DETECTING WILD YEASTS IN BREWERIES

Yeasts are an important group of micro-organisms that occur as contaminants in breweries. The term 'wild yeast' usually means any yeast not used intentionally and not under full control. It follows, therefore, that brewing yeasts themselves may become 'contaminants' within the brewery. This complicating situation can arise, for example, in breweries operating with more than one brewing strain, such as separate ale and lager yeasts, where undesirable cross contamination can occur.

The definition also includes many yeasts that are unable to grow extensively during beer processing and as such are relatively harmless, they merely serve as indicators of general hygiene and raw material problems. Wild yeasts take on greater significance when they are capable of producing spoilage which may take the form of haze (cloudiness), deposits and sediments, off-flavours and excessive carbon dioxide formation.

This paper is concerned only with conventional culture methods for the detection of wild yeasts in brewery quality control and does not include alternative procedures such as immunological or staining methods which are often used alongside cultural methods.

Wild yeasts of many different genera can be isolated from within the brewery. However, it is convenient to allocate them to one of two groups, in addition to brewing strains, namely: i) non-brewing Saccharomyces and ii) yeast genera other than Saccharomyces (Table 1). The significance of this classification is that wild Saccharomyces species may give rise to more problems with spoilage than the non-Saccharomyces. Being able to make this classification and, especially the choice of media, depends on a great deal on local circumstances and the type of processing the beer has undergone.

General methods

One of the more straightforward situations is the monitoring of beer pasteurisation, where the heated processing regime is designed to inactivate all organisms carried through in the beer. Any yeast surviving pasteurisation, whether it be brewers yeast or wild yeast, serves as a warning of process failure and therefore all that is required is a non-selective method for detecting yeasts in general. Many general purpose media will suffice in this case, of which WLN agar (Walker Laboratories Nutrient agar; Oxoid CM309/501) is commonly used, albeit often in a modified form, e.g., pH adjusted. WLN sometimes has the useful effect of differentiating strains of yeast on the basis of colony staining (see example in Figure 1), though this is by no means always the case and is therefore only of supplementary value.

The reliability of modern filters and pasteurisers almost invariably means the brewery microbiologist is seeking to detect low numbers of contaminants in heat-processed or sterile-filtered beer at packaging. A large sample volume of beer (typically 1 to 10 litres) must be membrane-filtered before incubation on nutrient media to achieve a realistic quantitative estimate of contamination. After a suitable period of incubation (2 to 7 days) the contamination level can be assayed by colony counting (Figure 2). Incubation times can be shortened significantly by scanning membranes for micro-colony development using a microscope. For this to be effective it is desirable to incorporate suitable stains in the agar before incubation.

Non-Saccharomyces yeasts

The majority of samples received by the quality control laboratories contain brewers yeast, often vastly in excess of any contaminants, so that the problem frequently becomes one of detecting a small proportion of wild yeasts against a background of brewers yeast. The solution of the problem is far easier when the contaminants are non-Saccharomyces than when they are Saccharomyces species. The most widely used medium for the former purpose is lysozyme agar (Oxoid CM191). This is used on the basis that yeasts have the ability to use lysozyme as sole nitrogen source whereas Saccharomyces species, and particularly brewing strains, do not normally grow on this medium. The need to ensure a washed inoculum and to regulate the quantity of brewers yeast plated means the procedure is somewhat tiresome in routine use, but even so it is still a very popular method in the industry.

An alternative to using lysozyme medium is to use Acridine/cycloheximide as a selective inhibitor.

However, this medium also requires careful standardisation to be effective and its use is not as widespread as lysozyme medium. Recently, another method has been proposed which relies on the selective inhibition of Saccharomyces (in particular brewing strains) by copper ions in a copper-supplemented agar formulation.

Addendum: The β-Lactam Antibiotics

In the last issue of Culture (Vol. 4 No. 2, September 1983) we provided a review of the nomenclature, structure and β-lactamases susceptibility of the wide range of β-lactam antibiotics available to us for characterisation. Since the article was written, information on a 'third generation' parenteral cephalosporin, ceftriaxone, has recently been made available to us.

Ceftriaxone

Ceftriaxone (Ro 13-9904, see above) shows potent in vitro and in vivo activity against a wide range of bacteria. Ceftriaxone is stable, as are other cephalosporins of the third generation, in the presence of most β-lactamases, a fact which is reflected in its activity against β-lactamase-producing organisms. The antibacterial spectrum and the activity in vivo of this antibiotic and its structure are very different from that of either cephalosporins or penicillins.

Reference


Allen Brown and Christopher Reading

Figure 1: Different colony types on WLN agar showing pale green and dark green staining yeasts.

Figure 2: Yeast colonies on a membrane filter.

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Campylobacter jejuni in food products

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The role of Campylobacter jejuni as a causative agent of acute enteritis is well established. As more and more reports come in it is clear that the infection is found in all areas of the world.1,2 It is most common in tropical under-developed countries where the majority of infected children under 9 months of age are symptomatic, whilst older infected children usually are asymptomatic.3-5 In developed countries the great majority of cases are symptomatic. This is probably due to a better control of the infection with fewer subclinical cases and hence less acquired immunity. The infection is usually mild, but large outbreaks of campylobacteriosis 1-4 may temporarily reduce the working capacity of the affected community. This is particularly true when outbreaks hit military units under field conditions.7

Source of infection

The infection is exceedingly common in Nature. Birds, domestic or wild, appear to be the most important source of infection. Reports from many countries indicate that about 80% of chickens and turkeys are infected and that they carry about 108 C. jejuni organisms per gram of faeces.6 The organisms seem to have a preference for the caeca (Figure 1) but whether or not chickens are the only persistent reservoir for the persistence of the infection is unknown. Most wild birds, including pigeons, migratory waterfowl, swans and flamingoes, are also infected.13,14 The organism has been isolated from most domestic animals4 and from numerous species of wild animals, ranging from cheetahs to crocodiles.14

Infected humans may also be a source of infection. In the acute phase there are about 105 C. jejuni/g ileal contents (N.E. Richardson, personal communication), falling off over the next 3-6 weeks until spontaneous bacteriological cure is attained.4 In tropical and sub-tropical countries the carrier state may last from a few weeks to more than a year.7 Young pets may also harbour the infection. From time to time they suffer from acute Campylobacter enteritis; the stools are liquid, often bloody and teeming with C. jejuni.6-9

Transmission of C. jejuni to man

Although man-to-man and pet-to-man infections have been reported, they are rare and of limited epidemiological importance. Outbreaks involving large populations are water born9 or milk born.10,11 The evidence is essentially epidemiological and circumstantial. The organisms have rarely been recovered from the in- vaded vehicles which supposedly have been contaminated with faecal material. Many smaller outbreaks have also been attributed to ingestion of raw milk.12,13 The great majority of clinical cases occur sporadically or in small clusters and appear to be due to consumption of infected, under-cooked food. Meat from domestic birds is probably the single most important source. Infection rates as high as 70-95% have been reported.12,13,14,15 About one third of the caecases are positive for C. jejuni after overnight soaking in chilled, chlorinated water.10 The organisms survive up to 7 days at 4°C on the skin and abdomen wall of the carcasses and up to 21 days in meals frozen at -25°C.17 There is no evidence that C. jejuni multiplies on the infected products — indeed their number dwindles with time.

How does meat poultry become infected? The answer has a bearing on the preventive measures that should be adopted to provide a clean consumer product. The seeding may take place in vivo secondary to bacteriemia9,12 or after slaughtering due to unhygienic handling of the offals. To distinguish between the two possibilities we examined 117 chicken livers purchased in retail outlets in New York City. Fifty-six livers (48%) were positive for C. jejuni (Table 1). More than half of these yielded growth from the surface only. When the organism was recovered from both surface and tissue, the tissue infection was invariably lighter than the surface infection. In the two cases where the organisms were isolated from the tissues only, no more than two colonies were observed. Moreover, no liver showed signs of abscesses or scar formation. It was concluded that unhygienic handling of poultry parts is the cause of the infection. Other types of meat are also infected. For example, Bolton et al.17 investigating C. jejuni infection of carcasses in English abattoirs found 32% infection of beef, 70% of sheep and 50% of pork. Other authors concur that the organism may be recovered from all these types of meat but have generally found a lower infection rate15-17. To the best of our knowledge C. jejuni has not been isolated from cured meat.

Detection of C. jejuni in meat products

The basic questions about detection are: should direct seeding of selective selective media be supplemented with enrichment procedures; and if so, how much do we gain? Some investigators employing enrichment media report improved recovery of the organism.3-4,6,9,36 but systematic inquiries are missing. This led us to examine the problem. First, we compared the sensitivity of selective and non-selective solid media for the isolation of wild strains of C. jejuni. This was accomplished by counting C. jejuni in selective filtrates of stools on the media under investigation. Blaser's medium (Oxoid: 10mg vancomycin, 2,500 U polymyxin B, 5mg trimethoprim, 20mg ampicillin, and 15mg cephalothin per litre) was used as selective to wild strains of C. jejuni, chocolate-agar supplemented Blaser's medium (Oxoid: 25,000 U bac­trin; 50mg cycloheximide; 10,000 U colistin sulphate; 10mg cephal­ozin sodium; and 5mg novobiocin per litre) was less sensitive but more selective. In experiments designed to identify the important components in a liquid medium we found that best results were obtained with thioglycolate broth supplemented with 5% lysed sheep blood. If this medium (TBS) was further supplemented with medium with 0.1ml of the same suspension, the sensitivity of the enrichment was approximately 50 cases/liter more than the sensitivity of the solid medium.

Conclusion

From a practical point of view, all meat should be considered to be infected with C. jejuni due to unhygienic handling and slaughtering. The organisms tend to diminish in number with time. Heat treatment, and particularly irradiation, diminish the rate of infection. Systematic examination of meat for C. jejuni is costly and of little epidemiological significance. In the rare cases where it may be justified to conduct a search for C. jejuni, the sen­sitivity of the methods should be increased significantly by the use of enrichment media.

References


Table 1. Site of C. jejuni in 117 chicken livers

<table>
<thead>
<tr>
<th>Site</th>
<th>Number positive for C. jejuni</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface and tissue</td>
<td>18 (15%)</td>
</tr>
<tr>
<td>Supernatant</td>
<td>2 (23%)</td>
</tr>
<tr>
<td>Tissue only</td>
<td>61 (62%)</td>
</tr>
<tr>
<td>Uninfected</td>
<td>70 (75%)</td>
</tr>
</tbody>
</table>

Figure 1: Caeca of chicken.
Serological Tests for Syphilis

J.D. Oriel MD, Consultant Physician, Department of Genito-Urinary Medicine, University College Hospital, London.

The accurate diagnosis of syphilis depends to a great extent on the results of laboratory investigation. In primary and secondary syphilis, material from the lesions can be examined for Treponema pallidum by dark-field microscopy, but in all stages of the disease the detection of circulating antibodies is a most valuable procedure.

Early Test Procedures

During the complex interaction between T. pallidum and its host, a series of antibodies is produced, some specific and others non-specific (Table 1). The first serological test for syphilis was introduced by Wassermann in 1906; this was a complement fixation test. The original antigen was an extract of syphilitic tissue from stillborn fetuses with congenital syphilis. It was thought at the time that the active component in the extract was T. pallidum, but it is now known to be a diphospholipid called cardiolipin which is present not only in treponemes but in many mammalian tissues as well. The Wassermann reaction (WR) is thus a non-specific serological test. In its most refined form it was performed with a chemically pure cardiolipin antigen supplemented by lecithin and cholesterol. Antibodies to cardiolipin have been called 'reagin', a term which has been condemned as misleading but which is still in general use. The WR held the field in syphilis serology for many years, but fixation tests are easier to perform and more sensitive for the detection of reagin. The Venereal Disease Research Laboratory test (VDRL), introduced in 1946, is the most popular of these. The patient's serum is reacted with a mixture of cardiolipin, lecithin and cholesterol on a slide and after a few minutes flocculation is detected with a microscope. The rapid plasma reagin test (RPR) is essentially the same test, but carbon particles are added to the antigen mixture and the results can be observed with the naked eye. The RPR can be used with autoanalyzer equipment; this automated reagin test by BFP reactions, a search was made for alternative serological cards which would be specific for treponemal disease.

More Specific Tests

The first of these was the Treponema pallidum immobilisation test (TPI), which was introduced in 1949. Live treponemes are incubated with the heat-inactivated test serum in the presence of complement and the preparation examined by dark-field microscopy; a positive reaction is reported if more than 50% of treponemes are immobilized. This test is specific for treponemal disease but it is expensive, hazardous and technically demanding; it can be performed only in specialist centres, and is now obsolete.

The fluorescent treponemal antigen-absorbed (FTA-ABS) test was developed in 1968. It is an indirect immunofluorescence procedure and uses T. pallidum as antigen. It is important that the test serum is first treated with a sonicate of T. pallidum treponemes to remove group-reactive antibodies, and the specificity of the test depends on the use of a really effective absorber. The FTA-ABS test is sensitive and specific; false-positive reactions are rare but are seen in some patients with collagen diseases. The test can be modified by the use of non-specific fluorescent-labelled anti-human globulins (e.g. anti-IgM) to identify the immunoglobulin class of the antibody. The FTA-ABS test is tedious to perform and is not suitable for screening large numbers of sera, but it is a useful confirmatory test and is of value in the diagnosis of primary syphilis (see below). The T. pallidum haemaggulination assay (TPHA) was introduced in 1966. Sheep or fowl erythrocytes coated with particles of T. pallidum are mixed with the test serum in wells; haemaggulination caused by specific immunoglobulins is observed.* Non-specific reactions are eliminated by pre-treatment of the serum with a extract of R. tetanum, rabbit testis and erythrocyte membranes. The TPHA test is highly specific, easy to perform and can be quantitated. It is increasingly used for the routine screening of sera — because of the cost of the reagents, a micro-method is usually employed.

Natural History of Syphilis

It may be of interest to outline the natural history of syphilis before the use of these serological tests in clinical practice is discussed. The incubation period of acquired syphilis is between 9 and 90 days. Primary syphilis is manifested by the appearance of a primary sore, or chancre, at the site of inoculation of T. pallidum: this may be on the penis, vulva, cervix or on extragenital sites such as the anus and mouth. There is usually an associated regional lymph node enlargement. Although these signs are local, in primary syphilis dissemination of treponemes through-out the body has already occurred. In secondary syphilis, which develops 8-12 weeks after the primary stage, this dissemination continues with immunological reactions resulting in a variety of widespread disease: malaise, fever, weight loss, skin rashes, enlargement of lymph nodes and disease of the heart, brain and central nervous system may all occur. If left untreated, secondary syphilis runs a fluctuating course for up to two years. The disease then becomes latent and there are no symptoms or signs for many years. In about one-third of the patients late syphilis then appears, characterised by disease of the cardiovascular system, the central nervous system or by gummatous destruction of various organs and tissues. During primary, secondary and latent syphilis it is possible for a mother to transmit the infection to the foetus in the uterus, resulting in congenital syphilis. When a person contracts syphilis, the first serological test to become reactive is the FTA-ABS test, followed by the reagin tests (VDRL or RPR tests). The TPHA becomes positive next, and the TPI test last.

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Table 1. Serological Tests for Syphilis

<table>
<thead>
<tr>
<th>Tests for Non-specific Antitreponemal Antibodies (Reagin Tests)</th>
<th>Wassermann Reaction (WR)</th>
<th>Venereal Disease Research Laboratory (VDRL) Test</th>
<th>Rapid Plasma Reagin (RPR) Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tests for Specific Antitreponemal Antibodies</td>
<td>T. Pallidum Immobilisation (TPI) Test</td>
<td>Fluorescent Treponemal Antibody-Absorbed (FTA-ABS) Test</td>
<td>T. Pallidum Haemaggulination Assay (TPHA) Test</td>
</tr>
</tbody>
</table>

| Table 2. Results of serological tests for syphilis in various conditions |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Condition                   | VDRL or RPR | TPHA | FTA-ABS |
| Primary syphilis            | ±             |      |       |
| Secondary syphilis          | ±             |      |       |
| Latent syphilis             | ±             |      |       |
| Late syphilis               | ±             |      |       |
| Non-venerial treponematoses | ±             |      |       |
| Biological false-positive reaction | +            |      |       |

This table shows the results of various serological tests for syphilis in different conditions. The VDRL and RPR tests are positive in primary and secondary syphilis, while the TPHA and FTA-ABS tests are positive in all stages of the disease.
Thus, the FTA-ABS and VDRL or RPR tests are useful for the serological diagnosis of primary syphilis, particularly if dark-field microscopy for *T. pallidum* is not practicable (Table 2). In secondary syphilis, all serological tests are strongly positive and the titre of antibodies in the VDRL and RPR tests is maximal. As the disease becomes latent this titre slowly falls, although the specific tests remain positive. In most patients with untreated late syphilis, reagin tests are reactive in low titre or they may even be negative; the TPHA and FTA-ABS tests however, are always positive. In a small proportion of patients with late syphilis the TPI test may give negative results.

What effect does antiretroviral treatment have on this serological pattern? The VDRL and RPR tests usually become negative one year after the effective treatment of primary syphilis and two years after treatment of secondary syphilis, but the specific tests may remain positive for many years. The FTA-ABS (IgM) test, which is always positive in untreated primary and secondary syphilis, usually becomes negative within 9 months of treatment. When patients are treated for latent or late syphilis, tests for cardioiogen antibody often show a decline in titre, but this may be slow, and in a few patients the titre becomes stationary and unaffected by further treatment. The specific tests remain positive indefinitely.

### Serological Tests in Clinical Practice

It is not possible to use a single serological test for the diagnosis of syphilis and for the follow-up of a patient after treatment. If a reagin test alone is used, many patients with latent and late syphilis may escape diagnosis; conversely, patients with BFP reactions may be wrongly suspected of having syphilis. The best combination is a quantitated reagin test (VDRL, RPR, or ART) and a TPHA test. An FTA-ABS test should be available for the diagnosis of early syphilis and for the resolution of discordant results between reagin and TPHA tests. For the assessment of the results of treatment, quantitated reagin tests are most useful; specific tests are of little value. The serological reactions to syphilis and other venereal treponematoses, such as yaws, are identical. Patients who have had yaws in childhood show positive specific tests and reagin tests which are weakly reactive or negative, a picture identical to that seen in patients with latent or late syphilis or a previously treated infection. This means that unless there are positive features in the history or physical examination it may be impossible to decide whether a patient from an area in which yaws is endemic has yaws or syphilis. It is customary to perform serological tests on all pregnant women and this, together with treatment of infected mothers, explains the very low incidence of congenital syphilis in developed countries. The interpretation of syphilis serology during pregnancy is no more difficult than in any other time, but it must be remembered that BFP reactions can occur during pregnancy, so serological screening must always include a specific test.

If a mother has been adequately treated for syphilis, and has not been reinfected since treatment, there is no risk of congenital syphilis in the baby. Occasions arise when there is doubt about the adequacy of treatment or the possibility of reinfection when it is important to ensure that the baby is not infected. Serological tests on the neonate will reflect those of the mother because of the transplacental passage of antibodies. These maternal antibodies normally disappear within 3 months of birth. Persistently positive serology, or a rising titre in the reagin tests, or a positive FTA-ABS (IgM) test, all suggest congenital infection. In some babies the onset of congenital syphilis may be delayed; therefore, it is customary to continue serological examination for the first 6 months of life if there is a possibility that the mother had untreated or inadequately treated syphilis before the baby's birth. The virtual disappearance of congenital syphilis from developed countries is one of the major successes of modern medicine and no relaxation of the present screening procedure should be contemplated.

### References


### Culture

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**Wild Yeasts in Breweries**

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Once again, inhibition of Saccharomyces includes many wild yeasts as well as culture yeast, though by comparison with lyase aga the incubation times are shorter and sample manipulation is minimal, making the procedure at least worthy of a closer examination. Often, though, non-Saccharomyces yeasts do not cause problems within the brewery and under these circumstances their selective detection serves as a measure of general brewery hygiene.

**Saccharomyces yeasts**

Over the years a number of media have been proposed for estimating wild Saccharomyces yeasts in the presence of brewing yeasts. Kato's medium employing crystal violet as selective inhibitor was probably one of the forerunners, though it has been reported subsequently to be unreliable on number of occasions. Schwarz Differential Medium (SDM) uses fuchsin sulphate as inhibitor but again its total reliability has been called into question. In a further variation, Li employed crystal violet and fuchsin sulphite into one medium and reported superior results compared with the individual inhibitors on their own. The efficiencies of these various differential media have been reviewed.

Longley and co-workers have taken a different approach to the problem of detecting Saccharomyces species by exploiting the well known obligate requirement of Saccharomyces cerevisiae under anaerobic growth conditions for both ergosterol and an unsaturated fatty acid. In fact almost all species of Saccharomyces have this requirement and grow adequately in the absence of oxygen on media supplemented with ergosterol and Tween 80 (I), whereas yeasts from other genera only produce microcolony. Thus, media can be made selective for Saccharomyces species in general using ETB and strictly anaerobic conditions. More importantly, media can be made selective for wild Saccharomyces using ETB-supplemented crystal violet agar or ETB-supplemented Lin's medium.1

**Summary**

The diversity of wild yeasts encountered in the brewery and their role in fermentation has been covered in previous articles and this contribution is in no way an attempt to cover the subject in detail. However, it is clear that wild yeasts can cause significant fermentation problems in the brewery and their use in fermentation is not recommended. In situations where wild yeasts are known to be a problem, a number of techniques have been developed to control the growth of wild yeasts. In this respect, a serious consideration of wild yeast control in the brewery is essential.

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**Wild Yeasts in Breweries**

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