# The Effect Of Chemotaxis On The Swarming Ability Of *Bacillus subtilis*: Critical Effect Of Glutamic Acid And Lysine

Lina Hamouche, Soumaya Laalami, Ghayas Lakkis, Ahmad Kobaissi, Ali Chokr, Harald Putzer, Kassem Hamze

**Abstract**: Bacterial cell differentiation constitutes an appropriate and efficient way to respond to an ever-changing environment. *Bacillus subtilis* is no exception where in some conditions planktonic cells differentiate into highly motile swarmer cells. The hyper-flagellated swarmer cells, located usually at the colony edge, move in a cooperative manner in order to reconnoiter new sites for colonization, this movement is called “swarming”. Whether chemotaxis plays a role in swarming migration remains controversial and therefore we examined the effect of amino acids (chemo-attractants), glutamic acid and lysine deprivation on swarming. Here we show that deprivation of glutamic acid in synthetic B-medium results in attenuated, defective and random swarming patterns, while deprivation of lysine leads to almost normal swarming. However, deprivation of both amino acids results in a major reduction in swarming. We also developed a method using swarm plates with a concentration gradient to measure chemotaxis. Using this approach, we found that *B. subtilis* swarmed normally towards 100 % glutamic acid but did not swarm towards medium lacking this amino acid. However, the bacteria swarmed to both sides of plates with a concentration gradient of lysine. Furthermore, our results indicated that these two chemo-attractants affect motility by modulating the expression of the *hag* gene. Thus, the absence of glutamic acid and lysine decreased the expression of *hag* during swarming, respectively by 36 % and 15 %.

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**Index Terms**: *Bacillus subtilis*, swarming, chemotaxis, single cell expression, flagellin, glutamic acid, lysine.

## 1 INTRODUCTION

*Bacillus subtilis* is a soil bacterium, considered as the model for Gram-positive bacteria. When growing on the surface of a synthetic medium (0.7 % agar), *B. subtilis* forms highly branched patterns in a process called swarming (Fraser and Hughes, 1999; Julkowska *et al*., 2004). The swarming process is a rapid and massive migration of cooperating groups of bacteria (Harshey, 2003; Julkowska *et al.,* 2004, 2005). The swarm develops in a series of specific steps including the secretion of surfactin at 11– 12 h, following inoculation and several generations of growth. After 12–13 h, groups of cells form ‘buds’ at the edge of the mother colony, which are abruptly expanded to form the initial monolayered dendrites. Extension of dendrites continues at approximately 3 mm/h, remaining as a monolayer for up to 18 h until multilayered growth (consolidation) commences from the base and spreads outwards (Julkowska *et al*., 2005; Hamze *et al*., 2009). Swarming of *B. subtilis* absolutely depends upon the presence of flagella, and, under most conditions, the production of surfactin (Kearns and Losick, 2003; Julkowska *et al*., 2004, 2005; Hamze *et al*., 2009). Surfactin is produced in the mother colony (MC) (Debois *et al*., 2008). Surfactin locally reduces surface tension, friction or viscosity at the air-water interface. In addition, surfactin modifies the agar surface, effectively extracting water from the agar matrix in order to maintain a depth of fluid that is sufficient for swarming. Surfactin is a cyclic lipopeptide (Peypoux *et al*., 1999) that is synthesized non-ribosomally, and spreads just ahead of the migrating bacteria throughout the swarming process (Julkowska *et al*., 2004, 2005; Debois *et al*., 2008). A peritrichous arrangement of flagella characterizes the great majority of bacteria that are able to swarm, where numerous flagella are disseminated randomly on the cell surface. Peritrichous flagella are of great importance for swarming movement. Flagella are packed together when rotated, to increase their rigidity and to generate a more effective force to move through the environment. Formation of the *B. subtilis* flagellum proceeds in a hierarchical, highly regulated manner controlled by specific regulatory genes (Aizawa *et al*., 2002). The large operon flgB-sigD contains 31 genes that encode proteins concerned with chemotaxis, proteins involved in flagellum biosynthesis, especially hook-basal body, as well as sigma factor D that activates the transcription of several flagellar genes (Fredrick and Helmann, 1996). The number of flagella is up regulated in swarmer cells as shown by Hamze *et al*. (2011) by measuring the pattern of *hag* expression (encoding flagellin). Flagellin is the main component of the flagellar filament, and the authors


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were able to identify three distinct subpopulations of cells: (i) long chains close to the edge of the MC with no detectable expression of *hag*; (ii) largely non-motile cells in dendrite stems, with intermediate levels of hag expression; and (iii) a population of highly motile cells, with hyper-expression of *hag* at the tip of dendrites, "the swarmers". Chemotaxis is the process by which cells sense chemical gradients (for example nutrients) in their environment and then move towards more favorable conditions. The chemotaxis system, composed of sensory receptor networking with components of a cytoplasmic phosphorylation cascade can alter the rotational bias of the flagella, allowing the bacteria to move toward attractants and away from repellents (Adler, 1966). A group of sensory transducers or transmembrane receptors (also referred to as methyl-accepting chemotaxis proteins or MCPs), have the capacity to detect stimuli. In *B. subtilis*, 10 MCPs have been identified, which distinguish attractants such as amino acids, sugars, and oligopeptides, as well as repellents like extreme pH, certain metal ions and hydrophobic amino acids. Ligand binding to these receptors ultimately leads to the appropriate change in rotation of the flagellum. (Garrity and Ordal, 1997; Kang *et al.,* 2010; Glekas *et al*., 2011). *B. subtilis* McpB is a class III chemotaxis receptor, methylated in response to ligand. McpB mainly plays the role of chemoreceptor for asparagine and glutamic acid (Kirby *et al*., 1999). Zimmer *et al*., (2000) showed that McpB is present as one or two isoforms in the absence or presence of asparagine, respectively. This suggested that McpB might predominantly have two methylated states and that the receptor is methylated selectively upon asparagine addition and removal. Probably this is also applicable to glutamic acid. Hanlon and Ordal (1994) showed that glutamic acid is an excellent chemo-attractant for *B. subtilis* in liquid medium. The chemoreceptor McpC plays also a major role in chemotaxis in *B. subtilis*, being required for taxis to the amino acids cysteine, proline, glycine, serine, lysine and valine. A *B. subtilis* mutant defective in McpC showed an almost total loss of taxis to all these and also arginine in liquid medium (Kristich *et al*., 2003; Müller *et al.*, 1997). From this point of view, it is extremely important to study if certain amino acids affect swarming of *B. subtilis* and we chose glutamic acid and lysine since they are components of B-medium used in previous swarming experiments. To investigate this issue, we carried out a comparative analysis of the development of these swarming communities of *B. subtilis* completely deprived of glutamic acid and lysine. In addition, we studied the effect of concentration gradients of glutamic acid or lysine on the swarming profile and in particular how this might affect the dendritic patterns. Finally, we compared the effect of glutamic acid and lysine on the level of the expression of *hag* in two conditions, in liquid medium and during swarming.

## 2 Materials and Methods

### 2.1 Strains and growth conditions.

Bacterial strains used in this study are listed in Table 1. Bacteria were grown with aeration at 37 °C in minimal B-medium composed of (all final concentrations) 15 mM $(NH_4)_2SO_4$, 8 mM $MgSO_4$ 7 H2O, 27 mM KCl, 7 mM sodium citrate $2H_2O$, 50 mM Tris/HCl, pH 7.5; and 2 mM $CaCl_2$ $2H_2O$, 1 µM $FeSO_4$ 7 $H_2O$, 10 µM $MnSO_4$. 4 $H_2O$, 0.6 mM $KH_2PO_4$, 4.5 mM glutamic acid, 862 µM lysine, 784 µM tryptophan and 0.5 % glucose were added before use (Antelmann *et al*., 1997).

For the thr auxotrophs, both liquid and solid media were always supplemented with 1 mM threonine. Antibiotics were added to plates at the following final concentrations: chloramphenicol, 5 µg ml-1; spectinomycin, 100 µg ml-1. In addition, minimal B-medium composed of all constituents but lacking glutamic acid or lysine or both glutamic acid and lysine were prepared and inoculated with bacteria.

**Gradient concentration plates**. The gradient plate consists of two wedge-like layers of media: a bottom layer of B-medium with 0 % glutamic acid or 0 % lysine agar and a top layer of normal B-medium agar. Glutamic acid or lysine in the top layer, diffuse into the bottom layer producing a gradient of chemical concentration from low to high across the plate. It is necessary to have both layers present in slant form until the gradient concentration from 0 to 100 % is achieved (Fig. 3).

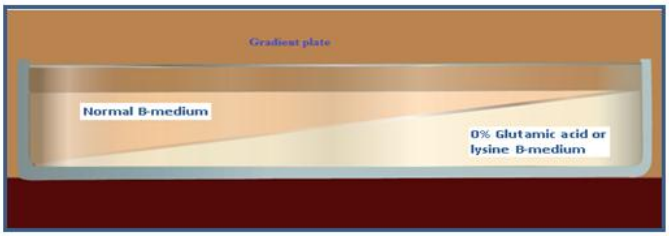


**Figure 3.** Gradient plate. A bottom layer (12.5 ml) of 0 % amino acid (glutamic acid or lysine or both) of B-medium agar and a top layer (12.5 ml) of normal B-medium agar. The amino acid in the top layer, diffuses into the bottom layer producing a gradient of chemical concentration from low to high and for that reason it is required that both layers must be in slant form until this gradient concentration is established across plate from 0 to 100 % of the intended amino acid.

***Table 1.*** *Strains used in this study*

| *Strain* | *Genotype* | *Reference, source or construction* |
|---|---|---|
| *OMG 900* | *trpC2 swrA amyE : : sfp+ cat* | *Julkowska et al., (2005)* |
| *OMG 992* | *trpC2 swrA thrC : : sfp+ erm amyE : : Phag–gfpmut3 spc* | *Hamze et al., (2011)* |

### 2.2 Conditions for dendritic swarming experiments.

For swarming on B-medium, 9 cm swarm plates containing 25 ml medium (0.7 % Bacto agar) were prepared 1 h before inoculation with drying restricted to 5 min before inoculation. Cultures for inoculation were prepared in 10 ml B medium inoculated from a single colony on an LB agar plate and shaken overnight at 37ºC. The culture was diluted to OD 570 ~0.1 and grown at 37ºC to OD 570 ~0.2. This procedure was repeated twice and finally the culture was grown to $T_4$ (4 h after the transition from exponential growth). The culture was diluted, and $10^4$ bacteria (2 µl) were placed at the center of a swarm plate and incubated at 30ºC (usually at a relative humidity of 70 %). Note that different types of swarm plates of B-medium (normal, 0 % glutamic acid, 0 % lysine or 0 % glutamic acid and 0 % lysine) were incubated for varying times in order to identify the terminal phenotype.

### 2.3 IMAGING.

Photographs of swarms were taken at the indicated times, either using an Epson 1600 Pro scanner at a resolution above 600 d.p.i. in transparent mode or by digital camera that should be 10 Mega pixels or above to get good resolution. In order to detect the surfactin ring, or at early stages in the swarming process (up to 16–17 h), when the bacteria normally form only a monolayer, plates should be photographed with reflected light, using a high resolution camera. Images were mainly captured using Sony 14.1 Mega pixels with dual shot processing, and figures were prepared using Adobe Photoshop software (version Cs5, 2011).

### 2.4 PHASE CONTRAST AND FLUORESCENCE MICROSCOPY.

Bacteria were examined *in situ* with different objectives appropriate for a phase-contrast/fluorescence microscope (Zeiss AxioImager M1). The microscope was fitted with an AxioCam camera (Zeiss).GFP was excited at 450–490 nm and the fluorescence collected in the range 515–565 nm (filter set 10, Zeiss). A range of low magnification air objectives (x 1.25, 5, 20 and 40) were used initially to establish the perspective of images before selecting an appropriate field for analysis at high magnification. We analyzed only the bacteria located on the tip of the dendrites. This was carried out as required with an x 100 oil objective (neofluar plan with numerical aperture1.3). For the latter, tips of dendrites were observed with a microscope coverslip placed gently over the required portion of the swarm. For the quantitative analysis of Phag–gfp expression along the dendrite with the microscope, images were captured using AxioVision software (release 4.6.3), false-colored, and analyzed with background subtraction, using Auto Measure associated with the AxioVision software. The results were expressed at the single-cell level, as the mean fluorescence intensity of an entire field of cells. The experiments, repeated three times, with dendrite tips analyzed from three different swarms, gave comparable results. For all quantitative single-cell measurements, between 500 and 900 cells per field "tip" were measured. We have also used another way to measure the fluorescence at the single-cell level, by scraping bacteria from the tip of the dendrite and dispersing them at low density in buffer for fluorescence measurements.

## 3 RESULTS

### 3.1 BACILLUS SUBTILIS GROWTH IN DIFFERENT B MEDIA

We decided to investigate if taxis of *B. subtilis* to amino acids, which are strong attractants and act through chemoreceptors (Sonenshein *et al*., 2002), would have any effect on swarming. In this study, we employed 2 amino acid ligands, glutamic acid and lysine that are in fact normal components of the minimal B-medium we used in our swarming assay. In liquid cultures, we inoculated *B. subtilis* strain OMG 900 in an assortment of B-media. The first having all components as described in materials and methods and termed Normal B-medium. The second medium was deprived of glutamic acid (0 % glutamic acid medium), while in the third we eliminated the lysine (0 % lysine). In addition, we prepared B-medium lacking both amino acids, called 0 % lysine 0 % glutamic acid. First, our results revealed that without either glutamic acid or lysine, there was little effect on growth of *B. subtilis*, strain OMG 900 (generation time around 87 minutes) similar to growth in normal B-medium (Fig.1). When both amino acids were absent (generation time 125 minutes) there appeared to be an extended lag but then growth proceeded close to the normal growth rate, although the mass yield was reduced. We wondered if without glutamic acid and lysine the medium was deficient in the nitrogen source. Therefore we added to B-medium lacking lysine and glutamic acid a triple quantity of $(NH_4)_2SO_4$. Indeed, this was sufficient to restore the normal growth profile (Fig. 1).

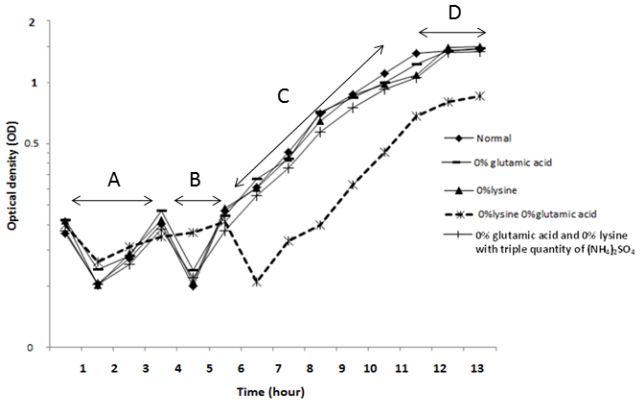


***Figure 1***. *Growth curves of B. subtilis in the presence and the absence of amino acids (glutamic acid and lysine). OMG 900 was grown in different types of B-medium with aeration at 37˚C. The bacterial growth curves are shown with bars for B-medium 0 % glutamic acid; triangles for B-medium 0 % lysine; dashed line with stars for B-medium 0 % lysine 0 % glutamic acid; crosses for B-medium 0 % lysine 0 % glutamic acid supplemented with 3x $(NH_4)_2SO_4$ and diamonds for normal B-medium. Optical density for bacterial growth is shown in the Y-axis. Maximum (OD) corresponds to almost 1.5 and generation time to 87 minutes in all types of B-medium except the 0 % lysine* 0 % glutamic acid medium, where generation time corresponds to 121 minutes. The X-axis represents the time in hours. Domain A corresponds to the first dilution of overnight pre-culture; domain B- second dilution from OD= 0.2 to 0.1; C- domain exponential growth; domain D- stationary phase.

### 3.2 EFFECT OF AMINO ACID DEPRIVATION ON *B. SUBTILIS* MIGRATION

OMG 900 inoculated on normal B-medium can fill the plate within 36 to 48 hours (Fig. 2A). On the same semi-solid agar, strain OMG 900 inoculated on B-medium 0 % glutamic acid, was able to swarm but there was reduction in swarm expansion and the pattern was disturbed. (Fig. 2B). The swarms do not reach the edge of plates, and after 48 hours it was still 1 to 1.5 cm from the edge of the swarm plates (Figure 2B). In contrast, on B-medium 0 % lysine the bacteria swarmed almost normally although there was some pattern changes in the branching in the centre of the swarm (Fig. 2C). We also examined the migration of *B. subtilis* on a B-medium lacking both amino acids. Surprisingly, Figure 2D clearly shows a synergistic effect with swarming much slower than with only glutamic acid absent. Branching of dendrites was also much reduced compared to the wild type or with lysine absence. As a control experiment, we inoculated OMG 900 in swarm plates lacking both amino acids but containing a triple amount of $(NH_4)_2SO_4$. In this case the same reduced swarming pattern was observed as on B-medium deprived of both

amino acids with the normal amount of $(NH_4)_2SO_4$ (data not shown). Thus, the reduced swarming in the absence of both amino acids was not the result of limited nitrogen source. These observations show that in the absence of glutamic acid and especially when lysine is also absent swarming is greatly reduced. Previous studies in liquid cultures have indicated that glutamic acid is a chemo-attractant for *B. subtilis* (Kirby et *al*., 1999). Now our results suggest that it is also an essential attractant on semi-solid medium.

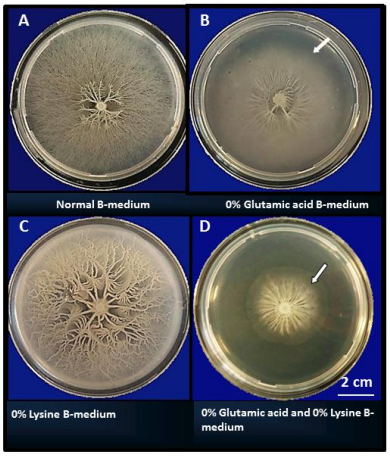


***Figure 2.*** *The effect of glutamic acid and lysine on the swarming pattern of Bacillus subtilis. OMG 900 was inoculated on different types of B-medium 0.7 % agar and incubated for 36 hours at 30˚C and 70 % of humidity. Plates were prepared and inoculated as described in materials and methods. (A) normal B-medium (B) B-medium lacking glutamic acid (C) B-medium lacking lysine (D) B-medium lacking glutamic acid and lysine. White arrows mark the limit of the bacterial swarm front. The typical dendritic swarming pattern is shown in A; B and D show reduced swarming speed and defective patterns.*

### 3.3 SWARMING VERSUS GRADIENT CONCENTRATIONS OF AMINO ACIDS

As described above, we showed that glutamic acid and lysine deprivation has an effect on swarming migration of *B. subtilis* OMG 900, suggesting that chemotaxis is involved in swarming. In order to define this apparent chemotaxis toward these amino acids, we inoculated strain OMG 900 in the middle of two-dimensional gradient swarm plates of glutamic acid. The gradient plate consists of two wedge-like layers of medium: a bottom layer of 0 % glutamic acid B medium agar and a top layer of normal B-medium agar (Fig. 3). The amino acid in the top layer diffuses into the bottom layer producing a gradient of glutamic acid concentration from low to high across the plate (from 0 to 100 %). Note that as a control normal plates with no gradient were also prepared in the same way with two layers of normal B-medium. We inoculated OMG 900 at the middle of gradient plates (see materials and methods) and after incubation we found that the *B. subtilis* strain in replicate experiments swarmed completely towards 100 % glutamic acid and never in the opposite direction. Nevertheless, the expanding surfactin zone was produced and observed on both sides (Fig. 4B). In contrast, when we inoculated strain OMG 900 in the middle of two-dimensional gradient swarm plates of lysine, swarming occurred normally on both sides, toward both the high and low concentrations of lysine (Fig. 4C). The results clearly confirmed a role for glutamic acid in swarming consistent with chemotaxis towards this acid.

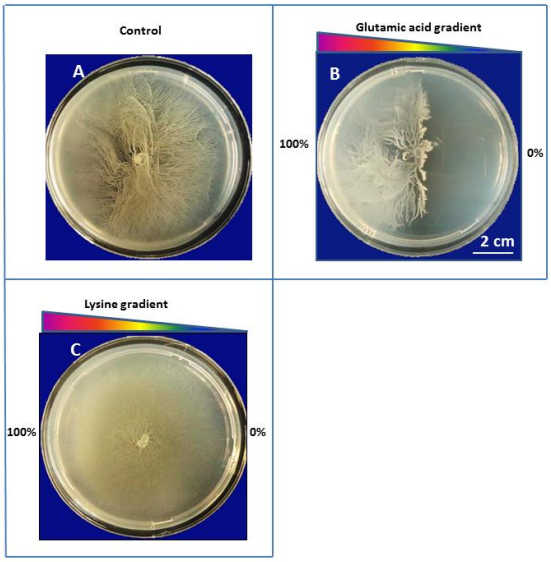


***Figure 4.*** *Dendritic migration of OMG 900 on amino acid B-medium gradient plates. Plates were prepared as described in materials and methods and shown in figure 3. Plates were incubated for 36 hours after inoculation and then photographed. (A) Plate with two layers of normal B-medium, used as control, shows dendritic swarming pattern over the whole plate. (B) Plate with one layer of B-medium lacking glutamic acid and the upper layer of normal B-medium, to produce a gradient (C) Plate with a gradient concentration of lysine. Gradient color scale indicates the concentration of glutamic acid and lysine from 100 % to 0 %.*

### 3.4 ANALYSIS OF HAG-GFP EXPRESSION IN SUBPOPULATION OF CELLS AT TIP OF DENDRITE

A previous study by Hamze *et al*., (2011) showed that flagella are essential for swarming on B-medium, and this is accompanied by an obvious gradient of expression of *hag–gfp* along the dendrites, with marked hyper-expression in the 1 mm zone of the tip. In this study we used the same strain and technique of single-cell quantitative analysis to compare the level of *hag-gfp* expression in the presence and the absence of glutamic acid and lysine. Our objective was to determine if these two amino acids have a role in flagella synthesis and hence in swarming. Elongation of dendrites until at least 1.5 cm (16-18 h after incubation) takes place as a monolayer before the transition to a multilayered pattern. Taking advantage of this monolayer, we examined *in situ* the expression of *hag-gfp* at the level of single cells in the tip region of the dendrite. On semi-solid agar, we measured fluorescence intensity in single cells from a series of images taken *in situ* at the tip of 1.5 cm dendrite with a fluorescence microscope at high magnification. In these images, the great majority of cells in the monolayer were quite well separated (Fig. 5). The results presented in Fig. 6B show the average fluorescence intensity per cell against the type of B-medium used. Our data revealed that the expression of *hag-gfp* decreased tips by 15 % in B-medium lacking lysine and by

36% in B-medium lacking glutamic acid compared to the expression of *hag-gfp* in normal B-medium. Very similar data were obtained in three separate replicate experiments. We also carried out experiments in liquid media, as a control. Our results showed no difference at the level of *hag-gfp* expression in the presence or the absence of glutamic acid or lysine (Fig. 6A). As we discuss below, the results of these measurements are consistent with our observations that swarming is reduced in the absence of the chemo-attractant glutamic acid.

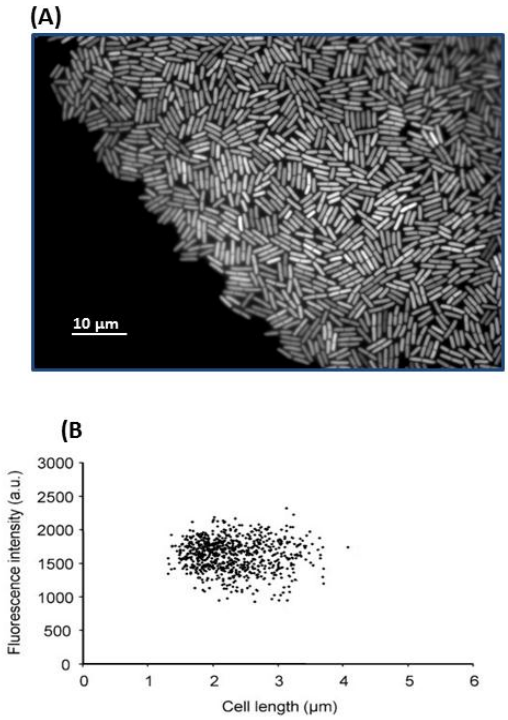


***Figure 5.*** *Single-cell, quantitative analysis in situ of Phag-gfp in dendrite tips. (A) Raw fluorescent image of the monolayer of cells at the tip of dendrite obtained in situ with the fluorescence microscope (100 objective). (B) Typical scatter plot of fluorescence intensity (a.u.) per unit surface area of cells versus cell length.*

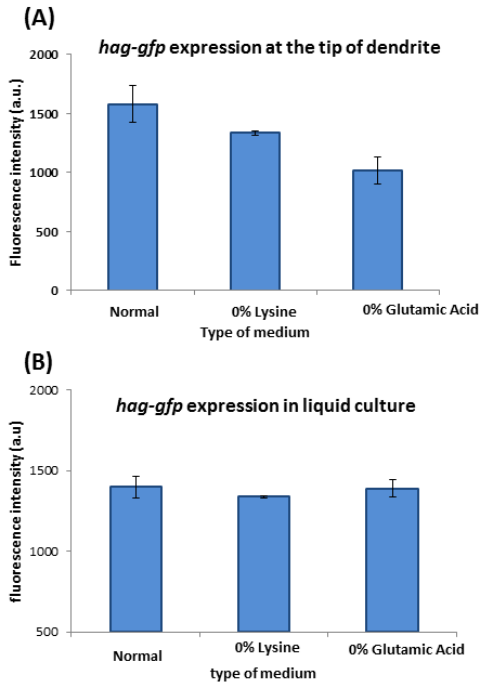


***Figure 6****. Quantitative analysis of hag-gfp fluorescence intensity in situ at tip region. Mean fluorescence intensity values of Phag-gfp are calculated after analysis of 500-900 cells from images taken at the tip region from triplicate experiments with high magnification (x100 objective). (A) the expression of Phag-gfp at the tip measured in the absence of lysine and in the absence of glutamic acid compared to the level obtained in normal B-medium. (B) Control experiment showing measurements of the expression of Phag-gfp in the three types of B-medium in liquid culture, at an O.D. of 1.*

## 4 Discussion

The original motive for undertaking these experiments was to attempt to gain a better understanding of the effect if any of chemotaxis on swarming migration of *B. subtilis*. Despite, previous studies that have reported that chemotaxis does not influence the swarming of bacteria because cells in a swarm are randomly reoriented and therefore do not display the basis of chemotactic orientation formed by running and tumbling behavior (Darnton et *al*., 2010). Here, we have shown that the growth rate of strain OMG 900 in liquid B-medium, lacking glutamic acid or lysine, was not affected. When *B. subtilis* was inoculated in B-medium lacking both glutamic acid and lysine there appeared to be a lag in starting growth but then the growth rate was the same as in normal B-medium. However, increasing the amount of $(NH_4)_2SO_4$ eliminated the delay in growth and the reduced growth yield (Fig. 1). This may indicate that the amino acids, especially glutamate, are important sources of nitrogen source. These results clearly indicated that glutamic acid or lysine deprivation had no effect on the growth of *B. subtilis,* and so they can be considered as important candidates to study a chemotaxis effect on swarming capabilities of *B. subtilis* depending on their proprieties as chemo-attractants. In the present work, we found that when glutamic acid is absent from the medium, *B. subtilis* swarms poorly. Speed was reduced and defective random swarming patterns were obtained (Fig. 2B). This

clearly shows that the absence of a strong chemo-attractant, like glutamic acid, was sufficient to attenuate the well-known dendritic swarming pattern in *B. subtilis.* At this point, it was interesting to test what would be the effect of deprivation of both glutamic acid and lysine on the swarming of *B. subtilis,* The results showed that deprivation of lysine has no effect but has an additive effect when glutamic acid is also absent. Thus, swarming is arrested prematurely (Fig. 2D). This phenotype can be explained by two hypotheses: nitrogen limitation or absence of chemo-attractants. The results shown in Fig.1 eliminate the first hypothesis because *B. subtilis* was able to grow normally in liquid medium lacking glutamic acid, and in B-medium lacking both glutamic acid and lysine with triple quantity of $(NH_4)_2SO_4$. Glutamic acid remains a key regulator for a successful typical swarming pattern, since the absence of glutamic acid, blocked normal swarming, and lysine may contribute to this in some way. Since deprival of lysine as well as that of glutamic acid reduced swarming additively, this may be explained if both amino acids influence the chemotaxis system by synergetic pathways. The flagella constitute the endpoint of the chemotaxis sensory system and flagellar rotation is changed when ligand bind to the receptors (Falke and Hazelbauer, 2001). Attractants like glutamate are known to be detected by a group of sensory transducers or transmembrane receptors (MCPs) which initiates a signal transmitted to the flagellar motor (Rao *et al*., 2008). In order to obtain more evidence for a role of glutamate chemotaxis in swarming of *B. subtilis,* we examined swarming with plates with a concentration gradient of amino acids from 100 % to 0 %. Interestingly, *B. subtilis* swarmed completely, with a normal pattern, toward the side having higher concentration of glutamic acid and never to the side of B-medium having the lower concentration. This confirmed that swarming of *B. subtilis* is dependent on glutamate. However, swarming was observed on both sides of the plates having a concentration gradient of lysine, which is consistent with our previous result. These results provide some evidence that glutamic acid modulates the swarming ability of *B. subtilis* in a chemotactic way. Still to be answered; why *B. subtilis* was able to swarm on B-medium lacking glutamic acid although with a defective pattern but not at all on the plate of B-medium having a concentration gradient of glutamic acid. We can envisage that the bacteria tried to swarm in B-medium fully deprived of glutamic acid because they have no other choice, while with the gradient plates there is a high concentration on one side that the cells can detect, apparently in a chemotactic manner. Based on the results obtained on plates with concentration gradient of amino acids and previous studies showing the importance of chemotaxis system on cell differentiation, maturity and production of flagella (Burkart *et al*., 1998; Mariconda *et al*., 2006). We measured the effect of glutamic acid and lysine (chemotaxis) on flagellar synthesis, by examining in situ the expression of hag, using *B. subtilis* carrying the *Phag–gfp* fusion (OMG 992) in normal B-medium and lacking glutamic acid or lysine. In liquid media, no significant difference was observed on the expression *of hag-gfp*. Whereas, on semi-solid medium, the results showed down regulation of *hag-gfp* expression, with a greater effect of glutamic acid (Fig. 6). These results support the role of the chemotaxis system on swarmer differentiation and the production of flagella, and indeed, several genes encoding proteins involved in chemotaxis and flagellar biosynthesis share the same operon flgB-sigD (Fredrick and Helmann, 1996). To conclude, this study shows in a comparative analysis approach the apparent effect of chemotaxis on the swarming profile of *B. subtilis*. Many experiments are still required to unlock and clarify this as yet intriguing relationship. Our results furnish new routes to examine swarming behavior in *B. subtilis*. Thus, the ability of highly potent amino acid attractants to control the swarming process provides an impetus to explore further the relationship between chemotaxis and swarming.

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