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Evaluation of critical level in Bacillus Cereus growth curve in milk products with different conditions based on experimental data and ComBase Predictive Models

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Evaluation of critical level in Bacillus Cereus growth curve in milk products with different conditions based on experimental data and ComBase Predictive Models

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Abstract

Predictive microbiology in food products empowered research application to achieve the high accurate evaluation of microbial risk in food products. According to previous research, *Bacillus cereus* selected as a serious problem in milk industry; because of the heat resistance characteristics which *Bacillus cereus* can causes two diverse syndromes of food poisoning (diarrhoea and vomiting), suspected foods contained between 10^6 and 0^9 cfu/g. In this study, two methods were used to evaluate, the first one is ComBase - *a Combined dataBase for predictive microbiology* - a common database on microbial responses to food environments and second one is *NeuroXL Predictor* (*neural network forecasting tool).* The study went on the interaction of temperature (6, 7, 8 and 9 °C), pH (6.6, 6.7 and 6.8), according to raw milk pH on the probability of pathogen growth. Thus, Over 74 recorded sample in ComBase remarkable date were collected for comparison. The prediction model showed a good performance for validation data were correctly classified. The predictions indicated an abrupt growth/no growth interfaces occurred at low levels of temperature and pH which could in high risk of production of toxin in milk products (10^5 cfu/g).

Introduction

Over the past few decades, major advances have occurred through the milk production system to enhance products quality from farm to table. In addition, the dairy industry has confronted with abundant of deficiency in supply chain, which could control by quality control system. Spoilage of pasteurized milk has been recognized from heat resistant gram-positive bacteria and spores, including those of the genus Bacillus. The incidence of these microorganisms in pasteurized milk can be explained by the presence of their heat-resistance spores in the raw milk or by milk recontamination, due to inadequately cleaned and sanitized vessels, pipe lines, and equipment due to clean-in-place CIP and packaging machines (Zacarchenco et al., 2000; Hayes and Boor, 2001; Christiansoon, 2001; Merin *et al*., 2002). In dairy farm, raw milk is most commonly contaminated under conditions of insufficient hygiene control system, Bacillus spp. are quite common in the agricultural environment (soils, dust, cattle feed and dung, and in guts of invertebrates) and may contaminate milk from various sources during the production, storage and processing (Janštová and Lukášová, 2001). This study scrutinizes the growth curve of bacillus cereus at a concentration level which capable to produce toxin in milk products.

Background and importance of Bacillus cereus in dairy products

Aerobic and facultative anaerobic spore-forming bacteria of the genus Bacillus bring a serious problem in dairy products; because of the heat-resistance features of spores and ability of vegetative cells to produce extra-cellular enzymes; they may cause milk product deterioration. B. cereus' ability to form endospores provides the ultimate organism surviving and spreading its benefits in a wide range within another microbial function (Jensen *et al*., 2003; Schoeni, 2005). Ability of spore-forming of Bacillus spp endure harsh condition during the heat-treatments processing, such as milk or juice pasteurization and spores are ubiquitous in raw milk, endure the pasteurisation treatment, and produce different enterotoxins(Christiansson *et al*., 1999). In addition, B. cereus is a widespread contaminant of raw milks, (Banykó and Vyletelová, 2009; Shaheen *et al*., 2010). At least one member of this genus, B. cereus, is a well–known in food poisoning list that may cause illness through the production of either an emetic (vomit-inducing) toxin or diarrhoeal toxins (enterotoxins) (Granum and Lund, 1997). The traditional approach to validate food safety is via challenge to control the any miserable test come into the food. That approach has long been regarded as cost effects, slow, authoritative facilities and microbiological skill. An alternative is to understand more fully the responses of the microbes of concern to the key controlling factors in the food environment, to build a database of microbial growth behaviour, and to develop the means to interpolate calculated microbial responses (Lund *et al*., 2000). However, B. cereus has been isolated from a variety of food products, including rice, pastry, vegetables and vegetable products, raw milk and milk products and ready-to-eat foods (Wijnands *et al*., 2006; Choma *et al*., 2000). The gradual evolution of food supply chain in dairy products, system has been associated with an extension of the shelf life as a perishable food. Generally, almost all viable bacteria present in milk are killed by a common heat-treatment (pasteurisation). However, approximately 0.1% of the natural microflora in pasteurised milk may survive in pasteurisation heat-treatment and support spoilage in the products, in addition, many of the strains can grow at 4–6°C (Temperature of milk storage) (Ranieri *et al*., 2009; Muir, 1996).

limitation Growth of Bacillus in the food chain

Bacillus cereus group strains shows expanded diversity, storing foods below 10°C prevents growth of strains that produce emetic toxin (responsible for the vomiting poisoning). Hence, reducing the storage temperature reduces the diversity of B. cereus population able to multiply below 10 centigrade. Published reports of B. Ceres foodborne poisoning shows that 10^5 - 10^6 cells or spores/g of food clearly can cause foodborne poisoning (Granum, 2005).

Emetic toxin producing

Bacillus cereus is powered by producing several toxins, evolving an emetic toxin, a necrotizing enterotoxin, phospholipases and haemolysins (Manzano *et al*., 2010; Lebessi *et al*., 2009). According to previous publication, B. cereus strains may be capable of producing diarrhoeal toxins under low temperatures condition (Soares *et al*., 2012; Finlay *et al*., 2000). Diarrhoeal infection prevail of enterotoxins production in the small intestine in the food Production of enterotoxins in foods by B. cereus (Granum and Lund, 1997; Ryser, 2012). Emetic intoxication occurs through ingestion of emetic toxin accompulished in the food. Thus determining conditions in the foods that would lead to production of cereulide by emetic B. cereus is important for appraisal risk of emetic intoxication. Cereulide is not easily destroyed by heat treatments. For example, it could resist 90 min at 126°C (Ryser, 2012; Ehling-Schulz *et al*., 2004; Rajkovic, 2006; Beuchat *et al*., 2011). It is also resistant to acid conditions. Cereulide became detectable at the end of the growth of B. cereus and its hardly can be eliminated from food Production of cereulide below 8°C is minimum (Häggblom *et al*., 2002). A wide range of foods have been implicated in poisoning from other Bacillus spp (Stenfors Arnesen *et al*., 2008), including various recipe dishes, pastries, dairy products, infant food formulae, sandwich, canned tomato juice. In all cases, suspected foods contained between 10^6 and 10^9 cfu/g. Significant numbers (>10^5 cfu/g) of B. cereus of an established food poisoning serovar should be isolated from the incriminated food, or vomitus of the affected persons (Granum, 2005; Clavel *et al*., 2004).

ComBase and neural network forecasting tool

ComBase (web-based resource for quantitative and predictive food microbiology) is used as a large database of microbial for response of pathogens and spoilage microorganisms to environmental factor (temperature, pH and salt concentration, etc). Although ComBase contains a vast amount of experimental records, one of the most significant application in ComBase is models predict the growth/survival of foodborne pathogens, predictive models for ComBase Predictor are based only on output from experiments recorded in laboratory under well controlled laboratory situation. The discrepancy of cell concentration quantity described by a mathematical curve (growth or survival) (Baranyi and Tamplin, 2004). Neural networks have been well established through the technology for solving prediction and classification problems, using training and testing data to build a model. The data involve historical data sets containing input with significant contributing factors (Haykin *et al*., 2009). The network uses the training data to learn how to find solution to the problem by previous data, so, the output strongly dependent on network information factors.

Methods

Data collection

The data used in this work were obtained from ComBase, a database (full recorded of excel version CB4.Xls kindly provided by the ComBase consortium (Baranyi and Tamplin, 2004). (UK, Institute of food research, USDA, ARS, Eastern Regional Research Centre, and Australian Food Safety Centre) consisting of 74 records of Bacillus cereus growth in milk. Bacillus cereus population changes in over time for each environmental condition which recorded in the database, graph illustrated based on temperature 9 °C (Figure 1), temperature 8°C (Figure 2), temperature 7°C (Figure 3).

Prediction and validation

The study went on the interaction of temperature (6, 7, 8 and 9 °C), pH (6.6, 6.7 and 6.8). Analysing over 74 conditions recorded in ComBase, remarkable date were chosen for comparison (ComBase,see www.combase.cc). The prediction model showed a good performance for validation data were correctly classified. The predictions indicated an abrupt growth/no growth interfaces occurred at low levels of temperature and pH which could in high risk of production of toxin in milk products (10^5 cfu/g). Then the Artificial Neural Newtwork (NeuroXL, www.analyzerXL.com) were used for the calculated on Significant point (10^5 cfu/g) input data in an attempt to design by recorded data in ComBase, (Figure 1) (Jeyamkondan *et al.*, 2001). The prediction graph according to ComBase illustrated based on temperature 9 °C (Figure 4), temperature 8°C (Figure 5), temperature 7°C (Figure 6).

Results and Discussion

One of the useful positive quality of prediction by computerized data is that the recorded data can be swiftly positioned, which give the enormous advantage even when the records of experimental test do not offer the entire original reports or the full description of an experiment. The objective of basing these studies on bacillus cereus growth according to experimental data and prediction modelling, to achieve the point of shelf life of milk products. According to reported data, Bacillus cereus was studied in the growth curve . in particular scrutinised based on pH (9.0; 8.0; 7.0; and 6.0) on the probability of growth. Out of the 76 conditions tested, 13 were chosen for model data for validation data. The predictions indicated an abrupt growth interfaces occurred between 4-10 centigrade. The Bacillus cereus population in BC_09_M2 and BC_09_M2 increased to 4.23 cfu/g after 100 hours then increased to 6 cfu/g after 146 hours approximately; however, in other sample BC_M1_09 and BC_M2_09 at the same temperature illustrated that Bacillus cereus population increased rapidly although these samples start at 0 stage in initial level and remained at this level until 28 hours, then after 24 hours the population achieved until 2.4, the most important point is that population increased sharply from 2.4 to 6 cfu/g ; 52 and 76 hours, respectively. So the growth data implies that based on literature report, the real infectious dose may fluctuate from concerning viable cell or spores/g 10^5 and 10^8 (Notermans and Tatini, 1993). However, figure 2 shows miscellaneous fluctuation points in bacillus cereus growth at 8 centigrade, which three of them shows similar growth (RecordID BC_08_M1, RecordID BC_08_M2 and RecordID BC_08_M3), the convenient point in a longer shelf life of milk products, based on shelf-life duration prolonged until 216 hours (3.46, 3.58 and 4.40, respectively). In contrast, ID_LA61k_2_20 Record and ID_LA64k_2_20 Record are achieved to 4.30 and 4.79 which their initial level was under 1. In comparing NeuroXL with ComBase, in temperature 7,8 and 9 NeuoXL predicted Bacillus cereus population at 10^5 cfu/g, 189.23, 281.44 and 300.81 hours respectively (figure 8). However, according to ComBase prediction model, in temperature 7 °C near the 10^5 cfu/g point (168.96 hours), its shows that similarity to NeuroXL prediction 189.23, according to temperature 7°C in experimental data maximum and minimum data (253.44, 168.95 respectively) shows that although the maximum achievement started with 2 initial level but in other experimental illustration shows when milk products stored at high-level contaminated, reached to the high level of Bacillus Cereus population faster (>10^5 cfu/g, critical level for consumer as a spoiled products). Figure 7 shows a plot comparing the recorded specific point in growth curve of Bacillus cereus near or above >10^5 cfu/g (experimental data from ComBase records) which associated with pH and temperature and initial level. These application could enhance the quality control system how to choose the raw material in dairy factory or declared ineligible raw material, for example, raw milk with high concentration of bacteria specially heat-resistance, pasteurization is not intended to pasteurized completely or deficiency in this process maybe could reduce the shelf life and could affect sensitive consumer (Children and Elderly), in contrast, its could be used as a sterilized milk under high-temperature treatment. In addition, with these modelling, quality control of dairy products adjusted their procedure and producing high quality products with less possibility of foodborne.

Abbreviation(s)

ComBase: *A Combined dataBase for predictive microbiology* (web-based resource for quantitative and predictive food microbiology)

Acknowledgement(s)

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Illustrations

Illustration 1

Figure 1: Growth curve of Bacillus cerus in milk at 9 centigrade recorded in ComBase, practical data.

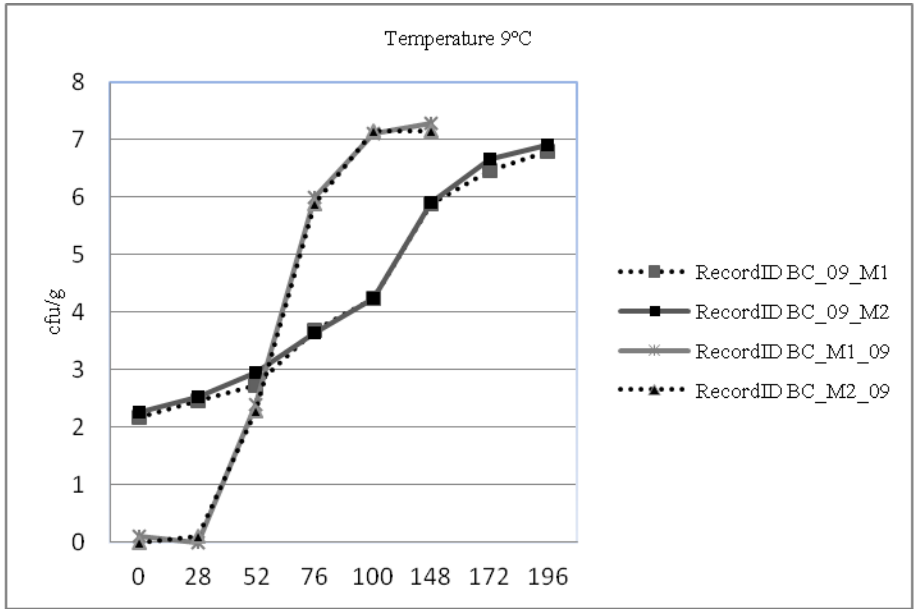

Illustration 2

Figure 2: Growth curve of Bacillus cerus in milk at 8 centigrade recorded in ComBase, practical data.

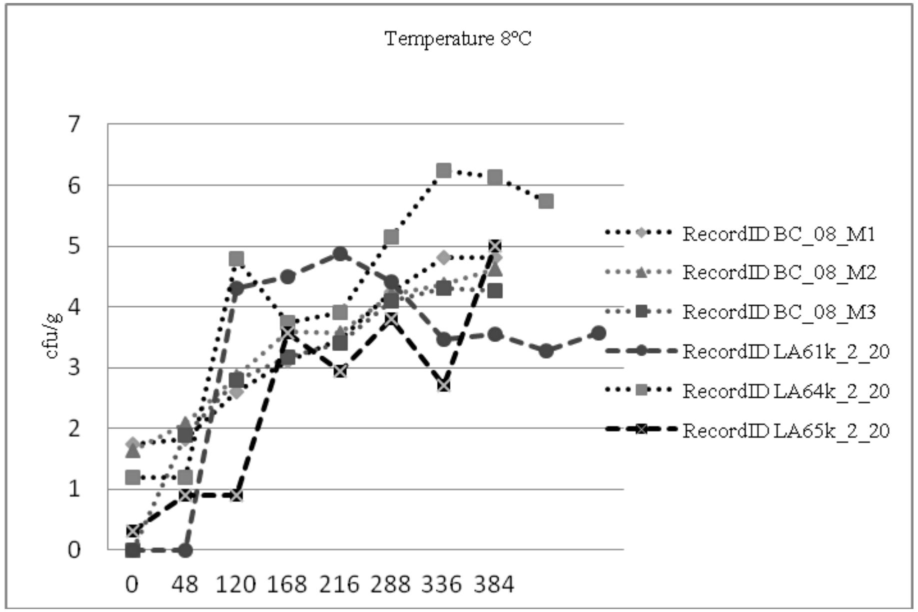

Illustration 3

Figure 3: Growth curve of Bacillus cerus in milk at 7 centigrade recorded in ComBase, practical data

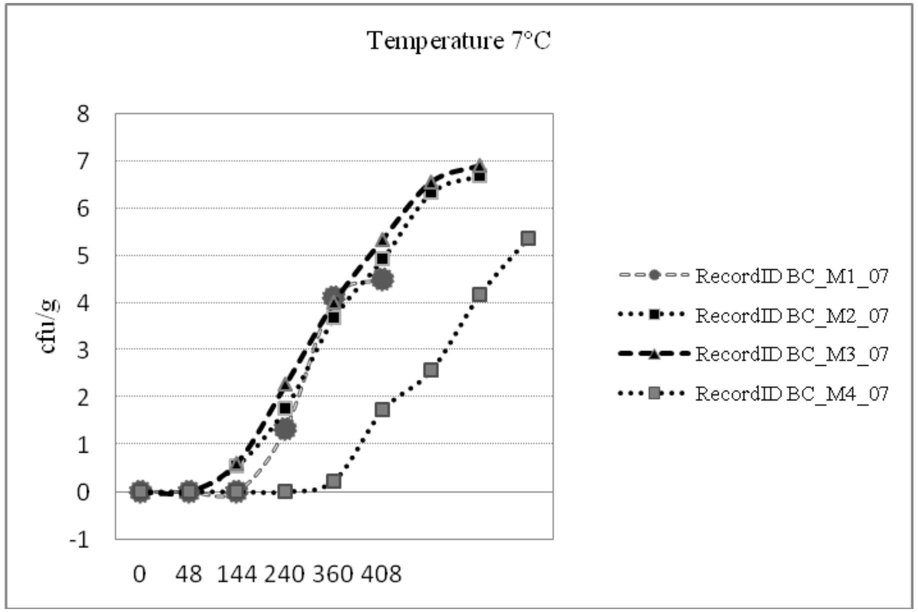

Illustration 4

Figure 4: Growth curve of Bacillus cerus in milk at 9 centigrade recorded in ComBase, prediction.

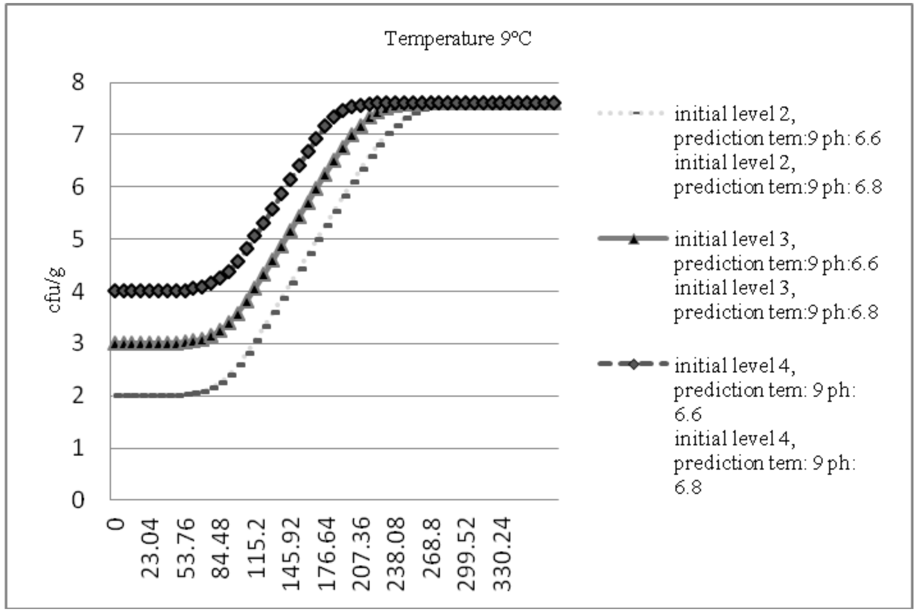

Illustration 5

Figure 5: Growth curve of Bacillus cerus in milk at 8 centigrade recorded in ComBase, prediction.

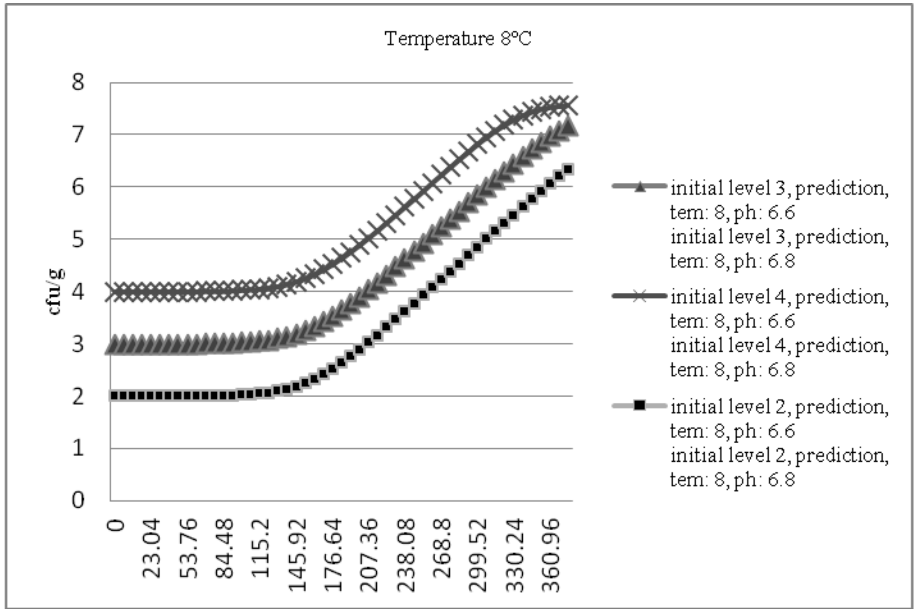

Illustration 6

Figure 6: Growth curve of Bacillus cerus in milk at 7 centigrade recorded in ComBase, prediction

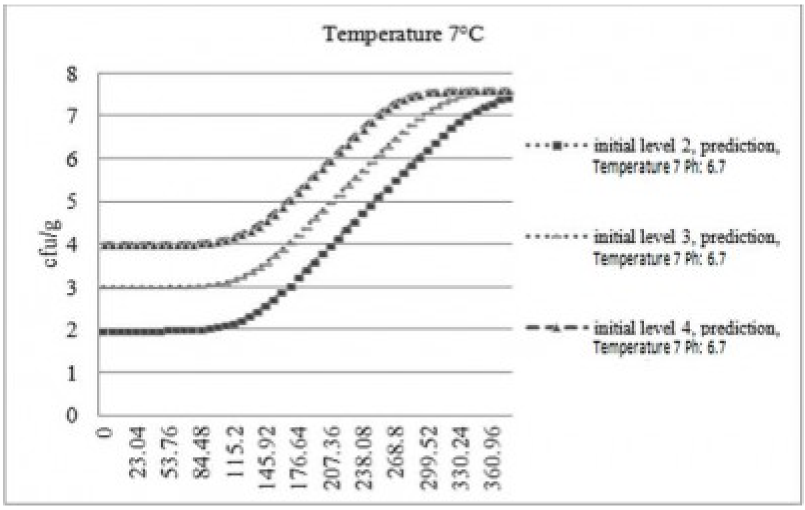

Illustration 7

Figure 7: All points near or above 10^5 cfu/g (experimental data from ComBase)

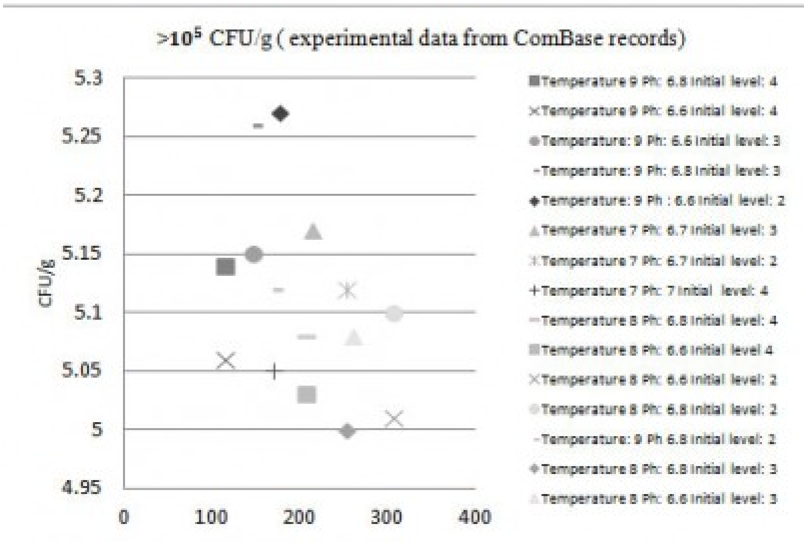

Illustration 8

Figure 8: prediction total; PredictorXL-(experimental data from ComBase records)