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Western Dairy Center

Project Report

Reporting Period January 1, 1997 — December 31, 1997

Principal Investigators: Bruce L. Geller, Associate Professor of Microbiology, Oregon State University

Co-Investigators:

Project Title: The Use of Bacteriophage-Receptor Genes of Lactococcus lactis to Devleop Bacteriophage-Resistance in Cheddar Cheese Starter Strains

Institution's Project #: 97081

Project Completion Date: 12/31/99

National Research Plan (1997): Priority: Goal: Tactic:
Cheese objective 4/Goal 4.2/Tactic 1,3 & 6

Modifications to Project/Budget:
None

Project Objectives: (Include any revisions to objectives)

1. Identify and isolate host genes other than pip that are required for infection by phage of the c2 species. 2. Identify a gene encoding a receptor for a phage of the p335 species. 3. Identify a gene encoding a receptor for a phage of the 936 species. 4. Construct a phage-resistant strain of L. lactis with defective copies of the receptor genes for phages of the c2, 936, and p335 species. 5. Evaluate the effects of receptor gene mutations on phage resistance, growth rate, acidification and coagulation of milk, and other cheese production traits.

Project Summary: (Suitable for inclusion in Center documents released to the public)

The proposed research examines early steps of bacteriophage infection of L. lactis, which include attachment of phage to the surface of cells, and entry of phage DNA into cells. Both of these steps are required for infection by phage. Our strategy of strain improvement is to prevent to phage from attaching or entering the host in the first place. To do this requires a knowledge of the host components required for attachment and phage entry. The outcomes of this proposal will enable the construction of new stains with defined mechanisms of phage-resistance. Host genes required for phage infection of L. lactis will be identified and isolated. We have previously isolated one such gene named pip (an acronym for phage infection protein). The protein encoded by pip (Pip) is a receptor for phage attachment and phage DNA entry into the host. We have constructed phage-resistant strains of L. lactis by replacing the pop gene with a defective version. There is evidence that host components in addition to pip are required for phage attachment and DNA entry. Isolating genes in addition to pip that are required for phage infection will enable the construction of new strains with alterations in two or more different host components. The strategy of combining multiple phage-resistance mechanisms will greatly decrease the chance that the strain will fail after introduction into commercial use. Genes will be isolated that also extend the range of resistance to phages that do not require pip. We propose to isolate genes that encode host receptors for two different types of small isometric-headed

phage (p335 & 936). Together with the phages that required pip, the p335 and 936 species of phage cause nearly all the starter failures in US cheese factories. Phages of the p335 species are particularly troublesome, as they have only recently emerged as a major problem, and less is known about their mechanism of infection. The isolated receptor genes will be inactivated and used to construct a new commerical strain with a combined phage-resistance defined by each of the inactivated genes (including pip). The phage-resistant strain will be evaluated for physiological characteristics important for making cheese.

1. Significant Progress against Objectives:

We have isolated spontaneous mutants of Lactococcus lactis that are resistant to phages of the p335 and 936 species. Specifically, strains of L. lactis 210 that are resistant to phage mm210 (p335 species), L. lactis NCK203 that are resistant to phage 31 (p335 species), and L. lactis LM2301 that are resistant to phage sk1 (936 species) were isolated by natural selection. We have characterized some of the above mutants. Strain 203 mutants all grow with a slightly longer doubling time than the parental strain. Complementaiton with a partial library of 203 genomic DNA did not yield any clones. We are constructing a better library from strain 203 and will try again. Strain LM2301 mutants have at least two distinct phage sk1-resistant phenotypes. One phenotype raises the minimal level of calcium required for infection to about 20mM from about 1 mN. This phenotype is not defective in the initial adsorption of phage to the host. Calcium is thought to be required for adsorption of the phage to the cell wall of the host. Complementation of one of these calcium mutants with a genomic library yielded a few clones, but none of these contained a complementing piece of chromosomal DNA. We interpret this to mean that these later isolates were spontaneous revertants to the phage-sensitive phenotype. Further work on these calcium mutants was halted, because they may be mutants with pleiotropic effects that the only indirectly affect phage infections through the calcium requirement. There is no evidence that calcium plays a role in adsorption to the membrane receptor. A second class of resistant mutant that is not affected by calcium concentrations and is not defective in the initial adsorbtion of phage is currently being complemented with our genomic library and screened for phenotypic reversion to phage sensitivity.

2. Significant Conclusions:

A phage sk1-resistant phenotype that raises the minimum calcium concentration required for phage infection to 20mM may not be complementable. The extra complexity of the calcium concentration-dependency appears to impose a risk that this phenotype may not lead to host factors that are directly related to phage infection. As a consequence of this, different phenotypes are now being complemented.

3. Anticipated Problems/Delays:

Publications:

None

Theses:

Published Abstract:

None

Presentations:
None

Patent/Invention Disclosures:
No patents/invention disclosures filed

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