

Chapter Five

5. Selection of lactic acid bacteria strains with antifungal capabilities

5.1 Introduction

The first stage in developing an antifungal silage inoculant is to screen a number of potential organisms for their ability to inhibit the fungal organisms that cause silage deterioration. In this chapter, the development of an assay to enable such a screen is described, and then the results of the screen are presented.

5.2 Assay development

The suppression of unwanted microbial growth by the application of more desirable organisms with the appropriate antimicrobial spectrum is a topic which attracts much interest. Unfortunately, it is often observed that organisms identified in the laboratory as being antimicrobial do not express this effect *in situ*. As an example, Pusey (1989) isolated a number of bacteria which possessed antifungal activity in culture, but when these bacteria were tested in field conditions (in this case, on fruit), they were found to have little or no effect.

Because of this, it is important that the assay system used replicates the expected *in situ* environment as closely as possible. For the purposes of the proposed screen, the choice of medium, assay technique and indicator organism were all factors in developing a assay system which both replicated the major factors expected in silage conditions, and allowed the maximum opportunity for any antifungal activity to be detected.

5.2.1 A novel medium

When a bioassay is used to screen for antifungal agents, the results can be critically dependent on the composition of the medium used. Both the indicator and the test strain are likely to be influenced by not only the nutritional makeup of the medium, but also by the physical structure, acidity, water activity, ionic strength, oxygen tension and presence of surfactants (Piard & Desmazeaud, 1991b, Vignolo *et al.*, 1995, Yang & Ray, 1994).

The facet of the natural substrate which is most difficult to replicate is its physical structure. This is because, in order to facilitate observation of microbial growth, the ideal laboratory medium should be translucent (in cases where colony growth within the medium could occur) and homogeneous (to minimise ambiguities and discrepancies across the test area). These are criteria which are obviously not fulfilled by most natural substrates. In the case of the present study, it is the anatomical structure of the plant material from which the silage is formed that provides the natural environment's physical structure. It is known that these structural properties have an influence on both the rate of fermentation and the types of microorganisms involved (Daeschel *et al.*, 1987) but, in order to detect microbial growth in such naturalistic conditions, the microbes must first be isolated from the material and grown on agar plates for enumeration. This is a time consuming process which is not suitable for screening large numbers of biocontrol organisms. Because of this, and for reasons discussed in Section 1.11, the test medium must be formulated as a homogenous preparation in agar.

A suitable agar test medium may be produced in two ways. The first method is simply to use the substrate on which the antifungal effect is to be expressed in practice. This involves taking the natural substrate and rendering it to make it amenable to laboratory procedures, for example by producing a liquid extract of the natural substrate which can then be incorporated into an agar gel. This would involve homogenisation followed by pressing and/or filtration, and sterilisation by a non-destructive technique (such as by ionising radiation). Because of the inherent variability of natural products, enough must be prepared from a single homogenous sample to last for the duration of the test procedure. An example of this method is provided by Tanaka & Ohmomo (1994), who

used alfalfa hay cubes (sterilised with ethylene oxide) to provide a medium for use in a model silage system. Their technique provides a repeatable system because alfalfa hay cubes are a fairly well standardised product, and because each cube provides 50 kg of dry substrate – sufficient for a large series of related experiments.

The second method is to prepare a wholly- or semi-synthetic medium which captures the essential elements of the natural substrate. This technique is hampered by the fact that unknown components of the natural substrate, often present only in small quantities, can be necessary as either precursors or stimulants of antimicrobial activity. For example rhizoctin, an antifungal compound produced by *Bacillus subtilis*, was originally observed in a complex culture medium. Its replacement with a defined medium caused a large reduction in activity (Kugler *et al.*, 1990). This problem can also occur in reverse – as a result of chemicals which may be present in the defined medium but are not present in the natural substrate. An relevant example is that of reuterin, which is a broad spectrum antibiotic compound synthesised from glycerol by *Lactobacillus reuteri* (Axelsson *et al.*, 1989). This is of particular concern when stored cultures are maintained frozen in glycerol.

Nevertheless, it was decided to use semisynthetic media for the present study. This was because it was felt to be the best way that a standardised medium could be prepared in unlimited quantities. To prepare such a medium, it is first necessary to know the chemical composition of the natural substrate. The composition of silage changes markedly during the fermentation process, so two media were designed; one which would simulate grass, and one which would simulate mature silage.

5.2.1.1 Synthetic grass medium

Woolford & Wilkins (1974) describe a semisynthetic medium designed to simulate crops before ensilage. They found that this medium, when incorporated into an artificial silo with a cotton wool matrix, gave rise to microbial growth which closely resembled natural silage. Two formulations were used, one which simulated crops likely to

generate high quality (low pH) silage, and one for crops likely to generate poor quality silage.

The problem of aerobic spoilage is particularly acute in silage which is of high quality, but this is because, in poor quality silage, the production of toxic metabolites from non-acidophilic organisms inhibits the growth of aerobic organisms (Section 1.5). These toxic metabolites would not be present in laboratory conditions, where only the test LAB and the indicator yeast are present. An effect that *is* likely to occur, however, is the inhibition of yeast by acidic products of the LAB themselves. As discussed in Section 1.4, a good silage inoculant must already be capable of a high acid output and so any antifungal inoculant must produce an effect above and beyond any acidifying capability. The formulation of Woolford & Wilkins which simulated crops likely to give a poor quality silage has a high buffering capacity and a lower sugar content, and as such is likely to minimise the effect of acid production. Because of this, it was this second formulation (known hereafter as Synthetic Grass Medium, SGM, and detailed in Section 2.2.9) which was used in the present study. It consists of peptone, yeast and beef extract, fructose, glucose and buffers.

The organic acid buffers used in SGM are metabolically active, and are likely to have an effect on the physiological behaviour of LAB. For example, they can be metabolised as an energy source. Woolford & Sawczyc (1984) analysed 21 strains of LAB for their ability to grow on yeast extract supplemented with malic, citric and succinic acids. They found that whereas all grew when supplemented with malic acid and 20 grew with citric acid, only 4 grew with succinic acid. Both malic and citric acid have been found by Kennes *et al.* (1995) to stimulate the growth of *L. plantarum*.

The degradation of citrate by LAB produces a number of unusual fermentation products, and because of this it has been extensively studied (Hugenholtz, 1993). It is initially converted to oxaloacetate and acetate by the enzyme citrate lyase. In *Lactococcus* spp. and *Leuconostoc* spp, oxaloacetate is then decarboxylated to pyruvate, from which a variety of related chemicals such as diacetyl, acetoin, butanediol and acetaldehyde may

be formed. In *Lactobacillus pentosus*, oxaloacetate is used as an electron acceptor, and the end product is succinate. *L. plantarum* is known to use both these pathways.

The degradation of malate by LAB has been studied because of its importance to the wine industry. At high concentrations, the malate anion may diffuse into the cell, where it is decarboxylated to lactate. Lactic acid diffusion is electroneutral, and the resulting pH gradient is used to provide metabolic energy (Olsen *et al.*, 1991).

5.2.1.2 Synthetic silage medium

There are no published descriptions of media designed to simulate silage, but the basic chemical composition of silage has been reported on a number of occasions. However as pointed out in Section 1.2, the constituents which were analysed, and the analysis techniques used, vary widely from one report to another. Not only that but, even where a particular constituent has been assessed in several reports, the amounts found vary widely, and this is a reflection of the inherent variability of silage. Because these components are not independently variable, it is not possible to simply take an average across a broad range of silage – the result would be unlikely to be representative of any natural silage. It therefore seems appropriate to take the single experiment in which the most comprehensive assessment is made and model a synthetic analogue on that. On this basis the most suitable analysis was that made by Rooke *et al.* (1985)

Rooke *et al.* (1985) analysed a range of silages which had been prepared in different ways. The most applicable to the aims of the present screen was silage B5, which was prepared from rye-grass after a 20 hour wilt and inoculated with *Lactobacillus plantarum* (in the form of the commercial preparation Siloaction). The various components analysed, along with the quantities found, are given in Table 5.1.

Of these components, the pH and the various organic acids are easiest to simulate. The pH is likely to have a profound effect on the outcome of an antifungal test, interfering with a variety of uptake and excretion mechanisms (Piard & Desmazeaud, 1991a), as well as modifying the toxicity of organic acids (Lindgren & Dobrogosz, 1990). There

Table 5.1. Composition of silage as reported by Rooke *et al.* (1985)

| Constituent | Quantity |
|----------------------------|------------------------|
| pH | 4.1 |
| Dry Matter | 184.0 g/kg silage |
| Water soluble carbohydrate | 51.0 g/kg DM |
| Formic acid | 11.0 g/kg DM |
| Acetic acid | 12.0 g/kg DM |
| Butyric acid | 4.0 g/kg DM |
| Lactic acid | 143.0 g/kg DM |
| Nitrogen | 26.3 g/kg DM |
| NH ₃ -Nitrogen | 4.6% of total nitrogen |

are, however, practical difficulties in obtaining a low pH. Agar sterilised by heating will hydrolyse if at a low pH, and there are also difficulties in obtaining acceptable growth rates at a pH of 4.1. For this reason, the pH of the SSM to be used in the assay was adjusted to 5.7.

Short chain fatty acids generally less toxic to yeast than to other fungi (Woolford 1975, 1984b), although butyric acid at low pH has a fairly high potency. Acetic acid is more toxic than lactic acid, and this can be explained by its higher pK_a (Lindgren & Dobrogosz, 1990). Middlehoven & Franzen (1986) found that most of the yeast strains they isolated from silage (with the exception of *S. dairensis*) could tolerate 5 g/l acetic acid at pH 4. The level of acetic acid found by Rooke *et al.* (1985) in silage is therefore not likely to inhibit the growth of the indicator yeast to be used in the assay. It may act synergistically with other toxins by producing stress in the yeast, increasing the potency of any antifungal activity produced by the LAB to be screened. The stress caused by these organic acids may also trigger the production of antimicrobial agents by the test LAB. Formic acid is much more toxic than the other organic acids found by Rooke *et al.*, and its presence has not been reported by other investigators. Because of this, it was removed from the formulation of SSM.

WSC are more difficult to accurately represent in a synthetic medium. This is because the generic term WSC covers a wide variety of mono and oligosaccharides. Ensuring that only the correct ones are incorporated is important because many organisms found

in silage have only a limited metabolic profile. For example, Middlehoven & Franzen (1986) noted that glucose and galactose are the only fermentable sugars for most yeast strains found in silage. Hickey *et al.* (1986), observed galactose fermentation in 6 strains of *Lactobacillus helveticus*, one of *Lactobacillus bulgaricus* and one of *Lactobacillus lactis*. They tested three strains of *L. bulgaricus* and one of *L. acidophilus* which were unable to use galactose.

A comprehensive breakdown of the WSC present in unopened silage (as determined by paper chromatography) is given by McDonald *et al.* (1960). They found the majority to be galactose or xylose, with small amounts of oligosaccharides, glucose, fructose and arabinose. Middlehoven & van Baalen (1988) found, using HPLC, relatively high levels of fructose (up to 5 g/kg) with no glucose or sucrose present after five days of ensilage. The discrepancy may be due to the lower sensitivity of the technique used by McDonald *et al.* Unfortunately, Middlehoven & van Baalen did not determine the levels of either galactose or pentoses in their silage. It is known that the pentose sugars xylose and arabinose are present in silage – it has been suggested by Brookes & Buckle (1992) that they originate from the breakdown of hemicellulose during the ensilage period. By amalgamating the analyses of McDonald (1960) and Middlehoven & van Baalen (1988), and assuming from Rooke *et al.* (1985) a total WSC concentration of 9 g/kg, it seems reasonable to use fructose, galactose and arabinose (in the ratio 2:1:1) in an artificial medium.

The peptide composition of the media is also likely to play a major role in antifungal activity. Many natural antifungal compounds are derivatives of amino acids or oligopeptides, and are taken up into the target organism *via* standard peptide transport mechanisms. The peptides in the medium will compete for this uptake and may interfere with the activity of the fungicide (Kugler *et al.*, 1990). Although there are no direct measurements of the amino acid and peptide composition of silage, the quantities involved can be estimated from the amount of nitrogen present. They can then be simulated by the incorporation of standard laboratory protein hydrolysates. Rooke *et al.* (1985) give a value of 26.6 g/kg DM for the nitrogen content of silage, but not all of this will be present in the form of oligopeptides and amino acids. Some will be inorganic,

and some will be in the form of larger proteins – and LAB have only slight proteolytic activity (Kandler & Weiss, 1990). Rather than using protein hydrolysates in sufficient quantity to simulate the total nitrogen content, it would be better to incorporate them in amounts which simulate the levels of oligopeptides in silage. In a two tonne silage with a LAB inoculant, Rooke *et al.* (1988) found that soluble nitrogen forms 58.8% of the total nitrogen. This soluble nitrogen consists mainly of free amino acids (Middlehoven & Franzen, 1986), with some contribution from ammonia, and so it is this fraction which should be used as a basis for the quantity of protein hydrolysates in an artificial medium. The contribution from ammonia – 47 g/kg according to Rooke *et al.* (1988) – can be simulated by the incorporation of ammonium chloride.

The dry matter content of silage is the most difficult to simulate. It is usually determined as that part of the silage remaining after toluene distillation, after corrections have been made for fermentation acids and alcohols (McDonald *et al.*, 1991). The SSM as formulated (Section 2.2.10) has a total DM content of 102.5 g/kg, as compared to 184 g/kg in the silage of Rooke *et al.* (1985). Thus the DM content (and, in all likelihood, the water activity) is low, which will tend to favour the growth of bacteria over yeast (Beuchat, 1983).

Because the toxicity of organic acids is pH dependent (Woolford, 1984b), the pH of the medium is a critical factor. LAB are potent producers of acids, but as discussed previously in relation to SGM, it is important that the production of acid does not interfere with the detection of other antifungal effects. For this reason, it is important that the medium is well buffered. The buffering capacity of SSM was measured (Section 3.2), and the results are shown in Figure 5.1. Standard buffers used for biological purposes generally have a β of around 0.03 and so, at the experimental pH of 5.7, SSM without sodium phosphate is only weakly buffered. The addition of 0.05 M sodium phosphate was found to considerably increase the buffering capacity at this pH. To determine whether this formulation was capable of supporting the growth of organisms found in silage, the growth capacity (Section 3.3) of a range of strains was tested. The results are shown in Table 5.2.

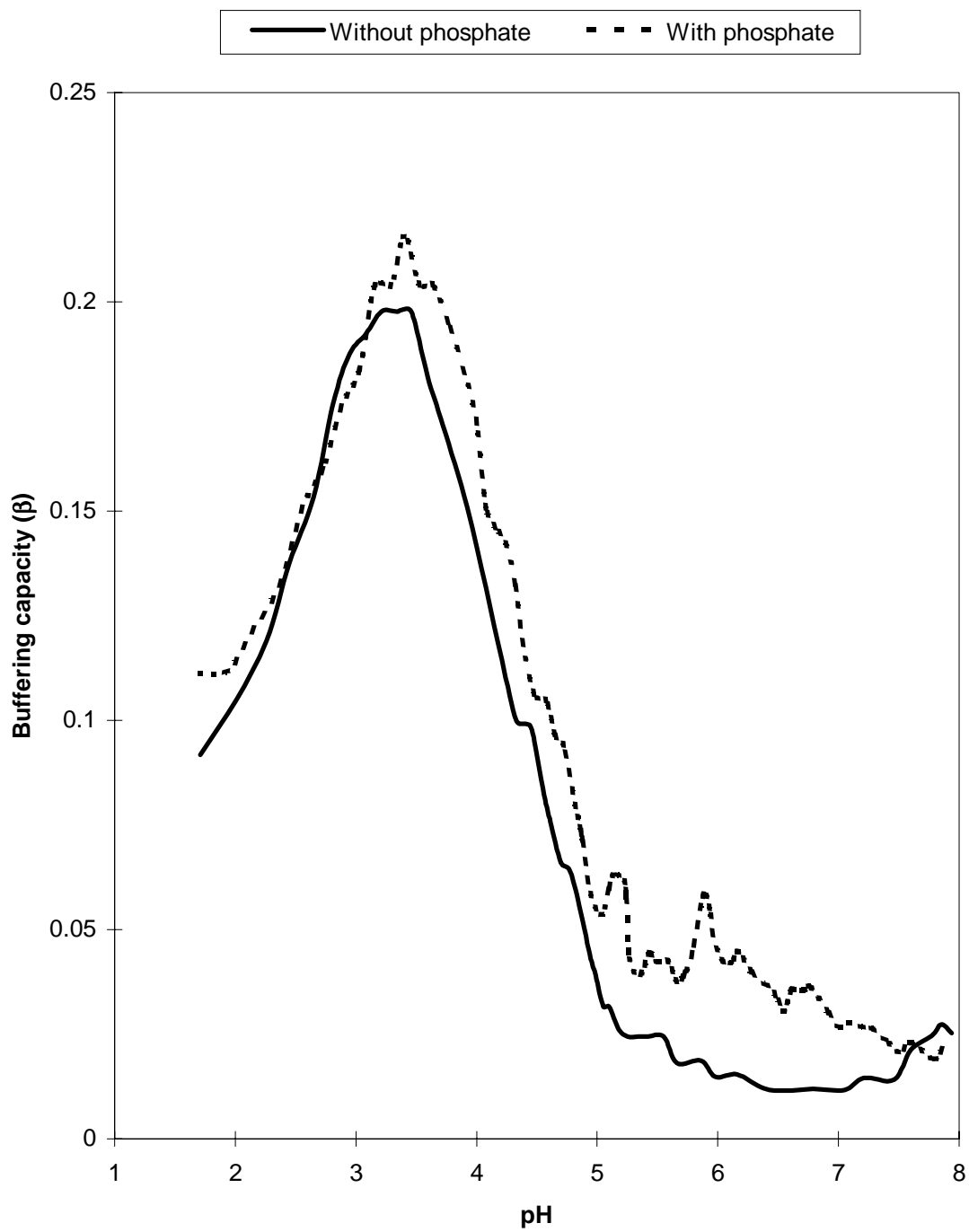


Figure 5.1. Buffering capacity of SSM with and without the addition of a phosphate buffer (0.05M).

Table 5.2. Growth of a selection of strains in SSM. The strains were inoculated into liquid SSM and examined for visible signs of growth after 48 hours.

| Strain | Growth | Strain | Growth |
|-------------------------------|--------|--------------------------------|--------|
| L4 | * | U156 | * |
| L5 | * | U195 | * |
| L6 | * | U204 | * |
| L7 | * | U258 | × |
| L8 | * | U259 | * |
| L9 | * | U275 | * |
| L10A | * | U276 | * |
| L10B | * | U346 | * |
| L11 | * | U384 | × |
| L12 | * | U388 | × |
| L14 | * | U389 | * |
| L15 | * | U392 | * |
| L16 | * | U398 | × |
| L17 | * | | |
| L19 | * | <i>Bacillus subtilis</i> CH201 | × |
| L20 | * | <i>B. licheniformis</i> CH200 | × |
| L21 | * | | |
| L25 | * | <i>S. cerevisiae</i> Y14 | * |
| | | <i>S. exiguus</i> | * |
| <i>L. plantarum</i> MTD/1 | * | <i>C. lambica</i> Y18 | * |
| <i>L. reuteri</i> NCIMB 11951 | * | <i>H. anomola</i> Y19 | * |

* Visible growth after 48 hours

× No visible growth after 48 hours.

5.2.2 A novel technique

The test used was based on the spot plate assay (Section 1.8), with some modifications. In the spot plate assay, a spot of agar containing the test strain is placed onto an agar plate, and it is then overlaid with agar containing the indicator strain. In the modified method, the test strain was spread over an agar plate, allowed to grow, and then overlaid with agar containing the indicator strain. A paper disc was placed between the two layers to prevent bacteria rising into the second layer containing the indicator yeast. This sacrifices one of the advantages of the spot plate assay, the ability to quantify the antifungal effect by the size of the inhibition zone, but it has several advantages over other methods used to screen for antifungal effects in live cultures:

1. Any observed inhibition is less likely to be due to nutritional competition, since the yeast is provided with fresh growth medium. Similarly, growth of the test organism is not restricted by growth of the indicator yeast.
2. The test is less sensitive to any antibacterial effects of the yeast.
3. It provides strong evidence that any observed inhibition is due to a diffusible agent.
4. It simulates more closely the situation found upon opening the silo where a large, established population of LAB is challenged by the sudden onset of yeast growth.
5. It is more sensitive to agents which may be produced only at low levels, since a large number of LAB will be present in the assay.

Initially the two stage protocol described in Section 3.5.1 was followed. Each LAB isolate was tested twice. Firstly, anaerobically on SGM – conditions designed to simulate freshly ensiled crops. Secondly, aerobically on SSM – designed to simulate the conditions found on opening the silo. The results of an initial, small scale screen based on this protocol was enough to demonstrate that LAB with an antifungal action were likely to be very rare. Because of this, the faster, one stage protocol described in Section 3.5.2 was adopted. This consisted of an anaerobic ‘ensilage’ phase during which the LAB were grown on SGM, followed by an aerobic phase in which the indicator yeast was overlaid. It was predicted that most of the strains tested would be able to inhibit yeast growth to some extent, simply as a side effect of their metabolism (the production

of organic acids, the drop in pH, and the depletion of sugars). Because of this, it was important that a control was adopted that allowed these effects to be taken into consideration, and it was decided to use *Lactobacillus plantarum* MTD/1, the strain which is currently used by Ecosyl Products Ltd as a silage inoculant, as a control organism. This because it is known to be a rapidly growing strain which produces large quantities of lactic acid, and it was important that any strain identified in the screen had a superior anti-yeast activity.

5.2.3 Identification of suitable indicator strains

As discussed previously (Section 1.9), a large number of yeast species have been implicated in the aerobic spoilage of silage, and an inoculant designed to minimise aerobic spoilage should ideally be active against them all. Unfortunately, there must always be a compromise between the number of LAB strains tested, and the number of indicator species they can be tested against. As a compromise, it was decided to use a two part screen. In the first, all the test LAB were screened against two species which, from literature reports, appear to be the most representative of the yeast responsible for aerobic spoilage – *Candida lambica* and *Saccharomyces exiguus*. LAB which showed activity against these species were put forward into the second part of the screen, which involved repeating the screening protocol with four new species; *Candida famata*, *Hansenula anomola*, *Geotrichum candidum* and *Candida holmii* (the imperfect form of *S. exiguus*). *G. candidum* is not a yeast, but it has been associated with aerobically spoiling silage. Its inclusion extends the scope of the test, as all the other indicator species used are members of the subfamily *Saccharomycetoidae*.

These yeast all have a widely varying metabolic profile, with some more able to assimilate the organic components of SSM and SGM than others. Their ability to use some of the major components as a sole carbon source is given in Table 5.3.

Table 5.3. Growth of representative silage yeast species on organic components of SSM and SGM.

| Species | Glucose | Galactose | Xylose | Succinate | Citrate | Lactate |
|----------------------------------|---------|-----------|--------|-----------|---------|---------|
| <i>C. lambica</i> | + | - | + | + | + | + |
| <i>S. exiguus/ C. holmii</i> | + | + | - | - | - | v |
| <i>C. famata</i> | + | + | - | - | - | v |
| <i>H. anomola</i> | + | v | v | - | + | + |
| <i>G. candidum</i> | v | + | + | ? | v | + |

Key: + definite growth
 v variable growth
 - no growth
 ? unknown

Adapted from Kreger-van Rij (1984) and Barnett (1983).

5.3 The assay

149 strains of lactic acid bacteria were obtained from a wide variety of sources, mainly isolated directly from silage, but also obtained from brewery research centres (Brewery Research Foundation International plc and Whitbread Research) and from the culture collections at Ecosyl Products Ltd and Cranfield Biotechnology Centre. The results of the one stage screen of these strains against *C. lambica* and *S. exiguus* are given in Table 5.4. Of the 149 strains tested, 20 were found to be more inhibitory to yeast than the control strain, *L. plantarum* MTD/1. Some of the strains were screened again, in order to verify the repeatability of the screening protocol. The results of the second screen are shown in Table 5.5. In general, the strains which proved positive in the first were less active in the second, especially towards *C. lambica*. Two strains which gave a positive result against *S. exiguus* in the first screen were negative in the second, but there were no positive results against *C. lambica* in the second screen.

12 of the strains selected in the first screen were screened once more in a third screen against a new selection of indicator yeasts, and the results of this screen are shown in Table 5.6. There was very little activity against any of the new indicator yeasts but three strains, LPC-22, M0042, and M0050, stood out as being active against two of the four indicator species.

LPC-22 is a strain, supplied to the Cranfield Biotechnology Centre by the Instituto de la Grasa, which was isolated from a green olive fermentation in Cordoba, Spain. The other two, M0042 and M0050, were isolated from silage provided by Bibby Agricultural. The alphanumeric strain designation denotes the region of the UK from which the silage sample was obtained, and the sample number. Thus M0042 and M0050 were obtained from the same region (Cheshire and surrounding area), and were the 42nd and 50th samples from that region. It is possible that they were obtained from the same farm, but that information was not available.

Table 5.4. Results of the first part of the screen for antifungal activity

| | | |
|------|-----|---|
| Key: | X | No effect compared with <i>Lactobacillus plantarum</i> MTD1 |
| | + | Some observable effect |
| | ++ | At least 2 cm ² of Petri dish devoid of yeast growth |
| | +++ | Complete inhibition |

Strains active against at least one strain are highlighted (Active = “++” or greater)

| Identification Number | Species (where known) | Indicator species | |
|--|-----------------------|-------------------|-------------------|
| | | <i>S. exiguus</i> | <i>C. lambica</i> |
| Strains isolated silage provided by Bibby Agricultural Ltd. | | | |
| A0010 | | + | X |
| A0012 | | X | X |
| A0013 | | X | X |
| A0014 | | X | X |
| A0015 | | X | X |
| A0016 | | X | X |
| B0030 | | X | X |
| B0031 | | X | X |
| B0033 | | X | X |
| B0034 | | X | X |
| B0035 A | | X | X |
| B0035 B | | X | X |
| D0035 | | X | X |
| D0036 | | X | X |
| E0009 | | X | X |
| F0003 A | | X | X |
| H0008 | | X | X |
| H0009 | | X | X |
| H0010 | | X | X |
| H0012 | | X | X |
| KBIC | | X | X |
| KBOC | | X | X |
| K0011 | | X | X |
| L0027 | | X | X |
| L0028 | | X | X |
| L0029 | | X | X |
| L0030 | | X | X |
| M0042 | | +++ | + |
| M0043 A | | + | X |
| M0046 | | X | X |
| M0048 | | X | X |
| M0049 | | + | X |
| M0050 | | +++ | + |
| Z0028 | | + | + |
| Z0029 | | + | + |

Table 5.4 (Cont.). Results of the first part of the screen for antifungal activity

| Identification Number | Species (where known) | Indicator species | |
|--|-----------------------|-------------------|-------------------|
| | | <i>S. exiguus</i> | <i>C. lambica</i> |
| Strains sourced from Brewery Research Foundation International: | | | |
| BSO 33 | | X | X |
| BSO 40 | | X | X |
| BSO 48 | | X | X |
| BSO 80 | | X | X |
| BSO 82 | | X | X |
| BSO 87 | | X | X |
| BSO 159 | | X | X |
| BSO 166 | | X | X |
| BSO 304 | | X | X |
| BSO 313 | | X | X |
| BSO 409 | | X | X |
| BSO 418 | | X | X |
| BSO 421 | | X | X |
| Strains isolated from silage provided by Farmlab: | | | |
| 20022/32 | | X | X |
| 20025/32 | | X | X |
| 20026/32 | | X | X |
| 20028/32 | | X | X |
| 20029/32 | | X | X |
| 20032/32 | | X | X |
| 30005/32 | | X | X |
| 50003/32 | | ++ | X |
| 50006/32 | | X | X |
| 50009/32 | | X | X |
| 50010/32 | | X | X |
| Strains isolated from silage provided by Lodge Farm: | | | |
| SI 7 | | X | X |
| SI 8 | | X | X |
| SI 9 | | X | X |
| SI 10 | | X | X |

Table 5.4 (Cont.). Results of the first part of the screen for antifungal activity

| Identification Number | Species (where known) | Indicator species | |
|--|---|-------------------|-------------------|
| | | <i>S. exiguus</i> | <i>C. lambica</i> |
| Strains sourced from Cranfield University Biotechnology Centre: | | | |
| 15 M1 | | + | X |
| 55/1 | | + | ++ |
| BOM 1 | | X | + |
| LPC 1 | | X | + |
| LPC 2 | | X | + |
| LPC 19 | | + | + |
| LPC 22 | | ++ | ++ |
| LPE 5 | | X | + |
| LPE 11 | | + | + |
| LPE 14 H7 | | + | + |
| LPP 2 | | X | X |
| LPS 21 | | X | X |
| LPS 22 | | X | + |
| SGL 1 | <i>L. plantarum</i> 343 | X | X |
| SGL 2 | <i>L. plantarum</i> 704 | X | X |
| SGL 3 | <i>L. casei</i> 55 <i>Rhamnosus</i> NCDO 86 | X | X |
| SGL 4 | <i>L. plantarum</i> 1193 | X | X |
| SGL 5 | <i>L. grayii</i> NCTC | X | X |
| SGL 6 | <i>L. innocua</i> 11 288 | X | X |
| SGL 7 | <i>L. immunocytogenes</i> NCTC 4883 | X | X |
| SGL 9 | <i>L. plantarum</i> 362 | X | X |
| SGL 10 | <i>L. plantarum</i> 340 | X | X |
| SGL 11 | <i>L. plantarum</i> NCDO 82 | X | X |
| SGL 12 | <i>L. plantarum</i> 352 | X | X |
| SGL 13 | <i>L. acidophilus</i> 1748 | X | X |
| SGL 14 | <i>L. pentosus</i> AB1 | X | X |
| Strains isolated from silage provided by Mount Pleasant Dairy Farm: | | | |
| SI 1 | | X | X |
| SI 2 | | ++ | X |
| SI 3 | | X | X |
| SI 4 | | X | X |
| SI 5 | | X | X |
| SI 6 | | X | X |
| SI 11 | | +++ | ++ |
| SI 14 | | X | X |
| SI 15 | | X | + |

Table 5.4 (Cont.). Results of the first part of the screen for antifungal activity

| Identification Number | Species (where known) | Indicator species | |
|--|--|-------------------|-------------------|
| | | <i>S. exiguus</i> | <i>C. lambica</i> |
| Strains isolated from silage provided by Solley's Dairy Farm: | | | |
| SI 12 | | X | X |
| SI 13 | | X | X |
| Strains sourced from Whitbread Research: | | | |
| WL 6 | | X | X |
| WL 11 | <i>Lactobacillus pastorianus</i> NCIMB 11719 | X | X |
| Strains sourced from Ecosyl Products Ltd.: | | | |
| | <i>Lactobacillus reuteri</i> NCIMB 11951 | X | X |
| L 4 | | ++ | X |
| L 5 | | ++ | + |
| L 6 | | + | X |
| L 7 | | ++ | X |
| L 8 | | + | X |
| L 9 | | X | X |
| L 10A | | X | + |
| L 10B | | +++ | X |
| L 11 | | + | X |
| L 14 | | + | X |
| L 15 | | + | X |
| L 16 | | ++ | X |
| L 17 | | ++ | + |
| L 18 | | ++ | ++ |
| L 19 | | + | X |
| L 20 | | ++ | X |
| L 21 | | + | + |
| L 25 | | ++ | X |
| U 156 | | X | X |
| U 162 | | ++ | X |
| U 195 | | X | X |
| U 204 | | X | X |
| U 258 | | X | X |
| U 259 | | X | X |
| U 275 | | ++ | X |
| U 389 | | ++ | X |
| U 398 | | ++ | X |
| U 392 | | ++ | X |

Table 5.4 (Cont.). Results of the first part of the screen for antifungal activity

| Identification Number | Species (where known) | Indicator species | |
|-----------------------|----------------------------------|-------------------|-------------------|
| | | <i>S. exiguus</i> | <i>C. lambica</i> |
| 0966 | | X | X |
| 0976 | | X | X |
| 1038 | | X | X |
| 1039 | <i>Lactobacillus fermentum</i> | X | X |
| 1040 | | X | X |
| 1041 | | X | X |
| 1042 | | X | X |
| 1044 | <i>Lactobacillus fermentum</i> | X | X |
| 1045 | | X | X |
| 1048 | <i>Lactobacillus fermentum</i> | X | X |
| 1050 | | X | X |
| 1053 | <i>Lactobacillus acidophilus</i> | X | X |
| 1055 | <i>Lactobacillus fermentum</i> | X | X |
| 1087 | <i>Lactobacillus salivarius</i> | X | X |
| 1094 | | X | X |
| 1100 | | X | X |
| 1155 | | X | X |
| 1156 | | X | X |

Table 5.5. Strains from the first part of the screen which were tested again.

Key: X No effect
 + Some observable effect
 ++ At least 2 cm² of Petri dish devoid of yeast growth
 +++ Complete inhibition

Strains active against at least one strain in both replications of the test are highlighted (Active = “++” or greater).

| Strain | Initial Test | | Retest | |
|--------|-------------------|-------------------|-------------------|-------------------|
| | <i>S. exiguus</i> | <i>C. lambica</i> | <i>S. exiguus</i> | <i>C. lambica</i> |
| 55/1 | ++ | + | + | + |
| LPC-22 | ++ | ++ | ++ | X |
| KBOC | X | X | X | X |
| L 5 | ++ | + | + | + |
| L 17 | ++ | + | ++ | X |
| L 18 | ++ | ++ | ++ | + |
| M0042 | +++ | + | ++ | + |
| M0050 | +++ | + | +++ | X |
| SI 11 | +++ | ++ | +++ | X |

Table 5.6. Activity of 12 selected LAB against further indicator strains.

Key: X No effect
 + Some observable effect
 ++ At least 2 cm² of Petri dish devoid of yeast growth
 +++ Complete inhibition

Strains active against at least one strain are highlighted (Active = “++” or greater)

| Test strain | <i>C. famata</i> | <i>C. holmii</i> | <i>H. anomola</i> | <i>G. candidum</i> |
|-------------|------------------|------------------|-------------------|--------------------|
| L5 | X | X | X | X |
| L7 | X | X | X | X |
| L10B | X | X | X | X |
| L18 | X | X | X | X |
| U392 | X | ++ | X | X |
| 55/1 | + | + | X | X |
| LPE 11 | X | X | X | X |
| LPC-22 | ++ | ++ | X | X |
| SI 8 | X | X | X | X |
| SI 11 | X | X | X | X |
| M0042 | ++ | ++ | X | X |
| M0050 | ++ | ++ | + | X |