

Brief Report

Sessile bacterium unlocks ability of surface motility through mutualistic interspecies interaction

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Summary

In addition to their common planktonic lifestyle, bacteria frequently live in surface-associated habitats. Surface motility is essential for exploring these habitats for food sources. However, many bacteria are found on surfaces, even though they lack features required for migrating along surfaces. How these canonical non-motile bacteria adapt to the environmental fluctuations on surfaces remains unknown. Here, we report a previously unknown surface motility mode of the canonical non-motile bacterium, *Dietzia* sp. DQ12-45-1b, which is triggered by interaction with a dimorphic prosthecate bacterium, *Glycocalyx alkaliphilus* 6B-8T. *Dietzia* cells exhibits 'sliding'-like motility in an area where the strain *Glycocalyx* cells was pre-colonized with a sufficient density. Our analysis also demonstrates that *Dietzia* degrade n-alkanes and provide *Glycocalyx* with the resulting metabolites for survival, which in turn induced directional migration of *Dietzia* towards nutrient-rich environments. Such interaction-triggered migration was also found between *Dietzia* and *Glycocalyx* strains isolated from other habitats, suggesting that this mutualistic relationship ubiquitously occurs in natural environments. In conclusion, we propose a novel model for such a 'win-win'

strategy, whereby non-motile bacteria pay metabolites to dimorphic prosthecate bacteria in return for migrating to seek for nutrients, which may represent a common strategy for canonically non-motile bacteria living on a surface.

Introduction

In addition to growing in a planktonic form, many bacteria also colonize surfaces, which is a process that requires attachment of bacterial cells to various surfaces and surface-associated motility (Henrichsen, 1972; Harshey, 2003; Maier and Wong, 2015). These movements allow bacteria to escape local stresses and explore their environment in search for nutrients (Henrichsen, 1972; Fraser and Hughes, 1999; Harshey, 2003). Motility is also involved in other bacterial processes occurring on surfaces, including biofilm formation, microbe-host interaction and bacterial cell morphogenesis (Henrichsen, 1972; Fraser and Hughes, 1999; Harshey, 2003). However, while these motility-related traits provide considerable selective advantage for bacteria living on surface, many bacterial species, called canonically non-motile bacteria, lack such migratory characteristics. How these canonically non-motile bacteria adapt to heterogeneous environments remains to be elucidated (Kearns, 2010; Ben-Jacob *et al.*, 2016).

Recently, several canonically non-motile bacteria have been described to move on surface by interacted with other species. For example, 'riding' on the swarming *Paenibacillus vortex* cells promote the dispersal of non-motile *Xanthomonas* (Hagai *et al.*, 2014) as well as *Escherichia coli* (Finkelshtein *et al.*, 2015) strains on solid surfaces. Gliding motility of *Capnocytophaga gingivalis* population plays an import role in long-range transport of those non-motile bacterial cells in a polymicrobial community (Shrivastava *et al.*, 2018). This type of social behaviour also contributes to shaping of spatial patterns of multi-species bacterial colonies (Xiong *et al.*, 2020). Together, these phenomena are called 'hitchhiking' strategies (Ben-Jacob *et al.*, 2016), which requires a highly motile population acting as a 'truck' carries non-motile

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'cargo' cells along. In addition, a recent case showed that two sessile soil strains, *Pseudomonas fluorescens* Pf0-1 and *Pedobacter* sp. V48 started co-migrating upon

initial close association (McCully *et al.*, 2019). However, due to the diversity of social interactions in microbial world, it still remains an open question whether there are

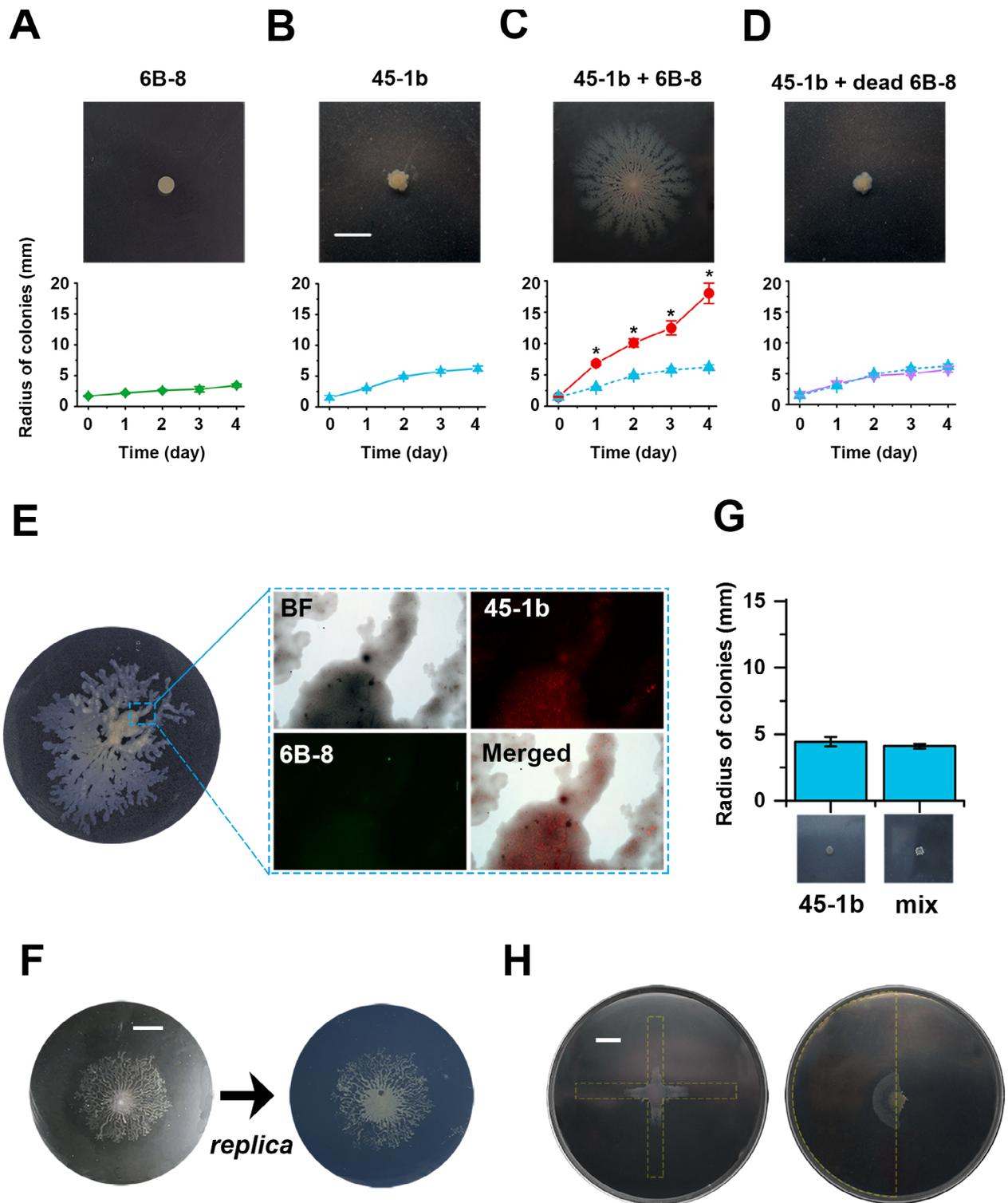


Fig. 1. Legend on next page.

other interaction modes that can trigger the surface motility of canonically non-motile bacteria.

Here, we report a previously unknown form of surface motility way of non-motile bacteria, *Dietzia* spp. that was induced by dimorphic prosthecate bacteria (DPB), *Glycocalxis* spp. Observations derived from this interaction is different from the previous manner. The presence of the DPB induced the *Dietzia* species migrate outward on a soft agar surface and form a dendritic fractal pattern, while the DPB earned essential carbon sources from the partner and survive better in the nutrient-limited environment. Therefore, this mode combined interaction-induced movement and by-product cross-feeding, which gives rise to a community-level 'win-win' strategy for both strains to live on surface-attached habitats.

Results and discussion

Interspecies interaction induces bacterial surface motility

Dietzia sp. DQ12-45-1b (45-1b) and *G. alkaliphilus* 6B-8T (6B-8) were both isolated from one oil reservoir in the Daqing Oilfield, China (Wang et al., 2011; Geng et al., 2015). Strain 45-1b is a Gram-positive, canonically non-motile bacterium with the ability to biodegrade a wide range of petroleum hydrocarbons (Wang et al., 2011). Strain 6B-8 is a Gram-negative bacterium that only utilizes a very narrow spectrum of carbon sources, excluding both alkanes and glucose (Geng et al., 2015) and does not show typical surface motility (Fig. 1A) on LB plate with 0.5% agar. While the strain 45-1b formed a smooth circular and static colony on 0.5% agar plates (Fig. 1B) containing hexadecane (C16) as the sole carbon source (henceforth called 'alkane agar plate'), the strain 6B-8 failed to grow alone under these conditions. Intriguingly, when we thoroughly premixed with 6B-8 cells with agar plate (containing C16 as the carbon source) and then inoculated strain 45-1b on the centre, we found that a fractal dendritic colony was developed with a spreading speed of $2.87 \pm 0.28 \mu\text{m min}^{-1}$ (Fig. 1C). Analysis by fluorescence microscopy showed that the dendritic pattern was mostly structured by cells of 45-1b

(Fig. 1E). Importantly, this pattern was observed only when live 6B-8 cells were present (Fig. 1D). Furthermore, replica plating analysis revealed that the colonies of 6B-8 that formed on 1.5% LB agar were identical to the fractal dendritic spreading path of the 45-1b colony on the alkane agar plate (Fig. 1F), which demonstrated that the pre-mixed 6B-8 cells grew at the sites at which 45-1b grew in the agar plate with C16 as the carbon source. Together, these results suggest that, assisted by 6B-8 cells, 45-1b cells migrate along the surface, thus exhibiting a behaviour characteristic of 'surface motility'. To investigate whether the movement of 45-1b was derived from the previous reported 'hitchhiking' strategies (Ben-Jacob et al., 2016) or 'social spreading' mechanisms (McCully et al., 2019), we performed a surface motility assay similar to these studies (Finkelshtein et al., 2015). In brief, we added an inoculum containing an equal mixture of 6B-8 and 45-1b to the agar plate and co-cultivated them with C16 as the sole carbon source. Unexpectedly, we found that 45-1b failed to move on the agar surface (Fig. 1G), indicating that the surface motility of 45-1b was not induced by the movement of 6B-8. Instead, 45-1b developed colonies only within the regions (e.g., semi-circle or cross) where *G. alkaliphilus* 6B-8T was previously overlaid (Fig. 1H). These results indicated that under this interaction mode, the 45-1b cells only migrated to where 6B-8 cells were present. This observation differs completely from the previously reported 'hitchhiking' strategy between non-motile and highly motile bacteria (Hagai et al., 2014; Finkelshtein et al., 2015; Shrivastava et al., 2018; Xiong et al., 2020), as well as the interaction mode of *P. fluorescens* Pf0-1 and *Pedobacter* sp. V48 (McCully et al., 2019), suggesting different underlying mechanisms.

Surface motility of Dietzia sp. DQ12-45-1b requires surfactants produced from alkane metabolism

To test whether the surface motility in 45-1b co-incubated with 6B-8 occurs under various environmental conditions, we incubated 45-1b cells on agar plates premixed with 6B-8 cells, each plate containing one of 23 different

Fig. 1. Surface motility of *Dietzia* sp. DQ12-45-1b induced by *Glycocalxis alkaliphilus* 6B-8T. A–D. Movement of 6B-8 (A) on a LB plate containing 0.5% agar and 45-1b on alkane agar plate in the presence of live (C), dead (D), or absence of (B) 6B-8. The lower panel depicts temporal changes in colony radii. Data represent mean \pm SD of three independent replicates. Dashed lines in (C) and (D) indicate changes in colony radii in (A) and statistical analyses were done by Student's *t*-test: **p* < 0.01. E. Fluorescent microscopic image shows that dendritic pattern was mostly structured by cells of 45-1b. 45-1b was labelled with DsRed, while 6B-8 was labelled with EGFP. BF: bright field; Merged: a composite of the other three images. F. Replica plate analysis shows that 6B-8 cells grow at sites where 45-1b cells grow on alkane agar plates. The left image shows the colony morphology of 45-1b on soft agar pre-mixed with 6B-8 using C16 as the sole carbon source. The right image shows the colony morphology of 6B-8 cells after blotting on LB agar containing $5 \mu\text{g ml}^{-1}$ kanamycin (6B-8 is kanamycin-resistant, while 45-1b is not). G. When the two strains were initially mixed together at equal amounts and inoculated onto a soft agar plate, no significant surface motility was observed; Statistical analysis by *t*-test compared with 45-1b (the movement of 45-1b in the absence of 6B-8), *p* = 0.27. H. Colonies of 45-1b cells only 'moved' at locations where 6B-8 cells were previously overlaid. The left image shows 6B-8 cells previously overlaid within the cross region indicated by dashed lines. The right image shows 6B-8 cells previously overlaid within the left half of the agar, as indicated by dashed lines. See Supporting Information S1 for detail about these assays.

compounds as sole carbon source (Table S1). While 45-1b cells can metabolize all of the 23 compounds, 6B-8 cells only metabolize acetic acid and α -ketoglutarate. Upon co-incubation with 45-1b cells on agar plates, we observed growth of 6B-8 cells for all 23 carbon sources, indicating that the growth of 6B-8 cells was supported by the metabolic by-products of 45-1b cells (Table S1). However, we only found surface

motility of 45-1b cells in agar plates containing three alkanes (i.e., dodecane, tetradecane and hexadecane; Table S2; Fig. 2A).

In one of previous studies, we reported 45-1b cells produce biosurfactants to emulsify alkanes (Wang *et al.*, 2013). To test whether biosurfactant production is the reason for alkane-dependence of this motility, we first acquired the biosurfactant-containing supernatant by

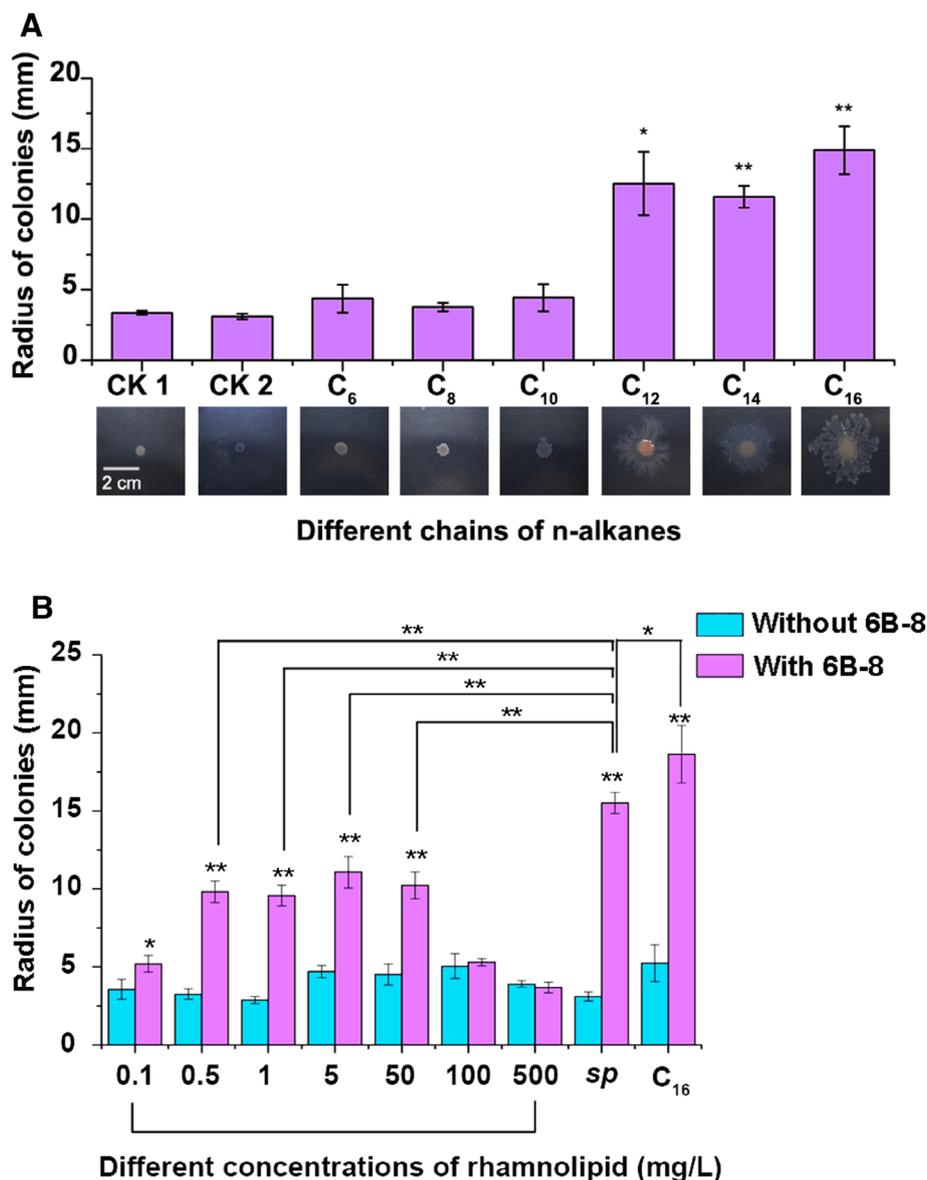


Fig. 2. Surface motility of *Dietzia* sp. DQ12-45-1b required surfactants produced from alkane metabolism. A. Effect of the carbon chain length of n-alkanes on induction of 45-1b surface motility. Data represent mean \pm SD of three independent replicates. The lower panel shows the colony pattern of 45-1b when co-cultured with 6B-8 using the corresponding carbon sources. CK1, in the absence of 6B-8 and using C16 as the carbon source. CK2, in the presence of 6B-8 but without carbon source addition. Statistical analysis was performed by comparison with CK1 using Student's *t*-test: * $p < 0.05$ and ** $p < 0.01$. B. Induction of 45-1b surface motility by bio-surfactant concentrations ranging from 0.1 to 50 mg l⁻¹ and *sp*: surfactant-containing supernatant. For incubation with rhamnolipid or *sp*, a 45-1b cell suspension was inoculated onto soft agar pre-mixed with 6B-8 cells and glucose as the sole carbon source. For C16 (positive control), a 45-1b cell suspension was inoculated onto soft agar pre-mixed with 6B-8 cells with C16 as the sole carbon source. See Supporting Information S1 for detail about these assays.

mono-culturing of 45-1b cells in liquid minimal medium containing C16 as the sole carbon source. We then incubated 45-1b on the agar plate both premixed with 6B-8 and overlaid the biosurfactant-containing supernatant on the surface, using glucose as the sole carbon source. Although glucose was not sufficient to trigger the interaction-dependent surface motility of 45-1b (Table S2), in the presence of the biosurfactant-containing supernatant, a turnover occurred where surface motility of 45-1b was observed (Fig. 2b, denoted as *sp*). To confirm that this turnover was due to the effects of the secreted biosurfactant, we overlaid solutions of rhamnolipid on the surface, and performed the similar assays. As shown in Fig. 2B, surface motility of 45-1b cells was observed with concentrations of rhamnolipid ranging from 0.1 to 50 mg l⁻¹. Together, these results indicated

that this interaction-triggered surface motility of 45-1b needs surfactants produced from alkane metabolism.

To investigate the motility way of 45-1b in this interaction, we analysed its genome (GenBank accession: GCA_009740915.1). However, its genome lacks any genes known to encode the active cell appendages, such as flagella, Type IV pili as well as rotary motor structures, suggesting that the migration of 45-1b was not derived from those active motility types, that is, swarming (Kearns, 2010), twitching (Maier and Wong, 2015) or gliding (Shrivastava *et al.*, 2018), but may be attributed to one type of passive motility that was characterized as appendage-independent, called sliding. Henrichsen (1972) defined sliding as a passive bacterial translocation created by expansive forces accelerated by surfactants that reduce surface tension (Henrichsen, 1972). This hypothesis is in

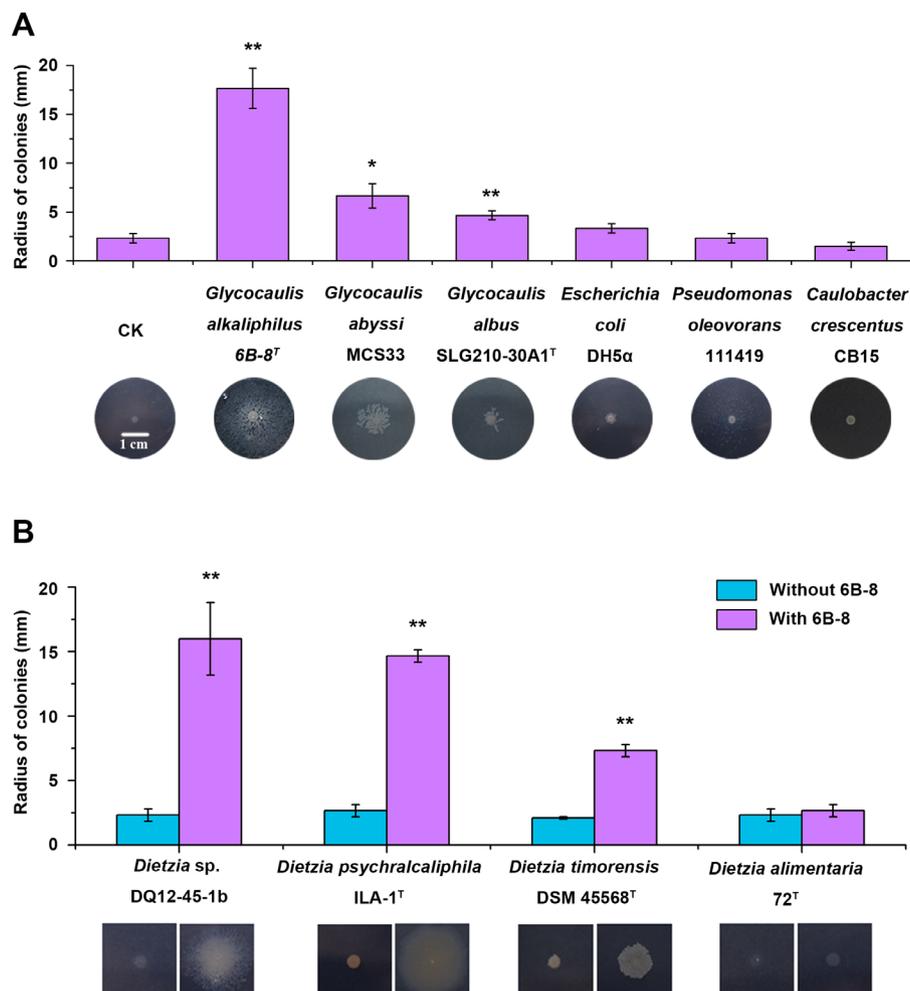


Fig. 3. Surface motility is induced in different *Dietzia* strains, and in response to various *Glycocalyx* strains. A. Only *Glycocalyx* strains were able to induce *Dietzia* surface motility. CK, the movement of *Dietzia* 45-1b in the absence of *Glycocalyx* 6B-8T. B. Other *Dietzia* strains also exhibited induction of surface motility by *G. alkaliphilus*, except for *Dietzia alimentaria* 72T, suggesting that induced surface motility may be universal to *Dietzia* strains. In all cases, data shown are representative of three independent experiments and the error bar represents the standard deviation. Statistical analysis by *t*-test compared with CK (A), or the movement of corresponding strains in the absence of *Glycocalyx* 6B-8T: **p* < 0.05 and ***p* < 0.01. See Supporting Information S1 for detail about these assays.

agreement with the fact that surface motility of 45-1b cells required biosurfactants. In addition, sliding motility commonly shapes dendritic colony morphology on agar plates (Holscher and Kovacs, 2017), a typical feature of our newly described interaction mode. Thus, we suggested that strain 45-1b exhibited a sliding-like motility in this interaction mode.

Both cell types yield from the interaction

Cell motility is usually associated with the search for nutrients. To better understand whether increased motility of 45-1b was associated with seeking nutrients, we developed a glucose gradient on an agar plate that was pre-mixed with 6B-8 cells and overlaid with the biosurfactant-containing supernatant, followed by inoculation of *Dietzia* 45-1b. As shown in Fig. S1A, 45-1b displayed chemotaxis towards the higher glucose concentration. This result suggested that, by interacting with 6B-8 cells, the 45-1b cells were able to move towards environments containing more nutrients (i.e., chemotaxis).

On the other hand, after co-incubating with 45-1b and replica blotting, the developed 6B-8 colony in LB plate showed a pattern that exactly matched the colony of 45-1b on the alkane agar plate (Fig. 1F), while much less visible colonies were observed after the same treatment but without co-incubation with 45-1b cells (Fig. S1B). This suggested that the strain 45-1b utilized C16 and simultaneously provided 6B-8 cells with essential nutrients, presumably for survival and/or growth. This idea was further supported by the observed growth of 6B-8 on supernatants from a monoculture of 45-1b cells in C16 liquid medium, and α -ketoglutarate, a derivative produced during C16 biodegradation by 45-1b (Fig. S1B). Taken together, our results indicate that both cell types yield from this interaction, resulting in a 'win-win' situation on surface-attached habitats.

The interaction mode is widespread across many ecological habitats

We finally assessed the prevalence of the described interaction. We first tested whether other bacteria or related DPB can assist in the migration of 45-1b cells. In these assays, *Glycocalyx* abyssii MCS33 (isolated from a deep-sea hydrothermal vent) and *Glycocalyx* albus SLG210-30A1T (isolated from oil-contaminated saline soil) also induced surface motility for 45-1b cells (Fig. 3A). Moreover, 6B-8 cells also induced surface motility of *Dietzia* psychrocaliphila ILA-1T (isolated from a drain of a fish product processing plant) and *Dietzia* timorensis DSM 45568T (isolated from soil; Fig. 3B). As these strains were isolated from a variety of

environments, we propose that this interaction mode is present across a wide range of ecological habitats.

Concluding remarks

Here, we showed that interaction with other species can trigger canonically non-motile bacteria to move on surfaces. Our results demonstrate that this interaction was not derived through a previously reported 'hitchhiking' strategy (6). Importantly, the surface motility of the sessile species required its partner pre-colonized the outward region of the starting point at sufficiently high density, which is also different from the interaction between *P. fluorescens* Pf0-1 and *Pedobacter* sp. V48 (McCully *et al.*, 2019). In addition, the presence of biosurfactants was also vital to this motility. Moreover, by interacting with 45-1b, 6B-8 cells get essential nutrients for survival/growth. Therefore, we proposed a previously unknown interaction mode that can promote the migration of canonically non-motile bacteria. This 'reciprocity' process is possibly widespread in many habitats, and presumably represents a prevalent 'win-win' strategy for bacteria to adapt to perpetually changing environments.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1: Supplementary Information

Figure S1. Interaction between the two strains is mutually beneficial. (a) 6B-8 cells survived better on an agar plate overlaid with the metabolite-containing supernatant (*sp*) or α -ketoglutarate, a metabolite of 45-1b degraded hexadecane. The agar plate overlaid with the minimum medium (MM) was used as control. Three independent replicated experiments were conducted, representative results are shown in the lower panel, depicting the colonies of 6B-8 growing on the LB agar plates. These results suggested that the metabolites produced by 45-1b assist 6B-8 cell survival /or growth. (b) When grown in a nutrient gradient environment containing 6B-8 cells, 45-1b grew and migrated towards high concentrations of nutrients. Soft agar plates were pre-mixed with 6B-8 cells; biosurfactant-containing supernatant was spread on this plate to induce surface motility, a strip of sterile paper (4 cm \times 0.35 cm) saturated with 20 μ l of a glucose solution (250 g/l) was placed on one side of the plate to form a nutrient gradient. Strain 45-1b was inoculated by dropping it on the surface of the soft agar. The ruler indicates the distance from the paper. Red dots represent the positions where 45-1b was inoculated. The binary image on the right shows the direction of surface motility. The arrows indicate the direction of the nutrient gradient.