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Relation between chemotaxis and consumption of amino acids in bacteria

Yiling Yang,[1,2] Abiola M. Pollard,[1,2] Carolin Höfler,[2] Gernot Poschet,[3] Markus Wirtz,[3] Rüdiger Hell[3] and Victor Sourjik[1,2]*

[1]*Max Planck Institute for Terrestrial Microbiology & LOEWE Center for Synthetic Microbiology (SYNMIKRO), Marburg, Germany.*
[2]*Zentrum für Molekulare Biologie der Universität Heidelberg, DKFZ-ZMBH Alliance, Heidelberg, Germany.*
[3]*Centre for Organismal Studies (COS), Universität Heidelberg, Heidelberg, Germany.*

Summary

Chemotaxis enables bacteria to navigate chemical gradients in their environment, accumulating toward high concentrations of attractants and avoiding high concentrations of repellents. Although finding nutrients is likely to be an important function of bacterial chemotaxis, not all characterized attractants are nutrients. Moreover, even for potential nutrients, the exact relation between the metabolic value of chemicals and their efficiency as chemoattractants has not been systematically explored. Here we compare the chemotactic response of amino acids with their use by bacteria for two well-established models of chemotactic behavior, *Escherichia coli* and *Bacillus subtilis*. We demonstrate that in *E. coli* chemotaxis toward amino acids indeed strongly correlates with their utilization. However, no such correlation is observed for *B. subtilis*, suggesting that in this case, the amino acids are not followed because of their nutritional value but rather as environmental cues.

Introduction

In order to grow and reproduce, heterotrophic bacteria must acquire nutrients from their environment. When nutrients are limited, chemotaxis would be advantageous, because it allows for motile bacteria to find nutritional sources by actively following gradients. Chemotaxis in bacteria has been extensively studied (Szurmant and Ordal, 2004; Wadhams and Armitage, 2004; Porter *et al.*, 2011; Hazelbauer, 2012). Typically, environmental stimuli are perceived through sensory complexes consisting of transmembrane chemoreceptors, an adaptor protein CheW and a kinase CheA (Sourjik, 2004; Hazelbauer *et al.*, 2008). In the cell, these complexes are organized into large clusters that are essential for amplification and integration of chemotactic signals. Binding of effectors to the receptor modulates the rate of CheA autophosphorylation. The phosphate group is then transferred to the response regulator CheY. Phosphorylated CheY (CheY-P) interacts with the flagellar motor changing either the rotational direction or speed of flagella rotation and causes reorientation (tumbling) of the cell. These proteins are the core of the chemotaxis pathway and are conserved among bacteria, with some variability (Szurmant and Ordal, 2004; Porter *et al.*, 2011). For example, binding of attractant to the receptor inhibits CheA activity in *Escherichia coli* but activates it in *Bacillus subtilis*, whereas interaction of CheY-P with the motor induces clockwise rotation in *E. coli* and counterclockwise rotation in *B. subtilis* (Fig. S1). Furthermore, whereas in *E. coli* the phosphoryl group from CheY-P is removed by a phosphatase CheZ that localizes at the sensory complexes (Wang and Matsumura, 1996; Sourjik and Berg, 2000; Cantwell *et al.*, 2003), in *B. subtilis* this function is performed by the motor-associated protein FliY (Szurmant *et al.*, 2004).

Amino acids are one of the most valuable nutrient sources for bacteria and can be utilized as sole nitrogen, carbon or energy sources (Halvorson, 1972). Previous studies have shown that most bacteria, including *E. coli* and *B. subtilis*, exhibit chemotaxis toward multiple amino acids (Mesibov and Adler, 1972; Van Der Drift and De Jong, 1974; Ordal and Gibson, 1977; Gotz *et al.*, 1982; Hedblom and Adler, 1983; Hugdahl *et al.*, 1988; Taguchi *et al.*, 1997), albeit with different preferences. In *E. coli*, amino acid attractants are sensed by the two major receptors Tar and Tsr, which have the highest affinity for aspartate and serine respectively. These two amino acids are also rapidly consumed by *E. coli* in complex medium (Pruss *et al.*, 1994; Chang *et al.*, 1999; Selvarasu *et al.*, 2009) and serve as precursors of other important intermediate metabolites (Reitzer, 2003; Keseler *et al.*, 2013). This indicates that the chemotactic and nutritional preferences of *E. coli* are

Accepted 19 March, 2015. *For correspondence. E-mail victor.sourjik@synmikro.mpi-marburg.mpg.de; Tel. (+49) 6421 28 21400; Fax (+49) 6421 28 21485.

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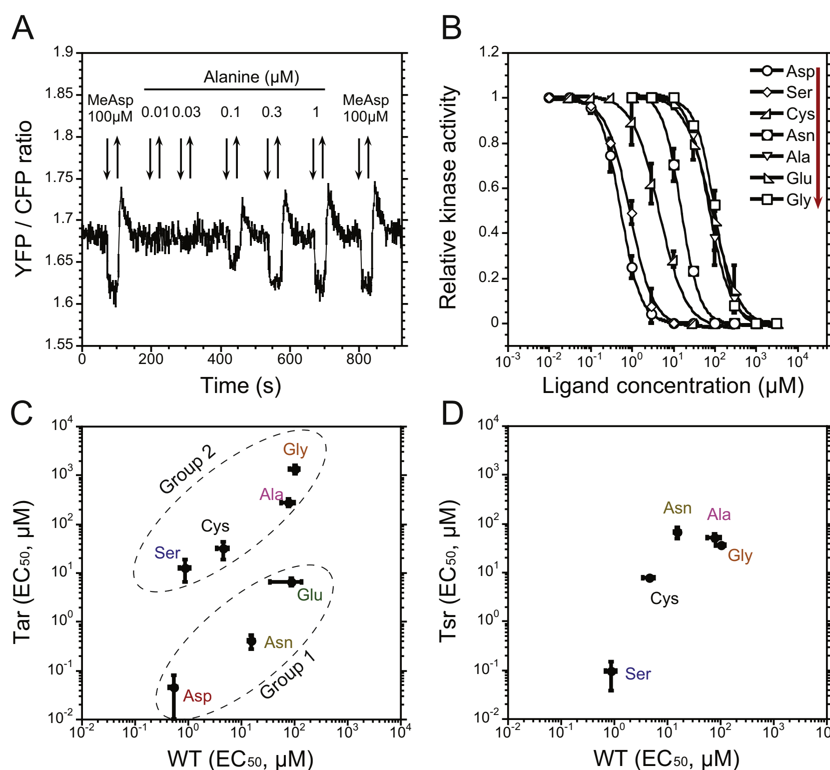

Fig. 1. FRET-based analysis of chemotactic responses of *E. coli* to amino acids.
A. Exemplary measurement of FRET response to alanine in the wild-type cells, using the phosphorylation-dependent interaction between CheZ-CFP and CheY-YFP. Buffer-adapted cells were stimulated with step-like addition and subsequent removal of indicated concentrations of amino acid (indicated by arrows). The response to a saturating stimulus, 100 μM α-methyl-DL-aspartate (MeAsp), was used as a positive control. Ratio of YFP to CFP fluorescence is proportional to the amount of FRET complex formed and thus to the kinase activity.
B. Dose responses of wild-type cells to amino acid attractants. Relative kinase activity, derived from the YFP/CFP ratio, was plotted relative to the steady-state activity in the buffer. Zero activity was obtained by a saturating stimulation with 100 μM MeAsp. Data were fitted using a Hill equation. Error bars here and throughout indicate standard errors.
C and D. Correlation between the values of EC_{50} in the wild type and in receptorless cells expressing Tar (C) or Tsr (D) as a sole receptor. Amino acid ligands sensed by Tar fall into two groups indicated by dotted lines, suggesting that Tar is primary sensor for group 1 and secondary sensor for group 2 amino acids.

related, despite the fact that the chemotactic signaling toward amino acids is decoupled from their uptake and metabolism (Mesibov and Adler, 1972; Kahane *et al.*, 1976).

Here we used pathway activity reporters based on fluorescence resonance energy transfer (FRET) to systematically quantify the chemotactic response of two model organisms, *E. coli* and *B. subtilis*, toward all 20 proteinogenic L-amino acids. We further investigated the utilization of amino acids during growth in chemically defined medium. Our results demonstrate that most of the amino acids that attract *E. coli* are also preferentially utilized during growth, with a strong correlation between the order of utilization and the chemoattractant potency. However, such correlation was not observed in *B. subtilis*, suggesting that it is not universal but may be rather related to the environment or physiology of an organism.

Results

Chemotactic response of E. coli *to amino acids*

To measure the intracellular response of the chemotaxis pathway in *E. coli*, we used a previously described FRET reporter that relies on phosphorylation-dependent interaction between CheY fused to yellow fluorescent protein (CheY-YFP) and CheZ fused to cyan fluorescent protein (CheZ-CFP) (Sourjik and Berg, 2002; Sourjik *et al.*, 2007) (Fig. S1A). Cells expressing this FRET pair were stimulated with serial dilutions of all 20 L-amino acids to measure the dose responses and to determine the EC_{50} – ligand concentration eliciting the half-maximum FRET response (Fig. 1A, Table S1). Mostly consistent with previous observations (Mesibov and Adler, 1972; Hedblom and Adler, 1983), wild-type *E. coli* cells showed response to seven amino acids in the sub-millimolar range (Fig. 1B, Table S1). Aspartate, serine and cysteine were the strongest attractants, with EC_{50} values below 10 μM. Asparagine, alanine and glutamate showed EC_{50} values of ~ 10–100 μM, whereas the EC_{50} for glycine was above 100 μM. In addition, weak attractant responses were also observed at millimolar concentrations of threonine and methionine (Table S1).

To further identify the roles of Tar and Tsr in the overall specificity of the amino acid response of *E. coli*, FRET measurements were also performed in strains expressing Tar or Tsr as the sole receptor. We observed that in the sub-millimolar range Tar mediates attractant responses to aspartate, asparagine, glutamate, serine, cysteine, alanine, methionine and glycine (Fig. S2A), whereas Tsr mediates attractant responses to serine, cysteine, glycine, alanine and asparagine (Fig. S2B). Interestingly, all of the Tsr-specific amino acids are also sensed by Tar, suggesting redundancy in the specificities of these two major receptors. This redundancy was not due to the impurities

of the amino acid solutions, as suggested previously (Hedblom and Adler, 1983), as identical responses were observed to synthetic amino acids.

To determine relative contribution of Tar and Tsr in the detection of particular amino acids in the wild type, we performed a correlation analysis between the EC_{50} values of the wild type and Tar- or Tsr-only cells. Such analysis for Tar clearly revealed two groups of amino acid attractants, one containing aspartate, asparagine and glutamate, and the other containing serine, cysteine, alanine and glycine (Fig. 1C). Although in both cases the EC_{50} values of Tar-only cells show a good correlation with the values of the wild type (Fig. S3A), the Tar values are lower for the first group and higher for the second group of amino acids. Since lower EC_{50} means higher sensitivity, these results suggest that the first group is exclusively (or primarily) detected by Tar. The observed higher sensitivity of Tar-only cells to these amino acids in comparison with the wild type is also consistent with previous studies for the Tar-specific attractant α-methyl-DL-aspartate (Sourjik and Berg, 2004) and can be explained by cooperative activation/inactivation of multiple receptors that are coupled in signaling teams (Mello and Tu, 2003; Sourjik and Berg, 2004; Keymer *et al*., 2006). This cooperativity results in signal amplification, which is stronger for the homogeneous teams that consist of only one type of receptor (Neumann *et al*., 2010).

The second group of amino acids is apparently detected primarily by Tsr, because Tsr-only cells sense all amino acids in group two with higher sensitivity than Tar-only cells, and clear correlation is observed between the Tsr and the wild-type specificity (Fig. 1D and Fig. S3B). Again, the sensitivity of the Tsr-only strain in detecting this second group of amino acids is higher than that of the wild type. Interestingly, although Tar is only a secondary receptor for serine, cysteine, alanine and glycine, it detects all of them and in the same order of sensitivity as Tsr. In contrast, Tsr detects only asparagine as a secondary ligand.

Tar-mediated response to serine

Although Tar responded to all of the amino acids that are sensed by Tsr, the response to serine was markedly different. Although the pathway activity in Tar-only cells was fully inhibited in presence of saturating concentrations of all other amino acid ligands, the response to serine saturated at ~ 20% of the initial activity (Fig. S2A). Moreover, Tar-only cells adapted rapidly to the initial serine stimulation, even at highest concentrations tested (Fig. S4). These differences suggested that the mechanism of serine sensing by Tar might be different from the conventional response mediated by ligand binding to the periplasmic sensory domain, as already proposed in a previous study (Neumann *et al*., 2010).

To determine whether serine response is mediated by the periplasmic domain of Tar, we used a 'pinhead' Tar construct that lacks the entire periplasmic domain but is nevertheless able to activate the kinase (Gosink *et al*., 2006) (Fig. S4A). Pinhead-Tar mediated response to serine with similar sensitivity and strength as the wild-type Tar (Fig. S4B). In contrast, responses of pinhead-Tar to other amino acid attractants were either severely weakened as compared with the full-length Tar or were entirely abolished (Fig. S4C–H). This result confirms that – in addition to the conventional sensing via the periplasmic domain of Tsr – serine is also detected by Tar through a mechanism that involves only the cytoplasmic domain.

Correlation between amino acid utilization and chemotaxis in E. coli

Wild-type *E. coli* is able to utilize several amino acids as carbon and nitrogen sources (McFall and Newman, 1996; Reitzer, 1996). Serine and aspartate, for which cells have the highest requirement (Reitzer, 2003), are the first amino acids consumed by *E. coli* in the complex medium (Pruss *et al*., 1994; Selvarasu *et al*., 2009). Because serine and aspartate are also the best amino acid attractants for *E. coli*, we investigated whether there might be a general correlation between chemotaxis and catabolism of amino acids. To address this question, we first tested utilization of amino acids in *E. coli* MG1655 culture grown in the minimal medium with an equal-molar (1 mM) mixture of all 20 amino acids and without other carbon or nitrogen sources. Changes in the levels of all amino acids in the medium could be monitored over time using liquid chromatography, except for tryptophan, which was not detected in our measurements. Our results showed that MG1655 cells are able to utilize at least seven amino acids during the time course of culture growth (Fig. 2A). In the early exponential phase, cells preferentially consume serine, cysteine and aspartate. Glutamine, glycine and glutamate were consumed during the late exponential phase, and alanine was consumed only after cells entered the stationary phase. A minor consumption of threonine and proline has also been observed, whereas the amounts of other amino acids either remained relatively constant or even increased, such as lysine (Fig. S5A). No amino acid degradation was detected in the absence of bacteria (Fig. S5B).

Comparison of these data with the set of amino acid attractants (Fig. 1B) showed nearly perfect overlap between the sets of chemoattractive and catabolized amino acids (summarized in Table S1). Among seven amino acids that are strong attractants (EC_{50} < 1 mM), only asparagine was not utilized from the mixture of amino acids under aerobic conditions (but see below). *Vice versa*, among seven efficiently utilized amino acids only glutamine is not an attractant for *E. coli*. The correlation

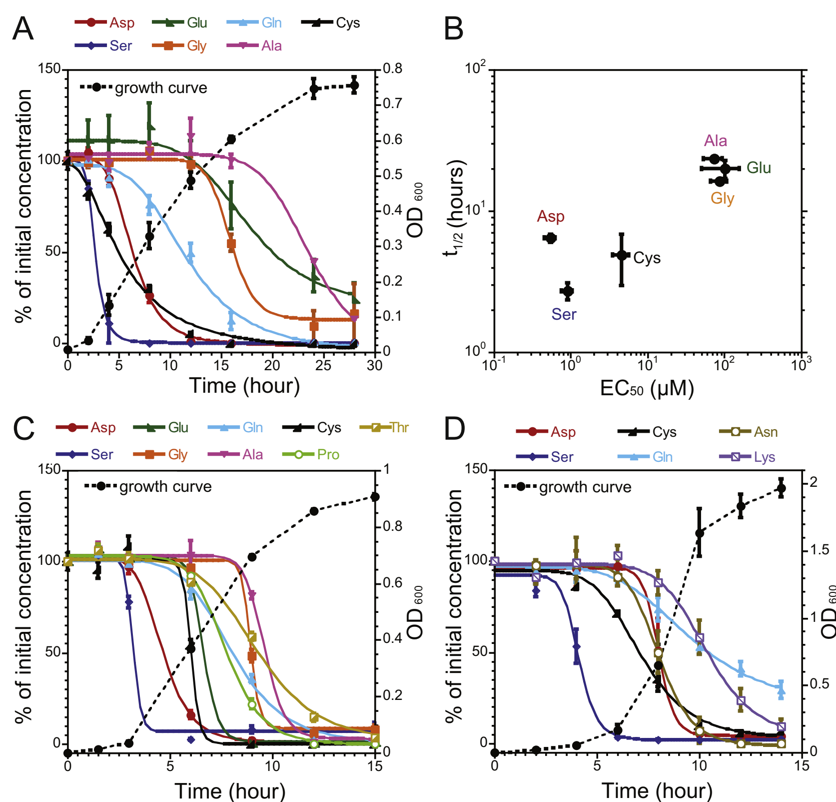

Fig. 2. Consumption of amino acids by *E. coli*.
A. Profile of amino acid uptake by *E. coli* MG1655 in minimal medium containing equimolar mixture of amino acids as the sole source of carbon and nitrogen. Profiles of amino acids with less than 50% utilization are shown in Fig. S5A.
B. Correlation between $t_{1/2}$ of amino acid uptake, derived from (A), and the EC_{50} of chemotactic response as determined in Fig. 1B.
C. Profiles of amino acid uptake by *E. coli* NCM3722 under the same growth conditions as in (A). Profiles of amino acids with less than 50% utilization are shown in Fig. S9A.
D. Profiles of amino acid uptake by *E. coli* NCM3722 in minimum medium under anaerobic conditions. Profiles of amino acids with less than 50% utilization are shown in Fig. S9B.

between the utilization and chemotaxis becomes even more pronounced when comparing the time point at which 50% of the respective amino acid is consumed, $t_{1/2}$, with the EC_{50} of the chemotactic response (Fig. 2B). These results suggest that stronger amino acid attractants are also consumed earlier during cell growth, indicating that chemotaxis to amino acids in *E. coli* has evolved to follow environmental gradients of catabolizable free amino acids.

To verify the generality of our conclusions, we further investigated how the consumption of amino acids by MG1655 is affected by the presence of other carbon (glucose or glycerol) or nitrogen (ammonia) sources (Fig. S6). Although the set of utilized amino acids was smaller in this case compared with the measurements performed with the mixture of amino acids as sole carbon and nitrogen source, three out of four amino acids that were consumed (serine, aspartate and glycine) are attractants and only one (glutamine) is not (cysteine was not measured in these experiments). We have further tested the capability of MG1655 to utilize individual amino acids as sole sources of carbon and/or nitrogen (Fig. S7A). Although none of individual amino acids could support efficient growth as sole carbon source, several amino acids could serve as nitrogen source. As expected, these are largely the same amino acids (serine, aspartate, cysteine, alanine, glutamine and glycine) that were utilized during growth on a mixture of amino acids and are (except glutamine) chemoattractants. Notably, asparagine (a potent attractant) could also efficiently support growth as a sole nitrogen source. In addition, several amino acids that are neither attractants nor utilized during growth on a mixture of amino acids (tryptophan, arginine and proline) could nevertheless serve as nitrogen sources. However, except glutamate (see below), none of the amino acids that could not serve as nitrogen sources were efficient attractants.

We further extended our analysis to *E. coli* strain NCM3722, a physiologically more robust parental strain of *E. coli* K-12 (Soupene *et al.*, 2003). As expected, NCM3722 showed chemotactic response to the same set of amino acids as MG1655, and also very similar EC_{50} values (Fig. S8), except for a more sensitive response to asparagine. The profile of amino acid utilization by NCM3722 (Fig. 2C and Fig. S9A) was also similar to that of MG1655, with only some differences in the order of consumption and more efficient utilization of proline and threonine. Consistently, NCM3722 could utilize largely the same amino acids as a sole nitrogen source as MG1655 (Fig. S7B), with addition of glutamate (another attractant), methionine (also a weak attractant) and lysine (not an attractant).

Finally, we also tested the utilization of amino acids by *E. coli* NCM3722 under anaerobic condition, which might better reflect the environment of the gut. Under these conditions, only six amino acids were utilized (Fig. 2D and

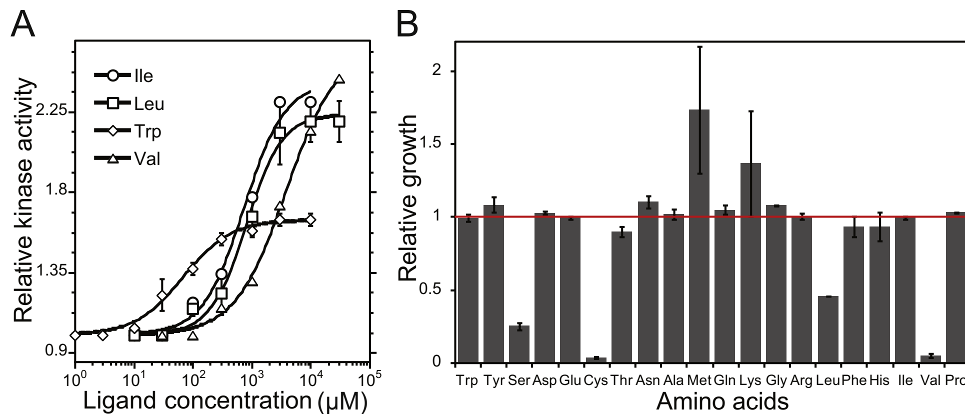

Fig. 3. Repellent responses and growth inhibition by amino acids in *E. coli*.
A. Dose responses to amino acid repellents. Measurements were done and plotted as in Fig. 1A and B. The response was normalized to the prestimulus pathway activity as in Fig. 1B.
B. Growth inhibition by amino acids. Cells were grown in the M9 minimal glucose medium containing individual L-amino acids at the final concentration of 1 mM. Culture density was determined after 8 h, and relative growth was quantified by normalizing OD_{600} values to the control culture with no addition of amino acids. Red line indicates the relative growth of control culture.

Fig. S9B), and the ones most rapidly consumed were the highest affinity attractants serine, aspartate and cysteine, and also asparagine – an attractant that was not utilized aerobically. Taken together, these results confirm that the correlation between chemotaxis and utilization of amino acids in *E. coli* is a general phenomenon, largely independent on the strain or growth conditions.

Repellent response and inhibition of growth

In addition to attractant responses, clear repellent responses were observed when wild-type cells were stimulated with isoleucine, leucine, tryptophan or valine. The values of EC_{50} for these responses were in the range of ~ 0.1–3 mM (Fig. 3A). Repellent responses to these amino acids were also observed in Tsr-only but not in Tar-only cells, with similar response strength as in the wild type, suggesting that they are Tsr-specific (Fig. S10). By using hybrid receptors that combine sensory domain of Tsr with the signaling domain of Tar or vice versa, we confirmed that repellent sensing is mediated by the sensory domain of Tsr (Fig. S11). Aspartate, glutamate, phenylalanine, histidine and glutamine also elicited weak repellent responses in Tsr-only cells, but no obvious repellent response was observed up to 1 mM of these amino acids in the wild type.

Because some of these amino acids are known to inhibit bacterial growth (Quay *et al*., 1977; De Felice *et al*., 1979), we investigated whether the observed repulsion might be related to this inhibitory effect. Indeed, significant growth inhibition was observed when 1 mM of serine, cysteine, leucine or valine was added to the culture (Fig. 3B). Thus, at least for leucine and valine, repellent response might have evolved to avoid toxic level of these non-catabolized amino acids. In contrast, although cysteine and serine can inhibit cell growth, they are rapidly catabolized by *E. coli* and hence rather serve as attractants. Nevertheless, *E. coli* has been shown to avoid chemotactic accumulation to high concentrations of these amino acids through a different mechanism (Neumann *et al*., 2014) (see *Discussion*).

Amino acid chemotaxis and utilization in B. subtilis

To further test the generality of the observed correlation between chemoattraction and catabolism of amino acids, we used another model system for bacterial chemotaxis, a Gram positive and soil-dwelling *B. subtilis*. Despite the large evolutionary distance between *B. subtilis* and *E. coli*, the core of the chemotaxis pathway in both organisms is well conserved (Fig. S1). Because *B. subtilis* lacks a homologue of CheZ and instead uses a cytoplasmic component of the motor FliY as the primary phosphatase for CheY-P, we constructed the *B. subtilis* FRET reporter of the chemotaxis pathway based on the phosphorylation-dependent interaction between CheY-YFP and CFP-FliY. Similar to the CheY-YFP/CheZ-CFP FRET pair for *E. coli*, CheY-YFP/CFP-FliY pair in *B. subtilis* allows monitoring changes of the intracellular CheA activity upon ligand stimulation (Fig. 4A). Expectedly, the FRET response to attractants in *B. subtilis* was opposite from that in *E. coli*, as the binding of attractant induces an increase in the kinase activity in *B. subtilis*.

Using this FRET reporter, we could detect attractant responses of *B. subtilis* to 18 out of 20 tested L-amino acids (Fig. 4A and B and Table S3). Lysine and arginine were the only amino acids that did not elicit clear responses at the tested concentrations. Cysteine, proline

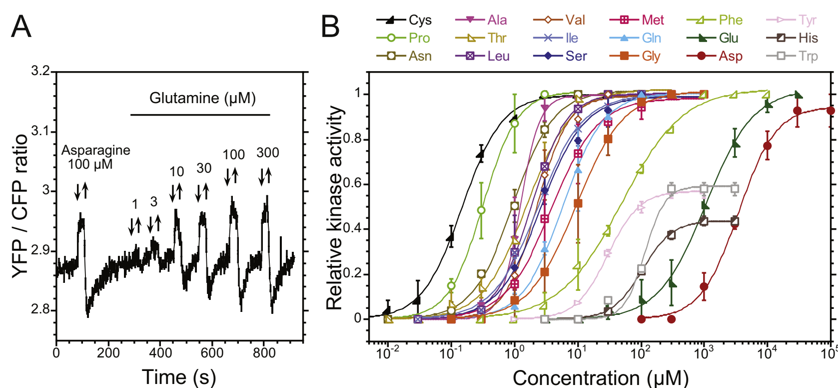

Fig. 4. Chemotactic response to amino acids in *B. subtilis*.
A. Exemplary FRET measurement of the chemotactic response in *B. subtilis* cells, using the phosphorylation-dependent interaction between CFP-FliY and CheY-YFP. Measurements were performed as in Fig. 1A. The response to a saturating stimulus of 100 μM asparagine served as positive control. Arrows indicate addition and removal of the indicated stimuli.
B. Dose responses to all amino acids attractants in *B. subtilis*, derived from FRET measurements performed as in (A).

and asparagine were most effective, with EC_{50} below 1 μM, and glutamate and aspartate were least effective with EC_{50} above 1 mM. Notably, the responses elicited by tyrosine, histidine and tryptophan were distinctly different, leading to lesser maximal activation of the pathway activity than other amino acids.

We further investigated the sequence of amino acid uptake in *B. subtilis*, showing that in the absence of other carbon and nitrogen sources, it can utilize nine amino acids during growth in minimal medium (Fig. 5). Fastest consumption was observed for asparagine and arginine, followed by glutamine, glutamate, serine, aspartate, alanine and cysteine. Proline was primarily consumed during the late exponential phase and stationary phase (Fig. 5A). The levels of other 10 amino acids remained relatively constant throughout the growth (Fig. S12A). Despite several differences in the order of consumption, the overall set of amino acids consumed by *B. subtilis* was similar to that of *E. coli*. The exceptions are arginine and asparagine, which are rapidly consumed by *B. subtilis* but are not catabolized by *E. coli* under aerobic conditions. A similar pattern of amino acid utilization was obtained for the parental wild-type strain of *B. subtilis* NCIB3610, although this strain could utilize several additional amino acids during the late exponential phase of growth (Fig. 5C and Fig. S12B).

In contrast to *E. coli*, however, no obvious correlation could be observed between the timing of uptake and chemotactic efficiency of amino acids in *B. subtilis* (Fig. 5B). For example, proline is a strong attractant but consumed at a later growth stage, whereas arginine is not an efficient chemoattractant but consumed rapidly. Some other strong amino acid attractants, such as leucine, isoleucine and valine, are not metabolized at all under the experimental conditions.

Discussion

Although most studied bacteria exhibit chemotaxis to amino acids and can also use them as nutrients, the relation between chemotaxis and consumption of amino acids remains largely unclear. In this study, we systematically characterized this relation for two model bacterial organisms, *E. coli* and *B. subtilis*. The chemotactic effectiveness of amino acids, quantified using FRET reporters of pathway activity for both organisms, was quite consistent with previous results from capillary assays (Mesibov and Adler, 1972; Van Der Drift and De Jong, 1974; Ordal and Gibson, 1977; Hedblom and Adler, 1983), further confirming the validity of the FRET assay for measurements of the chemotactic responses. Because of our observations that in the millimolar range many amino acids elicit apparently non-specific attractant responses in the absence of the receptor sensory domain (Fig. S4), we only consider seven amino acids – aspartate, serine, cysteine, asparagine, alanine, glutamate and glycine – to be specific attractants for wild-type *E. coli*. For *B. subtilis*, attractant response was observed for most amino acids, except glutamate, aspartate, arginine, and lysine that elicited only very weak or no response. Notably, these amino acids also showed least efficient chemotaxis in the capillary assays (Ordal and Gibson, 1977).

Importantly, the observed sets of the amino acid attractants are not expected to vary significantly between strains of *E. coli* or *B. subtilis* or to depend on growth conditions, and the two *E. coli* strains used in this study indeed showed nearly identical chemotactic responses to amino acids (Fig. 1 and Fig. S8). Bacterial chemotaxis system is known to function as a module (Hartwell *et al.*, 1999), largely independent of the other cellular processes. Moreover, in both *E. coli* and *B. subtilis* (as well as in other bacteria where amino acid chemotaxis has been characterized), the amino acid attractants are detected by specific receptors that are expressed as part of the chemotaxis module, meaning that the overall amino acid specificity is hardwired in the chemotaxis system. Nevertheless, within this set of amino acid attractants, relative preferences for individual amino acids might change due to changes in the relative expression of different receptors, e.g. dependent

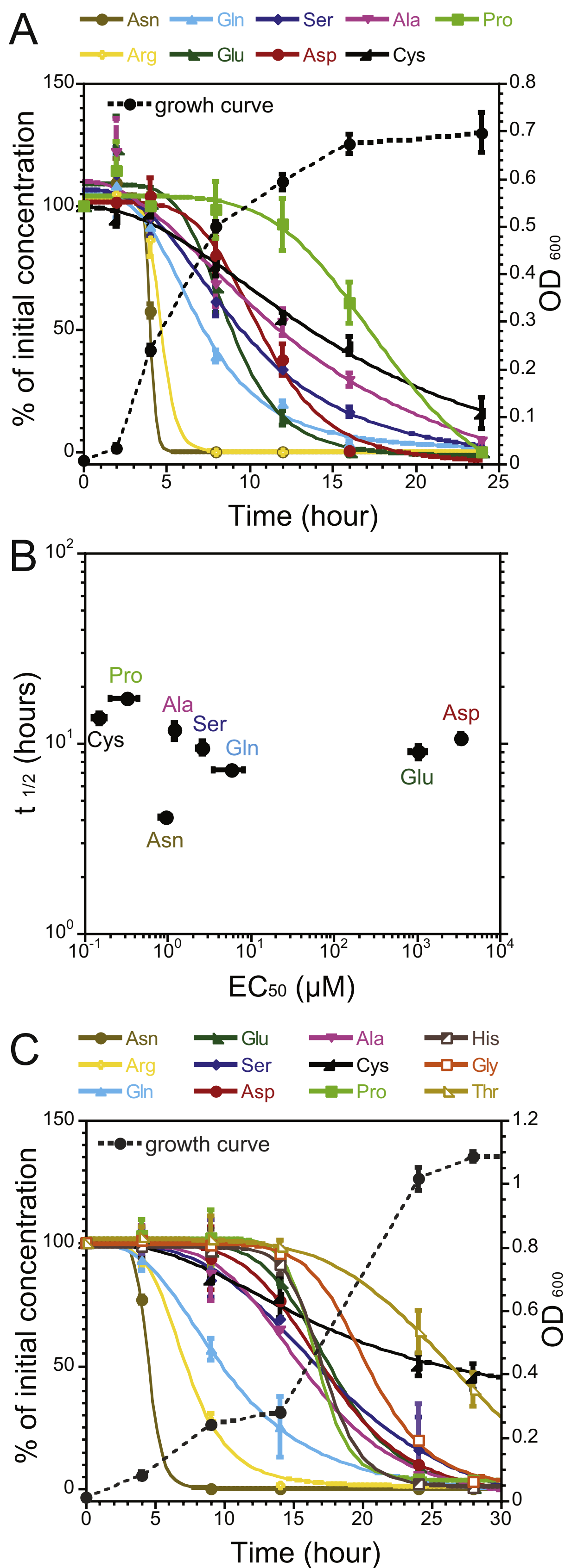

Fig. 5. Consumption of amino acids by *B. subtilis*.
A. Amino acid uptake profiles for *B. subtilis* cells grown in the minimal medium containing equimolar mixture of 20 l-amino acids as the sole source of carbon and nitrogen. Profiles of amino acids with less than 50% utilization are shown in Fig. S12A.
B. Correlation analysis between $t_{1/2}$ of amino acid uptake derived from (A) and the EC_{50} of chemotactic responses determined from Fig. 4B.
C. Amino acid uptake profiles for *B. subtilis* NCIB3610 grown under the same conditions as in (A). Profiles of amino acids with less than 50% utilization are shown in Fig. S12B.

on the growth stage of the culture (Salman and Libchaber, 2007; Sourjik *et al.*, 2007; Kalinin *et al.*, 2010; Neumann *et al.*, 2010).

In general, there was little similarity in the spectrum and efficiency of amino acid attractants between *E. coli* and *B. subtilis*. For example, aspartate, the best chemoattractant for *E. coli*, is very inefficient for *B. subtilis*, whereas leucine is a strong attractant for *B. subtilis* but repellent for *E. coli*. Moreover, the number of chemoattractive amino acids is much higher for *B. subtilis*, which may reflect differences in the habitat and in the life style. On average, among bacteria where the amino acid chemotaxis has been studied, the number of attractive amino acids is on average significantly larger in environmental than in intestinal bacteria (Table S4).

In contrast, *E. coli* and *B. subtilis* show very similar profiles of amino acid utilization, despite some strain- and specie-specific differences in the number of amino acids that can be utilized and in the order of consumption. Both *E. coli* and *B. subtilis* can utilize serine, aspartate, cysteine, glutamate, glutamine and alanine, and with lower efficiency proline and threonine. *B. subtilis* additionally consumes arginine and asparagine, whereas *E. coli* only consumes asparagine under anaerobic conditions. These findings are in general agreement with previous reports that used more complex media (Liebs *et al.*, 1988; Pruss *et al.*, 1994; Selvarasu *et al.*, 2009) and confirm strong evolutionary conservation of the metabolic network.

Most importantly, for *E. coli*, we observed a nearly perfect overlap between chemoattractive and catabolized amino acids (Table S1), with an additional correlation between the order of amino acid utilization and their efficiency as chemoattractants. This correlation was strongest with the amino acid utilization during aerobic growth on a mixture of amino acids as the sole carbon and nitrogen source, with six out of seven amino acids utilized by *E. coli* MG1655 (serine, aspartate, cysteine, glycine, glutamate and alanine) being attractants. Similar results were obtained for *E. coli* NCM3722, although this strain could additionally utilize, albeit less efficiently, several amino acids that were not attractants. Although fewer amino acids were utilized under anaerobic conditions, the strongest attractants serine, aspartate, cysteine and asparagine were also the first amino acids to be consumed

anaerobically. Thus, all of the efficient chemoattractive amino acids are also efficiently used, either aerobically or anaerobically, whereas none of the non-utilized amino acids are attractants. Although several of the least efficiently utilized amino acids are not attractants, these are presumably also least nutritionally valuable. The only interesting exception is glutamine, which is apparently nutritionally valuable but is not chemoattractive. Altogether, these results indicate that the chemotaxis system of *E. coli* has evolved to specifically locate sources of most nutritionally valuable amino acids under both aerobic and anaerobic conditions. Our conclusions are consistent with the theoretical analysis that suggested that chemotaxis is beneficial for bacteria to maximize their nutrient uptake even in a highly variable environment (Celani and Vergassola, 2010), such as that of the intestine.

Chemotaxis also apparently enables *E. coli* to avoid high concentrations of toxic amino acids, such as valine and leucine, which inhibit cell growth by affecting biosynthesis of other amino acids (Amos and Cohen, 1954; De Felice *et al*., 1979; Harris, 1981; Hama *et al*., 1990; 1991; Valle *et al*., 2008). Because high levels of valine may also be produced by bacterial biofilms (Valle *et al*., 2008), repulsion by valine could help to avoid nutrient-exhausted environment. An interesting dilemma is presented by serine and cysteine, which are valuable and rapidly catabolized nutrients but at the same time are growth-inhibitory. Serine and cysteine thus serve as highly efficient attractants sensed by Tsr, but cell accumulation toward toxic concentration of these amino acids is nevertheless prevented by a failure of Tsr to adapt to high levels of its ligands, which disables chemotaxis in the millimolar concentration range (Neumann *et al*., 2014).

The importance of the catabolized amino acids as attractants is further emphasized by both the receptor specificity and the observed redundancy of their detection by Tsr and Tar. Although Tsr is clearly a primary sensor for serine, cysteine, alanine and glycine, these amino acids are also sensed by Tar with the same order of preference. We speculate that such redundancy may be important to ensure detection of these attractants independent of cell density, because the ratio of Tar to Tsr is known to rise at later growth stages of bacterial culture (Salman and Libchaber, 2007; Kalinin *et al*., 2010). Expression of Tsr is highest at low cell density, which enhances chemotaxis toward its primary ligands serine and cysteine that are also the first amino acids to be catabolized. When Tsr is diluted at high cell density, the chemotactic preference is shifted to Tar ligands that are catabolized next, but certain sensitivity to serine and cysteine is maintained independent of Tsr expression because of their detection by Tar. Such redundant sensing may be a general feature of bacterial chemotaxis, because it is commonly found in bacteria (Hanlon and Ordal, 1994; Muller *et al*., 1997; Taguchi *et al*., 1997). Interestingly, whereas all other responses to amino acid attractants and repellents in *E. coli* are clearly mediated by the periplasmic sensory domains of receptors, the Tar-mediated serine response is apparently conferred directly by the cytoplasmic portion of Tar.

The strong correlation between metabolic and chemotactic preferences observed for *E. coli* suggests that in its natural environment *E. coli* is exposed to amino acid gradients of varying composition and that it benefits from being able to follow gradients with the highest metabolic value. However, this correlation for amino acids does not mean that *E. coli* exclusively uses chemotaxis to find nutrients, and *E. coli* chemotaxis toward both bacterial and mammalian signaling molecules has been demonstrated (Hegde *et al*., 2011; Pasupuleti *et al*., 2014). Furthermore, we show that *B. subtilis* is apparently attracted by a much broader spectrum of amino acids irrespective of their nutritional value. We thus speculate that – in contrast to *E. coli* – *B. subtilis* rather uses gradients of amino acids as general cues to locate sources of nutrients such as plant roots or to initiate symbiosis or pathogenesis (Caetano-Anolles *et al*., 1988; De Weert *et al*., 2002; Bacilio-Jimenez *et al*., 2003; Yao and Allen, 2006). This hypothesis is supported by the observation that such soil-dwelling bacteria as *Pseudomonas aeruginosa*, *P. fluorescens* or *Sinorhizobium meliloti* have similarly broad spectra of amino acid attractants (Burg *et al*., 1982; Gotz *et al*., 1982; Taguchi *et al*., 1997).

Experimental procedures

Strains and plasmids

All strains and plasmids used in this work are listed in Table S3. For *E. coli*, FRET pair CheY-YFP and CheZ-CFP was expressed from a bicistronic construct pVS88 (Sourjik and Berg, 2004). Strain VS104 [Δ(*cheY cheZ*)] (Sourjik and Berg, 2002) and NCM3722 were transformed with pVS88 were used as the wild type for FRET. Receptorless strain VS181 [Δ(*cheY cheZ*) Δ(*tar tsr tap trg aer*)] (Sourjik and Berg, 2004) was transformed with pVS88 and a plasmid expressing the receptor of interest. FRET reporter strain CH7-Bs for *B. subtilis* was constructed by genome integration of CFP-FliY-CheY-YFP at the *amyE* locus of *B. subtilis* 168.

Culture medium and growth conditions

For FRET experiments, *E. coli* cells were grown as previously described in TB (1% tryptone, 0.5% NaCl) at 34°C, 275 r.p.m., supplemented with appropriate antibiotics (100 mg ml^{-1} ampicillin; 17 mg ml^{-1} chloramphenicol) and inducers. Cells were harvested at OD_{600} of 0.6. *B. subtilis* cells were grown under the same conditions, except that the overnight culture was grown in Luria–Bertani (1% tryptone, 0.5% yeast extract and 0.5% NaCl), and the day culture was harvested at OD_{600} of 0.4.

For amino acid uptake experiments, *E. coli* MG1655 or NCM3722 were grown in modified M9 minimal medium

(47 mM Na_2HPO_4, 22 mM KH_2PO_4, 8 mM $NaCl_2$, 2 mM $MgSO_4$, 100 μM $CaCl_2$) supplemented with 1 mM of each 20 l-amino acids as the sole source of carbon and nitrogen. Alternatively, 0.4% glucose, 0.4% glycerol and/or 0.1% NH_4Cl were added to the medium, as indicated. For anaerobic growth of NCM3722, 0.4% glucose was added to the medium. *B. subtilis* 168 and NCIB3610 were grown in modified S7 minimal medium (4 mM KPO_4, 50 mM MOPS, 50 μM $MnSO_4$, 5 μM $FeSO_4$, 2 mM $MgSO_4$, 2 μM thiamine, 1 μM $ZnCl_2$, 700 μM $CaCl_2$) with the same amino acid supplementation as for *E. coli*. All bacteria were cultured at 37°C, 120 r.p.m. and sampling at regular time points for analyzing the residual concentration of each amino acid in the medium.

For the growth inhibition of *E. coli* by different amino acids, 1 mM of a single L-amino acid was added to the M9 medium (glucose as carbon source) in the beginning of culturing at 37°C, 120 r.p.m.

Growth of *E. coli* with individual amino acids as the sole carbon or nitrogen source was tested in the same modified M9 minimal medium, but 1 mM of one amino acid was added to each culture in the absence of any other carbon or nitrogen source, or in the presence of 0.4% glycerol (carbon source) or 0.1% NH_4Cl (nitrogen source). Cells were grown in 24-well plates, and OD_{600} values were detected with a plate reader (Tecan Infinite M1000, Tecan Deutschland GmbH, Crailsheim, Germany) after 48 h.

FRET assay

Measurements were performed on an upright fluorescence microscope (Zeiss Axio Imager.Z1, Carl Zeiss Microscopy GmbH, Jena, Germany) as previously described (Sourjik *et al*., 2007; Neumann *et al*., 2010), using either purified amino acids (Sigma or Applichem, > 98% purity) or synthetic amino acids (MP Biomedicals).

Analysis of amino acid utilization

Non-thiol-containing amino acids were quantified after specific labeling with the fluorescence dye AccQ-TagTM (Waters) according to the manufacturer's protocol. The resulting derivatives were separated by reversed phase chromatography on an Acquity BEH C18 column (150 mm × 2.1 mm, 1.7 μm, Waters) connected to an Acquity H-class UPLC system and quantified by fluorescence detection (Acquity FLR detector, Waters). The column was heated to 42°C and equilibrated with 5 column volumes of buffer A (140 mM sodium acetate pH 6.3, 7 mM triethanolamine) at a flow rate of 0.45 ml min^{-1}. Baseline separation of amino acid derivates was achieved by increasing the concentration of acetonitrile (B) in buffer A as follows: 1 min 8% B, 7 min 9% B, 7.3 min 15% B, 12.2 min 18% B, 13.1 min 41% B, 15.1 min 80% B, hold for 2.2 min, and return to 8% B in 1.7 min. Data acquisition and processing was performed with the Empower3 software suite (Waters). Cys was determined after labeling with monobromobimane (Calbiochem) as described before (Wirtz *et al*., 2004).

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