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A42

**EFFECT OF COPPER ON THE QUORUM SENSING SYSTEM  
OF *Pseudomonas capeferrum* WCS358**

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In agriculture, copper has largely been utilized for the control of phytopathogen fungi. However, copper tends to accumulate in soils resulting in negative biological effects. Although copper is one of the most studied metals, its effect on microbial interactions mediated by Quorum Sensing (QS) between rhizosphere microorganisms has not been evaluated up to date. QS systems are signaling mechanisms that control the microbial physiology in response to small signal molecules. *Pseudomonas capeferrum* WCS358 is a plant growth-promoting rhizobacterium whose QS system is regulated by the *N*-acyl homoserine lactones (AHLs) and is composed of the AHL synthase PpuI, the AHL receptor PpuR, the regulator RsaL and a second AHL receptor, PpoR. The aim of this work is the evaluation of the effect of copper on the expression of *ppuI*, *ppoR* and *rsaL*. Transcriptional fusion plasmids based on the pMP220 promoter probe vector were introduced independently into WCS358. The resulting strains were cultivated in solid and liquid medium with and without copper and  $\beta$ -galactosidase activity was measured. The activities of the *ppuI* and *ppoR* promoters were reduced in half or less in the presence of copper in liquid and solid medium indicating significant differences with the control condition without copper ( $p < 0.05$ , Tukey test). The promoter level of *rsaL* was reduced to a lesser extent than *ppuI* and *ppoR* in the presence of copper in liquid medium but no differences were observed in solid medium ( $p > 0.05$ , Tukey test). Results presented in this work show that copper modifies the expression of *ppuI*, *ppoR* and *rsaL*, suggesting a concomitant modification of the phenotypes controlled by the QS system in *P. capeferrum* WCS358.

A43

**RECEPTORS TNF $\alpha$ -RI AND TNF $\alpha$ -RII IN HOLSTEIN COWS NATURALLY INFECTED BY  
BOVINE LEUKEMIA VIRUS**

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Bovine leukemia virus (BLV) is a retrovirus that affects the immune system of infected cattle. Its target cell is the B lymphocytes specifically the CD5+ subset. The exposure of B cells to BLV generates the abnormal expression of cytokines and receptors. B cells are increasingly reactive to proliferative signals but they do not have the expected reactivity to the presented antigens. Because of the characteristics of the virus, the antiviral cytokines of the innate response are not efficient to control the viral infection. However, tumor necrosis factor (TNF $\alpha$ ) plays an important role in the pathogenesis of BLV infection. The functional activity of TNF $\alpha$  is mediated by two different surface receptors: TNF $\alpha$ -RI and TNF $\alpha$ -RII. TNF $\alpha$ -RI induces cell death through apoptosis, cytokine production and cytotoxicity; whereas TNF $\alpha$ -RII is involved in cell proliferation activities. The objective of this study was to determine the relationship between the expression level of TNF $\alpha$ -RI and TNF $\alpha$ -RII receptors with respect to infection or not by BLV in a population of Holstein cows. A blood sample was taken from 140 Holstein cows that belong to three specialized dairy farms located in the department of Antioquia. DNA and RNA were extracted from the samples. A nested-PCR was performed to amplify a viral env (gp51) gene region and obtain a 444 bp fragment. The level of expression of TNF $\alpha$ -RI and TNF $\alpha$ -RII receptors was determined by qPCR. The association of expression level of the receptors with the presence or absence of BLV was established using a t-test. A higher level of expression of the RI type receptor was found with respect to the RII receptor in all the cows evaluated. The expression level of the RI receptor was higher in the negative cows than in the BLV positive ones ( $P=0.0028$ ). On the other hand, there was no statistical difference between the expression level of the RII receptor between the positive cows and the BLV negative cows ( $P=0.560$ ). According to the results obtained, a higher level of RI receptor expression was obtained in healthy animals compared to infected animals, which may be related to a better ability to produce different cytokines and cytotoxicity, which induces the cell death of infected cells. No statistical difference was found between the level of expression of the RI receptor and RII in the positive cows, it is possible that if the level of expression of both receptors is similar, the cell proliferation or anti-apoptosis routes are promoted, maintaining viral replication.