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Variability of the Quorum Sensing System in Natural Isolates of *Bacillus* sp.

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Summary

Bacteria communicate with one another by (emitting and/or reacting) to chemical signals. These communications, also known as quorum sensing, enable cells to control gene expression in response to cell density at the intra- and inter-species level. While bacteria use common signaling themes, variations in the design of the extracellular signals, the signal detection apparatus, and the biochemical mechanisms of signal relay have allowed quorum sensing systems to be adapted to diverse uses. The quorum sensing systems that govern natural genetic competence in *Bacillus subtilis* involve the ComX pheromones and the ComP-ComA, two-component regulator. ComX is synthesized as an inactive precursor and is then cleaved and modified by ComQ before export to the extra-cellular environment. The *comQXP'* loci of a set of natural *Bacillus* isolates have been sequenced and a striking polymorphism that correlates with specific patterns of activation of the quorum sensing response was shown. The ComX molecules representing different pherotypes were purified and characterized by mass spectroscopy. The analyses revealed that ComX variants also differ at the level of posttranslational modification of a conserved tryptophane residue, which was found to be an isoprenoid. The striking variability found in competence quorum sensing systems might be important for the survival of these bacteria in nature to escape the inappropriate induction of competence by closely related strains, playing the role of a sexual isolation mechanism.

Key words: *Bacillus*, genetic competence, quorum sensing, polymorphism, sexual isolation

Introduction

Intercellular communication plays a pivotal role in the physiology and development of living organisms. Many bacterial species, long thought to live the life of single cell existence, coordinate their physiological responses at the population level. Bacteria produce extracellular signaling molecules (also called pheromones), which accumulate in the environment denoting the presence of relatively dense population of cells and thus appropriateness of coordinated group behavior. The binding of signaling molecule to cognate receptors (membrane or cytoplasmic) triggers a change in transcription of target genes, which leads to the change in physiology or behavior of the population. (Bacteria cell-cell communication has been reviewed in two recently published books *(1,2)* and several reviews (*3–6*)).

Signaling Molecules

In Gram-negative bacteria the most commonly identified signaling mechanism for extra-cellular communi-

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cation employs homoserine lactones (HSL). The signal enters the cell by diffusion and interacts with intracellular effector molecules. In Gram-positive bacteria peptide based signaling molecules seem to be the predominant mode of communication. The signaling mechanism can either require import of the signal and subsequent interaction with intracellular effector or a two-component signaling system that transduces the information across the membrane *(7)*. But there are exceptions to the rule. *Streptomyces* use a butyrolactone signal to regulate the antibiotic production in the stationary phase *(8)*. Recently, the LuxS QS system of *Vibrio harveyi* has attracted attention, because the homologues of *luxS* have been found in many different species *(3)*. *Vibrio harveyi* controls expression of luciferase by a quorum sensing system involving the two-component phosphorylation cascade that can be activated by two different signaling molecules, AI-1 and AI-2 *(9)*. AI-1 is a HSL *(10)* and AI-2, for which a biosynthetic pathway has been elucidated recently, is predicted to be a furanosyl borate diester *(11,12)*. The synthesis of AI-2 is dependent on the *luxS* gene, which encodes the AI-2 synthetase. Homologues of LuxS have been found in more than 40 species and AI-2 has been proposed to serve as a 'universal' signal for inter-species communication *(4)*.

Many important physiological responses are known to be regulated by quorum sensing. The role of QS in induction of bioluminescence in *Vibrio harveyi* and *V. fisheri* has been studied extensively, virulence genes have been shown to be regulated in many different bacteria including *Vibrio cholerae, Pseudomonas aeruginosa* and *Staphylococcus aureus*. Biofilm formation was affected in *Pseudomonas aeruginosa* if the QS system was inactivated. Production of antibiotics by *Streptomyces* and of bacteriocins by *Lactobacilli* has been shown to involve QS. Fruiting body development in *Myxobacteria* involves cell-cell signaling. Sporulation in *Bacillus subtilis* and also the transfer of genetic material in *Agrobacterium, Enterococcus, Bacillus* and *Streptococcus* are a few of the developmental processes, which are controlled by elaborate QS systems (reviewed in *(1,4)*). In this review we will focus on the quorum sensing system, which regulates the development of competence for transformation in *Bacillus subtilis*.

The Role of Quorum Sensing in Regulation of Genetic Competence in *Bacillus subtilis*

Genetic competence refers to the ability of bacteria to bind and take up DNA from the environment. Many bacterial species, both Gram-positive and Gram-negative have been reported to be naturally competent *(13)* but in only few (*Neisseria gonorrhoeae*, *Hemophilus influenzae*, *Streptococcus pneumoniae* and *Bacillus subtilis*) has this phenomena been studied extensively (reviewed in *(14)*).

The genetic competence of *B. subtilis*, which develops during the transition from exponential to stationary growth, is under temporal, nutritional and cell density control *(14,15)*. The response to crowding is controlled by two extracellular peptides: ComX *(16)* and CSF (competence and sporulation factor) *(17,18)* and is mediated by the ComA/ ComP two-component regulatory signal transduction system *(19)*. The ComX pheromone activates the histidine kinase ComP, leading to ComP autophosphorylation and the transfer of phosphate to the response regulator ComA *(16,18,20,21)*. On the other hand, CSF enters the bacterial cell through the oligopeptide permease Opp (SpoOK) and acts intracellularly by negatively regulating the activity of RapC, a putative ComA~P phosphatase *(17)*. The two pathways converge at the level of ComA phosphorylation, which they both control *(6,18,22)*. Phosphorylated ComA directly activates the transcription of *srfA (23)*, an operon required for the development of genetic competence in *B. subtilis (24–26)*. The *srfA* operon encodes the ComS protein *(25,27)* that positively affects the levels of ComK, the master regulator of competence *(28–31)*.

Polymorphism of Quorum Sensing Loci

The *comQ, comX, comP* and *comA* genes are encoded in this order on the chromosome *(32)*. A similar organization has been described for *agrBDCA* quorum sensing locus, which controls virulence in *Staphylococcus aureus (33)* and in the *comCDE* quorum sensing locus, which controls competence in *Streptococcus pneumoniae*. As expected, in *B. subtilis* and *S. aureus* the processing genes (*comQ* and *agrB*, respectively) are present while in *S. pneumoniae*, which produces an unmodified pheromone, the processing gene is absent *(7,34,35)*.

Genetic polymorphism in the quorum sensing loci of *S. aureus* and *S. pneumoniae* has been reported and has been shown to be associated with specificity in the quorum sensing response *(35–37)*. Recently, the sequencing of *comQXP'* loci from natural isolates of *B. subtilis* has also confirmed the presence of the striking polymorphism in these loci *(21,38,39)*. This polymorphism extends through *comQ*, *comX* and the N-terminal end of *comP*. Only about 56 % identity was found at the nucleotide level, while the C-terminal part of *comP* encoding the histidine kinase and the whole *comA* gene are highly conserved (more then 90 % identity). Sequences upstream of *comQ* comprising the *degQ* gene are also conserved *(21,38,39)*. Phylogenetic analyses of 13 quorum sensing loci of natural isolates revealed several distinct clusters or similarity groups, which were consistent for *comQ*, *comX* and the N-terminal part of the *comP* gene (Fig. 1). These results suggest that the three genes have co-evolved *(39)*. In addition, internal fragments of the *gyrA* and *rpoB* genes from 13 natural isolates were also

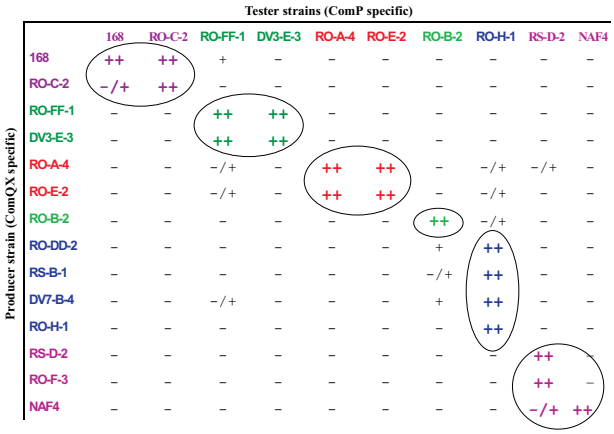

Table 1. Specific activation pattern is shown for 14 producer strains and 10 tester strains. Tester strains were grown in competence medium mixed with an equal volume of the indicated conditioned medium prepared by growing the producer strain to competence and then sterilizing the medium by filtration. Samples were collected at different time points and activity of β-galactosidase was determined. Symbols ++, 100 % activation or the same response as with homologous conditioned medium; + 50 % activation; -/+, weak but reproducible response; –, no activation.

Original strain[a]	*Bacillus* group[b]	*B. subtilis* producer strains[c]	*B. subtilis tester strains*[d]
168	168	BD2883*	BD2876*
NAF4	natto	BD2915*	BD2877*
RO-A-4	168	BD2938#	BM45
RO-B-2	mojavensis	BD2936*	BD2983#
RO-C-2	mojavensis	BD2937*	BD2963*
RO-DD-2	168	BD2948#	
RO-E-2	W23	BD2940#	BD3020#
RO-F-3	168	BD2946#	
RO-FF-1	168	BD2939*	BD2992#
RO-H-1	mojavensis	BD2913*	BD2962*
RO-PP-2	168	BD2950#	
RS-B-1	W23	BD2914*	
RS-D-2	168	BD2949#	BD3019
DV3-A-1	W23	BM42	
DV3-D-2	W23		
DV3-E-3	W23	BM43	BM50
DV7-B-4	W23	BM39	
IM-A-224	mojavensis		
IM-A-312	mojavensis		
IM-C-45	mojavensis		
IM-D-215	mojavensis		
RS-A-2	mojavensis		

sequenced *(39)*. These two genes showed a high degree of conservation (97 % identity at the nucleotide level) and phylogenetic trees of their sequences were not congruent with *comQXP'* phylogeny *(39)*. The authors concluded that the *comQXP* genes followed a different evolutionary path from the rest of the genome *(39)*. In addition, the *comQXP'* genes might have been acquired through horizontal transfer, because their GC content (29.48 %) is much lower than the values of 41.13 % obtained for *gyrA* and *rpoB* genes *(39)*, or 43.5 % reported for the entire *B. subtilis* 168 genome *(40)*.

Specificity of the *comQXP* Quorum Sensing System

In order to study the specificity of *comQXP'* systems from different natural isolates isolated from the Mojave and Gobi deserts *(41)* the *comQXPA* locus from these isolates was introduced into the laboratory strain *B. subtilis* 168 resulting in a set of isogenic producer strains. These strains secreted specific ComX pheromones but also carried the *srfA-lacZ* fusion, which enabled detection of competence induction by monitoring the activity of β-galactosidase. Next, tester strains were prepared by an in-frame inactivation of the *comQ* gene in the producer strains. These strains were not able to produce active pheromone but had the functional ComP receptor *(21)* and could respond to pheromone. All together 13 producer and 9 tester strains were constructed *(21,39)*. Using this system it was shown that each ComP sensor was specifically activated *in vivo* by its cognate pheromone and in some cases by a limited set of pheromones from other strains (Table1). The ComX -ComP pairs, which showed cross activation and therefore belonged to the same pherotype, were more closely related at the sequence level. This suggests that sequence may significantly contribute to the specificity of the response *(39)*. When an additional producer-tester pair (natural isolate *B. subtilis* DV3-A-1) was constructed, high reciprocal cross activation with the *B. subtilis* RO-FF-1 QS system was observed. This sugests that the RO-FF-1 and DV3-A-1 isolates may form a separate pherotype and that RO-FF-1 might not belong to the pherotype, which includes *B. mojavensis* RO-C-2 and *B. subtilis* 168 (Sabotic, Cepon, Mandic-Mulec, unpublished). Therefore, it is possible that an analysis of additional strains may reveal even higher number of pherotypes. In addition, it should be pointed out that some of the pherotypes are not completely closed. For example, RO-FF-1 is maximally activated by RO-FF-1 and DV3-E-3 pheromone (Sabotic, Cepon, Mandic, unpublished). But it can also be partially activated by 168 and to lesser extent by RO-A-4 and RO-E-2 (Table 1). The last three pheromones activate the *B. subtilis* RO-FF-1 tester strain only partially and never reach the potential of the cognate pheromone. On the other hand, the *B. subtilis* DV3-E-3 receptor is less promiscuous and can be activated only by its own ComX and by the very similar RO-FF-1 pheromone (Sabotic, Cepon, Mandic, unpublished). All together, the results of *in vivo* analyses show that natural isolates belonging to the same species or originating from the same ecosystem form different activation groups or pherotypes, which are not able to induce each other into competence *(39)*. The lack of cross activation between the strains of the same species may lower the probability for genetic exchange between strains of the same species. If sexual isolation occurs between isolates in one species it may be an important mechanism of speciation *(42)*.

The Biochemical Nature of ComX

ComX is synthesized as a 55 residue propeptide, which is processed and modified postranslationally in order to be active *(16,39)*. Also in other quorum sensing systems of Gram-positive bacteria postranslational modifications of signaling peptides have been observed. For example, the AgrD signaling molecule of *Staphylococcus aureus*, involved in regulation of virulence, has an intramolecular thiolactone bond *(43–45)*. Intramolecular lactone ring modification was also found in the gelatinase biosynthesis-activating pheromone of *Enterococcus faecalis (46)*. In contrast, the signaling peptide, which controls competence development in *Streptococcus pneumoniae*, has to be processed but is not modified *(47)*.

In *B. subtilis* two genes, *comX* and *comQ*, are required *(16)* and sufficient *(21)* for the production of an active ComX pheromone. Recently, a putative isoprenoid binding domain of ComQ was shown to be required for function *in vivo (48)*, which is consistent with

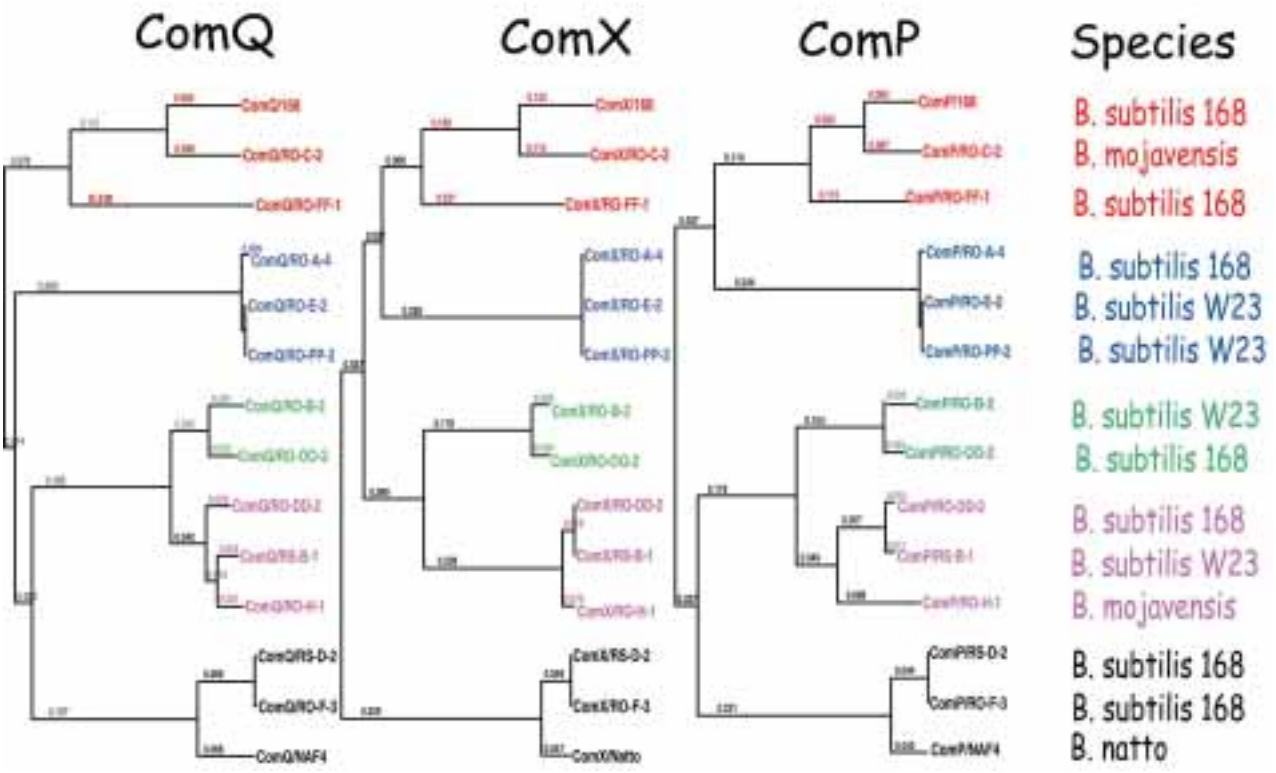

Fig. 1. The trees of *comQ*, *comX* and *comP'* sequences were drawn with NJPLOT software after multiple aligments of nucleotide sequences using the CLUSTALX software. The numbers at internal branches represent the bootstrap values estimated from 1000 resamplings. The results for 13 natural isolates and the laboratory strain *B. subtilis* 168 are shown. Five similarity groups or clusters can be depicted from these trees

the notion that the modification on ComX pheromone is likely to be an isoprenoid *(16)*. In addition, alanine-scanning mutagenesis of the final 9 codons of *comX* indicated that the tryptophane residue, proposed to be the site of modification, is important for the activity of ComX pheromone *(48)*. Recently, Ansaldi and Dubnau have purified ComX pheromones from 6 natural isolates belonging to 4 different pherotypes *(39)*, using an *E. coli* expression system *(21)*. Sequencing of these peptides revealed a striking variability in size (from 5 to 10 amino acids) and sequence (only the tryptophane residue was conserved in all peptides). In addition, they determined the mass of the purified peptides by mass spectrometry and observed that the actual mass was different from the calculated mass. This represented an evidence that purified ComX peptides were indeed modified *(39)*, as it was proposed previously for 168 ComX pheromone *(16)*. The calculated mass of modification was shown to be 206 Da, 136 Da or 120 Da. A modification mass of the same size was found on ComX pheromones from a given pherotype, whereas the masses of modifications differed between the pherotypes *(39)*. Finally, using an *in vivo* labeling system they have also shown that in all three cases the ComX modifications are isoprenoids, which is the first example of isoprenilated peptides used in bacterial cell-cell signaling *(39)*. Another isoprenylated molecule involved in microbial signal transduction is the a-factor in *Saccharomyces cerevisiae* that induces the mating process in a-type cells *(49)*. In some respects, ComX pheromones resamble the a-factor. Both control genetic exchange and their synthesis show certain similirities, as they are both sythesized as inactive precursors, which are then cleaved and modified by isoprenylations and exported to the extracellular environment *(39)*.

Conclusion

The ComQXPA quorum sensing system controls competence development in response to cell density but it also determines the pherotype specific induction of the DNA binding and uptake machinery. The specificity of the QS response in *Bacillus* encompasses a striking variability at the level of the sequence of ComQ, ComX and N-terminal part of ComP as well as variability at the level of isoprenylation of the ComX pheromone. The evolution of this locus is different as from the rest of the genome and the difference in GC content suggests that the loci might have been introduced by horizontal gene transfer. The lack of communication between the natural isolates of the same species, which affects their decision to become or not to become competent, may lower the frequency by which their genomes exchange. Therefore, the variability and with it connected specificity of the QS system described above may represent a novel mechanism of sexual isolation. This QS mechanism may

thus play a role in speciation, as suggested previously by Tortosa and Dubnau *(42)*.

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References

1. G. M. Dunny, S. C. Winans: *Cell-Cell Signaling in Bacteria*, American Society for Microbiology Press, Washington, DC (1999).
2. R. England, G. Hobbs, N. J. Bainton, D. M. Roberts: *Microbial Signaling and Communication*, Cambridge University Press, Cambridge, United Kingdom (1999).
3. B. L. Bassler, *Cell, 109* (2002) 421–424.
4. S. C. Winans, B. L. Bassler, *J. Bacteriol. 184* (2002) 873–883.
5. M. B. Miller, B. L. Bassler, *Annu. Rev. Microbiol. 55* (2001) 165–199.
6. B. A. Lazazzera, A. D. Grossman, *Trends Microbiol. 6* (1998) 288–294.
7. M. Kleerebezem, L. E. N. Quadri, P. Kuipers, W. M de Vos, *Mol. Microbiol. 24* (1997) 895–904.
8. S. Horinouchi: Gama-butirolactons that control secondary metabolism and cell differentiation in *Streptomyces.* In: *Cell-Cell Signaling in Bacteria*, M. G. Dunny, C. S. Winans (Eds.), American Society for Microbiology, Washington, D.C. (1999) pp. 193–207.
9. B. L. Bassler, *Curr. Opin. Microbiol. 2* (1999) 582–587.
10. J. G. Cao, E. A. Meighen, *J. Biol. Chem. 264* (1989) 21670–21676.
11. S. Schauder, K. Shokat, M. G. Surette, B. L. Bassler, *Mol. Microbiol. 41* (2001) 463–476.
12. X. Chen, S. Schauder, N. Potier, A. Van Dorsselaer, I. Pelczer, B. L. Bassler, F. M. Hughson, *Nature, 415* (2002) 545–549.
13. M. G. Lorenz, W. Wackernagel, *Microbiol. Rev. 58* (1994) 563–602.
14. D. Dubnau, *Annu. Rev. Microbiol. 53* (1999) 217–244.
15. D. Dubnau, C. M. Jr. Lovett: Transformation and Recombination. In: *Bacillus subtilis and Its Closest Relatives*, A. L. Sonenshein, J. A. Hoch, R. Losic (Eds.), American Society for Microbiology, Washington, D.C. (2002) pp. 453–471.
16. R. Magnuson, J. M. Solomon, A. D. Grossman, *Cell, 77* (1994) 207–216.
17. B. A. Lazazzera, J. M. Solomon, A. D. Grossman, *Cell, 89* (1997) 917–925.
18. J. M. Solomon, R. Magnuson, A. Srivastava, A. D. Grosman, *Genes Dev. 9* (1995) 547–558.
19. Y. Weinrauch, R. Penchev, E. Dubnau, I. Smith, D. Dubnau, *Genes Dev. 4* (1990) 860–872.
20. F. Piazza, P. Tortosa, D. Dubnau, *J. Bacteriol. 181* (1999) 4540–4548.
21. P. Tortosa, L. Logsdon, B. Kraigher, Y. Itoh, I. Mandic-Mulec, D. Dubnau, *J. Bacteriol. 184* (2001) 451–460.
22. B. L. Lazazzera, T. Palmer, J. Quisel, A. D. Grossman: Cell density control of gene expression and development in *Bacillus subtilis*. In: *Cell-Cell Signaling in Bacteria*, G. M. Dunny, C. S. Winans (Eds.), American Society for Microbiology, Washington DC (1999) pp. 27–46.
23. M. Roggiani, D. Dubnau, *J. Bacteriol. 175* (1993) 3182–3187.
24. J. Hahn, D. Dubnau, *J. Bacteriol. 173* (1991) 7275–7282.
25. M. M. Nakano, R. Magnuson, A. Myers, J. Curry, A. D. Grossman, P. Zuber, *J. Bacteriol. 173* (1991) 1770–1778.
26. M. M. Nakano, L. A. Xia, P. Zuber, *J. Bacteriol. 173* (1991) 5487–5493.
27. L. W. Hamoen, H. Eshuis, J. Jongbloed, G. Venema, D. van Sinderen, *Mol. Microbiol. 15* (1995) 55–63.
28. L. W. Hamoen, A. F. Van Werkhoven, J. J. E. Bijlsma, D. Dubnau, G. Venema, *Genes Dev. 12* (1998) 1539–1550.
29. K. Turgay, L. W. Hamoen, G. Venema, D. Dubnau, *Genes Dev. 11* (1997) 119–128.
30. K. Turgay, J. Hahn, J. Burghoorn, D. Dubnau, *EMBO J. 17* (1998) 6730–6738.
31. M. Persuh, K. Turgay, I. Mandic-Mulec, D. Dubnau, *Mol. Microbiol. 33* (1999) 886–894.
32. Y. Weinrauch, T. Msadek, F. Kunst, D. Dubnau, *J. Bacteriol. 173* (1991) 5685–5693.
33. J. Kornblum, B. Kreiswirth, S. J. Projan, H. Ross, R. P. Novick: A policistronic lucus regulating exoprotein synthesis in *Staphylococcus aureus*. In: *Molecular Biology of the Staphylococci*, R. P. Novick (Ed.), VCH Publishers, New York (1990) pp. 373–402.
34. H. L. Saenz, V. Augsburger, C. Vuong, R. W. Jack, F. Gotz, M. Otto, *Arch. Microbiol. 41* (2000) 463–476.
35. A. M. Whatmore, V. A. Barcus, C. G. Dowson, *J. Bacteriol. 181* (1999) 3144–3154.
36. G. Pozzi, L. Masala, F. Iannelli, R. Manganelli, L. S. Havarstein, L. Piccoli, D. Simon, D. A. Morrison, *J. Bacteriol. 178* (1996) 4540–4548.
37. G. Ji, R. Beavis, R. P. Novick, *Science, 276* (1997) 2027–2030.
38. L. S. Tran, T. Nagai, Y. Itoh, *Mol. Microbiol. 37* (2000) 1159–1171.
39. M. Ansaldi, D. Marolt, T. Stebe, I. Mandic-Mulec, D. Dubnau, *Mol. Microbiol. 44* (2002) 1561–1573.
40. F. Kunst, N. Ogasawara, I. Moszer, A. M. Albertini, G. Alloni, V. Azevedo, *et al., Nature, 390* (1997) 249–256.
41. M. S. Roberts, F. M. Cohan, *Evolution, 49* (1995) 1081–1094.
42. P. Tortosa, D. Dubnau, *Curr. Opin. Microbiol. 2* (1999) 588–592.
43. G. Ji, R. Beavis, R. P. Novick, *Proc. Natl. Acad. Sci. USA, 92* (1995) 11140–11144.
44. M. Otto, R. Süßmuth, G. Jung, F. Götz, *FEBS Lett. 424* (1998) 89–94.
45. P. Mayville, G. Ji, R. Beavis, H. Yang, M. Goger, R. P. Novick, T. W. Muir, P*roc. Natl. Acad. Sci. USA, 96* (1999) 1218–1223.
46. J. Nakayama, Y. Cao, T. Horii, S. Sakuda, A. D. Akkermans, W. M. de Vos, H. Nagasawa, *Mol. Microbiol. 41* (2001) 145–154.
47. L. S. Havarstein, G. Coomaraswamy, D. A. Morrison, *Proc. Natl. Acad. Sci. USA*, *92* (1995) 11140–11144.
48. K. B. Schneider, T. M. Palmer, A. D. Grossman, *J. Bacteriol. 184* (2002) 451–460.
49. P. Chen, S. K. Sapperstein, J. D. Choi, S. Michaelis, *J Cell. Biol. 136* (1997) 251–269.

Varijabilnost sustava za detekciju gustoće stanica u prirodnih izolata *Bacillus*

Sažetak

Bakterije međusobno komuniciraju kemijskim signalima (izlučuju ih i/ili reagiraju s njima). Ta komunikacija, nazvana i detekcija kvoruma, omogućuje stanicama da kontroliraju gensku ekspresiju kao odgovor na promjenu gustoće stanica unutar ili između vrsta. Dok bakterije koriste uobičajene signalne načine, varijacije u izgradnji ekstracelularnih signala, uređaj za njihovu detekciju i biokemijski mehanizmi prijenosa signala omogućili su sustavu za detekciju kvoruma da se prilagodi raznolikoj primjeni. Sustav za detekciju kvoruma koji kontrolira razvoj genetičke kompetencije u *Bacillus suptilis* uključuje feromone ComX i dvokomponentni regulacijski par proteina ComP-ComA. ComX se sintetizira kao inaktivni prekurzor, a djelovanjem ComQ cijepa se i modificira prije izlaska iz stanice. Sekvenciranje lokusa *comQXP´* iz srodnih prirodnih izolata *Bacillus suptilis* i *Bacillus mojavensis* potvrdilo je prisutnost velikoga genetskog polimorfizma unutar ovoga lokusa, što je u skladu s objavljenim rezultatima o specifičnom načinu aktivacije odgovora sustava za detekciju kvoruma. Molekule ComX, predstavljajući različite ferotipove, bile su pročišćene i identificirane masenom spektroskopijom. Analize su pokazale da se varijante ComX međusobno razlikuju na razini posttranslacijske modifikacije očuvanog triptofanskog ostatka, za koji je ustanovljeno da je izoprenoid. Izrazita varijabilnost nađena u sustavu za detekciju kvoruma važna je za opstanak tih bakterija kako bi izbjegle neodgovarajuću indukciju genske kompetencije od blisko srodnih sojeva, obavljajući time ulogu mehanizma za seksualnu izolaciju.