基于光学成像的益生菌体内示踪技术

王津^{1†}, 李萍^{2†}, 赵宁¹, 王志豪¹, 刘敬民¹, 王硕^{1*}

1. 南开大学医学院, 天津市食品科学与健康重点实验室, 天津 300350;

2. 天津科技大学食品工程与生物技术学院, 教育部食品营养与安全重点实验室, 天津 300457

† 同等贡献

* 联系人, E-mail: wangshuo@nankai.edu.cn

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摘要 益生菌是指对宿主健康有利而且能够促进人类健康的活的微生物, 主要通过调节胃肠道菌群、改善肠上皮细胞屏障功能、调节免疫功能等对宿主产生有益影响。过去, 益生菌在体内的存活情况是通过分析粪便中细菌的数量或是不同时间解剖动物胃肠道来进行益生菌计数。关于益生菌发挥益生作用的机理不清楚, 通过现代生物学技术对益生菌进行遗传标记可为解释益生作用机理提供参考。基于光学成像的益生菌体内示踪技术主要包括生物发光、传统荧光(荧光蛋白和荧光染料)和新型纳米材料3种技术, 本文评述了3种技术各自的特点和优缺点, 旨在为进一步发展和研究益生菌体内示踪技术提供理论和参考依据。

关键词 益生菌, 生物发光, 传统荧光, 新型纳米材料

联合国粮食及农业组织和世界卫生组织指出益生菌是一类活的微生物, 当以一定数量摄入益生菌后, 对宿主健康产生有益影响。研究表明, 益生菌可抵御病原菌侵入, 抑制病原菌繁殖, 改善肠上皮细胞屏障功能、维持胃肠道内环境稳态, 重建肠道菌群稳定^[1,2]。益生菌发挥益生作用主要通过产生优势菌群、生物夺氧和生物拮抗, 且通过产生有益物质和抗菌物质(如有机酸、过氧化氢和细菌素等)来调节宿主机体免疫功能。益生菌调节胃肠道菌群通过在胃肠道黏膜表面和表皮细胞的黏附和定殖, 阻止病原菌在肠道中的定殖和增长。常见的可作为益生菌的菌株有双歧杆菌(如长双歧杆菌、嗜热双歧杆菌)、乳酸菌(嗜酸乳杆菌、植物乳杆菌和罗伊氏乳杆菌)、芽孢杆菌(枯草芽孢杆菌)、酵母菌(酿酒酵母)等。

传统的监测益生菌在动物胃肠道定殖及饲喂益生菌在胃肠道存活情况的方法主要是通过采集粪便

样品进行分析益生菌数量, 或是通过在不同时间点宰杀实验动物, 解剖动物胃肠道器官, 对胃肠道部位内容物进行益生菌计数。然而粪便中益生菌的数量并不能真正反映该菌在动物胃肠道中的真正细菌增殖数, 而且通过解剖动物的方法也不能动态监测整个动物体内胃肠道益生菌移动和数量的变化和发展。最重要的一点是, 粪便中益生菌数量的检测无法辨别此菌是由动物饲喂摄入的还是来自于宿主本身胃肠道微生物定殖的益生菌。目前, 我们对益生菌发挥益生作用的机制还不清楚。益生菌被摄入后在胃肠道中如何黏附和定殖, 其在体内的菌群数量变化规律是什么, 是否增殖为优势菌群, 在胃肠道各个不同肠段部位中的分布情况是什么, 又对肠道内微生态产生怎么样的影响, 随着时间的变化有着怎么样的规律, 是否会在肠道内膜和上皮细胞上黏附和定殖, 是否进入小肠固有层和肠系膜淋巴结等, 这些相关

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问题微生物学家一直以来在不断地探讨和研究,但尚未得到解答^[3]。

因此,现代发展起来的基于光学成像的益生菌体内示踪技术对有益菌进行遗传标记,可为解释益生菌的益生作用机理提供参考。基于光学成像的益生菌体内示踪主要包括生物发光、传统荧光(荧光蛋白和荧光染料)和新型纳米材料等技术。近10年主要应用的是生物发光技术和荧光技术。两者的区别在于:生物发光是动物体内的自发光,不需要激发光源,一般需要底物的存在;而荧光发光不需要底物的参与,但需要外界激发光源的激发,从而发射出荧光,再通过检测器进行检测。纳米材料发光是近几年发展起来的新型益生菌示踪技术,主要包括长余辉纳米材料和上转换纳米材料等,运用纳米材料发光对益生菌体内分布进行示踪。本文主要评述了生物发光、传统荧光(荧光蛋白和荧光染料)和纳米材料3种技术各自在示踪益生菌上的应用。这些逐渐兴起的益生菌光学成像体内示踪技术具有无创性、可多次重复、实时活体成像、无放射性物质和安全等优势,势必会为益生菌的动态监测和作用机理研究提供良好的应用前景。

1 生物发光(bioluminescent imaging, BLI)示踪益生菌

生物发光指在活的生物体中,通过酶促反应发出可见光的现象,生物发光现象在细菌、昆虫、鱼、藻类等生物中广泛存在^[4]。发光反应的本质是酶促反应,在一定条件下,荧光素酶催化荧光素反应,并发出可见光(图1),发射光波可被高度敏感的电荷耦合CCD相机所检测^[5]。常用的方法是构建荧光素酶基因表达载体转化目标益生菌,并灌胃或注射到动物体内,观察时注入外源底物,目标益生菌即可发光,通过活体动物光学成像系统即可检测到生物发光。生物发光具有背景干扰小、无需创伤或损害生命、检测灵敏度高优势,广泛应用于食品、医药等研究中。目前已发现多种荧光素酶——荧光素发光反应体系,以下是常见的几种生物发光反应体系^[5](表1)。

1.1 细菌来源荧光素酶

自然界中存在许多可以发光的细菌,它们本身包含发光基因(*lux*),发光细菌表达的荧光素酶以长

图1 (网络版彩色)荧光素酶催化发光反应^[5]

Figure 1 (Color online) Luciferase-catalyzed luminescence^[5]

链脂肪醛和FMNH₂为底物,催化反应发出450~490 nm的蓝绿光。发光细菌多数来自于海洋,只有少数来自于陆地,目前已命名的发光细菌200多种,研究较多的发光细菌主要是明亮发光杆菌(*Photobacterium phosphoreum*)、费氏弧菌(*Vibrio fischeri*)、哈氏弧菌(*Vibrio harveyi*)、鳎鱼发光杆菌(*Photobacterium leiognathi*)等^[16,17]。当细胞含有细菌来源发光基因(*LuxCDABE*)时,在有氧条件下,无需添加底物,也无需外来光源的激发,细胞便能发出蓝绿光。

Cronin等人^[18]将细菌来源发光基因(*LuxCDABE*)导入双歧杆菌(*Bifidobacterium breve*),并在小鼠体内示踪监测,但发光量较小(细胞数量<10⁶^[19]),活体成像并未监测到发光信号,通过肠道体外成像才知双歧杆菌主要定殖于盲肠。de Andrés等人^[20]将*LuxCDABE*发光基因导入乳酸乳球菌(*Lactococcus lactis*)和唾液乳杆菌(*Lactobacillus salivarius*),在哺乳小鼠体内示踪其转移,用以研究母乳中微生物的组成。但细菌来源荧光素酶催化的发光反应发光波长较短,体内成像几乎检测不到的发光信号或发光信号较弱,只有定殖于肿瘤细胞,体内成像才可能监测到清晰的发光信号^[21],因此挖掘或改造得到发光信号更强的细菌来源荧光素酶基因,仍是研究的重点。

1.2 真核生物来源荧光素酶

真核生物来源荧光素酶主要指萤火虫(firefly)、叩头虫(click beetles)、海肾(renilla)等来源的荧光素酶,这些生物中包含有荧光素酶表达基因,可在一定的条件下,催化不同的底物发光。萤火虫来源荧光素酶以D-荧光素为底物,在ATP和Mg²⁺的共同作用下,催化反应发光;叩头虫来源荧光素酶的作用底物和条件与萤火虫的一致;而海肾来源荧光素酶以腔肠素(CTZ)为底物,部分发光反应受Ca²⁺浓度的影响。用真核生物来源荧光素酶基因作为标记基因虽然需要额外添加底物,但该反应不需要激发光,且发射波长较长,穿透组织成像效果较好,发光较稳定,在

表1 常用荧光素酶-荧光素反应体系^[5]

Table 1 Luciferases and luciferins commonly used in bioluminescence imaging^[5]

荧光素酶来源	底物	发光波长(nm)	反应特点	参考文献
细菌(LuxAB)	长链脂肪醛和还原型黄素单核苷酸(FMNH ₂)	450~490	操纵子LuxCDABE可以合成反应需要的底物,不需另外添加	[5~9]
萤火虫(Fluc), 叩头虫(CBRLuc/CBGLuc)	D-荧光素	537~620	需要ATP和Mg ²⁺ 的共同参与	[10,11]
海肾(RLuc)、Gaussia princeps (GLuc)、Aequorea Victoria 水母	腔肠素(CTZ)	470~487	发光效率高,部分反应的荧光素酶酶活受Ca ²⁺ 浓度影响	[9,10,12]
海萤(VLuc)	种虾素	460	常与其他发光系统共用	[9,10,13]
腰鞭毛虫	腰鞭毛虫素	—	发光反应受环境pH影响,中性条件下发光最好	[9,14,15]

体内成像应用中具有广阔前景. 目前已有较多真核生物来源荧光素酶在乳酸菌中表达,用以研究乳酸菌的益生特性,不同于荧光蛋白表达,荧光素酶表达常需根据目的菌株的密码子偏好性进行密码子优化,以提高表达量.

Lee等人^[22]通过Luc⁺标记乳酸乳球菌(*Lactococcus lactis*),首次在健康小鼠肠道中观测到发光的乳酸乳球菌,且发光信号维持2 h. 相同发光基因标记干酪乳杆菌(*Lactobacillus casei*),在小鼠体内示踪发现,口服摄入1 h后,干酪乳杆菌可通过胃部定殖于结肠和盲肠^[23].

Daniel等人^[24]将叩头虫(CBRLuc)、Gaussia(GLuc)和细菌(LuxCDABE)来源发光基因分别导入植物乳酸杆菌(*Lactobacillus plantarum*)和乳酸乳球菌(*Lactococcus lactis*)中,比较不同发光基因在两株益生菌中的发光特性,研究发现CBRLuc标记的菌株发光性最好(图2),通过单次和多次口服摄入2株益生菌,小鼠活体成像和肠道粪便成像可知,植物乳杆菌比乳酸乳球菌在小鼠肠道中定殖时间长,第6天在结肠和盲肠中仍能检测到发光植物乳杆菌. Karimi等人^[25]以CBRLuc发光基因标记罗伊氏乳酸杆菌(*Lactobacillus reuteri*),小鼠体内示踪罗伊氏乳酸杆

图2 乳酸乳球菌和植物乳杆菌在小鼠消化道中的转移。灌胃乳酸乳球菌-CBRLuc 10、45、90 和 240 min后,小鼠全身和肠道成像(1),灌胃乳酸乳球菌-Lux的小鼠成像(2),灌胃植物乳杆菌-CBRLuc的小鼠成像(3)。摄入益生菌前1 h灌胃D-荧光素底物,接种底物3 h后测发光信号^[24]

Figure 2 Transit of *L. lactis* and *L. plantarum* in the digestive tract of mice. One representative image of one mouse and its digestive tract are shown after 10, 45, 90 and 240 min in mice fed with *L. lactis*-CBRLuc (1), mice fed with *L. lactis*-lux (2), and mice fed with *L. plantarum*-CBRLuc (3). D-Luciferin was given intragastrically 1 h before administration of bacteria, and the bioluminescence signal was measured 3 h after inoculation of the substrate^[24]

菌,通过全身成像发现,摄入3 h后在胃里仍有较强的发光信号。

叩头虫来源红光发光基因(CBRLuc)发光波长较长,穿透组织能力强,发光时间久,是目前发现的最适合应运于体内成像的生物发光基因,对研究益生菌的免疫调节,抑制病原菌生长等功能具有重要作用。Daniel等人^[26]通过双荧光素酶标记法(CBRLuc和CBGLuc),在小鼠体内同时示踪植物乳杆菌和乳酸乳球菌,研究同时摄入两株益生菌,其在小鼠体内的相互作用;Bermudez-Brito等人^[27]利用CBRLuc标记植物乳杆菌,研究了植物乳杆菌对免疫调节的作用;Vågesjö等人^[28]以CBRLuc标记罗伊氏乳酸杆菌,研究了罗伊氏乳酸杆菌分泌物对伤口愈合的作用。

2 传统荧光示踪益生菌

传统荧光示踪技术指在外界激发光源的作用下,激发荧光物质到达高能量状态,然后发射出波长较长的发射光,传统荧光示踪技术主要包括荧光蛋白示踪和荧光染料示踪2种技术。

2.1 荧光蛋白示踪益生菌

自1962年,Shimomura等人^[29]首次从水母体中克隆出绿色荧光蛋白(green fluorescent protein, GFP)。

针对不同应用,通过对分离蛋白进行改造,研究人员现已研究出青色、橙色、红色等多种颜色荧光蛋白,如通过对绿色荧光蛋白和红色荧光蛋白(DsRed)进行突变,分别得到了发光性更好且更稳定的EGFP(enhanced green fluorescent protein)^[30],UKG^[31],Emerald^[32],Superfolder^[33]等绿色荧光蛋白和mRuby^[34],mCherry^[35],TagRFP 158T^[36],TagRFP^[37]等红色荧光蛋白。由于光穿透组织的能力主要取决于光的波长,波长越长,穿透组织的效果越好(波长>650 nm),在体内成像中,应用发射红色光谱的荧光分子效果更好^[38](表2)。荧光蛋白作为标记物,需要激发光才能使荧光基因达到较高的能量水平,然后发射出较长波长的发射光,此反应的背景光较强,但是发光强且稳定,不需要底物或其他条件的共同作用^[39,40],在体内成像应用中较广泛。荧光蛋白的表达不影响益生菌的生长及在动物体内的转移和定殖,对研究益生菌的功能特性具有重要意义。

2.1.1 绿色荧光蛋白

绿色荧光蛋白(GFP)的发现对生命科学的研究具有重要意义,钱永健先生在天然绿色荧光蛋白的基础上,对其进行了一系列的突变,得到了波长不同且发光强度更强的荧光蛋白,和其他2名科学家因此获得了2008年度诺贝尔化学奖^[48]。GFP蛋白无毒,在生

表2 部分红色荧光蛋白特性^[39]Table 2 Partial red fluorescent protein characteristics^[39]

荧光蛋白	激发峰(nm)	发射峰(nm)	量子产率	消光系数(M ⁻¹ cm ⁻¹)	发光强度(% DsRed)	参考文献
DsRed	558	583	0.79	75000	100	[41]
tdTomato	554	581	0.69	138000	160	[41]
mCherry	587	610	0.22	72000	27	[41]
mRaspberry	598	625	0.15	86000	22	[42]
TurboRFP	553	574	0.67	92000	104	[42]
RFP611	559	611	0.48	120000	97	[44]
mRuby	558	605	0.35	112000	66	[45]
AQ143	595	655	0.04	90000	6	[46]
IFP1.4	684	708	0.07	92000	11	[47]

物体中易表达且不影响生物体的正常功能,作为标记蛋白,其发光蛋白对目标蛋白的正常活性也没有影响,因此广泛应用于科学研究^[49]。

Yu等人^[50]以GFP标记德氏乳杆菌(*Lactobacillus delbrueckii*)和果糖乳杆菌(*Lactobacillus fructosus*),在鸡体内成像,示踪两株菌的定殖和转移,发现德氏乳杆菌可较长时间定殖于鸡肠黏膜表面。Wang等人^[51]以GFP标记乳酸乳球菌(*Lactococcus lactis*),在小鼠体内高表达GFP,示踪乳酸乳球菌,研究乳酸乳球菌在肠道中的定殖特性,发现乳酸乳球菌可通过小鼠的胃直达肠道。Han等人^[52]以GFP标记植物乳杆菌(*Lactobacillus plantarum*),研究羊口服植物乳杆菌在体内的通路,发现植物乳杆菌在羊体内主要定殖于羊的瘤胃,但不能定殖于十二指肠。

2.1.2 红色荧光蛋白

1999年首次报道红色荧光蛋白,其激发光和发射光的波长更长,可覆盖绿色荧光蛋白GFP不涉及的波长,穿透组织成像效果更好,背景光强较弱,在生物学研究中广泛应用^[49]。最早应用的红色荧光蛋白是从珊瑚中分离出来的DsRed,其发光强且稳定,但其荧光蛋白在细胞内表达时,会产生毒性,进行蛋白融合时,也易影响目标蛋白,科学家通过对其结构改造,已研究出了多种适合于不同成像技术的荧光蛋白^[39](表2),其中mCherry标记的红色荧光蛋白在益生菌研究中应用较广。

van Zyl等人^[53]以mCherry标记蒙氏肠球菌(*Enterococcus mundtii*)和植物乳杆菌(*Lactobacillus plantarum*),研究其在小鼠肠道中的转移和定殖,发现蒙氏肠球菌和植物乳杆菌主要定殖在结肠和盲肠(图3)。Russo等人^[54]以mCherry标记植物乳杆菌

(*Lactobacillus plantarum*)和发酵乳杆菌(*Lactobacillus fermentum*),研究2株益生菌在斑马鱼模型中的定殖,发现植物乳杆菌比发酵乳杆菌在斑马鱼体内的定殖能力强。

2.2 荧光染料示踪益生菌

在常规条件下监测有活性的或存活的微生物是比较困难的。平板计数技术是衡量细胞生存能力的标准方法,但并不能实时获得检测结果,于是用荧光染料来替代平板计数技术来测定细菌的生存能力,通过这些荧光染料对细胞进行染色标记,以实时监测细菌的活性和位置变化。目前运用于活体生物荧光成像的荧光染料包括香豆素类、萘酰亚胺类、罗丹明类等^[55-58]。

周佳等人^[59]报道了一种嘧啶类双光子荧光染料2-[(1E)-2-[4-(二甲氨基)苯基]乙烯基]-6-甲基-4(3H)-嘧啶,该染料与一般荧光染料相比有明显的优势,包括更大的组织穿透深度,较小的生物毒性和更稳定的发光性质。该染料在生物成像领域具有潜在的应用价值,并对复杂生理环境下具有稳定发光特性的双光子荧光染料的设计和合成有一定的指导意义。

Khoerunnisa等人^[60]设计合成出pH敏感型氟硼二吡(BODIPY)探针用于革兰氏阳性和革兰氏阴性菌的检测和成像(图4)。这一探针为两性离子荧光聚合物,在不同pH环境下有显著的荧光识别。因此,这种反应性聚合物是一种很有潜力的细菌检测方法。此外,如将这种两性离子聚合物黏附到载体上还可发展成一种便携式细检测装置。

尽管荧光染料研究较多,但因为其本身的设计缺陷,如光稳定性差、会出现光漂白现象等,而且生

图 3 植物乳杆菌 423 和蒙氏肠球菌 ST4SA 在小鼠体内的荧光成像。灌胃植物乳杆菌 423-pGKV223D 的小鼠荧光成像(a), 灌胃植物乳杆菌 423-cat-mCherry 的小鼠荧光成像(b), 灌胃蒙氏肠球菌 ST4SA-pGKV223D 的小鼠荧光成像(c), 灌胃蒙氏肠球菌 ST4SA-pCat-mCherry 的小鼠荧光成像(d)^[53]

Figure 3 Images produced by fluorescent cells of *L. plantarum* 423 and *E. mundtii* ST4SA in the abdomen. The mice were administered nonfluorescent *L. plantarum* 423 containing pGKV223D (a), fluorescent *L. plantarum* 423::cat-mCherry (b), nonfluorescent *E. mundtii* ST4SA containing pGKV223D (c), and fluorescent *E. mundtii* ST4SA containing pGKVnuc::cat-mCherry (pCat-mCherry) (d)^[S3]

物稳定性较差, 成像效果易受体内生物分子的影响。如何改善这些缺点是荧光染料研究的重点。荧光染料益生菌标记技术的改进或可参考以下几条思路: 将荧光染料进行表面功能化修饰, 以增加染料的生物稳定性; 结合两种或多种荧光染料制备成复合荧光染料, 以改善荧光染料的理化性质; 用纳米材料装载荧光染料, 可在增加荧光染料稳定性的同时还能提高荧光染料发光强度。

3 新型纳米材料示踪益生菌

基于纳米材料的光学生物成像技术由于其高度选择性、敏感性和非侵入性等优点,已广泛应用于生物医学领域^[61]. 进行光学生物成像的纳米材料主要包括长余辉纳米粒子、量子点纳米粒子、上转换纳米

粒子、适配体和磁性纳米粒子等^[62,63].

3.1 上转换材料

上转换材料能够在近红外光的激发下发射可见光. 镧系元素的掺杂使得这些纳米颗粒具有很宽的发射带, 并且无生物背景荧光、光稳定性强、细胞毒性低^[64]. 目前上转换材料已用来进行某些疾病诊断和治疗, 在生物成像和癌症诊断与治疗领域的报道很多^[65-67].

Li等人^[68]报道了DNA驱动的金上转换材料实时检测细胞内的microRNA, 检测灵敏, 选择性好. 这种上转换材料是细胞内分析的潜在生物探针, 若用于细菌的生物标记, 或为活体内的细菌示踪提供一种新的方法.

Jin等人^[69]报道了上转换纳米粒子与核酸适配体结合进行细菌检测的方法。该方法对目标细菌具有较高的专一性,能够快速灵敏地检测细菌、重金属和病毒,在环境监测、医学诊断和食品安全分析方面具有很大的潜力,并为益生菌检测提供了一条新的途径。

3.2 长余辉纳米材料

长余辉纳米材料成像是近几年来新兴起来的一种成像技术. 由于其成像光区在近红外光区, 长余辉纳米材料具有较强的穿透性, 在生物组织中的吸收也较少, 在体内成像中显现有明显的优势, 如无自荧光、低光漂白、信噪比高等^[70,71]. 将此材料进行功能化后, 在生物成像^[72]、疾病诊断^[73]和癌症治疗^[74]方面有广阔的发展前景.

Liu等人^[75]将Cr³⁺掺杂的铥镓锆(ZGGO)长余辉纳米材料用革兰氏阳性菌LTA抗体进行功能化,然后对罗伊氏乳杆菌进行标记,以监测其在口服后进入胃肠道的“行踪”,并通过动物光学成像来检测它们的生物分布(图5)。体内动物成像证明,经过纳米材料标记的罗伊氏乳杆菌经口服给药后能够实时、无损地检测罗伊氏乳杆菌在小鼠体内的分布。由于这种纳米材料尺寸可调节,能够重复激活,毒性低,光稳定性强,生物相容性好,可以长期对益生菌进行实时、无损检测。这一先进的功能化纳米材料为益生菌进入活体后的体内生物分布提供了一种新的视角,具有优良的实时监测和非损伤检测能力,并拓宽了基于先进功能化纳米材料的益生菌体内实时检

图4 在不同的pH条件下, 利用两性离子荧光聚合物BOD/BE-PSM对细菌进行检测^[60]

Figure 4 Scheme illustrating bacterial detection using zwitterionic fluorescent polymer BOD/BE-PSM under different pH conditions^[60]

图5 ZGGO纳米材料抗体探针在体内生物成像示意图^[75]

Figure 5 Schematic illustration of the utilization of the antibody ZGGO nanoprobe for orally administrated bioimaging^[75]

测和食品安全检测的思路。

Teston等人^[76]将细胞用具有长余辉性质和磁性的纳米材料进行标记, 在注射进生物体内后就可以进行实时追踪. 该方法结合了近红外光和磁靶向的优势, 从而保证材料具有长时间的示踪能力和稳定性. 若将此材料进行功能化也许能用于益生菌和其

他微生物的标记和体内示踪。

3.3 其他纳米材料

除了上述的光学纳米材料, 用于益生菌光学成像的纳米材料, 还包括生物传感器、磁性纳米材料、声学纳米材料等^[77~79].

RNA在细胞生理学中起着至关重要的作用,通过RNA适配体进行示踪来对细菌和哺乳动物细胞进行荧光成像,可以解决重要的生物学问题^[80,81]。然而传统的RNA适配体检测方法往往效果不好,很难满足检测的要求。在这种情况下,将纳米材料和适配体结合尤其重要。Sunbul等人^[82]将荧光探针与RNA适配体结合,以实时检测细菌的RNA转录,并对活细菌进行成像。这种模块化的RNA标记方法,可能发展成为不同颜色的、不同适配体的RNA适配体标记方法,并可能用于多重RNA适配体同时检测。

磁性纳米材料在磁共振成像、热疗和药物靶向输送等方面有巨大的作用^[83-85]。Martín等人^[86]报道了一种用氧化铁纳米粒子标记乳酸杆菌的方法,并且磁性在细菌分裂后还存在。与磁性细菌不同的是,这种人工磁化的细菌可以通过调整细菌培养条件和磁性纳米粒子的数量来调节细菌的磁性,磁性可进行量化,操作便捷。这种方法不仅适用于益生菌的体内成像,还适用于磁共振成像和药物输送等生物医学领域。

与传统成像方法不同,纳米材料进行生物成像有其独特的优势,如材料尺寸的可调节性,可根据不同的成像用途将纳米材料的尺寸进行设计;成像效果好,分辨率高;可进行多模态成像,方便科研人员和医务人员进行研究和疾病诊断等。虽然纳米材料益生菌标记技术潜力巨大,但目前的研究多是对纳米材料进行表面抗体修饰来标记益生菌,有可能存在标记脱落、标记稳定性差、标记时间短等问题。若能运用分子生物学方法利用细菌膜的内吞或将纳米材料导入到益生菌内甚至其细胞器中,那么这种标记方法的稳定性将大大增强。或者利用益生菌特有的酶、蛋白或其他特异性生物标志物将纳米材料在益生菌中原位合成,那么很容易将纳米材料导入益生菌内,而且对益生菌正常生理活性的影响会更小。

4 总结与展望

基于光学成像的益生菌体内示踪技术可通过对同一组实验动物对象在不同时间点进行记录检测,示踪益生菌在体内胃肠道的转移及变化,使得传统的分子和微生物学技术由体外转移到体内研究,避

免了在不同时间点处死动物和采集粪便等体外检测的烦琐,且无创伤性,提高了效率,所得的数据真实可靠、灵敏度高。生物发光、传统荧光、新型纳米材料3种技术示踪益生菌都有各自的优点和劣势。

生物发光技术背景干扰小,但是发光强度较弱,近几年通过对叩头虫来源红光发光基因(CBRLuc)的深入研究,用CBRLuc基因标记益生菌进行体内示踪,成为研究的热点^[24,25,27],但对于长时间的示踪监测,该技术仍有一定的局限性,挖掘发光波长更长,发光稳定的生物发光基因仍是研究的重点。传统荧光标记示踪益生菌,发光强且可持续发光,目前已有较多报道以绿色荧光蛋白或红色荧光蛋白标记益生菌进行体内示踪监测^[53,87],但该技术监测背景光较强,成像效果不理想,因此仍需改造开发新型荧光蛋白,满足不同发光成像需要。新型纳米材料荧光成像主要包括量子点、上转换材料、长余辉纳米材料等,其中长余辉纳米材料在停止激发后的发光现象甚至能维持几天,使得益生菌实时检测又有了新的成像技术^[75]。与传统荧光成像技术相比,由于不需导入荧光蛋白基因,纳米材料荧光成像的应用范围可能更广,但纳米材料仍存在成本较高和毒性等问题,发展低成本、无毒或毒性较小的纳米材料将成为最活跃的研究领域之一。

如何解决不同发光技术存在的问题,将是科研人员研究的方向。实验者可根据不同的研究目的,选择不同的示踪技术,同时还可根据发光物质的不同发光波长,同时示踪2种或多种益生菌,如以CBRLuc(620 nm)和CBGLuc(540 nm)分别标记植物乳杆菌和乳酸乳球菌,在小鼠体内同时示踪2种益生菌^[26];此外还可结合2或3种不同的发光技术,共同示踪益生菌,弥补不同报告系统的缺点,更加灵敏、持久的示踪益生菌,研究不同益生菌在体内的相互作用,及其在体内的转移和定殖等功能特性,这种双发光或多报告系统示踪益生菌将是今后研究的重点。

随着光学成像技术的发展,将会有越来越多的具有发光强度高、稳定性好的荧光物质或材料应运而生。相信基于光学成像的益生菌体内示踪技术在以后的医学、分子生物学、微生物学等科学领域会有很大的应用前景。

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Summary for “基于光学成像的益生菌体内示踪技术”

Tracer technology of probiotics based on optical imaging

Jin Wang^{1†}, Ping Li^{2†}, Ning Zhao¹, Zhihao Wang¹, Jingmin Liu¹ & Shuo Wang^{1*}¹ School of Medicine, Tianjin Key Laboratory of Food Science and Health, Nankai University, Tianjin 300350, China;² School of Food Engineering and Biotechnology, Tianjin University of Science and Technology, Key Laboratory of Food Nutrition and Safety, Ministry of Education, Tianjin 300457, China

† Equally contributed to this work

* Corresponding author, E-mail: wangshuo@nankai.edu.cn

Probiotics refers to live microorganisms, which is beneficial for host health mainly by adjusting the gastrointestinal tract flora, improving intestinal epithelial barrier function and regulating immune function. Common probiotic strains include *Bifidobacteria* (such as *Bifidobacterium longum*, *Bifidobacterium thermophilus*), *Lactobacillus* (such as *Lactobacillus acidophilus*, *Lactobacillus plantarum* and *Lactobacillus reuteri*), *Bacillus* (such as *Bacillus subtilis*), yeast *Bacteria* (such as *saccharomyces*) etc. In the past, probiotics were counted by analyzing the number of bacteria in feces or dissecting animals' gastrointestinal tracts at different times. The action mechanism of probiotics is still unclear. Genetic labeling of probiotics by modern biological techniques is one of important research methods and it provides a basis for researching the mechanisms of probiotics. Tracer technology of probiotics is based on optical imaging, which includes bioluminescence, traditional fluorescence (fluorescent protein and fluorescent dye) and nanomaterials fluorescence. The main applications in the past decade are bioluminescence technology and fluorescence technology. The difference between them is that bioluminescence refers to self-luminescence. It catalyzes the substrate reaction and emits visible light by expressing and secreting different sources of luciferase. This reaction does not require exciting light. The luciferase derived from bacteria does not require the addition of substrate through expressing *LuxCDABE*. The luminescent reaction catalyzed by luciferase derived from fungus has a longer luminescence wavelength and is more permeable to tissue for imaging, but this reaction requires the substrate. Fluorescence is the expression of different fluorescent proteins, which emit fluorescence under the stimulation of exciting light. This reaction does not require the participation of the substrate, but requires external exciting light, so the background interference is relatively intense. Nanomaterial luminescence is a new type of probiotic tracer technology in recent years. The fluorescence imaging of new nanomaterials mainly includes quantum dots, upconversion nanomaterials and persistent luminescence nanomaterials. Persistent luminescence nanomaterials can even maintain the luminescence phenomenon for a few days after stopping the excitation. Compared to traditional fluorescence imaging technology, fluorescent imaging of nanomaterials may have more extensive applications, due to the lack of introduction of fluorescent protein genes. However fluorescent imaging of nanomaterials still has some disadvantages, such as high cost and toxicity etc. The characteristics, advantages and disadvantages of the three techniques are reviewed in order to provide theoretical and reference basis for further development and research on the tracing technology of probiotics.

probiotics, bioluminescence, fluorescence, nanomaterials

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