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自生固氮菌对土壤钾的活化作用*

张 亮^{1,2} 袁 玲¹ 黄建国^{1†}

(1 西南大学资源环境学院, 重庆 400716)

(2 南京师范大学泰州学院生物技术与化学工程学院, 江苏泰州 225300)

摘 要 以土壤为钾源, 通过液培试验研究了 5 株自生固氮菌 (*Azotobacter* sp.) 对土壤钾的活化作用。结果表明, 自生固氮菌能分泌大量的氢离子, 大幅度降低培养液 pH, 使氢离子浓度提高 40 倍以上。自生固氮菌分泌有机酸的种类与数量因菌株不同而异, 这些有机酸包括甲酸、乙酸、草酸、丁二酸、柠檬酸、苹果酸和乳酸等。其中, 全部供试菌株均能分泌草酸和苹果酸。在自生固氮菌的培养液中, 钾浓度显著高于未接种的培养液, 但土壤矿物结构钾则显著降低。由于土壤是培养液钾的唯一来源, 说明自生固氮菌可促进土壤矿物钾溶解。相关分析表明, 土壤矿物结构钾与自生固氮菌的有机酸分泌总量呈显著负相关 ($r = -0.845^*$, $n = 6$), 与培养液 pH 呈显著正相关 ($r = 0.702^*$, $n = 6$), 说明自生固氮菌分泌的有机酸和氢离子可能溶解土壤矿物钾。考虑到自生固氮菌的草酸分泌量最大, 络合钙、镁、铁、铝的能力最强, 且与有机酸分泌总量呈极显著正相关 ($r = 0.990^{**}$, $n = 6$), 推测草酸的分泌在活化土壤无效钾的过程中起重要作用。接种自生固氮菌显著降低土壤无效钾, 活化能力因菌株不同而异, 其原因可能与有机酸分泌的数量和种类有关。

关键词 自生固氮菌; 土壤; 钾

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钾是植物生长所必需的大量营养元素之一。土壤全钾含量通常较高, 主要以铝硅酸盐含钾矿物的形式存在, 难于被植物吸收利用。我国人多地少, 复种指数高, 土地利用强度大, 有效钾含量普遍较低, 需要施肥补充。但是, 我国的钾肥资源贫乏, 需大量进口才能满足农业生产的需求^[1]。微生物不同程度地参与土壤钾的活化利用, 它们对土壤矿物钾的溶解作用日益受到人们的重视^[2]。

目前, 关于硅酸盐细菌 (Silicate bacteria) 活化土壤难溶性钾的研究较多^[3-4]。20 世纪 50 年代, 苏联学者亚历山大罗夫最先从土壤中分离获得了硅酸盐细菌, 可释放铝硅酸盐矿物中的钾^[5]。有研究表明, 在磁场作用下, 硅酸盐细菌能将含钾矿物中的结构钾迅速转化为可溶性钾, 提高其有效性, 溶钾量较对照高 32.53% ~ 41.80%^[6]。盆栽试验表明, 源于土壤的胶质芽孢杆菌 (*Bacillus mucilaginosus*)

K1 和 K3 菌株, 能将含钾矿物中的结构钾转化为速效钾, 供玉米吸收利用, 提高其产量^[7]。在土壤中, 硅酸盐细菌是一种特殊的根际微生物, 能够分泌草酸、乙酸、酒石酸、柠檬酸等有机酸, 可电离产生氢离子或直接络合长石、云母、花岗岩等硅酸盐含钾矿物中的铁、铝、钙、镁离子, 使之分解释放钾离子, 供植物吸收利用^[8-10]。

自生固氮菌不仅具有固氮和分泌植物生长激素的作用^[11], 而且还能分泌氢离子和有机酸 (如草酸、乙酸、柠檬酸等), 活化土壤中的难溶性磷酸盐^[12-13]。由于氢离子和有机酸溶解含钾硅酸盐矿物的机理类似磷酸盐, 推测自生固氮菌也可能活化土壤无效钾, 但相关研究甚少。本文选用从当地土壤中分离的 5 株自生固氮菌, 研究其对紫色土中无效钾的活化作用, 以探索自生固氮菌溶钾的可能机理, 多方面了解其生物学功能。

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† 通讯作者, E-mail: huang99@swu.edu.cn

作者简介: 张亮 (1987—), 男, 安徽太和人, 硕士, 主要从事资源环境微生物方向研究。Tel: 18860895619; E-mail: liangzai0061@126.com

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1 材料与方 法

1.1 供试材料

供试土壤为侏罗纪紫色砂泥岩发育的灰棕紫泥,采自重庆北碚区西南大学农场(29°49'18"N, 106°25'45"E)。蒸汽灭菌后的土壤 pH 6.15、有机质 14.96 g kg⁻¹、全氮 1.07 g kg⁻¹、全磷 0.39 g kg⁻¹、全钾 16.32 g kg⁻¹、速效钾 102.6 mg kg⁻¹。

自生固氮菌(*Azotobacter* sp.)从重庆市北碚灰棕紫泥菜地土壤中分离获得,保存于西南大学资源环境学院微生物实验室。取其中的 5 株为供试菌株,代号分别为 N01、N02、N03、N04 和 N05,25 °C 时的固氮酶活性变化于 4.5~23.2 μg C₂H₂h⁻¹mg⁻¹蛋白之间^[14]。

试验中有机酸标准品为色谱纯,其余试剂均为分析纯。有机酸标准液制备:精确称取草酸、柠檬酸、苹果酸、丁二酸、乳酸、甲酸、乙酸各 100 mg,用流动相溶解并定溶于 100 ml 容量瓶中,配置成 1.0 g L⁻¹的有机酸的标准混合溶液,再用流动相将标准溶液分别稀释至 0.01、0.02、0.04、0.06、0.08 和 0.10 g L⁻¹。

1.2 试验设计

首先,将 5 株自生固氮菌接种至自生固氮菌的专用固体培养基(培养基组成为:蒸馏水 1000 ml、琼脂 20 g、葡萄糖 10 g、KH₂PO₄ 0.2 g、MgSO₄·7H₂O 0.2 g、NaCl 0.2 g、CaSO₄·7H₂O 0.2 g、CaCO₃ 5.0 g、pH7.0~7.2,液体培养基去除琼脂即可),28 °C ± 1 °C 斜面培养 4 d。然后,将固体菌种接种至液体培养基中,摇床培养(28 °C ± 1 °C、60 r min⁻¹)3 d,离心收集,无菌水多次洗涤,除去分泌物等培养液成分,再用无菌水配制成自生固氮菌含量为 1 × 10¹² cfu ml⁻¹的菌悬液,备用。

风干土壤,磨细过 100 目筛,取 1.000 0 g 土壤置于两端开口直径为 1 cm 的塑料管中部,然后两端塞入玻璃纤维,将土壤夹在中间,再用微孔滤膜(孔径 0.22 μm)密封两端。水分子、无机离子、低分子有机酸等均可顺利进出与土壤接触,而土壤又不会穿过玻璃纤维和滤膜而进入溶液,自生固氮菌亦不能穿过玻璃纤维和滤膜进入土壤。

取 250 ml 三角瓶加入 50 ml 含磷量与原液体培养基相同的 NaH₂PO₄代替 KH₂PO₄,放入一个盛有土壤的塑料管作为钾源,蒸汽灭菌(121 °C, 30 min),冷却。为了避免在菌体繁殖生长过程中,产

生不可必要的干扰作用,保证快速释放大量的分泌物而产生作用效应,试验采用了大剂量接种,每 50 ml 培养液接入 1.0 ml 上述制备的自生固氮菌悬浮液,灭菌处理为对照。试验做 3 次,每次设置 5 次重复。摇瓶培养(28 °C ± 1 °C、60 r min⁻¹)7 d,备测有关项目。

1.3 测定项目与方法

用 PHS-3C 精密酸度计测定培养液 pH,火焰光度计测定钾离子浓度,高效液相色谱仪(日本 HITA-CHI 公司生产)测定有机酸浓度。色谱条件为:Diode Array L-7455 紫外检测器, Ion-300 有机酸分析专用柱(Phenomenex, Torrance, CA, USA),流动相:2.5 mmol L⁻¹硫酸,流速:0.5 ml min⁻¹,进样量:20 μl 样液(样液先经硫酸酸化),紫外检测波长:210 nm,柱温:35 °C,压力:3.1 × 10⁶ Pa。测定的有机酸包括草酸、柠檬酸、苹果酸、乳酸、丁二酸、甲酸、乙酸,其出峰时间依次为 9.57、11.52、13.31、14.53、15.95、17.47、20.72 min。

取出塑料管中的土壤,风干,依次用蒸馏水、1 mol L⁻¹醋酸铵和 1 mol L⁻¹沸硝酸提取水溶性钾、交换性钾和缓效钾,经上述溶液提取后的土壤用氢氧化钠 450 °C 熔融,获得矿物结构钾,用火焰光度计测定各提取溶液及土壤熔融液中的钾^[15]。

1.4 数据处理

用 Excel2003 对试验数据进行基本计算,SPSS18.0 进行统计分析,不同处理间的差异显著性用单因素方差分析进行比较,使用 Pearson 法进行双因素相关分析。

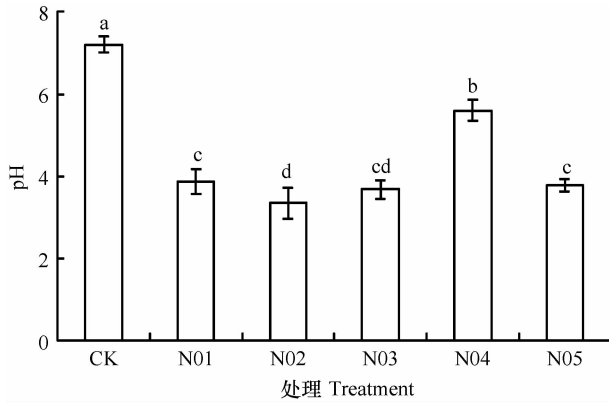
2 结 果

2.1 自生固氮菌对培养液 pH 的影响

图 1 可见,培养 7 d 之后,液体培养基的 pH 显著低于对照(不接种),但菌株不同,培养液的 pH 降幅也不同。接种 N02 之后,液体培养基的 pH 为 3.35,降幅最大;接种 N04,液体培养基的 pH 为 5.60,降幅最小;其余菌株培养液的 pH 介于 3.50 和 4.00 之间。

2.2 自生固氮菌液体培养基中有机酸的分泌

表 1 可见,在液体培养基中检测到甲酸、乙酸、草酸、乳酸、丁二酸、柠檬酸、苹果酸等 7 种有机酸。在不接种的液体培养基中(对照),检测到草酸、苹果酸和丁二酸,但含量极低,均可忽略不计。值得注意的是,自生固氮菌不同菌株分泌有机酸的种类



注:不同字母表示差异显著, $p < 0.05$,下同 Note: Different letters indicate significant differences at $p < 0.05$. Tnd the same below

图1 自生固氮菌对培养液 pH 的影响

Fig. 1 Effects of pH value in liquid culture mediums by *Azotobacter* sp.

和数量因菌株不同而异。在接种自生固氮菌的液体培养基中,草酸和苹果酸的含量显著高于对照,说明自生固氮菌能分泌草酸和苹果酸,其中草酸分泌量普遍最高。此外,大多数自生固氮菌能分泌丁

二酸和甲酸(N04除外),N01、N03、N05能分泌乙酸;N02、N03、N05能分泌柠檬酸;N02和N04能分泌乳酸。从5种有机酸的总量看,N03的分泌量最高,N01次之,N02和N05居第三,N04最低。

2.3 自生固氮菌对钾素转化的影响

图2可见,培养7 d之后,自生固氮菌培养液中的可溶性钾浓度均高于对照(不接种),培养液中可溶性钾浓度因菌株不同而异,变化于 $3.99 \sim 5.34 \text{ mg L}^{-1}$ 之间。

培养7 d之后,土壤水溶性钾、交换性钾、缓效钾和矿物结构钾的含量及变化见表2。接种自生固氮菌之后,土壤水溶性钾升高或无显著变化。接种N01、N02、N03和N04,土壤交换性钾显著提高,较对照土壤增加 $7.81\% \sim 22.91\%$,其余菌株与对照相似。除N05外,其余菌株显著提高土壤中的缓效钾含量,较对照增加 $4.41\% \sim 12.45\%$ 。接种N01和N03,土壤中的矿物结构钾含量显著降低,较对照降低 304.7 mg kg^{-1} 和 342.2 mg kg^{-1} ,其余菌株对矿物结构钾无显著影响。

表1 自生固氮菌培养液中有机酸的含量

Table 1 Contents of organic acids in liquid culture mediums grown with *Azotobacter* sp. (mg L^{-1})

处理 Treatment	草酸 Oxalic acid	苹果酸 Malic acid	丁二酸 Succinic acid	甲酸 Formic acid	乙酸 Acetic acid	柠檬酸 Citric acid	乳酸 Lactic acid	总浓度 Total content
CK	9.28d	8.77d	5.65c	ND	ND	ND	ND	23.70e
N01	76.15ab	39.01a	12.15a	11.99a	16.98a	ND	ND	156.3b
N02	49.25b	19.99c	8.56b	8.02b	ND	21.18ab	10.78a	117.8c
N03	82.17a	36.99a	12.43a	11.92a	5.87b	24.98a	ND	174.4a
N04	18.18c	19.39c	ND	ND	ND	ND	9.86a	47.43d
N05	41.89bc	27.34b	9.98ab	8.76b	2.07c	18.72ab	ND	108.8c

注:在同一列中,不同字母者表示差异显著($p < 0.05$),下同;ND表示未检测出 Note: Data followed by different letters in the column, are significantly different at $p < 0.05$. The same below. ND indicates not detected

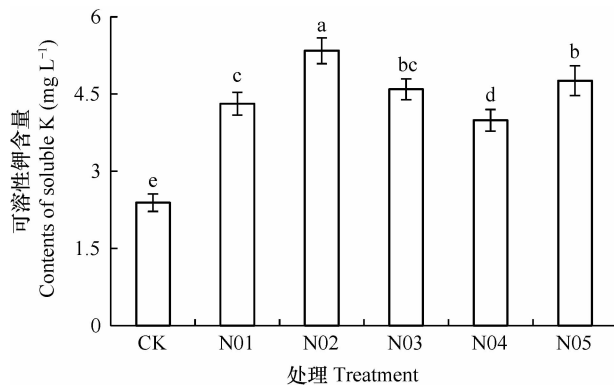


图2 自生固氮菌培养液中的可溶性钾含量

Fig. 2 Contents of soluble K in liquid culture mediums grown with *Azotobacter* sp.

土壤水溶性钾和交换性钾之和即为速效钾。全部接种菌株均增加了土壤速效钾含量,较对照增加 $4.10\% \sim 22.68\%$ 。

2.4 不同形态钾的相关性

表3可见,土壤矿物结构钾与土壤缓效钾、自生固氮培养液的草酸及有机酸总浓度呈显著负相关,相关系数 r 分别为 -0.895^* 、 -0.857^* 、 -0.845^* ($n = 6$),与培养液 pH 呈显著正相关,相关系数 0.702^* ($n = 6$)。自生固氮菌的草酸分泌量与有机酸分泌总量呈极显著正相关($r = 0.990^{**}$, $n = 6$)。此外,自生固氮菌培养液的有机酸总浓度与培养液 pH 呈显著负相关($r = -0.872^*$, $n = 6$)。

表 2 土壤含不同形态钾量的变化

Table 2 Changes in different K fractions in soil (mg kg^{-1})

处理 Treatment	水溶性钾 Water-soluble K	交换性钾 Exchangeable K	缓效钾 Slow effective K	矿物结构钾 Mineral K
CK	1.65ab	129.4d	325.3d	15 741a
N01	1.75a	139.5c	359.6ab	15 436b
N02	1.56ab	145.0b	339.7c	15 591ab
N03	1.73a	159.0a	365.8a	15 399b
N04	1.45ab	140.0bc	357.4b	15 535ab
N05	1.61ab	134.8cd	331.7cd	15 560ab

表 3 自生固氮菌解钾试验各形态钾的相关性

Table 3 Correlation coefficients between different K fractions in experimental of solubilizing K by *Azotobacter* sp.

钾形态 Potassium forms	水溶性钾 WSK	交换性钾 EK	缓效钾 SEK	矿物结构钾 Mineral K	草酸浓度 Oxalic acid	有机酸总浓度 TOA	pH
水溶性钾 WSK	1.000						
交换性钾 EK	0.249	1.000					
缓效钾 SEK	0.204	0.744	1.000				
矿物结构钾 Mineral K	-0.376	-0.751	-0.895*	1.000			
草酸浓度 Oxalic acid	0.689	0.731	0.654	-0.857*	1.000		
有机酸总浓度 TOA	0.630	0.731	0.602	-0.845*	0.990**	1.000	
pH	-0.243	-0.607	-0.405	0.702*	-0.804	-0.872*	1.000

注: WSK, 水溶性钾; EK, 交换性钾; SEK, 缓效钾; TOA, 有机酸总浓度; *, **, * 分别表示在 0.05 和 0.01 水平上相关性达到显著水平 Note: WSK, Water soluble K; EK, Exchangeable K; SEK, Slow effective K; TOA, Total organic acids; *, **, * show that correlations are significant at 0.05 and 0.01 level, respectively

3 讨论

培养自生固氮菌 7 d 之后, 供试菌株均显著降低培养液 pH, 至少降低了 1.60 个单位, 相当于氢离子浓度增加了 40 倍。但菌株不同, pH 的降幅也不一样。从有机酸的分泌情况看, 全部自生固氮菌均能分泌草酸和苹果酸, 大多数自生固氮菌能分泌丁二酸和甲酸 (N04 除外), N01、N03、N05 能分泌乙酸; N02、N03、N05 能分泌柠檬酸; N02 和 N04 能分泌乳酸。各有机酸的分泌量和有机酸分泌总量也因菌株不同而异。统计分析表明, 培养液的 pH 与

有机酸总浓度呈显著负相关 ($r = -0.872^*$, $n = 6$), 说明自生固氮菌分泌的有机酸是造成培养液 pH 降低的原因之一。在分泌的有机酸中, 绝大部分菌株分泌的草酸数量最大。

土壤钾主要以无机态存在, 包括速效钾 (水溶性钾和交换性钾)、缓效钾和无效钾 (矿物结构钾), 它们之间互相转化。在自生固氮菌培养液中, 钾浓度显著高于不接种, 由于土壤是培养液钾离子的唯一来源, 故培养液含钾量的增加必然来自于土壤钾的溶解, 自生固氮菌具有活化土壤中无效钾的作用。有学者在菜地土壤中分离到一株解钾能力较强的自生固氮菌, 通过盆栽试验发现, 不仅可以活

化土壤中的无效钾,而且能提高作物的产量^[16]。此外,在培养自生固氮菌的过程中,部分菌株提高土壤缓效钾含量,同时降低土壤矿物结构钾,二者之间呈显著负相关($r = -0.895^*$, $n = 6$)。说明在自生固氮菌的作用下,土壤矿物中的结构钾可能转化成为缓效钾,进而再转化为植物直接吸收利用的速效钾,自生固氮菌具有活化溶解土壤无效钾的能力。此外,有研究表明,接种自生固氮菌能显著促进高粱等作物的生长,并显著提高土壤有效钾的含量^[17]。看来田间接种自生固氮菌之后,不仅可以固氮,而且还能活化土壤无效钾,提高其生物有效性,改善土壤供钾能力^[18]。

Parmar 和 Sindhu^[19]指出,钾细菌能分泌有机酸,尤其是高分子有机酸,它们与矿物中的铁、铝、钙、镁发生络合作用,进而分解矿物释放钾离子。Zeng 等^[20]认为,钾细菌能分泌细胞外多糖和一些小分子的有机酸(甲酸、草酸、乙酸和乳酸等),活化矿物中的难溶性钾。此外,有研究表明,钾细菌既能分泌氢离子,又能释放有机酸,在两者共同作用下溶解钾矿石^[21-22]。因此,钾细菌在溶解硅酸盐类矿物时,能检测到甲酸、乙酸、草酸、柠檬酸、乳酸等多种有机酸,从而增加土壤有效钾,供植物吸收利用,增强土壤供钾能力^[23]。在本试验中,土壤矿物结构钾与自生固氮菌的草酸分泌量和有机酸分泌总量呈显著负相关($r = -0.857^*$ 、 $r = -0.845^*$, $n = 6$),推测它们分泌的草酸和有机酸可以溶解矿物钾。在自生固氮菌培养液中,草酸和柠檬酸的浓度远远高于其他有机酸。由于草酸能与 Ca^{2+} 、 Mg^{2+} 、 Fe^{3+} 和 Al^{3+} 等离子络合成稳定的草酸-Ca、草酸-Mg、草酸-Fe 和草酸-Al,其络合稳定常数($\log K_{\text{稳}}$)分别为 3.00、4.38、20.20 和 16.30^[24-25],草酸可能与含钾矿物中的钙、镁、铁、铝发生络合作用而使之分解,释放钾离子。故有理由认为,在活化土壤钾的过程中,自生固氮菌分泌的草酸至关重要,类似外生菌根真菌^[26]。此外,自生固氮菌还能分泌氢离子,所产生的酸溶作用也有益于分解含钾矿物。

4 结 论

自生固氮菌除固氮之外,某些菌株还能活化土壤中的无效钾,从而提高土壤钾的生物有效性。因菌株不同而表现出不同的活化能力,它们分泌的有机酸可能是溶解土壤矿物钾的原因之一。

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MOBILIZATION OF POTASSIUM IN SOILS BY AZOTOBACTER

Zhang Liang^{1,2} Yuan Ling¹ Huang Jianguo^{1*}

(1 College of Resources of Environment, Southwest University, Chongqing 400716, China)

(2 School of Biotechnology and Chemical Engineering, Taizhou College, Nanjing Normal University, Taizhou, Jiangsu 225300, China)

Abstract Potassium (K) is an essential nutrient element for plant growth. However, in most soils, K is low in both concentration and availability. Chemical K supplemented through fertilization is rapidly adsorbed by soil minerals or transformed by soil microbial organisms, resulting in low K use efficiency, as low as 20%~35%. Studies have found that *Azotobacter* sp., which can fix nitrogen from the atmosphere to supplement plants with nitrogen nutrient, also has the ability to mobilize soil K for plant use. The finding has aroused much attention the world over. However, little information is available to illustrate the mechanisms of *Azotobacter* mobilizing K in the soil. Five strains of *Azotobacter* sp., which were isolated from a gray brown purple soil in Chongqing, South China and coded as N01, N02, N03, N04 and N05, separately, were cultured a liquid medium in an experiment to study their K mobilization capacities. The medium contained 1L of H₂O, 10 g of mannitol, 0.176 g of NaH₂PO₄, 0.2 g of MgSO₄ · 7H₂O, 0.2 g of NaCl, 0.2 g of CaSO₄ · 7H₂O and 5.0 g of CaCO₃. Compared to the non-*Azotobacter* control, all the five *Azotobacter* treatments were found to be 40 times higher in concentration of hydrogen ions in the liquid media, as a consequence, pH dropped significantly after 7 days of incubation.

The *Azotobacter* strains all exude organic acids, including formic acid, acetic acid, oxalic acid, succinic acid, citric acid, malic acid and lactic acid, but vary in capacity and excretion of acids. Oxalic acid and malic acid was the most common ones all the five strains could exude. K concentrations in the liquid media were significantly higher than in the control, whilst the contents of soil mineral structure K were significantly lower in the *Azotobacter* treatments than in the non-*Azotobacter* treatment. Taking into account the soil being the sole K source, it is quite obvious that *Azotobacters* could promote dissolution of mineral K in the soil. Correlation analysis shows that content of soil mineral structure K is negatively related to the total organic acids *Azotobacters* exude ($r = -0.845^*$, $n = 6$), but positively related to pH of the liquid medium ($r = 0.702^*$, $n = 6$), which indicates that both the organic acids *Azotobacters* exude and hydrogen ions could dissolve soil K. Oxalic acid is the major organic acid *Azotobacters* exude in high volume and the most competent in complexing calcium, magnesium, iron and aluminum, and moreover, it is positively correlated with total organic acid ($r = 0.990^{**}$, $n = 6$), which suggests that oxalic acid exuded by *Azotobacter* might contribute directly to the mobilization of soil K. Meanwhile, soil mineral K was significantly reduced in all the *Azotobacter* treatments, to a varying extent, which depended on which strain of *Azotobacter* was used in this study, because the strains of *Azotobacters* vary sharply in exudation, in terms of amount and type of organic acids they exude. The findings demonstrate that inoculation of *Azotobacters* or intercropping with legumes is a good alternative to improve plant K nutrition, besides, *Azotobacters* and legumes can also help supplement the soil with nitrogen by fixing it from the atmosphere. Therefore, more efforts should be devoted to researches on the capability of *Azotobacters* of mobilizing soil K, and their associated nitrogen and K benefits to their host plants and neighbouring plants.

Key words *Azotobacter*; Soil; Potassium

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