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Short Communication

Changes in tRNA profiles in *Sinorhizobium meliloti* CCBAU 81024 during alkali-shock

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The changes of tRNA under alkali shock by the method of stable low-molecular-weight RNA profiles was firstly reported. In *Sinorhizobium meliloti* CCBAU 81024, there are a clear difference profiles obtained from cells exposed to alkaline shock. In contrast, the profiles from cells under alkaline stress conditions are undistinguishable from cells cultured at pH 7. It was shown that tRNA(s) play some roles in response to environmental stimuli, and the low-molecular-weight (LMW) RNA profiles can provide an inexpensive screening technique for measuring diversification of tRNA among the different controlled samples.

Key words: tRNA, alkali shock, low-molecular-weight (LMW) RNA profile.

INTRODUCTION

The separation of nucleic acids by electrophoresis has been traditionally applied in microbial taxonomy. In 1990, Hõfle (1990) proposed that tRNA profiles, which are part of the low-molecular-weight (LMW) RNA profiles, can be used to fingerprint bacteria. The band pattern of an tRNA profile therefore represents an essential part of the bacterial genome that can be used as a molecular fingerprint of the genotype of a specific strain. Since then, several authors have reported on differences in microbial populations. All previous studies have been carried out in specific groups of microorganisms and established the usefulness of LMW RNA profiles as a molecular marker for each microbial species. However, LMW RNA profiles are only important in taxonomy.

Bacteria are equipped with a variety of genetic mecha-nisms allowing them to cope with stressful situa-tions such as a sudden change in temperature, pH values, and oxidative stress (Storz and Hengge-Aronis, 2000). Using the DNA microarray technique, Akram and Schumann (2003) showed that more than 80 genes in *Bacillus subtilis* were induced at least threefold after the external pH was increased to 8.9. Factors which are able to change the abundance and distribution of the different tRNAs are present in each strain. Though there are up to 20% of the total RNA in bacterial cells is tRNA, the regulation of tRNA distribution on the genomic level remains unclear. tRNA distribution is governed by four processes: transcription, processing of precursor tRNA, degradation of precursor tRNA and degradation of mature tRNA.

Thus, we preliminarily focused on the abundant and distributional changes of tRNA in the *Sinorhizobium meliloti* CCBAU 81024 by LMN RNA fingerprint profiles between the alkali shock and alkali stress. Further

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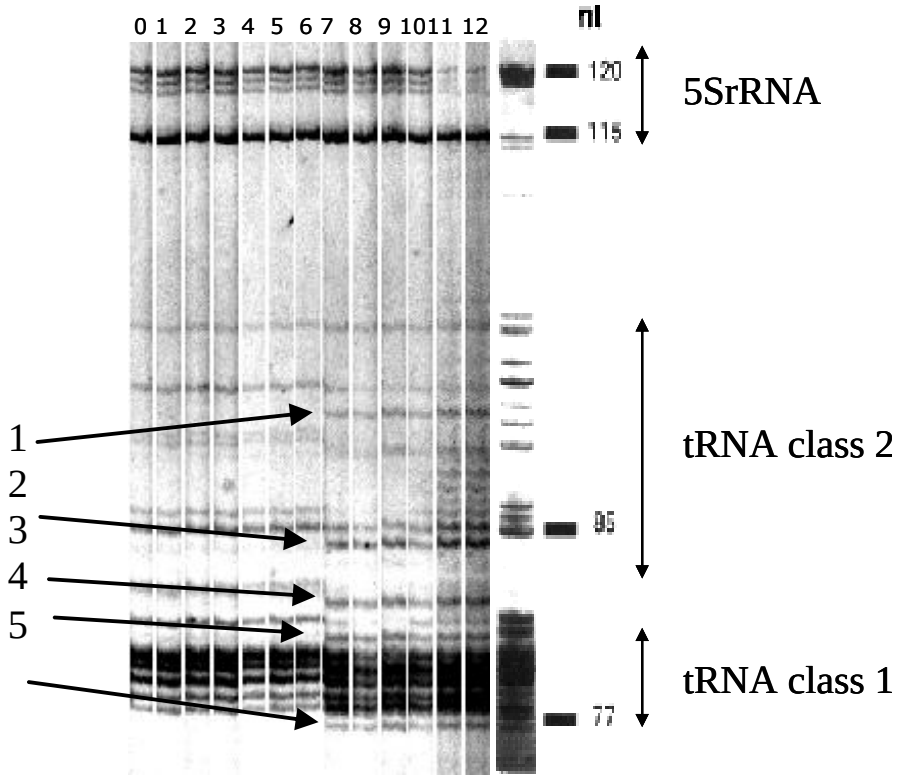

Figure 1. LMW RNA fingerprint profiles of the cells cultivated under aerobic condition in 200 ml TY liquid medium at pH 7.0 (lane 0), pH 8.0 (lane 1), pH 9.0 (lane 2), pH 10.0 (lane 3). LMW RNA fingerprint profiles of alkali shock at pH 8.0 for 10 min (lane 4), 20 min (lane 5) or 30 min (lane 6). LMW RNA fingerprint profiles of alkali shock at pH 9.0 for 10 min (lane 7), 20 min (lane 8) or 30 min (lane 9). LMW RNA fingerprint profiles of alkali shock at pH 10.0 for 10 min (lane 10), 20 min (lane 11) or 30 min (lane 12). The numbered arrows means the main changes on tRNA profiles.

studies on how and why the tRNA change will be done in our next work.

MATERIALS AND METHODS

Organisms, media and culturing

S. meliloti CCBAU 81024 which was isolated from Qinghai, China, is able to tolerate up to pH of 11.5. It was cultured at 28°C in TY medium at pH 7.0, 8.0, 9.0 and 10.0 to OD_{600} ~0.8, respectively. Cultures of pH 7.0 were adjusted with 2.0 N NaOH to pH 8.0, 9.0, or 10.0 and incubated at 28°C for 10, 20 or 3 0min. The different controlled cultures (1.5 ml) were harvested immediately by centrifugation at 15000 rpm for 1 min. The collected cells were stored at -80°C, prior to extraction of total RNA.

Extraction of RNA

For RNA extraction (Raúl, 2001), thawed cells were first resuspended in 200 µl of 0.1% N-lauroyl sarcosine sodium salt and collected by centrifugation at 15000 ×g for 1 min at 4°C. Then a 50 µl acetate/SDS solution were added and the mixture were heated at 100°C for 5 min. The suspension was diluted with 50 µl DEPC-H_2O and centrifuged at 10000 ×*g* for 5 min at 4°C. For further analysis, a total of 20 µl total RNA extract was collected and used.

LMW RNA profiles

Samples containing 5 µl total RNA were added to 5 µl of loading solution (300 mg/ml of sucrose, 460 mg/ml of urea, 10 µL/ml 20% SDS, 1 mg/mL xylene cyanol), 10 min of heating at 70°C, and applied in each well. LMW RNA profiles were obtained using staircase electrophoresis which was performed in 400 × 360 × 4 mm gels in a vertical slab unit (Poker Face SE 1500 Sequencer, Hoeffer Scientific Instruments, USA). After electrophoresis, the gels were silver-stained according to the method described by HAAS (1994).

RESULTS AND DISCUSSION

General analysis of LMW RNA profiles

In order to clearly identify the changed bands, we strictly controlled experimental conditions and repeated the experiments. The LMW RNA profiles of different samples were consistent in the three repetition. Figure 1 shows the patterns obtained. First, all these profiles contained the

the three well-distinguished groups expected (Hōfle et al., 1988), 5S rRNA, tRNA class 2, and tRNA class 1. Secondly, the tRNA profiles of the culture grown at pHs 7.0, 8.0, 9.0 or 10.0 showed no significant differences (Figure 1: lane 0, 1, 2, 3). The finding was consistent with Hōfle (1990) and Inna and Glenn (2013) who reported that the LMW RNA profiles were independent of the nutritional status of the bacterial cells in contrast to protein profiles and fatty acid pattern analysis. Thirdly, the tRNA profiles of cultures by alkali shock at pH of 8.0 for 10, 20, 30 min (Figure 1: lane 4, 5, 6) have no significant differences with cultures at pH of 7.0 (Figure 1: lane 0), which indicated that a higher stimulated intensity could turn on regulation of tRNA. Fourthly, changes of tRNA by alkali shock at pH 9.0 or 10.0 had been observed for the first time (Figure 1B and C), e.g. the bands of 1, 2, 3, 4 and 5, demonstrated that relevant cellular tRNA were up-regulated in several alkali shock treatments. So it is intriguing, why the changes of tRNA occurred for alkali shock rather than alkali stress. The significance of these findings was the uncovering of the changes of tRNA to deal with environmental variety and provide a new clue for other researchers.

How does the tRNA regulate gene expression?

Changes of tRNA species, as both relative percentage of tRNA and absolute concentration, have been reported in other aspect, e.g., during liver cell proliferation induced by partial hepatectomy (Qi and Heng, 2013). Changes in tRNA patterns have been also described during embryogenesis, differentiation aging and carcinogenesis (Giuseppina and Maria, 2010). There are two aspects of this problem which have to be addressed. On the one hand, the tRNA copy number was a factor affecting the formation of protein secondary structure. High tRNA copy number preferably coded for alpha-helix but less coded for coil (Luo and Li, 2003). Alpha-helix was a most stabile main-stranded conformation, so the increased proportion of alpha-helix in the protein could maintain the stability of structure and resist environmental stress. Moreover, differences in the abundance of isoaccepting tRNAs were observed between organisms, and also between the tissues of a given organism (Qi, 2013).

On the other hand, tRNA nucleoside modifications would affect tRNA structure and function in a number of subtle ways. The degree of modification could, in some cases, be varied in response to changes of the environment. Some of the clearest and most compelling evidence for functional roles of tRNA modification have been from studies which have demonstrated enhanced structural stabilization. *truB*-effected *ψ*55 modification of tRNA contributes to thermal stress tolerance in *Escherichia coli*, possibly by optimizing the stability of the tRNA population at high temperatures (Seonag et al., 2002). Base modifications at or near the anticodon are also known to affect the efficiency and specificity of decoding (Curan et al., 1998; Tineke and Jones, 2013). Some aspects of tRNA modification in archaea are strongly associated with environmental temperature and support the thesis that posttranscriptional modification is a universal natural mechanism for control of RNA molecular structure that operates across a wide temperature range in archaea as well as bacteria (Kathleen et al., 2003).

It should be noted that this study had examined only the changes of tRNA under the condition of alkali shock. The deeper implications will be investigated in future work. These results also provided a clue to explore the mechanism of stress response. In conclusion, despite its preliminary character, for the first time we indicated the function of tRNA in alkali shock. However, it was unknown whether the tRNA acted alone or in conjunction with factors.

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