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THE MICROBIOLOGICAL ECOSYSTEM OF TRADITIONAL FERMENTED SAUSAGES IN SERBIA – POSSIBILITY TO CREATE OUR OWN STARTER CULTURES*

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A b s t r a c t: Today in Serbia, according to the existing world trends, a growing number of meat industries are implementing in the production active starter cultures. Bearing in mind that in Serbia there is no commercial production of such cultures, the domestic industry is obliged to purchase such cultures from foreign manufacturers. Such cultures, are as a rule adapted for the needs of other markets and usually do not result in products which have traditional sensory characteristics which are acceptable to our customers.

The Project "Technological and protective features of autochthonous bacterial strains isolated from traditional fermented sausages and possibilities for their application in the meat industry" is financed by the Serbian Ministry of Science and Technology and has the aim to study the diversity of a number of bacteria such as: Lactobacillus, Micrococcus, staphylococcus and Streptococcus which carry out the fermentation in narrow diameter sausages "Levacka", "Sremska" and "Uzicka" from three different regions in Serbia , as well as to determine the possibility of their use within industrial conditions of production. Adequate selection and choice of bacterial strains, after their detailed morphological, biochemical, molecular and genetical, as well as potential technological, protective and probiotic features characterization would make the presumptions needed for a qualitative step forward in the production of our own starter cultures. With their regular use specific national products with distinctive sensory features to which our population is accustomed and with improved quality parameters would be obtained.

By realizing the scope the rationale for the use of autochthonous strains of LAB in the production of fermented sausages their authenticity will be preserved, uniform quality can be obtained, production mistakes avoided, the fermentation and maturation time shortened and at the same time the typical sensory features preserved and/or improved.

Key words: *traditional fermented sausages, isolates, LAB, starter cultures, meat industry*

Mikrobiološki ekosistem tradicionalnih fermentisanih kobasica u Srbiji – mogućnosti stvaranja sopstvenih starter kultura*

S a d r ž a j: Danas u Srbiji, a u skladu sa savremenim svetskim trendom, sve veći broj industrija mesa u svojoj proizvodnji primenjuje aktivne starter kulture. Obzirom da kod nas ne postoji njihova komercijalna proizvodnja, domaće industrije mesa su primorane da ih nabavljaju od stranih proizvođača. Takve starter kulture, po pravilu, prilagođene su potrebama drugih tržista pa najčešće ne daju proizvode sa tradicionalnim senzorskim svojstvima koja su najprihvatljivija za naše potrošače.

Projekat "Tehnološke i protektivne osobine autohtonih sojeva bakterija mlečne kiseline izolovanih iz tradicionalnih fermentisanih kobasica i mogućnosti njihove primene u industriji mesa", koji finansira Ministarstva zy nauku i tehnološki razvoj Republike Srbije,ima za cilj sagledavanje diverziteta različitih bakterijskih vrsta iz roda Lactobacillus, Micrococcus, Staphylococcus i Streptococcus, koji su nosioci fermentacije u kobasicama uskog dijametra („levačka", „sremska" i „užička") sa tri područja Srbije, kao i utvrđivanje mogućnosti njihove primene u industrijskim uslovima. Adekvatnom selekcijom i odabirom određenih sojeva bakterija, nakon njihovih detaljnih morfoloških, biohemijskih, molekularno-genetskih ispitivanja, kao i utvrđivanja potencijalno tehnoloških, protetektivnih i probiotskih svojstava, stvorile bi se pretpostavke za drugi, kvalitetan iskorak u pravcu sopstvene proizvodnje starter kultura. Njihovom primenom dobili bi se specifični nacionalni proizvodi sa karakterističnim i prepoznatljivim senzorskim svojstvima na koje je naše stanovništvo naviklo, sa, istovremeno, unapređenim parametrima kvaliteta.

Realizacijom postavljenih zadataka dokazaće se svrsishodnost korišćenja autohtonih sojeva BMK u proizvodnji fermentisanih kobasica, sačuvaće se autentičnost proizvoda, dobiće se ujednačen kvalitet, izbeći će se manje proizvodne greške, skratiće se proces zrenja i sušenja, a pri tome će biti očuvana i/ili unapređena karakteristična senzorska svojstva proizvoda.

Ključne reči: *tradicionalne fermentisane kobasice, izolati, BMK, starter kulture, industrija mesa*

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Introduction

The increasing manufacture of fermented products, after the Second World War, has conditioned the need for standardized and economical production on one side, and a safe product on the other. Nowadays, in order to fulfill these requirements the modern industry uses specially selected and chosen microorganisms, the so called starter cultures (*Caplice, Fitzgerald,* 1999). As during the traditional production of fermented meat products, the lactic fermentation is a spontaneous process, often uncontrolled and based on the activity of the "wild" epiphytic microflora, the quality of the products present on the market is variable and often lacking the specific sensory characteristics. The direction taken by these processes is guided by the accidentally present microflora which can give to the fermentation in an unwanted direction resulting in spoilage. The manufacture of good products with a standard quality is possible only if in the meat are the dominant useful homofermentative strains of LAB are present. If not, mistakes are not rare (*Coretti,* 1971, 1975).

The production of a safe product with standard uniform quality characteristics is an imperative for every serious producer. By respecting this principle on one side continuous production can be obtained and on the other customer confidence can be achieved. Starter cultures, which today are used in the meat industry, have the purpose not only to achieve desirable sensory characteristics, but by ensuring optimal microbiological processes to ensure a safe production.

The idea to inoculate *Lactobacillus* cultures for the production of fermented sausages was presented for the first time in 1940 by Jensen and Paddock (US Patent 2, 225, 783) as a way to shorten the maturation time and to obtain the desired quality and aroma. The first used starter cultures in the USA meat industry in 1955 were LAB, such as *Pediococcus cerevisiae* (Erkkila S., 2001). At the same time in Europe Niinivaara is (1955) used the Micrococcus M53 *M53 (Slavica Vesković, 2009).*

Lactic Acid Bacteria (LAB) as the carriers of lactic fermentation

The ability of LAB to ferment sugars down to lactic acid is the main principle which determines their use in the production of fermented meat products (Figure 1). Thus, the stability to ferment carbohydrates, which occurs at the level of fosforilated substrates, is the key feature of LAB (Slavica Vesković Moračanin, 2007). They produce lactic acid as the result of glucose breakdown during glucolysis or 6- phosphogluconate/ phosphoketolase reaction, depending if homo or heterofermentative bacteria are employed (*Kandler,* 1983; *Axelsson,* 1998). Homofermentative LAB genus *Lactococcus, Pediococcus, Streptococcus* and certain strains of *Lactobacilli* convert during anaerobic glucolysis 1mol of glucose into 2 moles of lactate, while the heterofermentative group of LAB (*Leuconostoc* and some *Lactobacillus*) during anaerobic glucose catabolism produce lactic acid, carbon dioxide, ethanol and half the energy (*Ros et al.,* 2002; *Caplice & Fitzgerald,* 1999).

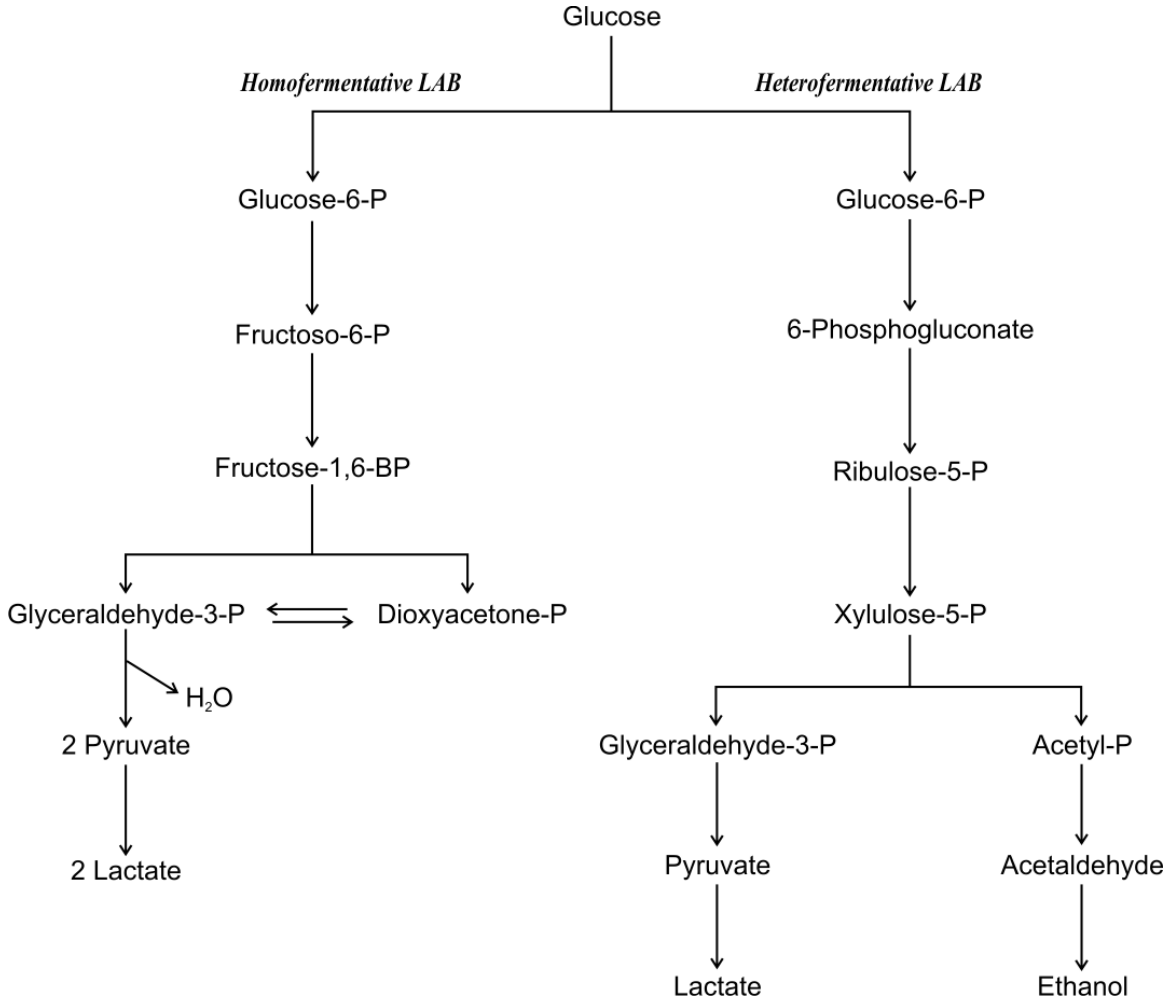
LAB besides having effects on the acidity by producing absolute and relative quantities of lactic and acetic acid, influence the taste of the final product by producing some substances which are under the sensitivity detection limit. By lowering the pH of the sausage filling during the fermentation process enzymes which regulate lypolisis (*Garcia et al.,* 1992; *Molly et al.,* 1996) and proteolysis (*Demeyer,* 1992) become activated.

Microorganisms, specially the catalase positive cocci, influence the aroma and taste of fermented sausages through direct breakdown of lipids and proteins into compounds which contribute to the desired sausage aroma. At the same time their nitrate – reducing activity results into the formation of a stable color (*Lücke, 2000*). The most important quality parameters affected by the starter cultures are shown in Table 1.

Nowadays a few important companies which produce starter cultures (needed for the meat industry) provide pure *Lactobacillus spp., Pediococcus acidilactici, P. pentosaceus, Staphylococcus xylosus* or *S. carnosus* (*Daly & Davis,* 1998, *Hammes et al.,* 1985) cultures.

LAB in biological food protection

The growing need for natural and safe food have lead to an increased interest for the use of bacteriocin- producing LAB bacteria which are used as protective cultures in the meat industry for the making of fermented products. The principle on which biological protection of these cultures (*Lindgren & Dobrogosr,* 1990) is achieved is based on lowering the number of unwanted spoilage bacteria, but without influencing the quality of the final product. The biological protection of LAB through the presence of bacteria and/or their metabolic products are achieved by: production of lactic acid or other volatile organic



Sheme 1. Metabolism of glucose in homo and heterofermentative LAB
Šema 1. Metabolizam glukoza kod homo- i heterofermentativnih BMK

Table 1. Effect of starter cultures on raw sausages
Tabela 1. Efekat starter kultura u sirovim kobasicama

Quality parameters	Mode of action	LAB	Catalase positive cocci
Color	- reduction of nitrates	-	+++
	- lowering of pH	+++	-
	- decreased O_2 content in the sausages (Eh)	-	++
	- H_2O_2 degradation	-	++
Aroma	- acid production	+++	-
	- proteolysis	-	+
	- lipless	-	++
	- rancidity (antioxidative)	-	++
Consistency	- lowering of pH	+++	-
Shelf time	- lowering of pH	+++	-
	- reduction of nitrates	-	++
	- suppression of unwanted microflora	++	-
Low content of residues	- reduction of nitrates	+	++

+++ very important role
++ important role
+ no importance

acids resulting in lowered pH; production of other primary metabolites such as hydrogen peroxide, carbon dioxide, diacetyl, reuterin and bacteriocine production which is a specific antibacterial compounds (*De Vuyst* and *Vandamme,* 1994) (Table 2).

tainty of the growth rate and metabolic intensities of the protective and unwanted bacteria.

However, even if many producers of starter cultures suggest that they can solve or even eliminate problems relative to hygienic standards of the basic components of meat products we have to be very cautious not to expect an immaculate product if we

Table 2. Metabolic products of LAB and their antimicrobial effect
Tabela 2. Metabolički produkti BMK sa antimikrobnim efektom

LAB products	Target microorganisms
Organic acids	
Lactic acid	Rotting and GR-ve bacteria, some fungi
Acetic acid	Rotting bacteria, clostridia, some yeasts and molds
Hydrogen peroxide (H_2O_2)	Pathogenic bacteria and bacterial contamination especially in high protein food
Enzymes	
Lactoperoxidase system with H_2O_2	Pathogenic bacteria and bacterial contamination (milk and milk products)
Lysosimes (tech recombinant RNA DNK)	Unwanted GR +ve bacteria
Low molecular weight metabolites	
Reuterin (3-OH-propionaldehide)	Wide spectrum bacteria, moulds and yeasts
Diacetyl	GR –ve bacteria
Fatty acids	Various bacteria
Bakteriocines	
Nisine	Some LA and GR+ve bacteria, specially those which are spore producing
Other bacteriocines	GR+ve bacteria, inhibitory spectrum

In order for the use of starter cultures to be justified it is needed that the used cultures fulfill the following conditions (*Holzapfel et alr.,* 1995, Slavica Vesković,2005, 2007, 2009): *they should not be harmful for the consumer* (i.e. do not produce toxins, biogenic amines or other metabolites which can harm human's health and are not part of pathogenic bacteria*); they must contribute towards desired effects in the product* (they have to be adapted for the product, must have a consistent protective effect, reliable metabolic activity and must suppress any undesirable microflora) and *must not have negative effects upon good manufacturing practice* (do not produce unwanted acids, gas, slime…).

At the same time from the ideal bacterial culture it is expected to be able to produce bacteriocines during the fermentation process. The formed bacteriocines must be stable in the meat matrix and inactivation by ingredients from the stuffing should not be allowed. The starter culture has to be preserved relative to other bacteriocine producing bacteria (*Slavica Vesković*, 2005, 2009).

The use of protective cultures bears some unknown elements, relative especially to the uncer-

have second rate raw materials. Bearing all of this in mind, the use of protective cultures has to be seen in the light of only an added security measure in the production processes in the meat industry.

Microflora of traditional fermented sausages

Following the modern trends a growing number of meat producers in Serbia are using active growing cultures. As in Serbia there is no commercial production of starter cultures, the meat industries are compelled to purchase from foreign producers. Such starter cultures are often adapted to the needs of other markets and do not result in products which have such quality to which our customers are used.

The use of LAB as starter cultures isolated from autochthonous fermented meat products would be the solution to this problem. Strains isolated from autochthonous fermented products would be the basis for a potential production of domestic starter cultures. This could be achieved independently or joined to existing established international companies.

The need of every modern state is to study the natural resources and have an overview on the possessed potentials. In Serbia, up to now there were no extensive studies on the diversity and characteristics of autochthonous microflora of traditionally fermented sausages. This resulted in insufficient data and knowledge needed for a direct application in the meat industry.

The justification of the project "Technological and protective features of autochthonous bacterial strains isolated from traditional fermented sausages and possibilities for their application in the meat industry", financed by the Serbian Ministry of Science and Technology, is based on the need to form a data bank of autochthonous LAB strains. Such a data bank would make the foundation for the production of starter cultures in Serbia.

In order to reach this goal in this research we have studied three different traditionally fermented sausages ("Uzicka", "Levacka" and "Sremska").

In the meat industry AD "Juhor" - Jagodina out of the raw material obtained by slaughtering the animals raised in the Levca region the "Levacka" and "Sremska" sausage were produced. The sausages were produced according to the standard (producer's specification) which is used in the regular production by AD "Juhor". Fermentation and smoking were based on traditional principles.

The "Sremska" sausage was made of pork meat, bacon and spices, while "Levacka" was made of equal quantities of pork and beef and firm fat tissue. The prepared stuffing was filled into pork's small intestine. The production process lasted for 21 days.

The traditional "Uzicka"sausage was manufactured in the household of Nikola Brkovic, on the Zlatibor slopes in the village of Kacer. "Uzicka" was made of beef and pork meat, minced beef, firm fat tissue, nitrites, salt and S/ (Alimenta) in a three month period (November 2008 – January 2009). The sausage filling was stuffed into beef's small intestines and the fermentation process lasted for 21 days. The characteristics, ingredients and procedures studied within the Project are shown in Table 3.

Besides the basic studies which encompassed procedures for the isolation and characterization of LAB at different stages of maturation extensive work on physical, chemical, microbiological and sensory characteristics of raw materials, spices salt and additives used in sausage production have been carried out. By doing so, in an indirect way

Table 3. Characteristics, ingredients and procedures for traditional fermented sausages
Tabela 3. Karakteristike, sastojci i procedura zrenja nih ispitivtradicionalnih fermentisanih kobasica

Type of fermented sausage	Dimensions and weight	Wrapping	Ingredients	Quantity (100 kg)	Duration of maturation
„Sremska" sausage	34-36 mm ø 24 cm length 300 g weight	*natural* (pork's small intestine)	Pork meat Pork shoulder Firm fat tissue Nitrite salt 1% Sugar – saccharose Ground hot and sweet pepper Black popper extract Garlic extract	45 kg 25 kg 30 kg 2.47 kg 330 g 140 g 70 g 35 g	Smoking - 3 days at 20°C, 66% RVV. Maturation - 21 days on 12 to 29°C, 58% - 80% RVV
„Levačka" sausage	34-36 mm ø 19 cm length 250 g weight	*natural* (pork's small intestine)	Pork meat Beef meat Firm fat tissue Nitrite salt 1% Sugar – saccharose Ground hot and sweet pepper Black popper extract Garlic extract	47 kg 20 kg 33 kg 2.5 kg 330 g 140 g 70 g 35 g	Smoking - 3 days at 20°C, 66% RVV. Maturation - 21 days on 12 to 29°C, 58% - 80% RVV
„Užička" sausage	40 mm ø 41 cm length 700 g weight	*natural* (beef small intestine)	Pork meat Beef meat Firm fat tissue Nitrite salt Sodium chloride Spice S77 Alimenta	70 kg 20 kg 10 kg 2.5 kg 300 g 850 g	Maturation - 21 days at 2 - 13°C, 64% - 88% RVV

it has been tried out to determine the presence and diversity of the characteristic achromous microflora in three different regions in Serbia (Zlatibor, Levac, and Pomoravlje). At the same time the physical and chemical sensory changes which occurred during the process of fermentation and maturation of traditional sausages. Not only, but all data relative to the manufacturing of pork meat, microclimatic changes during the production process (temperature, relative humidity and air currents) were collected and recorded.

The aim of the physical, chemical and microbiological studies was to register the changes which occurred during the fermentation and maturation process. The aim of the sensory studies was to establish when do start and with what intensity the desired sensory changes which are reflected in the final product.

In order to obtain reliable indicators and results during the Project all studies were repeated as triplicates in all three types of traditional sausages. Within each fermentation 50 LAB isolates were collected and 50 catalase positive cocci (staphylococci and micrococci). Resulting in a collection of 450 LAB isolates and 450 micrococci isolates which have been morphologically and biochemically studied and by API tests were closely identified.

The results of a number of papers related to studies of the microflora of traditional fermented sausages have shown that out of the total number of LAB the most predominant are the *Lb. sakei* (*Amor et al.,* 2005) and *Lb. curvatus*. *Lb sakei* making more than 55% of the total number of isolates (*Hugas & Monfort,* 1997). Both of these LABS have an interesting metabolic potential upon which the possibility of their application in the meat industry is based. A smaller part of the microflora of fermented sausages which mature spontaneously, are *Lb. plantarum*, *Lb. brevis*, *Lb. paracasei* i *Lb. buchneri*. Nowadays, some of them, specially *Lb. plantarum and, Lb. sakei* (commonly reffered to as the "good" technological bacterium due to their production of antimicrobic supstances – bacteriocines) and *Lb. curvatus* and *Lb. pentosus* are used as starter cultures in the producton of fermented sausages. Their use is the result of detailed studies, taxonomic determination, identification of morphologic, physiologic and functional biotechnologic properties.

For further projects it is planned that isolated LAB strains should undergo detailed morphological and biochemical studies, as well as molecular identification. Within all identified strains the most important technological and protective features will be studied. Thus, all isolates will be studied in order to enhance secondary metabolic compounds i. e. bactericines

LAB bactericenes are natural antimicrobial peptides or proteins with a very interesting potential application in the food industry, as bioprotectors (*Cleveland et al.,* 2001), with the aim to protect health (*Turcotte et al.,* 2004) with a simultaneous increase in shelf life (*Slavica Vesković, 2007, 2*007-1). Bacteriocines are polypeptides synthesized on ribosomes, have a potent bactericidal activity and are quickly digested by the proteases of the human alimentary tract (*Joerger et al.,* 2000).

In the published papers very often they are compared with antibiotics (*Hansen,* 1993; *Hurst*, 1981). However, being not equal to therapeutic antibiotics their use is seldom associated to allergic reactions in man (*Cleveland et al.,* 2001). What meant to the society the discovery by Alexandar Fleming (1929) of penicillin regard human health, from the aspect of food safety and natural protection is represented by the bacteriocines.

Bearing in mind the expressed bacteriocidic and bacteriostatic effects of bacteriocines on some pathogenic strains, in the last years their application within the meat industry has been reviewed. On the other side, direct consumers have a consistently negative approach on the question of the use of chemical additives in food production. As a result consumers are not sure on the use of treated foods, with the exception of fresh food. Such a trend on one side (so called green technology - *Ross et al.,* 2002) and the continuous development of modern protective technologies in the XX and XXI century have included the explatoation of biological protectors such as bacteriocines. However, for bacteriocines to be used in the food industry they must be approved as legal additives („GRAS" Generally Regarded As Safe). Up to now only nisine has this status.

Bacteriocines as bioprotectors can be used in food production in one of the following ways (*Schillinger et al.,* 1996):

- by adding them to the food LAB which produce bacteriocines within the product ("in situ" production);
- direct use and/or semi purified bacteriocines as additives
- use of previously fermented products containing bactericine producing bacterial strains.

Chosen strains of LAB with clear technological and/or protective features will be used for experimental production of fermented sausages in industrial conditions.

Instead of a conclusion

The predicted studies within the Project "Technological and protective features of autochthonous bacterial strains isolated from traditional fermented sausages and possibilities for their application in the meat industry" are aimed to improve the safety of food production, decrease of production costs, preservation and even improvement of sensory characteristics of traditionally fermented meat products, development of a national collection of LAB and a positive influence on the population's health.. The determination of critical factors on which depends the standard quality of traditionally fermented meat products is of great importance not only to Project participants, bat to the national public, as well.

Basic HACCP principles can not be implemented without adequate data on the physical and chemical, microbiological and sensory characteristics of all ingredients, standard operating procedures (SOP) for the technological process and modern methods for the control of individual production phases as well as for the control of the final products.

By isolation and adequate selection of domestic, epyphitic microflora and by forming a collection of LAB the fundations for the determination and their possible use in the production of autochtonous starter and/or protective cultures. In such a way can be mantained the preservation of the sensory characteristics of traditionally fermented sausages

It is known that due to their sensory characteristics traditional fermented sausages are highly rated not only on the domestic market, but there is also a great interest for them on foreign markets. In order to export products on foreign markets it is important to ensure a consistently good quality product with characteristic sensory features, all at a good price.

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