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专论与综述

土壤中微生物植酸酶的活性及其提高方法与应用

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摘　要：磷是有限不可再生资源，土壤缺磷是植物生长和农作物生产的主要限制因子之一。无机磷肥施入土壤后，极易被土壤固相吸附或与金属阳离子形成难溶性络合物或转化为有机磷，导致其生物可利用性降低。土壤磷主要以有机磷形式存在，占比 20%−80%。有机磷又以植酸(盐)为主要成分，占比约 50%。植酸不可被植物直接吸收利用，需在专一性酶植酸酶作用下经脱磷酸化水解释放磷供植物吸收。土壤植酸酶主要来源于微生物，易受温度、pH、土壤吸附、钙含量及钙磷比、底物含量和有效性等影响，导致酶活降低甚至失活。如何保持或提高土壤中植酸酶活性，进而提高土壤内源植酸磷的利用率，对降低外源磷肥施加和保障农业生产具有重要意义。本文综述微生物植酸酶的来源、分类与作用机制及土壤中植酸酶活性的影响因素，重点阐述保持或提高其活性的方法及实际应用效率。针对土壤植酸酶活性低和稳定性差的问题，对通过调控最适 pH 范围、提高热稳定性、将植酸酶负载于纳米材料和基因工程改造等改善植酸酶性质的方法进行展望。综述内容可为理解土壤中植酸酶活性的影响因素，进而提高土壤内源植酸磷的利用效率提供理论依据和技术参考，对减少外源磷肥施用、降低磷流失和土壤面源/水体污染风险及保障农业可持续发展具有一定的现实意义。

关键词：微生物；植酸；植酸酶；酶活性；影响因素；应用效率

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Microbial phytase activity in soils and methods to improve the activity and application: a review

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Abstract: Phosphorus (P) is a finite and non-renewable resource. Soil P deficiency is a main limiting factor for crop growth and agricultural production. Once entering into soils, inorganic P (P_i) in fertilizers is readily being fixed by soil solid phase, binds to metal ions to form the insoluble complex, or transform into organic P (P_o) which fails to be directly uptake by plants. Soil P mainly exists as P_o, which accounts for 20%–80% of total P. Phytic acid or phytate is the predominant form of P_o, accounting for about 50% of P_o. Phytate can not be directly uptake by plants, while can release P via dephosphorylation by the specific enzyme phytase. Phytase is mainly secreted by microbes and its activity is affected by temperature, pH, soil adsorption, Ca content, Ca/P ratio and substrate concentration and availability, leading to reduced activity or inactivation. Therefore, it is of great importance for reducing the application of exogenous P fertilizer and ensuring agricultural production to understand how to maintain or improve phytase activity in soils and improve the utilization of soil endogenous phytate. Thus, this paper reviews the sources, classification, and function mechanisms of microbial phytase and influencing factors of soil phytase activity, focusing on methods to maintain or improve phytase activity and the application efficiencies. In terms of the low activity and poor stability, methods to improve phytase properties including adjusting optimal pH range, improving thermal stability, loading phytase on nano-materials, and genetic engineering, are proposed. As such, this review is expected to help better understand the influencing factors of phytase activity in soil and provide theoretical and technical supports to improve endogenous phytic acid utilization efficiency. This is of great importance to reduce the application rate of P fertilizer, P loss, and risk of soil non-point source/water pollution and ensuring agricultural sustainable development.

Keywords: microbe; phytate; phytase; enzyme activity; influencing factor; application efficiency

磷(P)是植物生长发育必需的大量营养元素，影响植物生理生态，也是农业生产的限制性元素[1]。植物主要从土壤内源磷库和外源磷肥中获得磷。磷肥的主要生产原料磷矿石是有限不可再生资源[2]。磷肥进入土壤后极易被土壤强烈吸附，形成难以被植物利用的形态，在土壤中稳定存在，仅约 1%可被植物直接吸收利用[3]。由于磷肥利用率低，为保障农作物营养和产量，通常施用过量磷肥，长期施肥导致磷在土壤中大量积累，农田系统中磷流失可引起潜在水体污染风险[4]。

土壤有机磷是土壤磷的重要组成部分，土壤有机磷占总磷的 20%−80%[5]，是植物生长所需磷的重要供给源[6]。土壤有机磷主要为植酸(phytate)、磷脂和核酸等，其中植酸磷占比 50%−80%[7]。植酸磷不能被植物直接吸收利用[8]，

但研究表明，以植酸磷作唯一磷源时植物能正常生长，说明植酸磷是植物的潜在磷源[9]。土壤中植酸主要来源于动植物残体分解和无机磷转化。通常，植酸主要存在于谷物和种子中，是种子萌发所需磷和肌醇的储备库，种子中植酸磷占总磷的 80%[10]。每分子植酸含有 6 个磷酸基团和 12 个可解离质子，易螯合二价金属阳离子 Ca^{2+}、Mg^{2+}、Fe^{2+}、Zn^{2+}、Cu^{2+}等，形成难溶性或不溶性植酸盐络合物[11]，降低植酸和矿质元素的生物可利用性[12]，因此植酸是一种抗营养物质。土壤中植酸盐需在专一性酶植酸酶(phytase)的作用下，通过脱磷酸化水解反应释放无机磷[13]，但植酸酶酶活易受环境因子影响，导致水解效率低和土壤可利用态磷缺乏[14]。

植酸酶是一种磷酸单酯水解酶，是催化植酸及其盐类水解为肌醇与磷酸(盐)的一类酶的总称。植酸酶含量和活性被认为是影响土壤植酸盐水解和有效态磷含量的关键因素[10]。此外，植酸盐水解也可释放螯合的金属矿质元素，可同时为植物提供磷素和矿质元素，促进植物生长[15-16]。已发现微生物和植物根系均可分泌植酸酶，然而，土壤微生物和植物植酸酶含量与植物生长发育无显著相关性[17]，表明植酸酶进入土壤后其活性受土壤环境因子的显著影响。此外，植酸酶的水解效率也受水解底物——植酸盐的种类和有效性的影响。土壤植酸酶主要来源于微生物，产植酸酶的微生物主要包括真菌、细菌和酵母菌[18]。微生物植酸酶虽已进行工业化生产，但由于植酸酶种类及酶学性质等差异性造成其实际应用的局限性，主要表现为热稳定性差和活性低。如何提高植酸酶在土壤中的催化活性已引起关注，主要包括获得热稳定性植酸酶、提高酶在土壤中的稳定性和酶活性。本文基于前期研究，综述微生物植酸酶的来源与分类、水解作用机制及土壤中植酸酶活性的影响因素(温度、pH、土壤吸附、钙含量及钙磷比、底物浓度、含量和有效性等)，重点阐述保持或提高土壤中植酸酶活性的方法技术(纳米材料负载植酸酶、提高植酸酶 pH 适应范围、定点突变等)及实际应用效率，以期为微生物植酸酶应用于农业生产和提高土壤难利用态植酸磷的利用效率提供理论基础和技术参考，对降低外源磷肥施用和环境磷污染具有重要的现实意义。

1 微生物植酸酶的来源与分类

1.1 来源

植酸酶广泛分布于自然界，在动物、植物和微生物中均已发现，动物植酸酶含量低，植物植酸酶易受加工、贮存过程影响且难以提纯，而微生物植酸酶由于生长周期短、pH 和温度适应范围广(2.0−8.5，20−80 ℃)、活性高(811−1 800 U/mg)、易于生产、产量高且分离提纯较容易等，是植酸酶最易得和最经济的来源[19-20]。土壤中植酸酶主要由微生物分泌产生，主要包括真菌中的曲霉属(*Aspergillus*)，如黑曲霉(*Aspergillus niger*)、烟曲霉(*Aspergillus fumigatus*)、米曲霉(*Aspergillus orgaze*)、土曲霉(*Aspergillus terms*)、无花果曲霉(*Aspergillus ficuum*)等，以及青霉菌属(*Penicillium*)和毛霉菌属(*Mucor*)均可分泌高活性的胞外植酸酶[21-22]。其中，黑曲霉(*A. niger*)和烟曲霉(*A. fumigatus*)具有较强的植酸酶生产能力，被认为是植酸酶的主要生产菌[23]。例如，黑曲霉(*A. niger*)植酸酶活性可达 50−103 U/mg，具有较宽的 pH 适宜范围(2.5−7.5，最适 pH 为 4.6)，较好的热稳定性(25−70 ℃，最适温度为 60 ℃)[24-25]。烟曲霉(*A. fumigatus*)植酸酶活性为 23−28 U/mg[25]，具有较宽的 pH 适宜范围(2.5−8.0)、较好的热稳定性(50−90 ℃，最适温度 70 ℃)和较广泛的底物特异性(与一系

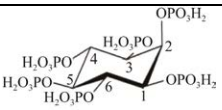
列在结构上与植酸相似的磷酸盐化合物，如苯基磷酸盐、对硝基苯基磷酸盐和磷酸烯醇丙酮酸均具有活性、易水解植酸为肌醇 2-单磷酸，中间产物无大量积累和易水解肌醇 1-单磷酸)[26]。细菌植酸酶通常在胞内，但肠杆菌属(*Enterobacter*)、芽孢杆菌属(*Bacillus*)和克雷伯氏菌属(*Klebsiella*)可产生胞外植酸酶[25,27-28]，如大肠杆菌(*Escherichia coli*)、枯草芽孢杆菌(*Bacillus subtilis*)和土生克雷伯氏菌(*Klebsiella terrigena*) (表 1)。其中大肠杆菌(*E. coli*)胞外植酸酶活性可达 811−1 800 U/mg、最适 pH 值为 4.5，最适温度 65 ℃[27]。枯草芽孢杆菌(*B. subtilis*)植酸酶活性为 9−15 U/mg，最适 pH 为 7.0，最适温度为 55 ℃[29,51]。微生物植酸酶由于具有较高的水解活性且易得，已通过生物技术进行广泛商业化生产[18]。

1.2 分类

植酸酶种类可根据不同标准进行分类，根据最适 pH 可分为酸性植酸酶(acidic phytase)和碱性植酸酶(alkaline phytase)；根据植酸水解位点特异性可分为 3-植酸酶(EC 3.1.3.8)、6-植酸酶(EC 3.1.3.26)和 5-植酸酶(EC 3.1.3.72)[52]；根据蛋白结构和催化机理可分为组氨酸植酸酶(histidine acid phosphatase phytase, HAP)、β-螺旋桨植酸酶(β-propeller phytase, BPP)、半胱氨酸植酸酶(cysteine phosphatase phytase, CP)和紫色酸性磷酸酶植酸酶(purple acid phosphatase phytase, PAP)[53] (表 1)。

表 1 微生物植酸酶的来源与分类

Table 1 Sources and classification of microbial phytase

微生物植酸酶种类 Microbial phytase species	微生物植酸酶来源 Microbial phytase sources	最适 pH Optimum pH	最适温度 Optimum temperature (℃)	结构特征 Structure characteristics	催化位置 Catalytic sites
3-植酸酶 (EC 3.1.3.8) 3-phytase	土生克雷伯氏菌[28] *K. terrigena*[28]	5.0	58	 由肌醇环和 6 个磷酸基团通过磷酸单酯键连接构成[31] Consisting of an inositol ring and 6 phosphate groups bound by a phosphate monoester bond[31]	植酸酶的植酸降解途径，肌醇依次从 C3、C4、C5、C6、C1 位游离磷酸基团生成 I(2)P[32] In phytic acid hydrolysis pathway by phytase, inositol generates I(2)P from free phosphate groups at C3, C4, C5, C6 and C1 sites[32]
	黑曲霉[24] *A. niger*[24]	4.6	60		
	烟曲霉[29] *A. fumigatus*[29]	7.5	40		
	卡氏德巴利酵母[30] *Debaryomyces castellii* CBS 2923[30]	–	–		
5-植酸酶 (EC 3.1.3.72) 5-phytase	反刍兽新月形单胞菌属[33] *Crotonomonas ruminants*[33]	5.0−5.5	45		植酸酶依次从植酸 C5、C4、C6、C3、C1 位释放磷酸基团，终产物为 I(2)P[33] Phytase releases phosphate groups from C5, C4, C6, C3 and C1 of phytic acid in order. The final product is I(2)P[33]
6-植酸酶 (EC 3.1.3.26) 6-phytase	隔孢伏革菌[34] *Peniophora lycii*[34]	5.0−5.5	45		肌醇从植酸 C6、C5、C4、C6、C3、C1 位释放磷酸基团，终产物为 I(2)P[35-36] Inositol releases phosphate groups from C6, C5, C4, C6, C3 and C1 of phytic acid and the final product is I(2)P[35-36]
组氨酸酸性磷酸酶植酸酶	无丙二酸柠檬酸杆菌[37] *Citrobacter amalonaticus* CGMCC 1696[37]	4.5	55	具有保守催化基元 RHGXRXP 和 HD[32]	氨基酸序列由 N-末端基元 RHGXRXP 和 C-末端基元 HD 组成，正确折叠时这两个远距离基元会聚形成 1 个特异的催化中心，起动两步反应水解磷酸单酯[40]

(待续)

(续表 1)

微生物植酸酶种类 Microbial phytase species	微生物植酸酶来源 Microbial phytase sources	最适 pH Optimum pH	最适温度 Optimum temperature (℃)	结构特征 Structure characteristics	催化位置 Catalytic sites
Histidine acid phosphatase phytase (HAP)	奥默柯达酵母菌[38] *Kodamaea ohmeri* BG3[38]	5.5	65	Conserved catalytic units RHGXRXP and HD[32]	The amino acid sequence consists of an N-terminal unit RHGXRXP and a C-terminal unit HD. When correctly folded, these two remote units converge to form a specific catalytic center and initiate a two-step hydrolysis of phosphate monoester[40]
	微细正青霉[39] *Eupenicillium parvum* BCC 17694[39]	5.5	50		
	大肠杆菌[27] *Escherichia coli*[27]	4.5	65		
β-螺旋桨植酸酶 β-propeller phytase (BPP)	枯草芽孢杆菌[29] *Bacillus subtilis*[29]	7.0	55	由 β-折叠片组成，类似六叶螺旋桨[42] Composed of β-sheets, similar to a six-bladed propeller[42]	BPP 具有 2 个不对称磷酸基团结合部位，“切割部位” (cleavage site, CS)和“亲和部位” (affinity site, AS)。AS 促进植酸分子结合，而 CS 负责水解磷酸基团[43] BPP has two asymmetric phosphate binding sites, ‘Cleavage site’ (CS) and ‘Affinity site’ (AS). AS promotes binding of phytic acid molecules while CS is responsible for hydrolysis of phosphate groups[43]
	解淀粉芽孢杆菌[41] *Bacillus amyloliquefaciens*[41]	7.0−8.0	70		
半胱氨酸磷酸酶植酸酶 Cysteine phosphatase phytase (CP)	反刍兽新月形单胞菌[44] *Selenomonas ruminantium* SrPf6[44]	2.6−6.0	50−55	氨基酸序列包含活性部位基元 HCXXGXXR (T/S)，并与 CP 类的蛋白酪氨酸磷酸具有相似性[45] The amino acid sequence contains the active site motif HCXXGXXR (T/S) and is similar to CP tyrosine phosphates[45]	形成一个含保守半胱氨基酸(C241)的磷酸基团结合环(phosphate-bindingloop, P-loop)，其中 C241 不可逆氧化使 P-loop 由非活性的开放构象转变为活性的闭合构象[46] Forms a phosphate-binding loop (P-loop), containing a conserved cysteic amino acid (C241), in which irreversible oxidation of C241 converts the P-loop from an inactive open conformation to an active closed conformation[46]
紫色酸性磷酸酶植酸酶 Purple acid phosphatase phytase (PAP)	烟草(*Nicotiana tabacum*)根系分泌 PAPhy[47] *N. tabacum* root-exudated PAPhy[47]	5.0−5.5	45	7 个残基(D、D、Y、N、H、H、H)包含在 5 个保守基元(DXG/GDXXY/GNH(ED)/VXXH/GHXH)中[50] 7 residues (D, D, Y, N, H, H, H) contained in 5 conserved units (DXG/GDXXY/GNH (ED)/VXXH/GHXH)[50]	保守基元(DXG/GDXXY/GNH(ED)/VXXH/GHXH)[50] Conserved units (DXG/GDXXY/GNH(ED)/VXXH/GHXH)[50]
	拟南芥[48] *Arabidopsis thaliana*[48]	4.5	25−37		
	新洋葱伯克霍尔德菌[49] *Burkholderia cenocepacia*[49]	−	−		

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2 植酸酶的作用机理

植酸酶可专一性水解植酸分子中的磷酯键，水解过程分步进行，逐个释放磷酸基团，始于完全磷酸化的六磷酸肌醇(IP6)，过程中形成肌醇五磷酸酯至肌醇一磷酸酯中间产物(IP5−IP1)，终产物为肌醇和磷酸根[54]。

不同来源植酸酶的水解作用机理不同，起始水解酯键不同。微生物 3-植酸酶首先从植酸的第 3 碳位点开始水解酯键释放无机磷，继而依次释放其他碳位点磷，最终酯解植酸分子，作用过程为：植酸→D/L-1,2,4,5,6-五磷酸肌醇→D/L-1,2,5,6-四磷酸肌醇→D/L-1,2,5-三磷酸肌醇、D/L-1,2,6-三磷酸肌醇、D/L-2,5,6-三磷酸肌醇→D/L-2,5-二磷酸肌醇、D/L-1,2-二磷酸肌醇、D/L-2,6-二磷酸肌醇→2-磷酸肌醇[35,55] (图 1)。

来源少数细菌的 5-植酸酶可优先从植酸的第 5 碳位点开始水解酯键释放无机磷，其作用过程为：植酸→1,2,3,4,6-五磷酸肌醇→D/L-1,2,3,4-四磷酸肌醇、D/L-1,2,3,6-四磷酸肌醇→D/L-1,2,3-三磷酸肌醇→D/L-1,2-二磷酸肌醇→2-磷酸肌醇[55] (图 2)。

6-植酸酶主要来源于植物，在担子菌中也有报道[32,56]。6-植酸酶首先在植酸的第 6 碳位点开始水解催化而释放无机磷，其作用过程为：植酸→L-1,2,3,4,5-五磷酸肌醇、D-1,2,4,5,6-五磷酸肌醇→L-1,2,3,4-四磷酸肌醇、D-1,2,5,6-四磷酸肌醇→L-1,2,3-三磷酸肌醇、D-1,2,6-三磷酸肌醇→D/L-1,2-二磷酸肌醇→D/L-1-磷酸肌醇、D/L-2-磷酸肌醇→肌醇[55] (图 3)。

3 土壤中植酸酶活性的影响因素

植酸酶活性以一定 pH 值(5.5)和温度(37 ℃)条件下每分钟从植酸底物中释放无机磷的含量来表征，表示为 U。土壤微生物植酸酶活性因分子结构不同而产生差异[57]，且不同来源植酸酶活性的影响因素也存在较大差异。微生物植酸酶活性与植物磷营养无显著相关性，表明植酸酶进入土壤后其活性降低，主要影响因素为温度、pH 值、土壤吸附、钙含量及钙磷比、底物种类、含量和有效性等[58]。

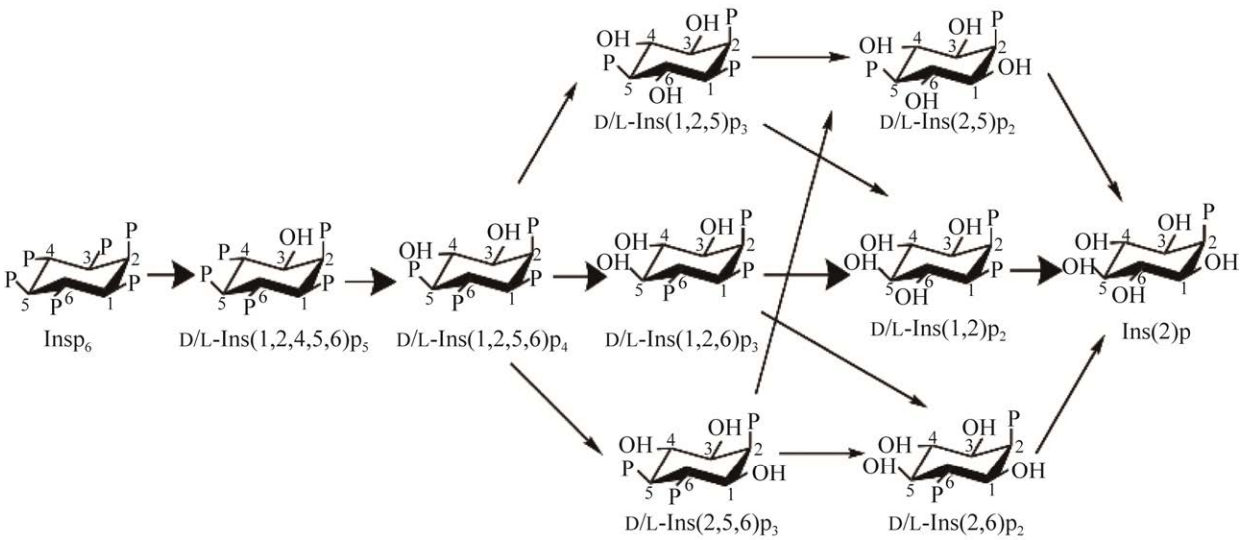

图 1 3-植酸酶对植酸的脱磷酸化过程

Figure 1 Dephosphorylation of phytate by 3-phytase.

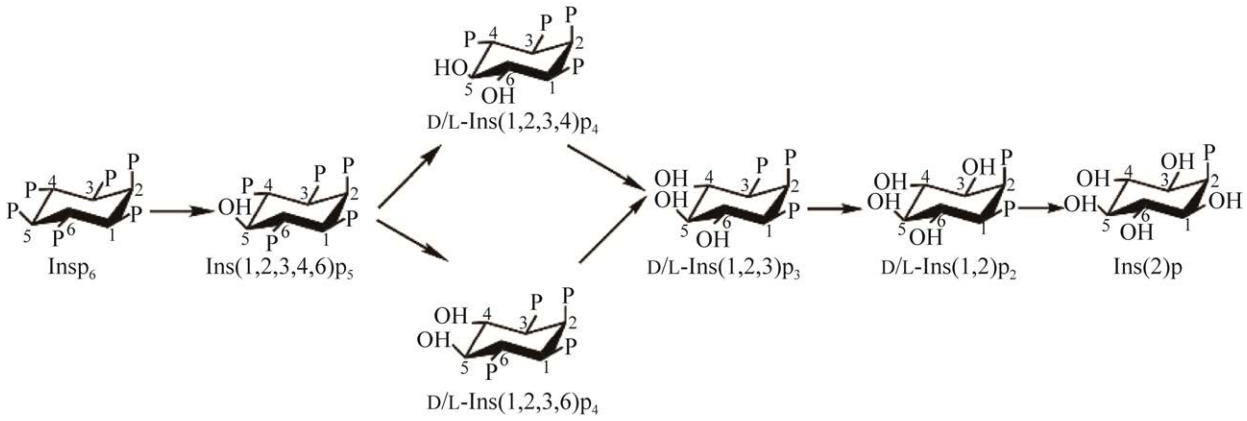

图 2　5-植酸酶对植酸的脱磷酸化过程

Figure 2　Dephosphorylation of phytate by 5-phytase.

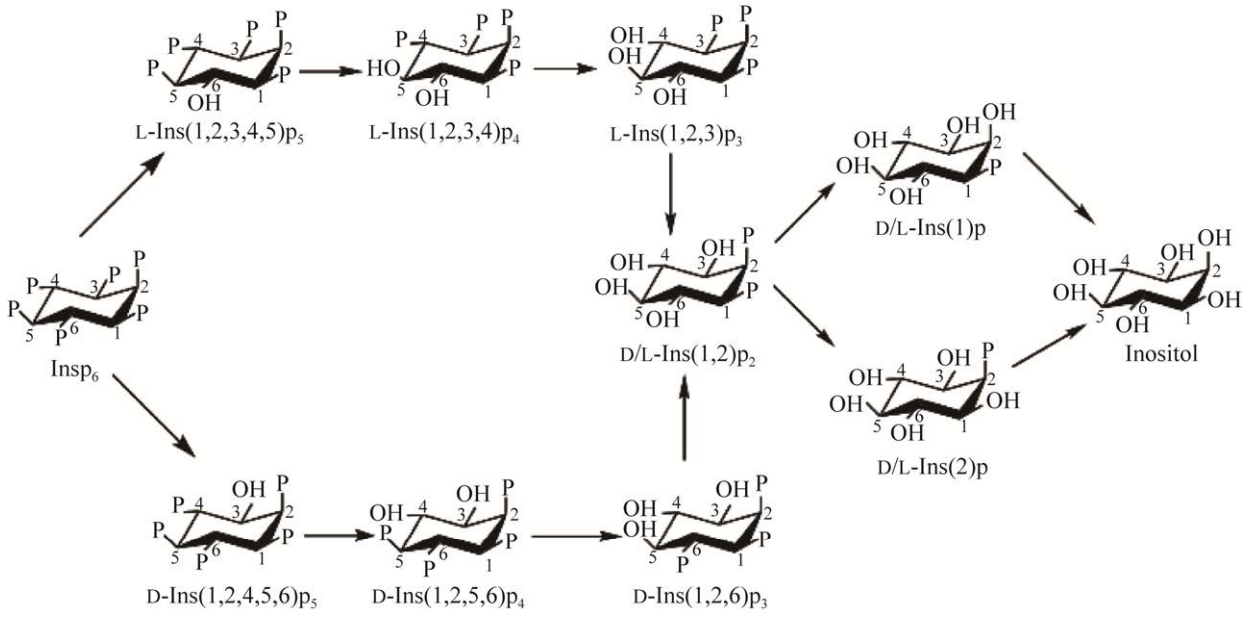

图 3　6-植酸酶对植酸的脱磷酸化过程

Figure 3　Dephosphorylation of phytate by 6-phytase.

3.1　温度

温度是影响植酸酶活性的关键因素[59]。植酸酶一般在 25−70 ℃范围内具有活性[60]，最适温度一般为 44−60 ℃[61]。不同来源植酸酶的活性对温度适应性差异较大。植物性植酸酶最适温度为 45−60 ℃[62]，微生物植酸酶一般为 45−55 ℃[63]，其中土生克雷伯氏菌(*K. terrigena*)植酸酶最适温度为 37 ℃和 55 ℃[64]，但土壤放线菌(*Actinomycete*)胞外植酸酶最适温度为 70 ℃[65]。在一定温度范围内，酶活性随温度升高而提高，但当温度升高到一定值(80 ℃)时，酶活性降低至失活。例如，无花果曲霉(*A. ficunm*)植酸酶于 80 ℃加热 3 min 后酶活降低了 82%，加热 5 min 后其活性降为 0[66]。尹德胜等[67]研

究表明，pH 7.8 条件下，无花果曲霉(*A. ficunm*)植酸酶最适温度为 50 ℃，50−55 ℃范围内活性较高(>90%)，65 ℃时酶活性降至 70%，70 ℃时降至 55%。Wyss 等[68]研究表明，75 ℃时烟曲霉(*A. fumigatus*)和黑曲霉(*A. niger*)的植酸酶活性降至 63%−73%，85 ℃时降至 31%−51%。也有报道指出，黑曲霉(*A. niger*) 9701 植酸酶具有较好的热稳定性，90 ℃加热 15 min 后仍保留 75%的酶活性，30 min 后酶活仍可保持 84%[28]。烟曲霉(*A. fumigatus*)植酸酶基因在黑曲霉(*A. niger*) NW205 中表达后，获得的重组酶在 100 ℃加热 20 min 后酶活仅损失 10%，具有极好的热稳定性[69]。

3.2 pH 值

植酸酶 pH 值活性范围一般为 2.0−8.5，通常植酸酶活性最佳的 pH 值范围为 4.5−6.0[70]。不同来源植酸酶的最适 pH 值差异较大。植物性植酸酶活性 pH 值范围为 4.0−6.0[71]，最适 pH 值为 5.5，<4.0 或>7.0 植酸酶基本失活[62]，而豆科植物种子、百合花粉、香蒲植酸酶在 pH 8.0 时仍具有活性[17]。微生物植酸酶 pH 活性范围较宽，黑曲霉(*A. niger*)植酸酶具有较宽的 pH 适宜范围(2.5−7.5，最适 pH 值为 4.6)[24]。无花果曲霉(*A. ficunm*)植酸酶最适 pH 值为 7.8，pH 9.0 时酶活降至 7%[67]。Greiner[72]研究表明，成团泛菌(*Pantoea agglomerans*)植酸酶在 pH 2.5−7.5 和温度 4 ℃条件下较稳定，最适 pH 值为 4.5，10 d 时酶活性仍可保留 95%以上(pH 4.5)，但 pH<2.0 或 pH>8.0 时酶活性迅速下降，如 pH 1.5 时剩余酶活为 85%，pH 9.0 时酶活在 24 h 内降低了 70%。

3.3 土壤吸附

土壤中植酸酶的稳定性和活性受土壤颗粒/有机矿物表面吸附的影响[73]，植酸酶的吸附可降低其对底物的亲和力，从而降低其有效活性。例如，将植酸酶添加到土壤中后，其活性在 8 min 内迅速下降至初始活性的 25%以下，并且活性随时间的延长持续下降[74]。黑曲霉(*A. niger*)游离植酸酶活性为 80% (pH 4.5−5.5)，土壤吸附后酶活降至 37.3%[75]。将黑曲霉(*A. niger*)植酸酶与针铁矿和赤铁矿混合，植酸酶活性随时间延长显著降低，吸附 24 h 后酶活降低了 50%[76]，吸附后植酸酶发生构象变化从而导致部分或全部发生不可逆失活。通常情况下，土壤对植酸酶的吸附在 pH 4.5 时最高，随着 pH 升高，吸附逐渐减少，pH 7.5 时植酸酶完全存在于土壤溶液中[77]。植酸酶稳定性和活性受土壤类型和土壤矿物组成的影响。例如，吸附 28 d 后，沙质土壤中植酸酶活性可保留 40%，而黏土中活性仅为 5%[77]。此外，被黏土矿物吸附后，植酸酶活性最适 pH 值升高[78]，因最适 pH 值变化进而影响植酸酶在土壤中的稳定性和活性，导致对植酸盐的水解效率发生变化[79]。

3.4 钙含量及钙磷比

Ca^{2+}维持细胞结构稳定性和细胞内离子平衡，作为重要的第二信使偶联胞外信号与胞内生理反应，是植物代谢和生长发育的主要调控因子[80]。植酸酶基因呈 Ca^{2+}端依赖催化特性，但高 Ca^{2+} (5 mmol/L)抑制植酸酶的活性，10 mmol/L Ca^{2+}导致植酸酶完全失活，产生这一现象的原因为过量 Ca^{2+}占据酶的活性位点，阻碍植酸酶与植酸底物的接触和反应[81]。研究表明，植酸酶活性受 Ca^{2+}质量浓度的影响，当 Ca^{2+}质量浓度为 1%−2%时酶活较低(445−450 U/mL)，Ca^{2+}质量浓度为 3%时酶活最高(480 U/mL)，Ca^{2+}质量浓度为 4%时酶活降为 420 U/mL[82]。类似地，当 Ca^{2+}质量浓度增加至 0.1%、0.4%和 0.9%时，植酸酶活性分别降低了 52%、67%和 85%[83]，表明随 Ca^{2+}质量浓度升高，Ca^{2+}对植酸酶产生竞争性抑制，因此高质量浓度 Ca^{2+}不利于植酸酶活性发挥。钙磷摩尔比为 1.1:1−1.4:1

时植酸酶活性较高，提高钙磷摩尔比导致植酸酶活性降低[84]。例如，钙磷摩尔比由 1.4:1 提高至 2:1 时，植酸酶活性降低了 7.4%[85]；钙磷摩尔比由 2:1 降至 1.1:1 时，植酸酶活性提高了 5%−12%[86]；钙磷摩尔比由 2.1:1 降至 1.2:1 时，植酸酶活性提高了 16%[87]。产生该现象的可能原因是：(1) 钙与植酸可形成植酸钙沉淀或钙-植酸络合物[88]，导致植酸可利用性和有效性降低，影响植酸酶对植酸的水解效率[87]，从而导致植酸酶活性降低；(2) 高钙竞争植酸酶的活性位点，直接降低植酸酶的活性和水解效率。

3.5 底物种类、含量和有效性

植酸酶活性受水解植酸盐底物种类、含量和有效性的影响。Greiner[72]通过对 14 种底物的水解效率(37 ℃, pH 4.5)分析发现，成团泛菌(*P. agglomerans*)植酸酶对不同底物的活性存在差异，其中对植酸、葡萄糖-1-磷酸酯、氢化钠-焦磷酸盐和 1-磷酸萘酯表现出较高活性，米氏常数 K_m 分别为 0.34、0.26、0.98 和 1.10 mmol/L，酶转化数 k_{cat} 分别为 21、101、15 和 6.1 s^{-1}，总催化效率 k_{cat}/K_m 分别为 61 765、384 615、15 406 和 5 596 L/(mmol·s)。此外，植酸酶活性受底物浓度的影响，酶促反应速率与底物浓度密切相关，当酶浓度、温度和 pH 恒定、底物浓度较低(0−4.8 mmol/L)时，酶促反应速率与底物浓度呈正相关，底物浓度提高至 4.8−6.6 mmol/L 时反应速率基本无变化，维持平稳状态；随底物浓度继续升高，酶促反应速率增幅下降，底物浓度达到一定临界浓度(6.6 mmol/L)时反应速率下降，原因是植酸酶被底物浓度饱和，达到反应平衡，表明高底物浓度对植酸酶的催化活性具有不利影响[89]。

土壤中植酸盐的活动性和有效性也对植酸酶的水解效率具有一定影响，土壤中植酸盐的有效性受有机质、黏土类型、pH 和金属氧化物等因素的影响[90-91]。其中，黏土类型影响植酸的吸附强度，如伊利石、高岭石和蒙脱石、针铁矿[92]、赤铁矿[93]、氢氧化铝对植酸具有很强的吸附作用[94]，植酸在针铁矿上的吸附量显著高于无机磷(3.8−12.7 *vs*. 2.4−4.6 μmol/m^2)，其与无结晶铝氧化物的结合诱导形成稳定的植酸铝沉淀($\log_{10}K_{13-16}$=8.84−20.1)[95-96]。植酸与重金属离子的络合作用表现为 Al^{3+}>Fe^{3+}>Mg^{2+}>Fe^{2+}>Ca^{2+}[97]，其稳定常数 $\log_{10}K_{13-15}$ 分别为 12.2−20.1、8.89−18.2、8.76−10.5、7.71−10.5 和 8.3−8.4[98]。此外，随 pH 值升高，植酸吸附量则降低。例如，pH 4.5 时植酸在铁氢化物表面的吸附量为 2.12 μmol/m^2[99]，pH 值升高至 6.5 时吸附量降低了 50%[96]，这是因为植酸与 Fe、Al、Ca 和 Mg 的吸附/络合反应受 pH 影响，酸性(pH 5.0)条件下易与 Fe/Al 形成沉淀，碱性条件(pH 7.5)下易与 Ca/Mg 形成沉淀[79,100-101]。

4 提高植酸酶活性的方法及其应用

4.1 纳米材料负载植酸酶

纳米材料负载植酸酶可降低土壤颗粒对植酸酶的吸附，提高植酸酶在土壤溶液中的稳定性，使其发挥活性。近几年的研究表明，羟基磷灰石(hydroxyapatite, HA)作为一种无机固体材料，具有良好的生物相容性、强亲和力和无毒等特点，可制成纳米颗粒，通过快速吸附将植酸酶负载固定，固定率近 100%且负载后酶活未损失[102]。HA 中 Ca^{2+} 与酶的羧酸基团螯合反应迅速，产生高度稳定的固定化酶，对植酸酶在土壤中发挥活性具有一定应用前景。与游离植酸酶相比，固定化/负载植酸酶在一定 pH 和温度范围内可保持较高活性，在高温下可保持较高的稳定性。例如，相较于游离植酸酶，负

载酶在pH 3.0时活性由40%提高至90%,pH 7.0时活性由23%提高至38%；80−90 ℃时，游离植酸酶发生变性，20 min内完全失活，而负载植酸酶在80 ℃、3 h和90 ℃、20 min时，活性仍可保持60%和40%[103]。纳米材料负载植酸酶目前主要应用于饲料添加剂以提高饲料中植酸磷的利用率,减少家禽(特别是单胃动物)未消化植酸磷向环境中的排放[104] (表2)。后续可考虑将纳米材料负载植酸酶应用于土壤，提高植物/作物对土壤植酸磷的利用率，以降低外源磷肥施加，同时降低土壤和水环境的磷污染风险。

近年来，石墨烯在生物工程中的应用取得一定进展，在立体选择性和生物溶解度方面表现出一定优势，纳米技术和生物技术结合交叉表现出一定应用前景。氧化石墨烯(graphene oxide, GO)具有单层Sp2杂化结构,可用于有机生物大分子的固定化(表 2)。GO 纳米载体具有较大比表面积，相较于游离植酸酶，负载植酸酶活性在42 ℃和85 ℃分别提高40倍和20倍，GO固定化技术已用于开发生产耐热植酸酶[105]。

此外，壳聚糖包埋和纳米纤维负载也可提高植酸酶在土壤中的稳定性和活性(表2)。例如，钱浩[106]通过体外壳聚糖包埋植酸酶纳米颗粒，发现负载酶的适宜温度变广，70 ℃加热30 min后，游离植酸酶活性为21.4%，而负载酶活性为63.3%；90 ℃时，游离植酸酶失活，负载酶活性仍可保持11.4%。有研究发现，与游离植酸酶相比，纳米纤维负载酶的活性提高，米氏常数K_m由34 μmol/L提高至56 μmol/L，最大反应速率V_{max}由120 μmol/(min·mg)提高至401 μmol/(min·mg),但最适pH和温度无显著变化；纳米纤维固定化效率为193%，固定化植酸酶的催化活性可达50.4%，植酸酶活性的提高有助于开发用于生物修复和生物转化的技术和材料[107]。例如，王成波[108]以戊二醛为交联剂，用壳聚糖固定植酸酶，利用共价结合方法对酶的固定化进行研究，结果表明，壳聚糖负载植酸酶酶活可达1 665 U。同时，纳米材料负载植酸酶作为一种高效的固定化糖蛋白表达系统具有良好的应用前景，在畜禽和水产养殖的应用可产生较高的经济效益，且可保护生态安全，改善生态环境[124]。

4.2 提高植酸酶对土壤pH的适应范围

可通过提高植酸酶对土壤pH的适应范围，以保持植酸酶在不同土壤中的活性。Bei 等[109]通过重组从黑曲霉(*A. niger*) NRRL3135和烟曲霉(*A. fumigatus*) ATCC13073 获得突变株植酸酶片段，获得突变株A23D5F7和AB345F7，其最适pH范围提高至2.5−5.5和5.0−5.5,活性高达35−40 U/mg和80−90 U/mg。微生物植酸酶可促进土壤稳定态有机磷向活性有机磷和无机磷转化,从而提高土壤磷的生物有效性[19](表2)。Li 等[110]将无丙二酸柠檬酸杆菌(*Citrobacter amalonaticus*)植酸酶基因在毕赤酵母(*P. pastoris*)细胞中进行表达，使其植酸酶活性由 300 U/g提高至6 413 U/g，最适温度为60 ℃，酶活pH稳定性显著提高，活性pH范围由4.0−6.0扩大至1.6−6.0，且酶活性由70%提高至90%。

4.3 定点突变

定点突变是一种更快更有效提高植酸酶热稳定性和催化效率的方法。随着现代分子生物学技术的发展，采用基因工程技术手段构建植酸酶基因工程菌，可获得高产、高活性植酸酶菌株(表 2)。例如，Shivange 等[111]通过突变莫氏耶尔森氏菌(*Yersinia mollaretii*)植酸酶,突变株活性达993 U/mg，且热稳定性和pH稳定性也提高，58 ℃加热20 min后，突变株活性是野生型的2.4倍(55% *vs*. 23%)，强酸性条件下(pH 2.8),突变株活性是野生型的2.38倍(74% *vs*. 31%)。

表 2　提高土壤植酸酶酶活的方法与应用前景

Table 2　Methods and application prospect of improving phytase activity in soil

方法 Methods	材料 Materials	技术 Technology	酶活特性 Characteristics of enzyme activity	应用 Application	参考文献 References
纳米材料负载植酸酶 Nanomaterial loaded phytase	羟基磷灰石 Hydroxyapatite (HA)	吸附、固定 Adsorption and fixation	负载酶在 pH 3.0 时活性由 40%提高至 90%，pH 7.0 时活性由 23%提高至 38%；80−90 ℃时，游离植酸酶发生变性，20 min 内完全失活，而负载酶在 80 ℃、3 h 和 90 ℃、20 min 时，活性仍可保持 60%和 40% The activity of loaded enzyme is increased from 40% to 90% at pH 3.0 and from 23% to 38% at pH 7.0; At 80−90 ℃, the free phytase is denatured and completely inactivated within 20 min, while the activity of the loaded enzyme remained 60% and 40% at 80 ℃, 3 h and 90 ℃, 20 min	土壤改良剂 Soil amendment	[103-104]
	氧化石墨烯 Graphene oxide (GO)		负载植酸酶活性在 42 °C 和 85 °C 分别提高 40 倍和 20 倍 The activity of loaded phytase is increased by 40 and 20 times at 42 °C and 85 °C, respectively	开发耐热植酸酶 Develop heat-resistant phytase	[105]
	壳聚糖 Chitosan	包埋，壳聚糖纳米粒将植酸酶包封 Embedding, chitosan nanoparticles encapsulate phytase	负载酶适宜温度变广，70 ℃加热 30 min 后，游离植酸酶活性为 21.4%，负载酶活性为 63.3%；90 ℃时，游离植酸酶失活，负载酶活性仍可保持 11.4% The optimum temperature of the loaded enzyme is wide. After heating at 70 ℃ for 30 min, the free phytase activity is reduced to 21.4% and loaded enzyme activity remains 63.3%; At 90 ℃, free phytase was inactivated and the loaded enzyme activity remains 11.4%	生物修复和生物转化 Bioremediation and biotransformation	[106]
			负载酶的活性提高，米氏常数 K_m 由 34 μmol/L 提高至 56 μmol/L，最大反应速率 V_{max} 由 120 μmol/(min·mg)提高至 401 μmol/(min·mg)，固定化植酸酶的活性可达 50.4% The activity of loaded enzyme is increased, with K_m increased from 34 μmol/L to 56 μmol/L, the maximum reaction rate V_{max} increases from 120 μmol/(min·mg) to 401 μmol/(min·mg), and the immobilized phytase activity reaches 50.4%		[107]
			植酸酶酶活可达 1 665 U Phytase activity reaches 1 665 U		[108]
提高 pH 适应范围 Improve pH adaptations	黑曲霉 *A. niger*	基因重组 Genetic recombination	重组植酸酶的最适 pH 范围提高至 2.5−5.5 和 5.0−5.5，活性高达 35−40 U/mg 和 80−90 U/mg The optimal pH range of recombinant phytase is increased to 2.5−5.5 and 5.0−5.5, and the activity reaches 35−40 U/mg and 80−90 U/mg	促进土壤稳定态有机磷向活性有机磷和无机磷转化 Promote soil stable organic P transformation to active organic P and inorganic P	[109]
	无丙二酸柠檬酸杆菌 *C. amalonaticus*	在酵母(*P. pastoris*)表面表达 Express on yeast (*P. pastoris*) surface	植酸酶活性由 300 U/g 提高至 6 413 U/g，酶活的 pH 稳定性提高，pH 范围由 4.0−6.0 提高至 1.6−6.0，且酶活性由 70%提高至 90% Phytase activity increases from 300 U/g to 6 413 U/g, pH stability increases from 4.0−6.0 to 1.6−6.0 and the enzyme activity increases from 70% to 90%		[19,110]

(待续)

(续表 2)

方法 Methods	材料 Materials	技术 Technology	酶活特性 Characteristics of enzyme activity	应用 Application	参考文献 References
定点突变 Fixed point mutation	莫氏耶尔森氏菌 *Yersinia mollaretii*	定点突变 Fixed point mutation	突变株活性达 993 U/mg，58 ℃加热 20 min 后，突变株活性是野生型的 2.4 倍(55% *vs*. 23%)，强酸性条件下(pH 2.8)，突变株活性是野生型的 2.38 倍(74% *vs*. 31%) The activity of the mutant reaches 993 U/mg, which is 2.4 times that of the wild type (55% *vs*. 23%) after heating at 58 ℃ for 20 min, and 2.38 times that of the wild type (74% *vs*. 31%) under strong acidic conditions (pH 2.8)	土壤改良剂 Soil amendments	[111]
	大肠杆菌 *E. coli* AppA	定点突变 Fixed point mutation	突变株活性是野生型的 1.5 倍(175 *vs*. 117 U/mg)，且最适温度由 65 ℃提高至 75 ℃，90 ℃加热 15 min 后植酸酶活性是野生型的 3.14 倍(44% *vs*. 14%) The activity of the mutant is 1.5 times that of the wild type (175 *vs*. 117 U/mg), and the optimal temperature increases from 65 ℃ to 75 ℃, mutant phytase activity is 3.14 times that of the wild type after heating at 90 ℃ for 15 min (44% *vs*. 14%)	土壤改良剂 Soil amendments	[32,112]
		随机突变 Random point mutation	突变株 I408L 活性高于野生型(1 653 *vs*. 1 610 U/mg)，85 ℃热处理 5 min 后活性是野生型的 1.83 倍(51.3% *vs*. 28%) The activity of mutant I408L is higher than that of wild type (1 653 *vs*. 1 610 U/mg), with the activity being 1.83 times that of wild type (51.3% *vs*. 28%) after heating at 85 ℃ for 5 min		[113]
	大肠杆菌 *E. coli*	易错 PCR Error prone PCR	突变株 AppA2 的热稳定性提高 25% (80 ℃, 10 min)，催化效率较野生型[660 和 1 065 *vs*. 423 L/(μmol·s)]提高 56%和 152% The thermal stability of mutant AppA2 is increased by 25% (80 ℃, 10 min), and the catalytic efficiency is increased by 56% and 152% compared to wild type (660 and 1 065 *vs*. 423 L/(μmol·s))		[114]
		定点突变 Fixed point mutation	突变株 AppA2 (D144N/V227A 和 D144N/V227A/G344D)的热稳定性提高 15% (80 ℃, 10 min)，突变株催化效率是野生型的 1.9 倍和 2.9 倍[1 205 和 1 654 *vs*. 445 L/(μmol·s)]，其中 D144N/V227A 活性(1 385 U/mg)比野生型(1 003 U/mg)提高 38% The thermal stability of mutant AppA2 (D144N/V227A and D144N/V227A/G344D) was increased by 15% (80 ℃, 10 min). The catalytic efficiency is 1.9 and 2.9 times that of the wild type (1 205 and 1 654 *vs*. 445 L/(μmol·s)). The activity of D144N/V227A (1 385 U/mg) is 38% higher than the wild type (1 003 U/mg)		[115]
		定点突变 Fixed point mutation	突变株在 80 ℃加热 10 min 后，酶活分别为 31.4%和 70.5%，热稳定性比野生型提高 39.1% After heating at 80 ℃ for 10 min, the enzyme activity of the mutant is 31.4% and 70.5%, respectively, and the thermal stability is 39.1% higher than the wild type		[116]

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(续表 2)

方法 Methods	材料 Materials	技术 Technology	酶活特性 Characteristics of enzyme activity	应用 Application	参考文献 References
	黑曲霉 *A. niger*	定点突变 Fixed point mutation	突变株在 80 ℃热处理 10 min 后其活性分别为 466 688、438 366 和 503 217 U/mg The activities of the mutant are 466 688, 438 366 and 503 217 U/mg after heating at 80 ℃ for 10 min	构建高产、高活性的超级产植酸酶菌株 Develop high yield and high phytase activity strain	[117]
		定点突变 Fixed point mutation	突变株在 80 ℃时稳定性最高，其酶活分别提高 24%和 22.6% The mutant shows the highest stability at 80 ℃, and its enzyme activity is increased by 24% and 22.6%, respectively	土壤改良剂 Soil amendments	[118]
	黑曲霉 N25 *A. niger* N25	定向改造 Directional transformation	突变株 PP-NP^{ds}-12D 热稳定性较高，在 70、80 和 90 ℃水浴处理 10 min，热稳定性分别提高 35%、40%和 31%，突变株 PP-NP^{ds}-12G 催化活性较高，酶活性达到 112 957 U/mL，是原始菌株(513.4 U/mL)的 220 倍以上 The thermal stability of the mutant PP-NP^{ds}-12D is improved by 35%, 40% and 31% after heating at 70, 80 and 90 ℃ for 10 min, respectively. The catalytic activity of the mutant PP-NP^{ds}-12G reaches 112 957 U/mL, which is more than 220 times of the original strain (513.4 U/mL)	筛选热稳定性好、催化效率高的植酸酶突变株 Screen mutants with high thermal stability and high catalytic efficiency	[119]
	黑曲霉 YM-01 *A. niger* YM-01	原生质体紫外诱变 Ultraviolet mutagenesis of protoplasts	植酸酶活性达到 2 204 U/mL，突变株 YM-16 酶活比 YM-01 提高 61.2%，且热稳定性提高，90 ℃高温处理 10 min 后，YM-16 酶活为 226 U/mL The phytase activity of the mutant YM-16 is 2 204 U/mL. The enzyme activity of the mutant YM-16 is 61.2% higher than YM-01, and the thermal stability is improved, which is 226 U/mL after heating at 90 ℃ for 10 min	土壤改良剂 Soil amendments	[120]
	黑曲霉 MA021 *A. niger* MA021	紫外、亚硝基胍单独和复合处理 UV/Nitrosoguanidine separate or mix-treatment	植酸酶活性达到 2 950−3 015 U/mL，是原始菌株的 3.6 倍 Phytase activity is 2 950−3 015 U/mL, which is 3.6 times that of the original strain		[121]
	解淀粉芽胞杆菌 DSM 1061 *B. amyloliquefaciens* DSM 1061	定点突变 Fixed point mutations	突变株 D148E 活性提高 35%，催化效率是野生型的 1.5 倍，pH 5.0−8.0 范围内活性高于野生型(7.0−20.6 *vs*. 6.0−15 U/mg)，D148E 活性在 65 ℃时高达 20.6 U/mg。在 40−75 ℃的温度范围内活性增加，突变株 D148E 的活性高于野生型(2.5−20.6 *vs*. 2−15 U/mg) The activity of mutant D148E phytase is increased by 35%, and the catalytic efficiency is 1.5 times that the wild-type, with activity is higher than wild-type (7.0−20.6 *vs*. 6.0−15 U/mg) at pH 5.0−8.0. The activity of D148E phytase reaches 20.6 U/mg at 65 ℃, which is higher than the wild type (2.5−20.6 *vs*. 2−15 U/mg) at 40−75 ℃	土壤改良剂 Soil amendments	[41]
	放射型根瘤杆菌 *A. radiobacter*	紫外-氯化锂诱变 UV-lithium chloride mutagensis	植酸酶活性达到 18.5 U/mL，比原始菌株酶活提高 47.7% Phytase activity is 18.5 U/mL, which is 47.7% higher than that of the original strain		[122-123]

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Rodriguez 等[112]通过突变大肠杆菌(*E. coli*) AppA 植酸酶，突变株活性是野生型的 1.5 倍(175 U/mg *vs*. 117 U/mg)，且最适温度由 65 ℃提高至 75 ℃，90 ℃加热 15 min 后植酸酶活性是野生型的 3.14 倍(44% *vs*. 14%)[29]。Zhu 等[113]对大肠杆菌(*E. coli* AppA)植酸酶进行突变，突变株 I408L 活性高于野生型(1 653 *vs*. 1 610 U/mg)，且热稳定性显著提高，85 ℃热处理 5 min 后活性是野生型的 1.83 倍(51.3% *vs*. 28%)。Kim 等[114]通过易错 PCR 获得植酸酶 AppA2 突变株，与野生型相比，AppA2 的热稳定性提高 25% (80 ℃, 10 min)，催化效率较野生型[660 和 1 065 *vs*. 423 L/(μmol·s)]提高 56%和 152%。类似地，Kim 等[115]从大肠杆菌(*E. coli*)中获得了植酸酶 AppA2 突变株，与野生型相比，AppA2 (D144N/V227A 和 D144N/V227A/G344D)的热稳定性提高 15% (80 ℃, 10 min)，突变株催化效率是野生型的 1.9 倍和 2.9 倍[1 205 和 1 654 *vs*. 445 L/(μmol·s)]，其中 D144N/V227A 活性(1 385 U/mg)比野生型(1 003 U/mg)提高 38%。Fei 等[116]通过突变大肠杆菌(*E. coli*)植酸酶，在 80 ℃加热 10 min 后，野生型 AppA 和突变株的酶活分别为 31.4%和 70.5%，突变株的热稳定性比野生型提高 39.1%。

Tang 等[117]通过突变黑曲霉(*A. niger*)植酸酶，获得突变株(Q172R、Q172R/K432R 和 Q368E/K432R)，80 ℃热处理 10 min 后其植酸酶活性分别为 466 688、438 366 和 503 217 U/mg。Hesampour 等[118]通过突变黑曲霉植酸酶(*A. niger* PhyA)，突变株 P9 和 P12 在 80 ℃时稳定性最高，植酸酶酶活分别提高 24%和 22.6%。廖燕[119]通过突变黑曲霉(*A. niger*) N25 植酸酶，突变株 PP-NP^{ds}-12D 热稳定性较高，在 70、80 和 90 ℃水浴处理 10 min，热稳定性分别提高 35%、40%和 31%，PP-NP^{ds}-12G 催化活性较高，酶活性达到 112 957 U/mL，是原始菌株(513.4 U/mL)的 220 倍。为进一步应用蛋白质工程技术改善植酸酶酶学性质奠定基础，筛选出热稳定性好、催化效率高的植酸酶突变株。叶明等[120]对土壤中分离的黑曲霉(*A. niger*) YM-01 菌丝体进行原生质体紫外诱变，诱变后酶活达到 2 204 U/mL，突变株 YM-16 酶活比 YM-01 提高 61.2%，且热稳定性提高，90 ℃高温处理 10 min 后，YM-16 酶活为 226 U/mL，进一步对其液态产酶发酵条件进行优化，可应用于土壤酶制剂(表 2)。陈红歌等[121]对黑曲霉(*A. niger*) MA021 进行紫外、亚硝基胍单独处理和复合处理，获得植酸酶高产菌株 U-12-10，其植酸酶活性达到 2 950−3 015 U/mL，是原始菌株的 3.6 倍。

Xu 等[41]对解淀粉芽胞杆菌(*B. amyloliquefaciens*) DSM 1061 植酸酶进行突变，突变株 D148E 活性提高 35%，催化效率是野生型的 1.5 倍，在 pH 5.0−8.0 范围内活性高于野生型(7.0−20.6 *vs*. 6.0−15 U/mg)，在 65 ℃时高达 20.6 U/mg，40−75 ℃温度范围内活性增加，且突变株 D148E 的活性高于野生型(2.5−20.6 *vs*. 2−15 U/mg)。王陶等[122]对放射型根瘤杆菌(*Agrobacterium radiobacter*)进行紫外-氯化锂复合诱变，筛选出产植酸酶活性高的菌株，酶活达 18.5 U/mL，比原始菌株酶活提高 47.7%。

植酸酶对土壤具有一定改良作用，向土壤中施加外源微生物植酸酶制剂或植酸酶生产菌，可有效提高土壤中植酸的分解效率和磷利用率[123] (表 2)。例如，添加微生物植酸酶制剂后，土壤中植酸含量降低了约 20.5%，同时有效磷含量提高 1.22 倍[125]。此外，添加微生物植酸酶可促进土壤植酸磷转化和植物生长。曲博等[126]通过向土壤添加外源微生物植酸酶，发现土壤中活性有机磷含量提高 1.73 倍，且使黄瓜苗株高、玉米苗干重和株高以及茼蒿苗叶绿素含量分别提高 32.4%、74.8%、48.1%和 30.6%，

3 种植物幼苗的生物量与微生物植酸酶的添加量呈显著正相关[127]。因此，微生物植酸酶作为生物菌剂，可提高土壤内源植酸盐的利用效率，减少外源磷肥施用，降低磷流失的环境污染风险，促进农业可持续发展。

5 结论与展望

环境磷污染已引起广泛关注，尤其是农业活动过度施肥及动物产生的高磷粪便使大量磷进入土壤，具有潜在的污染土壤和水体风险。无机磷肥施入土壤后极易被土壤吸附或与金属离子形成难溶性络合物或转化为有机磷(植酸盐为主要成分)，导致其生物可利用性降低。植酸酶是水解植酸盐的关键酶，在此过程中植酸酶的热稳定性和活性是影响植酸盐水解和磷释放的重要因素。因此，通过阐述土壤中微生物植酸酶活性的影响因素(温度、pH、土壤吸附、钙含量及钙磷比、底物浓度、含量和有效性等)，重点综述提高植酸酶活性和稳定性的方法技术(纳米材料负载植酸酶、提高植酸酶 pH 适应范围、定点突变等)及应用效率。

植酸酶的适当选择是其应用成功的关键，微生物植酸酶在实际应用中仍具有一定局限性，为此，可加强几方面研究：(1) 了解微生物植酸酶的来源，致力于筛选出热稳定性好、活性高的产植酸酶微生物；(2) 理解土壤中植酸酶活性的影响因素，进而寻求方法提高植酸酶在土壤中的活性；(3) 调节植酸酶最适 pH 范围、提高植酸酶热稳定性、将植酸酶负载于纳米材料和基因工程改造等改善植酸酶性质；(4) 研究植酸酶的作用机制，进一步为微生物植酸酶应用于农业生产及提高土壤难利用态植酸磷的利用效率提供理论基础和技术参考，对降低农业生产成本、减少环境磷污染、控制过量磷肥施用导致的潜在面源污染及水体富营养化风险具有重要的现实意义。

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