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Mini-Review

On the safety of *Kluyveromyces lactis* – a review

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Introduction

For many centuries, yeasts have been indispensable for the preparation of a broad range of food products such as, for example, bread. Men have known how to use yeast for the preparation of beer for thousands of years, but it was not until the 1870s that Pasteur discovered that fermentation is a result of the action of living organisms. This discovery led to the first introduction of pure yeast cultures in the brewing industry in 1883 by Emil Christian Hansen, and some years later in the baking industry. Ever since, more and more pure cultures of micro-organisms ranging from bacteria to yeasts and fungi have proved to be very useful for a broad range of industrial applications. Some of them have a long record of industrial use and have been subject to intensive studies regarding the biochemical processes in which they participate, as well as all kinds of aspects concerning the safety of their use.

In this work we review the history and the safety aspects of the use of one of these organisms, the yeast *Kluyveromyces lactis.*

History and taxonomy

Originally, the yeast that is now known as *K. lactis* was called *Saccharomyces lactis,* but since it appeared to be unable to cross-breed with the yeast *S. cerevisiae,* it was grouped under the genus *Kluyveromyces* in 1965 and renamed *K. lactis* (Dombrowski) van der Walt (van der Walt 1970). Under this name the yeast is deposited at the Centraalbureau voor Schimmelcultures in The Netherlands. In 1984, the classification of the species became as follows (van der Walt and Johannson 1984):

Family: Saccharomycetaceae
Subfamily: Saccharomycetoideae
Genus: *Kluyveromyces*
Species: *K. marxianus* var. *lactis*

Note that generally the shorter name *K. lactis* is used.

As early as in 1889, Beijerinck reported his findings on *S. kefyr* (Beyerinck 1889), in his opinion identical to *S. lactis.* In 1910 Dombrowski isolated this species from raw milk (Dombrowski 1910). A number of other authors have also reported on the occurrence of *S. (K.) lactis* in dairy products, amongst others in the Russian fermented milk product *koumiss* (Lang 1970), in starter cultures for yoghurt (Rizvanov 1960), in Greek cheeses (Picci 1960), in *laban zeir* – a type of buttermilk (El-Sadek 1965), in Cheddar cheese (Cooke and Brazis 1968), in Damietta types of cheese (Ghoniem 1968), in Roquefort cheese (Devoyod and Sponem 1970) and in Camembert (Jabarit 1970).

Industrial uses

In the 1950s and 1960s, *K. lactis* biomass was manufactured on whey, where it uses lactose as a source of carbohydrate to produce an alimentary yeast with high nutritional value in food and feed (Vrignaud 1971). The ability of *K. lactis* to utilize lactose is also used for the production of lactase. This *K. lactis* enzyme, which hydrolyses milk lactose into its constituent monosaccharides glucose and galactose, is the most widely used commercial preparation for the production of low-lactose milk for intolerant populations (Woychik and Holsinger 1976; Nijpels 1980; Bacques and Simonpieri 1987; Hussein et al. 1989). The lactase preparation from *K. lactis* has been affirmed as Generally Recognised As Safe (GRAS) by the United States Food and Drug Administration (FDA) as a direct ingredient of food (Randolph 1984). It has also been found economically feasible to use *K. lactis* for microbiological recycling of whey in countries where millions of tons of this product are still regarded as industrial waste and dumped into sewage systems (Foda et al. 1988). Finally, in the past decade, *K. lactis* has proved to be a very efficient host for the production of heterologous proteins. This latter aspect is treated separately below.

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Safety aspects

As already mentioned above, *K. lactis* has been affirmed as a GRAS organism for lactase production by the U.S. FDA. The FDA confirms that: "*K. lactis* is a normal, even necessary component of many cultured dairy products", and that "no reports of toxicity or pathogenicity have ever been associated with the presence of *K. lactis* in food" (Randolph 1984). Thus, *K. lactis* is not pathogenic for healthy individuals. When defence mechanisms are severely impaired, however, any host can be infected even by micro-organisms with a GRAS status, and therefore also by yeasts normally occurring in foods. However, the incidence appears to be very low. Nielsen et al. (1991) described the detection of 118 episodes of fungemia in a university hospital in Copenhagen over a 5-year period, as a result of performing about 50000 blood cultures. All 117 patients involved had underlying diseases. *Candida* was isolated in the vast majority of cases (93%) and Saccharomycetaceae in six cases (5%). Only one of these last isolates belonged to the genus *Kluyveromyces,* and was described as *K. marxianus.*

K. lactis itself was used in the form of an inactivated powder as a health food and protein supplement for various groups of people in the period from 1964 to 1971. Used as such, the yeast was consumed over periods of 1–60 days at doses up to 30 g per day. When consumed by, for example, infants, sick persons, and under-fed persons, high nutritional qualities and a broad range of therapeutic effects could be demonstrated with no problems whatsoever with acceptability in any of the groups (Vrignaud 1971; Gervais 1973).

We have shown at Gist-brocades in live microcosm experiments (surface water, soil, and sewage water), that the *K. lactis* strains used for the production of lactase and chymosin (see below) show a significantly reduced viability as compared to the wild type (van der Kleij 1992). A recombinant strain of *K. lactis* producing the milk-clotting enzyme chymosin (see below) was tested for pathogenicity according to the standard operating procedures of Gist-brocades. For that purpose, it was administered to mice by the intravenous, intraperitoneal, nasal, subcutaneous and intracerebral route. The intraperitoneal and intravenous application was done in both normal and immune-suppressed mice. After 1, 3, 7, 14 and 21 days two animals of each treatment group were sacrificed. The heart, brains, kidney, spleen, liver and lungs were examined for pathological lesions, and by microbiologial isolation methods checked for the presence of the yeast. In addition, the remaining animals were investigated daily for symptoms of disease. The results clearly showed that *K. lactis* is not a pathogenic organism (van Lambalgen 1987).

It is found that certain strains of *K. lactis* produce a protein that specifically inhibits cell division of other sensitive yeast species in the G1 phase of the cell cycle. Sensitivity seems to be correlated with haploid-specific functions in the cell. The ability to secrete this inhibitor as well as the immunity against it is encoded by two linear, double-stranded DNA plasmids (for a recent r view see Stark et al. 1990). Since the *K. lactis* strai employed for industrial purposes do bot possess the plasmids, they do not produce this yeast specific inhib tor.

Recombinant strains

The use of various yeast systems for the commerci production of heterologous proteins, and the advar tages of these systems as expression systems for con plex proteins have recently been reviewed (Buckho et al. 1991). With regard to *K. lactis,* the most prom nent example so far of the production of a heterol gous protein is that of chymosin. This aspartyl pr tease, which is responsible for the coagulation of mil in the fourth stomach of milk-fed calves, was synthe sised and secreted as fully active prochymosin (van de Berg et al. 1990). Many studies have been performe to compare the genetically engineered bovine chym sin derived from *K. lactis* with its natural counterpa isolated from calf stomachs. No differences in milk clotting activity of the two preparations could be de tected under any of the environmental conditions stud ied (O'Sullivan and Fox 1991). Also, with regard t general composition or ripeness of different types c cheese prepared with the two preparations, it was con cluded that recombinant calf chymosin can be used a a substitute for calf rennet (Prokopek et al. 198 Chamba 1989).

Permission to produce chymosin from recombinan *K. lactis* strains for use in cheese manufacture has bee granted in a number of countries worldwide includin The Netherlands. Recently, chymosin production wit *K. lactis* has been affirmed as GRAS by the FDA. Th *K. lactis* strain used as a host for chymosin expressio is a direct descendant of that used for lactase produc tion described above. In addition to the reduced viabil ity in different live microcosms (see above), we hav shown at Gist-brocades that the chymosin productio strain cannot compete with its parental strain or th wild type in either of these environments. Further- more, the chymosin production strain was shown t possess a reduced mating efficiency (van der Klei 1992). The latter further reduces the chance of transfe of genetic material from these strains. The host organ- ism has a long history of safe use in the food industr (see above), and the inserted sequences are well char- acterised and in essence limited to those necessary fo expression and secretion of the protein (van den Berg et al. 1990). The chymosin-producing strain can there- fore be considered to meet the Good Industrial Large Scale Practice (GILSP) criteria as formulated by the Organisation for Economic Co-operation and Devel- opment (OECD) (1986). Also Fleer et al. have re- ported on the use of industrial strains of *Kluyvero- myces* yeasts. In their case, *K. lactis* is used for high level secretion of recombinant human proteins such as human serum albumin (Fleer et al. 1991a) and inter- leukin-1β (Chen et al. 1990; Fleer et al. 1991b). These

human proteins are expressed from multi-copy vectors based on the 2 μ-like plasmid pkD1 from *K. drosophilarum*. Thus, besides being used for the production of food enzymes, *K. lactis* seems to become a prominent host for the production of pharmaceutical proteins as well.

Conclusions

As described above, *K. lactis* is a yeast that is generally regarded as a safe organism for use in lactase and chymosin production. Characteristic for such a type of organism is the fact that men and the environment have been exposed to it for many years and in significant doses, and that industry has used it for so many years that it knows how to handle it and feels safe with it. This also means that *K. lactis* offers the possibility of developing GILSP processes for heterologous gene expression, as long as the genes to be expressed encode non-hazardous proteins. Production of these proteins can then be performed in such a way that there is no risk for health or the environment, even when the organisms are fermented in production facilities originally designed for the fermentation of non-engineered organisms.

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