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(71) Applicant(s):

HUNAN YANDI BIOENGINEERING CO., LTD. [CN/CN]; No.8, Mingri Road, National Hi-tech Development Zone (Tianyuan District) Zhuzhou, Hunan 412007 (CN) *(for all designated states)*

(72) Inventor(s):

YU, Jia; No.8, Mingri Road, National Hi-tech Development Zone (Tianyuan District) Zhuzhou, Hunan 412007 (CN)
ZHANG, Ruihua; No.8, Mingri Road, National Hi-tech Development Zone (Tianyuan District) Zhuzhou, Hunan 412007 (CN)
WANG, Yulan; Building No.39, 1000 Zhang Heng Road Shanghai 200120 (CN)
ZHAN, Hao; No.8, Mingri Road, National Hi-tech Development Zone (Tianyuan District) Zhuzhou, Hunan 412007 (CN)
CHEN, Sheng; Building No.39, 1000 Zhang Heng Road Shanghai 200120 (CN)
WANG, Wei; No.8, Mingri Road, National Hi-tech Development Zone (Tianyuan District) Zhuzhou, Hunan 412007 (CN)

(74) Agent(s):

MSJ LAW OFFICE; Maggie Dong Room 301B, Building 358, No.3663, North Zhongshan Road, Putuo District Shanghai 200062 (CN)

(54) Title (EN): METHODS FOR EXTRACTING AND PURIFYING *NOSTOC SPHAEROIDES* KUTZING PHYCOBILIPROTEIN, AND PURIFIED PHYCOERYTHRIN

(54) Title (FR): PROCÉDÉS D'EXTRACTION ET DE PURIFICATION DE LA PHYCOBILIPROTÉINE DE *NOSTOC SPHAEROIDES* KÜTZING ET PHYCOÉRYTHRINE PURIFIÉE

(54) Title (ZH): 一种葛仙米藻胆蛋白的提取、纯化方法及纯化的藻红蛋白

(57) Abstract:

(EN): Provided is a method for purifying a *Nostoc sphaeroides* Kutzing phycoerythrin, the method comprising: (1) selecting an anion exchange column for being passed through; (2) formulating a PB buffer solution, i.e., buffer A, and a PB-NaCl buffer solution, i.e., buffer B; (3) dissolving a coarsely extracted *Nostoc sphaeroides* Kutzing phycobiliprotein powder with the PB buffer solution, i.e., buffer A, and subjecting same to centrifugation and filtration; (4) washing a pump, and balancing the ion exchange column with the PB buffer solution, i.e., buffer A; (5) directly loading the *Nostoc sphaeroides* Kutzing phycobiliprotein dissolved in the PB buffer solution, i.e., buffer A, and prepared in step (3), carrying out gradient elution with the PB-NaCl buffer solution, i.e., buffer B, and collecting an eluent; (6) cleaning the ion exchange column with a salt solution, and then reversely rinsing same with a base solution; and (7) subjecting the eluent to ultrafiltration concentration and dialysis to obtain a purified *Nostoc sphaeroides* Kutzing phycoerythrin aqueous solution. In addition, further provided is a method for extracting a *Nostoc sphaeroides* Kutzing phycobiliprotein.

(FR): La présente invention concerne un procédé de purification d'une phycoérythrine de *Nostoc sphaeroides* Kützing, le procédé consistant à : (1) sélectionner une colonne échangeuse d'anions pour la faire passer à travers ; (2) formuler une solution tampon PB, c'est-à-dire le tampon A, et une solution tampon PB-NaCl, c'est-à-dire, le tampon B ; (3) dissoudre une poudre de phycobiliprotéine de *Nostoc sphaeroides* Kützing grossièrement extraite avec la solution tampon PB, c'est-à-dire le tampon A, et la soumettre à une

centrifugation et à une filtration ; (4) laver une pompe et équilibrer la colonne échangeuse d'ions avec la solution tampon PB, c'est-à-dire, le tampon A ; (5) charger directement la phycobiliprotéine de *Nostoc sphaeroides* Kützing dissoute dans la solution tampon PB, c'est-à-dire le tampon A et préparée à l'étape (3), réaliser une élution par gradient avec la solution tampon PB-NaCl, c'est-à-dire le tampon B et collecter un éluant ; (6) nettoyer la colonne échangeuse d'ions avec une solution saline, puis effectuer un rinçage inverse de celle-ci avec une solution de base ; et (7) soumettre l'éluant à une concentration par ultrafiltration et à une dialyse pour obtenir une solution aqueuse de phycoérythrine de *Nostoc sphaeroides* Kützing purifiée. De plus, l'invention concerne en outre un procédé d'extraction de la phycobiliprotéine de *Nostoc sphaeroides* Kützing.

(ZH): 提供了一种葛仙米藻红蛋白的纯化方法,包括:(1)选用阴离子交换柱过柱;(2)配置PB缓冲液BufferA和PB-NaCl缓冲液Buffer B;(3)葛仙米藻胆蛋白粗提粉末用PB缓冲液BufferA溶解,离心,过滤;(4)洗泵,用PB缓冲液BufferA平衡离子交换柱;(5)将步骤(3)制备的溶解于PB缓冲液BufferA中的葛仙米藻胆蛋白直接上样,PB-NaCl缓冲液Buffer B梯度洗脱,收集洗脱液;(6)用盐溶液清洗离子交换柱,然后用碱溶液反向冲洗;(7)对淋洗液进行超滤浓缩,透析,得到纯化的葛仙米藻红蛋白水溶液。此外,还提供了一种葛仙米藻胆蛋白的提取方法。

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Declarations:

Declaration made as to the identity of the inventor (PCT Rules 4.17(i) and 51bis.1(a)(i))

Declaration made as applicant's entitlement, as at the international filing date, to apply for and be granted a patent (Rules 4.17(ii) and 51bis.1(a)(ii)), in a case where the declaration under Rule 4.17(iv) is not appropriate

Declaration made as applicant's entitlement, as at the international filing date, to claim the priority of the earlier application, where the applicant is not the applicant who filed the earlier application or where the applicant's name has changed since the filing of the earlier application (Rules 4.17(iii) and 51bis.1(a)(iii))

Declaration of inventorship (Rules 4.17(iv) and 51bis.1(a)(iv)) for the purposes of the designation of the United States of America