Food Microbiology 37 (2014) 41–43

Contents lists available at SciVerse ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Starter cultures for cereal based foods

Markus J. Brandt*

Ernst Böcker GmbH & Co. KG, Ringstrasse 55-57, 32423 Minden, Germany

ARTICLE INFO

Article history:
Available online 22 June 2013

Keywords:
Starter cultures
Sourdough

ABSTRACT

Fermented cereals play a significant role in human nutrition in all parts of the world where cereals grow. These fermentations are started spontaneously or there have been traditional techniques developed in order to keep starter cultures for these processes alive. With the growing impact of industrial microbiology during 20th century this traditional starter culture propagation was replaced often, especially in the dairy industry, by the use of pure, frozen or freeze-dried cultures grown on microbial media. In contrast to the production of ethanol from cereals, in sourdough a pasteurization step before inoculation is avoided due to gelatinization of starch and inactivation of endogenous enzymes. Therefore cultures must be competitive to the relatively high microbial load of the cereal raw materials and well adapted to the specific ecology determined by the kind of cereal and the process conditions. Less adapted cultures could be used, but then the process of back-slopping of cultures is limited. Although cereal fermentations take the biggest volume among fermented foods, only for sourdoughs commercial cultures are available.

1. Introduction

Fermented cereals play a significant role in human nutrition in all parts of the world where cereals grow. Among all food fermentations (e.g. milk, meat, fish, soy or wine) cereal fermentations reach the highest volume (Hammes et al., 2005). Depending on the water content during processing and on the cereal-specific enzyme activities a broad range of fermented cereal foods and beverages were developed by mankind (Nout, 2009; Steinkraus, 2002):

- Alcoholic beverages like beer, sake or chicha with amylolysis performed by malted grains or fungi.
- Fermented porridge like uji, pozol, kenkey or gari. Often these porridges act as an intermediate product for further processing, for example sourdough for baked goods like bread or injera.

Cereal fermentations can be traced back to ancient times and Pliny the elder (Historica naturalis 18,26) described procedures to obtain a starter cultures and the backslopping process. During vintage, he describes that three days fermenting grape must is kneaded either with millet or wheat bran and dried in the sun and used as starter culture for bread baking. For other times in the year he recommends the boiling of a barley-water mixture and waiting until it gets sour. Alternatively dough from the previous day should be used to inoculate the dough, which is still today in use for a lot of cereal fermentations. In medieval times this knowledge on fermentations got widely lost. Around the 15th century in Europe again the use of yeasts from alcoholic fermentations for bread leavening baking got be ramped. However the success of a fermentation was rarely calculable. This changed by the start of the research on industrial microbiology by Pasteur and others in the second half of 19th century. An important breakthrough was the application of pure-culturing techniques and the construction of the necessary apparatus for industrial use by Emil Christian Hansen in 1885 (Hansen, 1895). With this procedure it was possible to obtain yeasts for beer or spirit production without contaminants. It can be seen as a predecessor of the autoclave. Although the Hansen procedure is with some slight modifications still in use in breweries today, another important microbiologist of that time – Max Delbrück – did not believe to the success of Hansen's procedure, due to its high investment and energy costs. In 1895 (Henneberg, 1909) he proposed a system of "natural pure culturing" as an alternative to Hansen's system of "artificial pure culturing" as it was denominated by Delbrück. He proposed, probably influenced by Darwin, "More generally, a natural pure culture to denote the selection under natural conditions as a result of various mutual struggle, living side by side under the same conditions for the benefit of one or more of these organisms." Thus, not sterilization with subsequent inoculation of the desired microbes should be used, due to its high costs, but a control of process parameters will lead to the desired microbiota – the base of microbial ecology. These two concepts are still in use today in the production of starter cultures for cereal

* Tel.: +49 (0)571837990; fax: +49 (0)5718379920.
E-mail address: markus.brandt@sauerteig.de.

0740-0020/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fm.2013.06.007

fermentation and it is the aim of this paper to describe the specialties of applied starter cultures for cereal fermentations in industrial scale.

2. Cereals as raw material for fermentations

Cereal fermentations are, except for beer wort, usually carried out on non-heated raw materials. Compared to the dairy industry, where nearly all the milk is at least pasteurized before fermentation, cereal starter have to be much more competitive. Flours have a total aerobic cell count up to 10^7 cfu/g, 10^3 cfu/g coliform bacteria and 10^7 cfu/g bacilli, respectively (Berghofer et al., 2003). A pasteurization step is not possible, due to the gelatinization of starch and the inactivation of endogenous cereal enzymes, which are required for fermentation. Part of the microbial load is removed during the milling process by separating the bran, but also this surface layers are useful in fermented form for nutrition (Poutanen et al., 2009). Mixing a flour with water results after 24 h in a cell count of enteric bacteria at about nearly 10^8 cfu/g (Hochstrasser et al., 1993). Enteric bacteria produce often undesired cheesy flavor compounds. Therefore a high competitiveness and short lag-phases are desirable for starter cultures. For production of leavened baked goods mainly wheat, rye and spelt are used, in some regions also barley and oats. Rice is used either alone or in combination with wheat for steamed buns in Asia (Luangsakul et al., 2009) or used in combination with legumes for idli (Nout, 2009). Kisra and injera are pancake-like breads produced from sorghum or tef, respectively (Haard et al., 1999).

3. Types of starter cultures in use

The most traditional technique in use for cereal fermentation is backslopping: It is the inoculation of the raw material with a small amount of dough from a previous successful fermentation. Sometimes from that are simple starter preparations created by addition of further flour, resulting in a reduced physiological activity of the microorganisms. These preparations can be stored short-time, i.e. days or weeks. Typical for this starter class are also the Ragi-type starters (according to Holzapfel, 2002). Fermented product from a previous batch is used to form balls from rice flour and water. Fermentation is performed at the air, where a gradual desiccation occurs. These dried balls are used as inoculum for other fermentations. In companies, where a microbial laboratory exists, e.g. breweries, strains are ordered from specialized strain collections and propagated inhouse on wort. Thus, the only cereal fermentation where commercial starter preparations are applied, is sourdough. Table 1 summarizes the different procedure in use for starting cereal fermentations. Natural pure cultures ("Reinzucht" starters) are derived from continuously propagated fermentations, they are sold after thickening of the sourdough. Similar to that are the so-called "baking- ferments" (Freund, 2006), where the propagated starter preparation is selected from several batches of spontaneous sourdoughs. Starter cultures which are aiming on leavening by yeasts are often sold as liquid doughs. Defined cultures are pure cultures which are propagated in microbial media and subsequently frozen or freeze dried (Vogel et al., 2011). There are several criteria by which cereal starter cultures can be classified. One is the kind of substrate used for propagation. Defined starter cultures are usually grown on microbial media and baker's yeast is usually (with the exception of some "organic" yeasts) propagated on molasses supplemented with ammonium. On the other hand, "Reinzucht" and "Ragi"-type starters are propagated on a cereal substrate. This type of cultures has a shelf life of around 4–8 weeks, whereas frozen or dried cultures are stable up to two years. In most areas of the world parts of a previous fermentation are used as starters for cereal fermentations, or spontaneous fermentation is applied. In industrialized countries, due to improved reproducibility of fermentation and resulting bread quality, mainly commercial starter cultures are in use.

There are not much reports on the start of commercialization of such cereal starter cultures. Interestingly a first commercial sourdough starter preparation was sold in Scotland and Ireland as "Parisian Barm". According to Boutroux (1892, cited after Maurizio, 1917) it is a milky liquid, containing yeasts and bacteria for leavening of bread. In Germany, commercial "Reinzucht" starter cultures were available from 1910 (Brandt, 2010). Table 2 gives an overview, which species of lactic acid bacteria and yeasts are sold today as single or mixed-strain cultures for sourdough.

Table 1
Type of starters used in various cereal fermentations.

	Alcoholic beverages	Vinegar	Acid fermented beverages	Acid fermented gruels	Leavened baked goods
Spontaneous	x		x	x	x
Backslopping	x	x	x	x	x
Sterile strain propagation (partly from strain collections)	x				
Commercial starter cultures					x

4. Composition of cultures for different aims in sourdough fermentation

Main aim of sourdough fermentation is either acid production or leavening. It depends on the final bakery product, what composition of the starter culture should be applied. In rye processing, where a drop of pH in bread dough is necessary in order to obtain an elastic bread crumb, a single-strain lactic acid bacteria might be enough, if additional baker's yeast is used for leavening. For a three-stage-process with enhanced acetic acid concentration, a combination of yeasts and lactic acid bacteria is required. Acetic acid may be formed by heterofermentative lactic acid bacteria if electron acceptors, e.g. fructose are available. Acetic acid is mainly produced during first hours of fermentation until free fructose gets limited in the dough. Therefore in three-stage-sourdough process acetic acid concentrations are higher as in one-stage-process. Additional fructose can be liberated by yeast invertase from fructooligosaccharids and saccharose (Brandt and Hammes, 2001). If in

Table 2
Lactic acid bacteria and yeasts used in commercial starter preparations.[a]

	Lactic acid bacteria	Yeasts
Frozen/ freeze-dried/ spray-dried preparations	*Lactobacillus brevis*, *L. plantarum*, *L. sanfranciscensis*, *L. casei*, *L. delbrueckii*, *L. fermentum*, *Pediococcus pentosaceus*, *P. acidilactici*	*Saccharomyces cerevisiae*, *S. cerevisiae var. chevalieri*, *Torulaspora delbrueckii*
Cereal-based preparations	*L. sanfranciscensis*, *L. pontis*, *L. crispatus*, *L. brevis*, *L. casei*, *L. plantarum*, *L. fermentum*, *L. paracasei*, *L. helveticus*, *L. paralimentarius*, *Leuconostoc lactis*	*Candida milleri*, *S. cerevisiae*, *S. pastorianus*

[a] Poitrenaud, 2003; Hammes and Vogel, 2007.

wheat baked products the aim of fermentation is limited to improvement of flavor, the use of lactic acid bacteria is sufficient. On the other hand, especially in southern Europe exist some baked goods with unique properties, which should be leavened solely by sourdough, e.g. Pane Pugliese and Panettone. For these breads a starter culture rich in yeast is used, usually called “natural yeast”. The fermentation conditions used for the propagation of sourdoughs favor the growth of yeasts over lactobacilli: Temperatures between 18 and 22 °C and a high amount of backslopping (Ottogalli et al., 1996).

In general, if backslopping procedures shall be used, a strict compliance with the process parameters recommended by the culture producer is necessary. Böcker et al. (1990) studied over a period of 10 month the microbial composition of a continuously on rye dough propagated starter culture and found always the same two *Lactobacillus sanfranciscensis* strains. The genome of one of that strains was compared by Ehrmann et al. (2011) with the genome of an isolate from 2006 from the same sourdough starter culture. The sizes of the circular chromosome sequences differed only in 577 bp and were highly similar. That confirms *L. sanfranciscensis* as stable element in this sourdough starter preparation.

Meroth et al. (2003) investigated the development of microorganisms during sourdough fermentations applying several different process parameters. After 18 refreshment *L. sanfranciscensis*, which was incorporated in the starter culture, was still dominant, due to the correct process parameters for this organism. Similar results are reported from Siragusa et al. (2009). For an industrial type II fermentation, Gänzle and Vogel (2002) showed the stable persistence of *Lactobacillus reuteri* over 10 years or 50,000 generations. Not only the process parameters, but also the kind of cereal is of importance. Cereals are different regarding the availability of sugars and the amylolytic or peptidolytic enzymes. That influences strongly the competitiveness of the used starters (Vogelmann et al., 2009) and therefore the selection of a starter culture should be fit to the intended cereal for use.

For the economic production of frozen or dried starter cultures for food fermentations are, beside the physiological properties relevant for bread quality (e.g. flavor, taste, exopolysaccharide production, etc.), a good growth in a transparent fermentation broth and high survival rates during freezing or drying, necessary. For industrial cultures sold on a cereal base as sourdough, other criteria are more important. In multiple strain starters the lactic acid bacteria need similar growth rates and should not compete with yeasts on substrate. Furthermore they have to fit the intended fermentation process (temperature, amount of mother dough) and the kind of cereal for which they are used.

5. Conclusion

Supported by metabolomics studies, the knowledge on the microbiota of cereal fermentations and how it can be influenced by process conditions and the cereal substrate, respectively, is rapidly increasing. Strangely only a small part of this knowledge was transferred into industrial application. Beside sourdough, for cereal fermentations commercial starters are not in use and the main part of it is performed by traditional procedures.

References

Berghofer, L.K., Hockhing, A.D., Miskelly, D., Jansson, E., 2003. Microbiology of wheat and flour milling in Australia. International Journal of Food Microbiology 85, 137–149.

Böcker, G., Vogel, R.F., Hammes, W.P., 1990. *Lactobacillus sanfrancisco* als stabiles Element in einem Reinzucht-Sauerteig-Präparat. Getreide, Mehl, Brot 44, 269–274.

Boutroux, L., juin 1892. Annales de chimie et de physique 6. ser. T. 26.1.

Brandt, M.J., 2010. Zur Geschichte des Reinzuchtsauerteiges. Cereal Technology 64, 194–195.

Brandt, M.J., Hammes, W.P., 2001. Einfluß von Fructosanen auf die Sauerteigfermentation. Getreide, Mehl und Brot 55, 341–345.

Ehrmann, M.A., Behr, J., Böcker, G., Vogel, R.F., 2011. The genome of *L. sanfranciscensis* after 18 years of continuous propagation. In: 10th Symposium on Lactic Acid Bacteria, Egmond an Zee, Netherlands. Abstract book, A005.

Freund, W., 2006. Starterkulturen und Sauerteigprodukte. In: Brandt, M.J., Gänzle, M.G., Spicher, G. (Eds.), Handbuch Sauerteig. Behr's Verlag, Hamburg, pp. 353–375.

Gänzle, M.G., Vogel, R.F., 2002. Contribution of reutericyclin production to the stable persistence of *Lactobacillus reuteri* in an industrial sourdough fermentation. International Journal of Food Microbiology 80, 31–45.

Haard, N.F., Odunfa, S.A., Lee, C.H., Quintero-Ramirez, R., Lorence-Quiñones, A., Wacher-Radarte, C., 1999. Fermented Cereals. A Global Perspective. In: FAO Agricultural Service Bulletin, 138. FAO, Rome.

Hammes, W.P., Brandt, M.J., Francis, K.L., Rosenheim, J., Seitter, M.F.H., Vogelmann, S.A., 2005. Microbial ecology of cereal fermentations. Trends in Food Science and Technology 16, 4–11.

Hammes, W.P., Vogel, R.F., 2007. Sauerteig. In: Holzapfel, W. (Ed.), Mikrobiologie der Lebensmittel – Lebensmittel pflanzlicher Herkunft. Behr's Verlag, Hamburg, pp. 285–314.

Hansen, E.C., 1895. Untersuchungen aus der Praxis der Gärungsindustrie, I. Heft. R.Oldenbourg, Munich.

Henneberg, W., 1909. Gärungsbakteriologisches Praktikum, Betriebsuntersuchungen und Pilzkunde. Paul Parey, Berlin.

Hochstrasser, R.E., Ehret, A., Geiges, O., Schmidt-Lorenz, W., 1993. Mikrobiologie der Brotteigherstellung. II. Mikrobiologische Untersuchungen an unterschiedlich hergestellten –Spontansauerteigen aus Weizenmehl. Mitteilungen aus dem Gebiete der Lebensmittel-untersuchung un Hygiene 84, 356–381.

Holzapfel, W.H., 2002. Appropriate starter culture technologies for small-scale fermentation in developing countries. International Journal of Food Microbiology 75, 197–212.

Luangsakul, N., Keeratipibul, S., Jindamorakot, S., Tanasupawat, S., 2009. Lactic acid bacteria and yeasts isolated from the starter doughs for Chinese steamed buns in Thailand. Food Science and Technology 42, 1404–1412.

Maurizio, A., 1917. Die Nahrungsmittel aus Getreide, Erster Band, first ed. Paul Parey, Berlin.

Meroth, C.B., Walter, J., Hertel, C., Brandt, M.J., Hammes, W.P., 2003. Monitoring the bacterial population dynamics in sourdough fermentation processes by using PCR-denaturing gradient gel electrophoresis. Applied Environmental Microbiology 69, 475–482.

Nout, M.J.R., 2009. Rich nutrition from the poorest – cereal fermentation in Africa and Asia. Food Microbiology 26, 685–692.

Ottogalli, G., Galli, A., Foschino, R., 1996. Italian bakery products obtained with sourdough: characterization of the typical microflora. Advances in Food Science 18, 131–144.

Poitrenaud, B., 2003. Commercial starters in France. In: Kulp, K., Lorenz, K. (Eds.), Handbook of Dough Fermentations. Marcel Dekker, New York, pp. 197–230.

Poutanen, K., Flanders, L., Katina, K., 2009. Sourdough and cereal fermentation in a nutritional perspective. Food Microbiology 26, 693–699.

Siragusa, S., Di Cagno, R., Ercolini, D., Minervini, F., Gobbetti, M., De Angelis, M., 2009. Taxonomic structure an monitoring of the dominant population of lactic acid bacteria during wheat flour sourdough type I propagation using *Lactobacillus sanfranciscensis* starters. Applied and Environmental Microbiology 75, 1099–1109.

Steinkraus, K.H., 2002. Fermentations in world food processing. Comprehensive Reviews in Food Science and Food Safety 1, 23–32.

Vogel, R.F., Hammes, W.P., Habermeyer, M., Engel, K.K.D., Eisenbrand, G., 2011. Microbial food cultures - opinion of the senate commission on food safety (SKLM) of the German research foundation (DFG). Molecular Nutrition & Food Research 55, 654–662.

Vogelmann, S.A., Seitter, M.F.H., Singer, U., Brandt, M.J., Hertel, C., 2009. Adaptability of lactic acid bacteria and yeasts to sourdoughs prepared from cereals, pseudocereals and cassava and use of competitive strains as starters. International Journal of Food Microbiology 130, 205–212.