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学位授与の要件	環境生命科学研究科 農生命科学専攻 (学位規則第4条第1項該当)
学位論文の題目	Diverse roles of a siderophore in a plant-growth promoting methylotroph, <i>Methylobacterium aquaticum</i> strain 22A (植物生育促進メチロトロフ細菌 <i>Methylobacterium aquaticum</i> 22A 株におけるシデロフォアの多様な役割)
論文審査委員	教授 馬 建鋒 教授 ガリス イバン 准教授 植木 尚子 准教授 谷 明生
学位論文内容の要旨	
<p><i>Methylobacterium</i> species are facultative methylotrophic bacteria predominant in the phyllosphere. They have been identified as plant growth-promoting bacteria although their actual mechanism has not been well investigated. On the other hand, they have two methanol dehydrogenases, MxaF and XoxF, which are dependent on either calcium or lanthanides (Lns) as cofactor, respectively. Lns exist as insoluble minerals in nature, and their solubilization and uptake is proposed to require a siderophore-like substance (lanthanophore). This study aimed to reveal the roles of siderophore in <i>Methylobacterium aquaticum</i> strain 22A in Ln uptake, bacterial physiology, and plant growth promotion.</p> <p>The strain 22A genome contains an eight-gene cluster putatively encoding staphyloferrin B (sbn)-like siderophore. The growth of $\Delta sbnCF$ was complemented with citrate or spent medium of the wild type of strain 22A or other strains of the genera, suggesting that the gene cluster is indeed involved in siderophore synthesis. The plant growth promotion effect was prominent in low iron conditions for duckweed and <i>Arabidopsis thaliana</i>. $\Delta sbnCF$ mutant failed to promote the growth, the plant iron content was increased by the wild type strain 22A and significantly reduced by $\Delta sbnCF$ treatment. Hence, the plant growth promotion effect by strain 22A was partly explained by siderophore that can assist plant in iron acquisition under iron depleted condition. The culture supernatant of the strain 22A wild type could solubilize insoluble Ln oxide (La_2O_3) as opposed to the mutant spent media. The promoter of <i>sbnA</i> was activated by the presence of high concentration of $LaCl_3$ (90 μM) under 5 μM $FeSO_4$, suggesting that $LaCl_3$ created iron scarcity, increasing the need for siderophore synthesis. $\Delta mxaF$ can grow on methanol only in the presence of Ln, due to intact XoxF. Importantly, $\Delta sbnCF$ and $\Delta mxaF\Delta sbnCF$ could not grow on methanol, suggesting the critical role of the siderophore in methylotrophy. Interestingly, siderophore was not required for methylotrophic growth in the presence of citrate but was indispensable as a lanthanophore in the absence of citrate. Citrate also chelates Ln for uptake in strain 22A. The sbn siderophore also plays a role in maintaining cellular Ln homeostasis based on the overactivation of <i>xoxF</i> promoter in the presence of $LaCl_3$ in a sbn siderophore mutant. Furthermore, this study showed the siderophore is involved in biofilm formation, mitigation of hydrogen peroxide, heavy metal tolerance of strain 22A. In silico analysis revealed the existence of diverse types of siderophore synthesis genes among <i>Methylobacterium</i> species. Some types of siderophore are distinctly conserved in phylogenetic clades. Co-inoculation of 58 <i>Methylobacterium</i> type strains with $\Delta sbnCF$ resulted in growth restoration by 21 strains, suggesting that siderophores may be exchangeable among the <i>Methylobacterium</i> species in the ecosystem. Among 12 genes for TonB-dependent receptors (TBDRs), the ones for the sbn siderophore-iron complex, iron citrate, and Ln were identified. The <i>tonB_Ln</i> was found to be involved in Ln transport, and its expression is repressed in the presence of high concentration of Ln. <i>Methylobacterium</i> type strains contain diverse types of TBDRs. <i>Methylorubrum</i> species have an exclusive TBDR for Ln uptake that have been identified as LutH in <i>M. extorquens</i> strain AM1, while TonB_Ln identified in this study in strain 22A was randomly conserved across the species. The TonB_sbn was discovered to be homologous to TonB_LCC of strain AM1 and 52 others in other <i>Methylobacterium</i> genomes.</p> <p>This study identified the genes for a carboxylate-type siderophore in strain 22A. The siderophore plays important roles in plant growth promotion, methylotrophy, iron and Ln chelation, and bacterial physiology. This study also contributed toward expanding the knowledge the diversity of siderophore and TBDRs in <i>Methylobacterium</i> species.</p>	

論文審査結果の要旨

植物が放出するメタノールを利用して生育出来る*Methylobacterium*属細菌は、植物葉上の微生物群集の中で優占化し、植物の生育を促進する。生育促進の要因の一つとして、不溶性の鉄を溶解するシデロフォアの働きが考えられた。また、本属細菌はカルシウム依存のMxaF, ランタノイド(Ln)に依存するXoxFという二つのメタノール脱水素酵素(MDH)を持っている。XoxFはLn依存の酵素として初めて発見されており、これらの発現はLnの有無とLnの細胞内への取り込みに依存する。Lnは鉄と同様に不溶性なため、その取り込み機構は鉄の取り込みと同様にTonB-dependent receptorによる機構が存在すると考えられていた。

本研究では*M. aquaticum* 22A株において、シデロフォア合成遺伝子を同定し、シデロフォアが鉄の溶解に関わるだけでなく、植物の生育促進に関わること、Lnの溶解と取り込みにも関わること、メタノール生育及びMDHの発現調節にも関わること、また細胞の酸化ストレスや重金属耐性、バイオフィーム形成など様々な機能に関わることを遺伝学的に証明した。さらに22A株が持つ12個のTonB-dependent receptorのうち、シデロフォア鉄、クエン酸鉄、Lnの取り込みに関わる輸送体を、それぞれの遺伝子破壊株作製により同定した。また、それらが本属細菌基準株の系統間で異なる保存性を持つことなどを明らかにした。

本研究の成果はFrontiers in Microbiology誌に掲載された。シデロフォアの精製と構造決定には至らなかったが、植物共生細菌のシデロフォアの多様な機能を明らかにした点、特にLn取り込みにも関わることの発見が学術的に高く評価できる。国内・国際学会で研究成果を発表しており、演習科目での他の研究分野の発表にも積極的に質問議論し、学位発表でも複雑なデータを簡潔にまとめて発表出来た。Patrick Otieno Juma氏は学位の授与に値する十分な研究成果をあげ、またその過程で十分な研鑽を積んだことを学位論文審査委員として認める。