

魏斯氏菌的研究进展

Recent progress of *Weissella*

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摘要:对魏斯氏菌属的分离鉴定、生物学特性、耐酸特性、抑菌特性、产胞外多糖特性、益生特性、组学研究及其在食品与医药方面的应用进行了综述,并为魏斯氏菌属的开发利用提供了建议。

关键词:魏斯氏菌;分离鉴定;特性研究;组学研究

Abstract: Summarizes *Weissella* spp. including the isolation and identification, biological properties, acid tolerance, bacterial inhibition, extracellular polysaccharide production, probiotic properties, histological studies, and food and pharmaceutical applications, and provides suggestions for the development and utilization of *Weissella* spp.

Keywords: *Weissella*; isolation and identification; characterization; histological studies

魏斯氏菌是一种乳酸菌,分布广泛。其不仅能产生细菌素、有机酸等抗菌物质,还能产生胞外多糖,因而在医药、食品等行业的关注度较高^[1]。研究^[2]表明,魏斯氏菌能够缩短食品发酵进程并对食品风味物质的产生起到贡献作用,其中食窦魏斯氏菌和融合魏斯氏菌还具有抑制牙周病的潜力。魏斯氏菌还是一种潜在的益生菌^[3],但有些魏斯氏菌也会引发疾病,Flaherty等^[4]研究案例显示融合魏斯氏菌会引发心内膜炎,Liu等^[5]从患病的虹鳟鱼身上分离出6株魏斯氏菌。研究主要从魏斯氏菌的生物学特性、耐酸特性、抑菌特性、产胞外多糖特性、益生特性等性质进行阐述,并对其应用情况进行概括,以期对魏斯氏菌的开发应用提供理论依据。

基金项目:吉林省科技发展计划技术创新引导项目(编号:20220402053GH);国家自然科学基金地区科学基金项目(编号:31660452)

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收稿日期:2021-12-26 **改回日期:**2022-05-13

1 魏斯氏菌的分离鉴定

魏斯氏菌属于厚壁菌门(*Firmicutes*)、芽孢杆菌纲(*Bacilli*)、乳杆菌目(*Lactobacillales*)、明串珠菌科(*Leuconostocaceae*)、魏斯氏菌属(*Weissella*)^[1]。截至目前已被分离和鉴定的魏斯氏菌共有25个种,分别是独魏斯氏菌(*Weissella soli*)、*Weissella diestrammenae*、*Weissella koreensis*、坎氏魏斯氏菌(*Weissella kandleri*)、大米魏斯氏菌(*Weissella oryzae*)、食窦魏斯氏菌(*Weissella cibaria*)、融合魏斯氏菌(*Weissella confusa*)、*Weissella thailandensis*、赫伦魏斯氏菌(*Weissella hellenica*)、类肠膜魏斯氏菌(*Weissella paramesenteroides*)、鲸魏斯氏菌(*Weissella ceti*)、*Weissella halotolerans*、绿色魏斯氏菌(*Weissella viridescens*)、微小魏斯氏菌(*Weissella minor*)、葡萄魏斯氏菌(*Weissella uvarum*)、*Weissella beninensis*、雁魏斯氏菌(*Weissella fabalis*)、*Weissella fabaria*、*Weissella ghanensis*^[6]、*Weissella cryptocerci*^[7]、*Weissella bombi*^[8]、*Weissella jogaejeotgali*^[9]、*Weissella muntiaci*^[10]、*Weissella sagaensis*^[11]和*Weissella coleopterorum*^[12-13]。通过查询NCBI网站,利用MAGA软件构建了魏斯氏菌的系统发育树(图1)。

由于魏斯氏菌与明串珠菌的生物学特性非常相似,且共同存在于多种发酵食品中,因此通过表型难以区分魏斯氏菌与明串珠菌。Zamudio-Maya等^[14]通过在MRS培养基中加入2,3,5-三苯基氯化四氮唑(TTC)的方式从沿海沼泽沉积物中分离出单一菌落,并根据菌落表型进行分类鉴定,共鉴定出37个菌落,分属7种不同的表型,其中魏斯氏菌在2种表型中具有较好的同源性。目前尚未探索出能够精准筛选出魏斯氏菌的培养基。

现今许多学者利用分子技术对魏斯氏菌进行分类,如核糖体DNA扩增片段限制性内切酶分析(ARDRA),随机扩增多态性DNA标记(RAPD),扩增片段长度多态性(AFLP)等技术^[15]。Adesulu-Dahunsi等^[16]通过ITS-

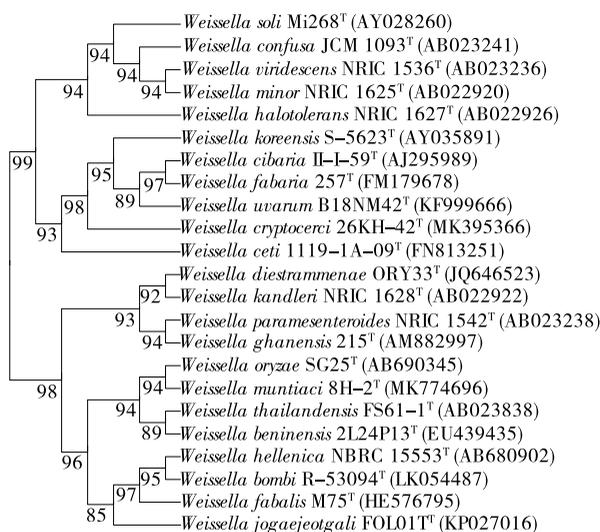


图 1 魏斯氏菌的系统发育

Figure 1 Phylogenetic tree of *Weissella* bacteria

PCR 和 ARDRA 技术,对植物乳杆菌、戊糖片球菌和融合魏斯氏菌进行了区分,结果表明 ITS-PCR 和 ARDRA 技术可用于乳酸菌的常规鉴定和快速区分。

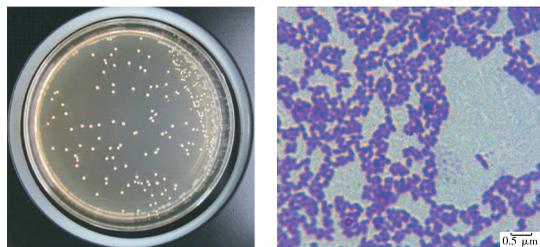
2 魏斯氏菌的特性

2.1 生物学特性

魏斯氏菌是一种异型发酵乳酸菌,其 G+C 含量为 37%~47%,在显微镜下呈现不规则短棒状,常以成对或短链形式存在,菌落形态通常表现为微隆起的光滑不透明的白色小圆点(见图 2)^[17-20]。魏斯氏菌呈革兰氏阳性、过氧化氢酶阴性,无芽孢形成,在兼性厌氧环境中生长情况最佳。大部分魏斯氏菌最适的生长 pH 为 3.0~9.5,最适生长温度为 30~37 °C^[6,20-21]。魏斯氏菌可发酵葡萄糖、麦芽糖、蔗糖等糖类,通过磷酸己糖和磷酸酮酶途径异型发酵葡萄糖产生乳酸^[1]。并能够以碳水化合物为发酵底物来维持生长,可产生细菌素、过氧化氢、胞外多糖和双乙酰等生物活性物质^[15,22-25]。综上,魏斯氏菌在食品、医药等领域有着巨大的开发潜力。

2.2 产酸特性

魏斯氏菌具有良好的产酸特性,有助于提高发酵制

图 2 魏斯氏菌的菌落形态和显微状态^[20]Figure 2 The colony and micrograph shape of *Weissella*

品的品质,改善发酵制品的风味。Cennet 等^[2]从传统鹰嘴豆发酵面团中分离出大量的融合魏斯氏菌,并发现它产生的大量丁酸和乙酸乙酯不仅能保持面团的特性还能改善面团香气。Lee 等^[26]对泡菜的微生物发酵特性进行研究,发现魏斯氏菌能利用丙酮酸和乙酰磷酸发酵碳水化合物形成乳酸、醋酸、二氧化碳和乙醇,这些物质能够有效改善泡菜的风味。Mercha 等^[27]从摩洛哥骆驼奶中分离出融合魏斯氏菌、食窦魏斯氏菌等菌株,发现这些菌株均能快速酸化,对胃液和胆盐的耐受性均较高,有助于其在乳制品行业的应用。

2.3 抑菌特性

魏斯氏菌发酵产生的某些抗菌物质能够直接抑制病原菌的生长,如细菌素、有机酸等,还可以通过与病原菌竞争定植位点,调节细菌环境平衡等方式起到抑菌作用。单核细胞增多性李斯特菌、金黄色葡萄球菌、沙门氏菌、大肠杆菌等都是较为常见的病原菌,许多研究都将其作为指示菌进行抑菌试验。Teixeira 等^[28]从巴西不同地区生产的乳制品及生产环境当中分离筛选出 26 株菌株,其中大多菌株对常见致病菌表现出拮抗作用,最低抑制率为 60%。其中 20 株菌株能够通过有机酸抑制靶标,12 株能产生双乙酰。Yu 等^[23]对从泡菜中分离出的 2 株食窦魏斯氏菌进行了益生菌特性及抗菌潜力研究,发现它们对常见病原菌也均有较高的抗黏附活性,表现出中等的生长抑制作用。赵冬兵等^[29]从南京板鸭中筛选出 1 株希腊魏斯氏菌,在 pH 2~12 时均有较好的抑菌效果,对革兰氏阳性菌有明显的抑制作用,具有作为生物防腐剂的潜力。综上,魏斯氏菌能够通过抑制细菌生长在一定程度上保证食品安全。

细菌素主要由乳酸菌生产,被认为是安全的天然抗菌剂和防腐剂^[30]。关于细菌素的研究,Fusco 等^[1]进行了相关总结(见表 1)。Tenea 等^[3]分离出 1 株产细菌素菌株——融合魏斯氏菌 Cys2-2,发现其产生的细菌素对大肠杆菌、沙门氏菌和志贺氏菌具有明显的抑制作用,作用机理为通过破坏靶细胞膜的完整性发挥抑菌作用。Lee 等^[36]对食窦魏斯氏菌 CMU 进行研究,发现其对金黄色葡萄球菌有抑制作用,且能够改善口腔内细菌环境,减少牙龈沟的微生物数量,降低患龋齿的风险,改善口臭,提高整体口腔健康。Xia 等^[37]从马粪中分离出的 4 株融合魏斯氏菌对马常用抗生素的敏感性各不相同,但均表现出耐药性,能成功抑制肠炎沙门氏菌、大肠杆菌和金黄色葡萄球菌的生长。Dey 等^[38]研究发现融合魏斯氏菌 DD_A7 能够限制耐药多药 ESBL 阳性大肠杆菌生长,且具有减少宿主细胞中炎症细胞因子的能力。这些研究表明魏斯氏菌能够通过产生抗菌物质,调节细菌环境,有效抑制致病菌,在食品和医药方面均有着很好的应用潜力。

2.4 产胞外多糖特性 胞外多糖具有凝胶、增稠、乳化等多种功能,还可以用作稳定剂^[39-40]。魏斯氏菌能产生大量的胞外多糖^[41],但胞外多糖的产量可能会受到环境因素的影响。

表 1 魏斯氏菌细菌素的类别、名称、对细菌素具有活性的生物体

Table 1 Bacteriocinogenic *Weissella* strains, class, name, organisms against which the bacteriocins were active and relevant reference

细菌素名称	分类	生产菌株	敏感指标菌株	参考文献
Weissellicin 110	Unclassified	<i>W. cibaria</i> 110	<i>Lactobacillus sakei</i> JCM 1157, <i>L. sanfranciscensis</i> JCM 5668, <i>L. homohiochii</i> JCM 1199, <i>L. coryniformis subsp. coryniformis</i> , JCM 1164, <i>L. acetotolerans</i> JCM 3825- <i>Weissella halotolerans</i> JCM1114, <i>W. kandleri</i> JCM 5817, <i>W. paramesenteroides</i> JCM 9890, <i>Leuconostoc lactis</i> JCM 6123	[31]
Weissellin A	Class IIA	<i>W. Paramesenteroides</i> DX	<i>Bacillus cereus</i> LMG13569, <i>Clostridium sporogenes</i> NCTC533, <i>C. thiaminolyticum</i> ATCC15579, <i>Enterococcus faecalis</i> NCTC8176, <i>Lactobacillus brevis</i> ATCC8287, <i>L. bulgaricus</i> LMG13551, <i>L. casei</i> ATCC344, <i>L. curvatus</i> ATCC51436, <i>L. jensenii</i> ATCC25258, <i>L. plantarum</i> CECT220, <i>L. sakei</i> CECT906T, <i>Lactococcus lactis</i> LM0230, <i>Lact. lactis</i> ATCC11454, <i>Lact. lactis</i> IL1403, <i>Lact. lactis subsp. cremoris</i> MC1363, <i>Leuconostoc mesenteroides</i> ATCC19254, <i>Listeria innocua</i> ATCC BAA-680D, <i>List. monocytogenes</i> ATCC19111, <i>Micrococcus luteus</i> CECT241, <i>Pediococcus acidilactici</i> ATCC25740, <i>P. pentosaceus</i> ATCC 33316, <i>P. pentosaceus</i> LMG13560, <i>Staphylococcus carnosus</i> LMG13564	[32]
Weissellicin L	Unclassified	<i>W. hellenica</i> 4-7	<i>L.monocytogenes</i> ATCC 19111, <i>L. sakei subsp. sakei</i> JCM 1157, <i>L. bulgaricus</i> ATCC 11842, <i>W. paramesenteroides</i> ATCC33313, <i>W. hellenica</i> ATCC 51523, <i>W. viridescens</i> ATCC 12706, <i>S. thermophilus</i> ATCC 19258	[33]
Weissellicin D	Unclassified	<i>W. hellenica</i> D1501	<i>L.lactis</i> ssp. <i>lactis</i> , <i>Lactobacillus fermentum</i> ATCC 14931, <i>Lb. sake</i> , <i>Lb. plantarum</i> 70810, <i>Lb. bulgaricus</i> ATCC 7830, <i>Lb. helveticus</i> Mb2-1, <i>Lb. paracasei</i> , <i>Lb. curvatus</i> , <i>Lb. brevis</i> , <i>Pediococcus pentosaceus</i> CGMCC1.2695, <i>Streptococcus thermophilus</i> CGMCC1.6472, <i>Staphylococcus aureus</i> ATCC 6538, <i>Bacillus subtilis</i> ATCC 6633, <i>B. cereus</i> ATCC 11778, <i>Pseudomonas aeruginosa</i> , <i>Listeria monocytogenes</i> CMCC 54004, <i>Micrococcus luteus</i> CMCC28001, <i>Saccharomyces cerevisiae</i> ATCC 26603, <i>Debaromyces hansenii</i> ATCC 4143, <i>Kluyveromyces marxianus</i> , <i>Candida albicans</i> CMCC 28001, <i>Mucor</i> CICC 2521	[34]
Weissellicin M	Unclassified	<i>W. hellenica</i>	<i>aL. lactis</i> ssp. <i>lactis</i> ATCC 19435T, <i>L. lactis</i> ssp. <i>lactis</i> NCDO 497, <i>Lactobacillus sakei</i> ssp. <i>sakei</i> JCM 1157T, <i>Lb. plantarum</i> JCM 1149T, <i>Weissella cibaria</i> JCM 12495T, <i>W. hellenica</i> JCM 10103T, <i>W. paramesenteroides</i> JCM 9890T, <i>W. confusa</i> JCM 1093T, <i>Pediococcus pentosaceus</i> JCM 5885, <i>P. dextrinicus</i> JCM 5887T, <i>P. acidilactici</i> JCM 8797T, <i>Enterococcus faecium</i> JCM 5804T, <i>E. durans</i> NBRC 100479T, <i>E. faecalis</i> JCM 5803T, <i>Streptococcus bovis</i> JCM 5802T, <i>Str. dysgalactiae</i> ssp. <i>Dysgalactiae</i> JCM 5673, <i>Bacillus coagulans</i> JCM 2257T, <i>B. circulans</i> JCM 2504T, <i>B. subtilis</i> ssp. <i>subtilis</i> JCM 1465T, <i>B. cereus</i> JCM 2152T, <i>Kocuria rhizophila</i> NBRC 12708, <i>Listeria innocua</i> ATCC 33090T, <i>Leuconostoc mesenteroides</i> ssp. <i>mesenteroides</i> JCM 6124T	[35]
Weissellicin Y	Unclassified	QU 13		

魏斯氏菌产生的胞外多糖具有良好的黏度、增稠、稳定等性能,因而在食品应用中具有良好的前景。Kim 等^[42]从泡菜中分离出赫仑魏斯氏菌 SKkimchi3,其产生的胞外多糖中的 β -葡聚糖可以用作安全的食品添加剂。Galli 等^[22]从鹰嘴豆酸面团中分离出 1 株融合魏斯氏菌 Ck15,其产生的胞外多糖能够提高面团的黏度,为提高豆类面粉在烘焙产品中的利用提供了一种潜在的途径。有学者研究环境因素对胞外多糖产量的影响,以此优化胞外多糖制备条件。Hu 等^[43]研究了温度对食窦魏斯氏菌 10M 产胞外多糖的影响,发现在 20 °C 时寡糖产量较高,在 15 °C 时葡聚糖产量最大。陈媛等^[44]采用响应面法对食窦魏斯氏菌 SJ-02 产胞外多糖的发酵条件进行优化,结果表明以 MRS 为基础培养基,用 1.5% 大豆蛋白胨替代蛋白胨,同时菌株接种量为 3%、在 37 °C 下培养 34 h 为最优条件,产量可提高 17%。Lakra 等^[45]研究发现食窦魏斯氏菌 MD2 产生的果聚糖有助于提高线虫的抗氧化能力和延长线虫的寿命,为开发功能性乳酸菌食品提供了思路。

此外,魏斯氏菌产生的胞外多糖还具有抑菌、益生等特性。姜静等^[46]从自制水果发酵液中得到高产胞外多糖的融合魏斯氏菌 H2,产量为(40.21±3.63) g/L,且产生的胞外多糖具有良好的乳化性、抑菌性能和抗氧化活性。Zhao 等^[47]发现融合魏斯氏菌 XG-3 产生的胞外多糖为葡聚糖,该胞外多糖具有良好的抗氧化活性、热稳定性以及刺激益生菌生长的能力。Kibarab 等^[48]研究结果表明由食窦魏斯氏菌 EIR/P2 产生的葡聚糖具有抗氧化、抗菌特性,对生物膜的形成有 70% 的抑制作用,能提高人类牙周韧带成纤维细胞(hPDLFCs)的活力。基于以上特性,食窦魏斯氏菌 EIR/P2 产生的葡聚糖有可能成为促进牙周愈合和再生的天然药物。

魏斯氏菌属在产胞外多糖方面有着突出的表现,对于食品以及医药方面均有很大的应用潜力,但目前对胞外多糖产量的提高及胞外多糖理化性质的研究相对较少。

2.5 益生特性

益生菌是一类通过合理摄取后能够对机体产生有益作用的微生物,其主要功能是调节肠道稳态,保持人体肠道环境健康^[49]。魏斯氏菌因其抗菌活性及对胃肠道的耐受性,成为一种潜在益生菌^[50-51]。

Yu 等^[52]发现,食窦魏斯氏菌 JW15 有较好的胃肠道耐受性,且对肠上皮细胞有黏附性,对病原体具有抑制活性,还能够通过提高自然杀伤细胞(NK)活性增强人体免疫功能,是一种优良的益生菌。李文等^[53]研究结果表明绿色魏斯氏菌 ZY-6 和希腊魏斯氏菌 WS-419 均显示出较好的耐酸性和胆盐耐受性。Kinjal 等^[54]从不同水果中分离出的 2 株菌株 FX5 和 FX9 能在低 pH 环境下存活,且表现出很强的抗菌活性,具有潜在的益生菌特性。Lakra 等^[55]从发酵面糊中分离得到的融合魏斯氏菌 MD1 和食

窦魏斯氏菌 MD2 在人工胃肠道环境中均表现出较强的生存能力、抑菌活性和抗氧化活性,对常规抗生素均无溶血活性和敏感性。Anandharaj 等^[56]从发酵的黄瓜中分离出 3 株魏斯氏菌,它们能够降低胆固醇含量。以上研究均说明魏斯氏菌具有成为益生菌的潜力。

目前对魏斯氏菌益生作用的研究和应用相对较少,但现有研究表明魏斯氏菌是一种潜在的益生菌,存在很大的研究空间。

2.6 其他

Li 等^[57]研究发现绿色魏斯氏菌 ZY-6 具有毒性,可以通过吸附去除 3 种蔬菜和水果汁中 69.45%~79.91% 的镉,因此绿色魏斯氏菌 ZY-6 可作为环境、人类饲料和动物饲料中镉的有效生物吸附剂。农药的过度使用会对生态系统以及人类健康造成影响,Hamoud 等^[58]探究了融合魏斯氏菌对毒死蜱农药的耐受力和降解能力,该菌株对 200 μ g/mL 毒死蜱的耐受性良好,在含不同浓度毒死蜱的无葡萄糖 MRS 培养基中能够显著生长。该菌株还能够耐受胃肠道环境,对病原菌有较好的抑菌活性,对毒死蜱农药具有解毒作用。Lee 等^[59]发现食窦魏斯氏菌生物转化的乳清蛋白显著降低了 3T3-L1 细胞中甘油三酯的积累。魏斯氏菌不仅在食品和医药上有着良好的前景,在农业等方面的研究前景也非常广阔。

3 魏斯氏菌的组学研究

随着技术的发展,人们利用组学技术对菌株进行了更加深入的分析。截至 2021 年 10 月,完成全基因组测序并上传至 NCBI 网站的魏斯氏菌菌株有 23 种,基因组大小多数在 2 Mb 左右,GC 含量为 37%~47%,蛋白质基因数量为 1 000~3 000。

Wang 等^[60]从人类粪便中分离和纯化了 31 个菌株,利用基质辅助激光解吸电离飞行时间质谱(MALDI-TOF MS)和 16S rRNA 基因测序相结合的方法进行鉴定,对获得的菌株进行一系列试验,最终筛选出 3 株潜在的益生菌菌株,鉴定为融合魏斯氏菌。Mun 等^[61]通过基因组学对 *W. koreensis* SK 菌株的表型和基因组特征进行了鉴定,发现 *W. koreensis* SK 可以利用精氨酸替代碳源来获取能量。Mänberger 等^[62]对食窦魏斯氏菌菌株 92 的基因组进行了分析,发现了与低聚木糖、阿拉伯低聚糖和 β -葡萄糖苷酶运输、水解和代谢相关的基因簇。Jeong 等^[63]对 *W. koreensis* 进行了基因组学、代谢组学和转录组学的分析,发现 *W. koreensis* 对万古霉素具有内在抗性,并含有潜在的溶血素基因。Quattrini 等^[64]对 23 个食窦魏斯氏菌和 7 个融合魏斯氏菌的基因组进行了比较分析,结果发现它们没有专门的致病基因,这项研究对今后魏斯氏菌的开发与利用提供了良好的依据。

利用各种组学手段对魏斯氏菌进行研究能够更加深入地解析其生理和代谢机制,挖掘出与耐酸、抑菌、产胞

外多糖等重要性状相关的功能基因,为魏斯氏菌的高效利用和规模化生产提供帮助。

4 魏斯氏菌的应用

魏斯氏菌是重要的发酵菌种,可用于各种发酵蔬菜、奶酪、酱油、发酵鱼制品的生产,在食品领域具有重要的应用价值。Benhoua 等^[30]将分离出的融合魏斯氏菌 W4 应用到酸奶发酵生产中,发现酸奶的凝乳质地得到了改善,且表现出与工业添加剂相同的效果。徐亚洲等^[65]在灭菌后的泡菜原材料中接种了食窦魏斯氏菌 NCU034005,发现该菌能使发酵泡菜具有独特的风味,且与自然发酵泡菜风味差异显著,可作为泡菜风味改良的菌种。曲红叶等^[66]通过温度梯度驯化和添加相容性溶质驯化,获得了可耐低温的护色食窦魏斯氏菌,制备出高活力的直投式护色食窦魏斯氏菌产品,可应用在低温腌制肉制品中。这些研究为魏斯氏菌在食品方面的应用提供了强有力的支持,将魏斯氏菌作为发酵剂应用到食品中,除了能够缩短发酵进程,提高食品风味以外,还可作为食品添加剂和功能性食品对其进行开发。

魏斯氏菌在医药方面也有着良好的应用潜力。融合魏斯氏菌 DD_A7 具有在宿主细胞中减少炎症细胞因子的能力^[33],Kibarab 等^[48]研究表明食窦魏斯氏菌 EIR/P2 产生的胞外多糖为葡聚糖,50 mg/mL 的葡聚糖表现出杀菌效果,对生物膜的形成有 70% 的抑制作用。此外,通过 MTT 分析发现葡聚糖在提高人牙周膜成纤维细胞分化能力方面有一定功效。结合 2.4 中提到的研究结果可知,食窦魏斯氏菌有抗氧化、降低胆固醇、抑菌、改善口腔健康等功能。这些研究为魏斯氏菌在医药方面的应用提供了强有力的支持。

5 结语

魏斯氏菌具有多种生物学特性,因而成为食品、医药等领域的研究热点。近年来,对于魏斯氏菌的研究多集中于分离鉴定、所产成分的特性分析和制备条件优化,对于耐酸、抑菌、产胞外多糖等相关功能基因的研究鲜有报道,魏斯氏菌作为潜在益生菌在实际应用开发方面仍有很大的空间。后续可以通过基因组学、转录组学以及多组学联用的方法深入地研究相关功能的基因及机制。

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