae	Increased aerobic synthesis of ethanol	8
Tremella fuciformis	Increased cell density	65
Yarrowia lipolytica	Enhanced specific growth rate, oxygen uptake rate, respiratory activity, and secreted ribonuclease production	2
Filamentous fungi		
Acremonium chrysogenum	Increased cephalosporin C production	13
Plant		
Bead plants	Increased nitrogenase activity and total nitrogen content during symbiosis with the engineered *R. etli* strain expressed VHb	50
Hyoscyamus muticus	Enhanced growth	60
Nicotiana tobaccum	Enhanced growth, reduced germination and flowering time periods, and increased chlorophyll and nicotine production	16
	No lag-phase and improved cell growth	57
Animal		
Chinese hamster ovary	Enhanced human tissue plasminogen activator production	47

長，主要的原因乃 VHb 對於氧具有獨特的動力學性質 [56, 61]，如表 3 所示，VHb 與其它生物的血紅素相比，它對氧的結合速率常數 k_{on}（rate constant of oxygen association）稍大一點，但是對氧的解離速率常數 k_{off}（rate constant of oxygen dissociation）卻異常的大 [44]，這異常大的 k_{off} 讓 VHb 比肌血紅素與其它的血紅素更容易釋放出氧。當細胞內有 VHb 存在時，有效的溶氧量等於真實的溶氧再加上 VHb 所吸附的氧 [25]，因此，讓細胞處於比外界含有更高溶氧的環境，因而有利於細胞的生長與代謝。一些研究也顯示出 VHb 的表現對細胞的功能與組成有所影響 [7, 25, 52]，它可以（1）增加最終氧化酶細胞色素 *o* 的濃度與比活性；（2）增加質子通量（proton flux），提高細胞膜內外的△pH；（3）提升 30% ATPase 的活性；（4）增加 65% 的 ATP 轉換率（turnover rate）。這些數據證實了 VHb 能增加細胞內溶氧的濃度，因而提升細胞色素 *o* 的比活性，亦增加質子幫浦傳送（pumping）效率與 ATP 產生，進而大幅提升電子轉移鏈的效率與活性。此外，研究核糖體與 tRNA 的量、細胞色素、能量參數、NAD(P)H 轉換率與氧氣利用率等也支持

表 3. 血紅素與氧反應的動力學常數與平衡常數 [4, 5, 55]

Protein	k_{on}, μ M^{-1}·s^{-1}	k_{off}, s^{-1}	K_d, nM
Muscle Mbs			
Sperm whale	19	10	526
Aphrodite	170	360	2118
Aplysia	15	70	4667
Busycon	47.5	71	1495
Bacteria and algae			
Chlamydomonas Hb		0.014	
Mycobacterium HbN	25	0.199	8
Nostoc cyanoglobin	390	79	203
Vitreoscilla Hb	78	5600	71795
E. coli flavoHb	38	0.44	12
Alcaligenes flavoHb	50	0.2	4
Yeast			
Saccharomyces flavoHb	17	0.6	35
Nematodes			
Ascaris Hb	1.5	0.004	2.7
Ascaris Mb	1.2	0.23	192
Pseudoterranova Hb	1.1	0.0035	3.2
Trematodes Hbs			
Paramphistomum	108	0.033	0.3
Gastrothylax	205	0.4	2
Dicrocoelium	300	30	100
Symbiotic plant Hbs			
Soybean LegHba	120	5.6	47
Parasponia HbI	165	15	91
Casuarina HbII	41	5.5	134
Nosymbiotic plant Hbs			
Barley Hb	7.1	0.027	3.8
Rice HbI	68	0.038	0.6
Arabidopsis HbI	75	0.12	1.6
Arabidopsis HbII	1.07	0.14	131
Clam gill Hbs			
Lucina HbI	190	61	321
Lucina HbII	0.39	0.11	282
Lucina HbIII	0.29	0.075	259
Insect Hbs			
Gastrophilus	10	2.4	240
Chironomus HbIII	300	218	727
Annelid RBC Hb			
Glycera II Hb	186	1800	9677
Mammalian			
Sheep Hb	2	30	15000
Human Hb	3	40	13333
Human Mb	14	11	786

這一種假設 [7, 43, 51-53]。

VHb 的第二種作用機制是與氧結合後的 VHb 會影響細胞內一些對於氧化還原敏感性蛋白質的活性，這些蛋白質可能是感測器、調控因子或甚至是呼吸性酵素等，因而使細胞會更有效率地使用與保存能量。或者，除了這兩種作用機制外，VHb 有著一個完全不同的功能或不只一個功能，例如，最近從 *E. coli* 細胞中發現了一個黃素血紅素，它是一種一氧化氮雙加氧酶（Nitric oxide dioxygenase），它可以將一氧化氮氧化成硝酸鹽，以保護細胞免於受到自由基的破壞 [18]。

五、VHb 之標的物

在 *E. coli* 與 *Vitreoscilla* 細胞中，使用免疫金膠法（immunogold）標示 VHb 在細胞內的位置，實驗結果顯示出 VHb 是位於細胞質內並且集中靠近於細胞膜，也就是細菌的呼吸膜上 [49]。VHb 對 *Vitreoscilla* 細胞膜的解離常數 K_d 是 6.5 μM，大於哺乳動物血紅素對 *Vitreoscilla* 細胞膜之 K_d 約 10 倍。而將 *Vitreoscilla* 細胞色素 *bo* 放入人工合成膜中，測量 VHb 對此蛋白脂質體（proteoliposome）的解離常數 K_d 是 6.2 μM。在微氧環境中，將 VHb 外加於呼吸膜與細胞色素 *bo* 蛋白脂質體中，刺激了泛醌氧化酶（ubiquinol oxidase）的活性 [49]，這些研究證實了 VHb 與細胞色素 *bo* 間的作用會影響細胞的生理作用。利用酵母菌雙雜合系統（yeast two-hybrid system） 技術，以 VHb 當誘餌去測試 *Vitreoscilla* 細胞中每一個細胞色素 *bo* 的次單元體，結果發現其中只有次單元體 I 與 VHb 有結合作用，如圖 4 所示，VHb 與細胞色素 *bo* 的次單元體 I 的結合現象也同樣發生在重組 *E. coli* 與 *Pseudomonads aeruginosa* 細胞中 [45]。而細胞色素 *bo* 次單元體 I 含有 binuclear center 結構可將氧還原成水，這些數據都支持了 VHb 能提供氧到最終氧化酶的證據，也可以解釋 VHb 在 *Vitreoscilla* 與其他宿主細胞中的所產生的正面效應現象。

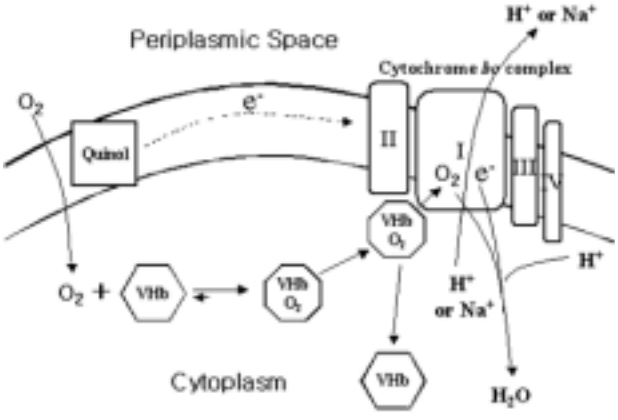

圖 4. VHb 與細胞色素 *bo* 泛醌氧化酶之次單元體 I 間的作用 [45]

六、國內研究現況

Pichia pastoris 爲一株嗜甲醇酵母菌，近年來已發展成爲高度表現的蛋白質生產系統。在進行 *P. pastoris* 高密度細胞培養並使用強啓動子 AOX1（alcohol oxidase 1）表現異源蛋白質時，氧氣的需求是相當高，因爲除了它是一株好氧性酵母菌外，當它代謝唯一碳源－甲醇時更需要氧氣的參與。因此將 VHb 表現在 *P. pastoris* 中以解決氧氣需求的實際問題。研究結果發現，將 VHb 表現在 *P. pastoris* 進行呼吸作用的粒腺體（mitochondria）胞器中而進行微氧細胞培養時，VHb 可以提高細胞的生長密度。而當 VHb 表現於 *P. pastoris* 進行甲醇代謝的胞器－過氧化氫體（peroxisome）中時，VHb 能增加目標蛋白質半乳糖苷酶（β-galactosidase）比活性達 4 倍 [10, 64]。

在 *E. coli* 中表現 D 型胺基酸氧化酶（D-amino acid oxidase）時通常會導致細胞存活率明顯地下降，這都歸因於表現出來的 D 型胺基酸氧化酶消耗了細胞內的 D-alanine，而 D-alanine 是細胞壁的組成之一，所以造成細胞生長不好。在 *E. coli* 中同時表現 VHb 與 D 型胺基酸氧化酶時，不但可以降低 D 型胺基酸氧化酶對細胞的毒性，而且還可以明顯的減緩因爲 T7 lysozyme 與 D 型胺基酸氧化酶合力所造成 BL21(DE3)pLysS 細胞的溶解現象。因此，細胞可以達到較高的生長密度，而 D 型胺基酸氧化酶的活性表現也高出 1.5 倍。VHb 能減輕 D 型胺基酸氧化酶對細胞的毒性，乃因 VHb 與氧的結合降低了細胞內自由氧的濃度，因而減緩了 D 型胺基酸氧化酶的活性與反應，也因此降低了它對細胞的毒性 [9]。

七、結語

以細胞爲工廠生產製造抗生素、蛋白質藥物、抗體或疫苗時，通常不易達到高產量的產物，醱酵與回收純化技術的改良雖可提高產量，但亦有其極限。而代謝工程技術可以提高產量，但醱酵過程中若進行高密度細胞培養時，則會遇到氧氣供應不足的問題，此時，可利用 VHb 細菌血紅素工技來解決，讓細胞在低氧濃度時更容易自胞外取得氧氣，使細胞仍能繼續生長，持續生產而提高產量，正如同 *Vitreoscilla* 細菌的特性一樣。此外，細菌血紅素還能提高生物降解的能力。因此，細菌血紅素工技可視爲新一代基因工程的重要應用。

參考文獻

1. Andrews, S. C., D. Shipley, J. N. Keen, J. B. Findlay, P. M. Harrison and J. R. Guest (1992) The haemoglobin-like protein (HMP) of *Escherichia coli* has ferrisiderophore reductase activity and its C-terminal domain shares homology with ferredoxin $NADP^+$ reductases. *Federation of European Biochemical Societies Letters*, 302, 247-252.
2. Bhave, S. L. and B. B. Chattoo (2003) Expression of *Vitreoscilla* hemoglobin improves growth and levels of extracellular enzyme in *Yarrowia lipolytica*. *Biotechnology and Bioengineering*, 84, 658-666.
3. Boerman, S. J. and D. A. Webster (1982) Control of heme content in *Vitreoscilla* by oxygen. *Journal of General and Applied Microbiology*, 28, 35-43.
4. Bolognesi, M., D. Bordo, M. Rizzi, C. Tarricone and P. Ascenzi (1997) Nonvertebrate hemoglobins: Structural bases for reactivity. *Progress in Biophysics and Molecular Biology*, 68, 29-68.
5. Bulow, L., N. Holmberg, G. Lilius and J. E. Bailey (1999) The metabolic effects of native and transgenic hemoglobins on plants. *Trends in Biotechnology*, 17, 21-24.
6. Cheah, U. E., W. Weigand and B. C. Stark (1987) Effect of recombinant plasmid size on cellular processes in *Escherichia coli*. *Plasmid*, 18, 127-134.
7. Chen, R. (1994) Energetic effect of *Vitreoscilla* hemoglobin expression in *Escherichia coli*: An on-line ^{31}P NMR and saturation transfer study. *Biotechnology Progress*, 10, 360-364.
8. Chen, W., D. E. Hughes and J. E. Bailey (1994) Intracellular expression of *Vitreoscilla* hemoglobin alters aerobic metabolism of *Saccharomyces cerevisiae*. *Biotechnology Progress*, 10, 308-313.
9. Chien, L. J., J. M. Wu, I. C. Kuan and C. K. Lee (2004) Coexpression of *Vitreoscilla* hemoglobin reduces the toxic effect of expression of D-amino acid oxidase in *E. coli*. *Biotechnology Progress*, 20, 1359-1365.
10. Chien, L. J. and C. K. Lee (2005) Expression of bacterial hemoglobin in the yeast, *Pichia pastoris*, with a low O2-induced promoter. *Biotechnology Letters*, 27, 1491-1497.
11. Chung, J. W., D. A. Webster, K. R. Pagilla and B. C. Stark (2001) Chromosomal integration of the *Vitreoscilla* hemoglobin gene in *Burkholderia* and *Pseudomonas* for

the purpose of producing stable engineered strains with enhanced bioremediating ability. *Journal of Industrial Microbiology and Biotechnology*, 27, 27-33.

12. Damiano, D. and S. S. Wang (1985) Novel use of a perfluorocarbon for supplying oxygen to aerobic submerged cultures. *Biotechnology Letters*, 7, 81-86.
13. DeModena, J. A., S. Gutierrez, J. Velasco, F. J. Fernandez, R. A. Fachini, J. L. Galazzo, D. E. Hughes and J. F. Martin (1993) The production of cephalosporin C by *Acremonium chrysogenum* is improved by the intracellular expression of a bacterial hemoglobin. *Bio/technology*, 11, 926-929.
14. Dikshit, K. L., R. P. Dikshit and D. A. Webster (1990) Study of *Vitreoscilla* globin (*vhb*) gene expression and promoter activity in *E. coli* through transcriptional fusion. *Nucleic Acid Research*, 18, 4149-4155.
15. Dogan, I., K. R. Pagilla, D. A. Webster and B. C. Stark (2006) Expression of *Vitreoscilla* hemoglobin in *Gordonia amarae* enhances biosurfactant production. *Journal of Industrial Microbiology and Biotechnology*, in press.
16. Farres, J. and P. T. Kallio (2002) Improved cell growth in tobacco suspension cultures expressing *Vitreoscilla* hemoglobin. *Biotechnology Progress*, 18, 229-233.
17. Fish, P. A., D. A. Webster and B. C. Stark (2000) *Vitreoscilla* hemoglobin enhances the first step in 2.4-dinitrotoluene degradation *in vitro* and at low aeration *in vivo*. *Journal of Molecular Catalysis, B, Enzymatic*, 9, 75-82.
18. Gardner, P. R., A. M. Gardner, L. A. Martin and A. L. Salzman (1998) Nitric oxide dioxygenase: An enzymic function for flavohemoglobin. *Proceedings of the National Academy of Science of the United States of America*, 95, 10378-10383.
19. Geckil, H., B. C. Stark and D. A. Webster (2001) Cell growth and oxygen uptake of *Escherichia coli* and *Pseudomonas aeruginosa* are differently effected by the genetically engineered *Vitreoscilla* hemoglobin gene. *Journal of Biotechnology*, 85, 57-66.
20. Hardison, R. (1998) Hemoglobins from bacteria to man: Evolution of different patterns of gene expression. *Journal of Experimental Biology*, 201, 1099-1117.
21. Ho, C. S., L. K. Ju and R. F. Baddour (1990) Enhancing penicillin fermentation by increased oxygen solubility through the addition of *N*-hexadecane. *Biotechnology and Bioengineering*, 36, 1110-1118.
22. Holmberg, N., G. Lilius, J. E. Bailey and L. Bulow (1997) Transgenic tobacco expression *Vitreoscilla* hemoglobin exhibits enhanced growth and altered metabolite production. *Nature Biotechnology*, 15, 244-247.
23. Iijima, M., H. Shimizu, Y. Tanaka and H. Urushihara (2000) Identification and characterization of two flavohemoglobin genes in *Dictyostelium discoideum*. *Cell Structure and Function*, 25, 47-55.
24. Kallio, P. T. and J. E. Bailey (1996) Expression of *vhb* encoding *Vitreoscilla* hemoglobin (VHb) enhances total protein secretion and improves the production of α-amylase and neutral protease in *Bacillus subtilis*. *Biotechnology Progress*, 12, 31-39.
25. Kallio, P. T., D. J. Kim, P. S. Tsai and J. E. Bailey (1994) Intracellular expression of *Vitreoscilla* hemoglobin alters *Escherichia coli* energy metabolism under oxygen-limited conditions. *European Journal of Biochemistry*, 219, 201-208.
26. Kang, D. G., J. Y. H. Kim and H. J. Cha (2002) Enhanced detoxification of organophosphates using recombinant *Escherichia coli* with co-expression of organophosphorus hydrolase and bacterial hemoglobin. *Biotechnology Letters*, 24, 879-883.
27. Kang, D. G., Y. K. Kim and H. J. Cha (2002) Comparison of green fluorescent protein expression in two industrial *Escherichia coli* strains, BL21 and W3110, under co-expression of bacterial hemoglobin. *Applied Microbiology and Biotechnology*, 59, 523-528.
28. Khosla, C. and J. E. Bailey (1988) The *Vitreoscilla* hemoglobin gene: Molecular cloning, nucleotide sequence, and genetic expression in *Escherichia coli*. *Molecular and General Genetics*, 214, 158-161.
29. Khosla, C. and J. E. Bailey (1988) Heterologous expression of a bacterial haemoglobin improves the growth properties of recombinant *Escherichia coli*. *Nature*, 331, 633-635.
30. Khosla, C. and J. E. Bailey (1989) Characterization of the oxygen-dependent promoter of the *Vitreoscilla* hemoglobin gene in *Escherichia coli*. *Journal of Bacteriology*, 171, 5995-6004.
31. Khosla, C., J. E. Curtis, J. DeModena, U. Rinas and J. E. Bailey (1990) Expression of intracellular hemoglobin improves protein synthesis in oxygen-limited *Escherichia coli*. *Bio/technology*, 8, 849-853.

32. Khosravi, M., D. A. Webster and B. C. Stark (1990) Presence of the bacterial hemoglobin gene improves α-amylase production of a recombinant *Escherichia coli* strain. *Plasmid*, 24, 190-194.
33. Khosravi, M., W. Ryan, D. A. Webster and B. C. Stark (1990) Variation of oxygen requirement with plasmid size in recombinant *E. coli*. *Plasmid*, 23, 138-143.
34. Kolb, A., K. Igarashi, A. Ishihama, M. Lavigne, M. Buckle and H. Buc (1993) *E. coli* RNA polymerase, deleted in the C-terminal part of its alpha-subunit, interacts differently with the cAMP-CRP complex at the lacP1 and at the galP1 promoter. *Nucleic Acids Research*, 21, 319-326.
35. Konz, J. O., J. King and C. L. Cooney (1998) Effects of oxygen on recombinant protein expression. *Biotechnology Progress*, 14, 393-409.
36. Lin, J. M., B. C. Stark and D. A. Webster (2003) Effects of *Vitreoscilla* hemoglobin on the 2,4-dinitrotoluene (2,4-DNT) dioxygenase activity of *Burkholderia* and on 2,4-DNT degradation in two-phase bioreactors. *Journal of Industrial Microbiology and Biotechnology*, 30, 362-368.
37. Liu, S. C., D. A. Webster and B. C. Stark (1995) Cloning and expression of the *Vitreoscilla* hemoglobin gene in *Pesudomonas*: effects on cell growth. *Applied Microbiology and Biotechnology*, 44, 419-424.
38. Liu, S. C., D. A. Webster, M. L. Wei and B. C. Stark (1996) Genetic engineering to contain the *Vitreoscilla* hemoglobin gene enhances degradation of benzoic acid by *Xanthomonas maltophilia*. *Biotechnology and Bioengineering*, 49, 101-105.
39. Magnolo, S. K., D. L. Leenutaphong, J. A. DeModena, J. E. Curtis, J. E. Bailey, J. L. Galazzo and D. E. Hughes (1991) Actinorhodin production by *Streptomyces coelicolor* and growth of *Streptomyces lividans* are improved by the expression of a bacterial hemoglobin. *Bio/technology*, 9, 473-376.
40. Membrillo-Hernandez, J. and R. K. Poole (1997) Bacterial flavohaemoglobins: A consensus sequence and identification of a discrete enterobacterial group and of further bacterial globins. *Federation of European Microbiological Societies Microbiology Letters*, 155, 179-184.
41. Minas, W., P. Brunker, P. T. Kallio and J. E. Bailey (1998) Improved erythromycin production in a genetically engineered industrial strain of *Saccharopolyspora erythraea*. *Biotechnology Progress*, 14, 561-566.
42. Nasr, M. A., K. W. Hwang, M. Akbas, D. A. Webster and B. C. Stark (2000) Effects of culture conditions on enhancement of 2,4-dinitroluene degradation by *Burkholderia* engineered with the *Vitreoscilla* hemoglobin gene. *Biotechnology Progress*, 17, 359-361.
43. Nilsson, M. P., P. T. Kallio, J. E. Bailey, L. Bulow and K. G. Wahlund (1999) Expression of *Vitreoscilla* hemoglobin in *Escherichia coli* enhances ribosome and tRNA levels: A flow field-flow fractionation study. *Biotechnology Progress*, 15, 158-163.
44. Orii, Y. and D. A. Webster (1986) Photodissocation of oxygenated cytochrome *o*(s)(*Vitreoscilla*) and kinetic studies of reassociation. *Journal of Biological Chemistry*, 261, 3544-3547.
45. Park, K. W., K. J. Kim, A. J. Howard, B. C. Stark and D. A. Webster (2002) *Vitreoscilla* hemoglobin binds to subunit I of cytochrome *bo* ubiquinol oxidases. *Journal of Biological Chemistry*, 277, 33334-33337.
46. Patel, S. M., B. C. Stark, K. W. Hwang, K. L. Dikshit and D. A. Webster (2000) Cloning and expression of *Vitreoscilla* hemoglobin gene in *Burkholderia* sp. strain DNT for enhancement of 2,4-dinitrotoluene degradation. *Biotechnology Progress*, 16, 26-30.
47. Pendse, G. J. and J. E. Bailey (1994) Effect of *Vitreoscilla* hemoglobin expression on growth and specific tissue plasminogen activator productivity in recombinant chinese hamster ovary cells. *Biotechnology and Bioengineering*, 44, 1367-1370.
48. Poole, R. K. (1994) Oxygen reactions with bacterial oxidases and globins: Binding, reduction and regulation. *Antonie van Leeuwenhoek*, 65, 289-310.
49. Ramandeep, K. W. Hwang, M. Raje, K. J. Kim, B. C. Stark, K. L. Dikshit and D. A. Webster (2001) *Vitreoscilla* hemoglobin. Intracellular localization and binding to membranes. *Journal of Biological Chemistry*, 276, 24781-24789.
50. Ramirez, M., B. Valderrama, P. Arredondo-Peter, M. Soberon, J. Mora and G. Hernandez (1999) *Rhizobium etli* genetically engineered for the heterologous expression of *Vitreoscilla* sp. Hemoglobin: Effects on free-living and symbiosis. *Molecular Plant-Microbe Interactions*, 12, 1008-1015.
51. Tsai, P. S., V. Hatzimanikatis and J. E. Bailey (1996) Effect

of *Vitreoscilla* hemoglobin dosage on microaerobic *Escherichia coli* carbon and energy metabolism. *Biotechnology and Bioengineering*, 49, 139-150.

52. Tsai, P. S., P. T. Kallio and J. E. Bailey (1995) Fnr, the global transcriptional regulator of *Escherichia coli*, activates the *Vitreoscilla* hemoglobin (VHb) promoter and intracellular VHb expression increases cytochrome *d* promoter activity. *Biotechnology Progress*, 11, 288-293.
53. Tsai, P. S., M. Nageli and J. E. Bailey (1996) Intracellular expression of *Vitreoscilla* hemoglobin modifies microaerobic *Escherichia coli* metabolism through elevated concentration and specific activity of cytochrome *o*. *Biotechnology and Bioengineering*, 49, 151-160.
54. Wakabayashi, S., H. Matsubara and D. A. Webster (1986) Primary sequence of a dimeric bacterial haemoglobin from *Vitreoscilla*. *Nature*, 322, 481-483.
55. Weber, R. E. and S. N. Vinogradov (2001) Nonvertebrate hemoglobins: Functions and molecular adaptations. *Physical Review*, 81, 569-628.
56. Webster, D. A. (1988) Structure and function of bacterial hemoglobin and related proteins. In: *Advances in Inorganic Biochemistry*, 7, 245-265. G. L. Eichhorn and L. G. Marzilli Eds. Elsevier Science Publishing Company, New York, NY.
57. Webster, D. A. and C. Y. Liu (1974) Reduced nicotinamide adenine dinucleotide cytochrome *o* reductase associated with cytochrome *o* purified from *Vitreoscilla*. Evidence for an intermediate oxygenated form of cytochrome *o*. *Journal of Biological Chemistry*, 249, 4257-4260.
58. Wei, M. L., D. A. Webster and B. C. Stark (1998) Genetic engineering of *Serratia marcescens* with bacterial hemoglobin gene: Effects on growth, oxygen utilization, and cell size. *Biotechnology and Bioengineering*, 57, 477-483.
59. Wei, M. L., D. A. Webster and B. C. Stark (1998) Metabolic engineering of *Serratia marcescens* with bacterial hemoglobin gene: Alternations in fermentation pathways. *Biotechnology and Bioengineering*, 59, 640-646.
60. Wilhelmson, A., P. T. Kallio, K. M. Oksman-Caldentey and A. M. Nuutila (2005) Expression of *Vitreoscilla* hemoglobin enhances growth of *Hyoscyamus muticus* hairy root cultures. *Planta Medica*, 71, 48-53.
61. Wittenberg, J. B. and B. A. Wittenberg (1990) Mechanisms of cytoplasmic hemoglobin and myoglobin function. *Annual Review of Biophysics and Biophysical Chemistry*, 19, 217-241.
62. Wittenberg, J. B., F. J. Bergensen, C. A. Appleby and G. L. Turner (1974) Facilitated oxygen diffusion. The role of leghemoglobin in nitrogen fixation by bacteroids isolated from soybean root nodules. *Journal of Biological Chemistry*, 249, 4057-4066.
63. Wittenberg, J. B., M. Bolognesi, B. A. Wittenberg and M. Guertin (2002) Truncated hemoglobins: A new family of hemoglobins widely distributed in bacteria, unicellular eukaryotes, and plants. *Journal of Biological Chemistry*, 277, 871-874.
64. Wu, J. M., T. A. Hsu and C. K. Lee (2003) Expression of the gene coding for bacterial hemoglobin improves β-galactosidase production in a recombinant *Pichia pastoris*. *Biotechnology Letters*, 25, 1457-1462.
65. Zhu, H., T. W. Wang, S. J. Sun, Y. L. Shen and D. C. Wei (2006) Chromosomal integration of the *Vitreoscilla* hemoglobin gene and its physiological actions in *Tremella fuciformis*. *Applied Microbiology and Biotechnology*, in press.

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