2003 MAFMA FINAL REPORT

Project Title:	**Naturally occurring Antifungal Agents from Lactic Acid Bacteria**
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Objective Summary

The objectives of this project were to search for and screen food grade lactic acid bacteria and other biological agents for antifungal activity, and to develop these food grade biological agents for use in preventing mold invasion, growth and mycotoxin production in stored foods.

Objective Accomplishments

A total of 312 bacteria were isolated from a variety of products, and from these, 144 were Gram positive and catalase negative, fitting the criteria for lactic acid bacteria (LAB). Of the 144 isolates, 65 (45%) came from dairy products and 79 (55%) from fermented or fresh plant foods.

All the isolates obtained (144) from dairy products and fresh or fermented plant foods were screened for antifungal activity using *Fusarium graminearum* as the indicator of antifungal activity. Each Petri dish was inoculated with four bacterial isolates at 12 points, 3 for each isolate, and the score for each isolate was the result of the observation of the effect of these three bacterial colonies on the mold growth, based on the scale proposed in Table 1 for mycelial growth. The whole experiment was repeated, and the average scores obtained from both experiments for each day of observation are given in Table 2, along with the source of the isolate, for those isolates that presented an initial inhibitory activity of 5 (Clear zones around bacterial colonies), and remained with a score of at least 4 (Clear zone over bacterial colony with inhibition around), after 3 days of observation, which accounts for 52 (36%) from the initial 144 LAB isolates.

Table 1. Scale for scoring mold growth and sporulation as measures of antifungal activity of LAB using the drop plate technique.

Mycelial Growth		Spore Formation	
0	No inhibition	0	No inhibition
1	Slight inhibition of growth over bacterial colony	1	Slight inhibition over bacterial colony
2	Slight inhibition of growth over and around bacterial colony	2	Inhibition over bacterial colony
3	Inhibition of growth over and around bacterial colony	3	Inhibition over and around bacterial colony
4	Clear zone over bacterial colony with inhibition around	4	Complete/Strong inhibition all over the plate
5	Clear zones around bacterial colonies (halo formation)		

Table 2. Scores attributed to the antifungal activity of LAB isolates with strong antifungal activity, during the period of observation, based in the growth inhibition.

Isolate	Source	1st Day	2nd Day	3rd Day
131	Cottage Cheese	5	5	5
141	Cottage Cheese	5	5	4
189	Mozzarella Cheese	5	4	4
193	Mozzarella Cheese	5	5	5
207	Sauerkraut 1.26%	5	5	4
208	Sauerkraut 1.26%	5	5	4
209	Sauerkraut 1.26%	5	5	4
210	Sauerkraut 1.26%	5	4	4
211	Sauerkraut 1.26%	5	5	4
222	Sauerkraut 1.25%	5	5	5
223	Sauerkraut 1.25%	5	5	4
224	Sauerkraut 1.25%	5	4	4
225	Sauerkraut 1.25%	5	4	4
226	Sauerkraut 1.25%	5	5	4
227	Sauerkraut 1.25%	5	4	4
250	Kimchi 1	5	5	5
252	Kimchi 1	5	5	5
253	Kimchi 1	5	5	5
255	Kimchi 1	5	5	4
256	Kimchi 1	5	5	5
257	Kimchi 1	5	5	5
258	Kimchi 1	5	5	5
259	Kimchi 1	5	5	5
264	Kimchi 2	5	5	5
265	Kimchi 2	5	5	5
266	Kimchi 2	5	5	4
267	Kimchi 2	5	5	5
268	Kimchi 2	5	5	5
269	Kimchi 2	5	5	5

270	Kimchi 2	5	5	5
275	Kimchi 4	5	5	5
276	Kimchi 4	5	5	5
278	Kimchi 4	5	5	4
280	Kimchi 5	5	5	4
290	Kimchi 6	5	5	5
292	Kimchi 6	5	5	5
295	Kimchi 6	5	5	5
322	Sour cream	5	5	5
336	Cultured buttermilk	5	5	5
337	Cultured buttermilk	5	5	5
349	Hot Kimchi	5	5	5
350	Hot Kimchi	5	5	5
350B	Hot Kimchi	5	5	5
351B	Hot Kimchi	5	5	5
352	Hot Kimchi	5	5	5
353	Hot Kimchi	5	5	5
354	Hot Kimchi	5	5	5
358	Hot Kimchi	5	5	5
359	Hot Kimchi	5	5	5
360	Hot Kimchi	5	5	5
361	Hot Kimchi	5	5	5
362	Hot Kimchi	5	5	5

Among those isolates reported in Table 2, which are strong inhibitors of *Fusarium graminearum*, 13% (7) came from dairy products and 87% (45) from fermented plant foods. It appears that the isolates from fermented plant foods were more strongly antifungal and better able to inhibit mold growth than those isolates from dairy products, at least for *Fusarium graminearum*.

It is possible that isolates from the same source belong to the same species, and even to the same lineage. Therefore, all 144 isolates obtained will be kept and stored at -80^{o}C, and from those with strongest antifungal activity against *Fusarium graminearum*, at least 2 or 3 from each different source will be used in further tests, as this work will continue. In future experiments, the isolates will be tested against a broader range of molds and yeasts to identify those with the broadest antifungal activity for potential applications in protection of foods from mold growth, with eventual extension of shelf-life of products susceptible to fungal spoilage.

To accomplish this, mold growth or lack of growth will be measured by techniques, such as determining mycelial dry weights and measurements of colony diameters, which allow a more quantitative comparison of the inhibitory activity of the LAB isolates. The range of fungi that will be used in these experiments will also be expanded to include *Alternaria sp., Penicillium sp., Cladosporium sp., Phoma sp., Aspergillus niger, Penicillium roqueforti, Penicillium expansum, Saccharomyces cerivisiae,* and *Rhodoturula sp.*

After identifying the isolates of interest, further tests will be conducted to determine the nature of the inhibitory activity (competitive growth, production of active

metabolites, or other). Tests using only the supernatant of cell cultures, such as well assays tests, could be used to rule out the effect of competitive growth and the effect of low pH, since the pH of the supernatant can be neutralized.

The amount of time involved in doing the work did not permit the isolation and identification of antifungal metabolites produced by the lactic acid bacteria. However this work is continuing and these activities are planned for futures studies.

Unexpected Findings

There were no unexpected findings, though the finding that the lactic acid bacteria from plant sources appeared to have stronger antifungal activity than lactic acid from dairy sources is interesting.

Short Term Impact:

The short term impact of this work is that it showed that lactic acid bacteria from plant sources and plant based foods appear to have stronger antifungal activity than lactic acid bacteria from dairy sources. This is a useful finding that suggests that future studies should focus efforts and resources on plants and plant based food sources for isolation of antifungal lactic acid bacteria.

Long Term Impact:

The finding that lactic acid bacteria from plant based sources have strong antifungal activity has potential commercial applications for development of new antifungal agents that can be used to diminish food losses and ensure food safety. This will result in long term benefits to consumers and the agricultural and food industries.