Unveiling CO_2 stimulon underlying the microbial community

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Report:

In order to widely assess the influence of high CO_2 atmosphere on the bacterial cellular state, comprehensive transcriptional and protein expression analyses were carried out. The results demonstrated that the condition remarkably affected the expression level of 20-50 genes in each organism and that each strain exhibited a different expression profile. Short time exposure to high CO_2 atmosphere not only suggested the presence of a specific genetic regulatory system but also inferred that the condition causes cellular lesion. Isolation of multiple bacterial strains exhibiting a high-CO_2-dependent colony morphotype demonstrated the general occurrence of CO_2-dependent phenomenon.

Research objectives

CO_2 is an effective indicator of biological activity that probably serves as the most general quormone (a specific small molecule being an indicator of the maturation level of the microbial community) in the natural environment. The author hypothesizes that CO_2 is received by various microorganisms and affects their mode of action, thus constituting a basis supporting the development of the bacterial ecosystem.[1-5] This study aims at understanding the influence of a general environmental factor on the microbial community from the viewpoint of genetic regulation by comprehensively analyzing the effect of CO_2 on bacterial gene expression. In particular, the study tries to elucidate the whole picture of CO_2 stimulon, the genetic group whose expression varies simultaneously along with the change in CO_2 level, to clarify that CO_2 is the most general and effective metabolic indicator and serves as a key factor that determines the mode of microbial community structure. To date, the argument in microbial ecology is mostly based on limited evidence from the observation on a certain physiological property of a specific organism and the change in microbial content, and has not yet figured out the principle fundamental to the development of the microbial community. This study brings forth the new understanding in microbial ecology by elucidating the role of CO_2, a key factor fundamental to the microbial physiology and community structuring.

Methods:

To study the cellular response to the change in CO_2 concentration, expression comparison analyses were carried out at an RNA and protein level by using bacterial cells incubated under normal conditions (Air) and at 5% CO_2 air (high-CO_2). For RNA analysis, the transcripts extracted from the cells cultivated for 24 h under the two conditions were subjected to RNAseq and RT-PCR analyses. For protein analysis, the cells were precultured

and then incubated for 1 h under air and high- CO_2 condition to study the response in a short period. Proteins from intercellular and extracellular fractions were subjected to a comprehensive MS analysis. The transcriptional analysis was carried out in five model bacterial organisms including those affiliating *Bacillus*. The protein analysis was done with regard to *Bacillus subtilis*.

In addition to the aforementioned expression analysis, new bacterial strains exhibiting a different colony morphotype under air and high-CO_2 condition were screened. Isolates obtained mainly from water samples by cultivation under high-CO_2 conditions were replica-plated and their colony morphotypes were compared after cultivation under the two conditions at 28°C.

The aforementioned experiments were carried out using buffered medium to prevent the pH shift due to the addition of high CO_2.

Results:

(1) Transcriptional analysis.

Transcriptional profiles under air and high-CO_2 conditions were obtained for 6 strains that exhibited a CO_2-dependent phenotype in the preliminary observation. As a result of comparison analysis, distinctive transcriptional upregulation was observed in all strains with regard to 20-50 genes both under air and high-CO_2 conditions. The constituents of the upregulated gene group were diverged, and no common gene was identified. Any specific regulatory mechanism that commonly controls the CO_2-dependent gene expression has not yet been deduced. Meanwhile, the CO_2-dependent transcription was confirmed with respect to some of the genes of *B. subtilis* exhibiting a positive or negative response to high- CO_2 through RT-PCR analysis. These genes included those involved in the amino acid metabolism. The fact that the genes identified by the abovementioned RNAseq analysis included those for primary metabolism inferred that the increase in CO_2 concentration stimulates the signal transduction connected to the metabolic control.

(2) Protein analysis.

The transcriptional analysis demonstrated the occurrence of a complex transcriptional profile specific to each organism. Based on this result, the next trial was performed by focusing on a short-period cellular response at the protein level. A single batch of *B. subtilis* cells was divided into two fractions and incubated under air and high-CO_2 conditions for 1 h, and the resultant cultures were subjected to the comprehensive protein expression analysis using mass-spectrometry. The analysis successively detected proteins exhibiting marked dependence on CO_2, however, those included innercellular proteins such as ribosomal proteins. This raised the possibility that the exposure to high CO_2 atmosphere causes cellular lesion and leakage of innercellular contents. On the other hand, the high CO_2-dependent reduction of certain proteins such as the cell wall-binding protein suggested the occurrence of a specific repression mechanism responding to the high CO_2 environment.

(3) Screening for CO_2-dependent bacterial strains.

About 500 natural isolates were studied for the dependence of their colony phenotype on high CO_2. Of these, ca. 30 strains exhibited distinctive dependence. The major phenotypes affected by the CO_2 level were the production of a sticky matrix (a possible extracellular polysaccharide), pigment production, and colony morphology. The *Bacillus* strains used in the aforementioned analysis also showed a marked dependence of their phenotype.

Conclusion:

This and the previous studies indicate that many bacteria are variously affected by high CO_2. The influence caused by CO_2 probably includes the change due to the adaptive response of the cell, which is based on the signaling network controlling gene expression by sensing the change in CO_2 concentration. On the other hand, the evidence obtained in this study suggests that the change in CO_2 concentration causes other events that significantly affect the cell homeostasis such as cellular lesion and metabolic flow exchange due to the influence on the carboxylating or decarboxylating enzymatic reactions as well as the shift of pH. CO_2 stimulon should be recognized as a global system that includes the responses to these indirect factors affected by CO_2 change. Therefore, the understanding of the whole picture of the system should be carried out through the identification of each factor and relating signal network.

References:

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